ASSAYS FOR DETECTING ANTIBODIES SPECIFIC TO THERAPEUTIC ANTI-IGE ANTIBODIES AND THEIR USE IN ANAPHYLAXIS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. provisional applications Serial No. 61/255,052, filed October 26, 2009, which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] The present invention relates generally to the fields of methods and reagents for detecting anti-drug antibodies of IgE isotype to therapeutic anti-IgE antibodies, and methods for assessing risk of anaphylaxis.

BACKGROUND

[0003] IgE is a member of the immunoglobulin family that mediates allergic responses such as asthma, food allergies, type I hypersensitivity and the familiar sinus inflammation suffered on a widespread basis. IgE is secreted by, and expressed on the surface of B-cells or B-lymphocytes. IgE binds to B-cells (as well as to monocytes, eosinophils and platelets) through its Fc region to a low affinity IgE receptor, known as FcεRII. Upon exposure of a mammal to an allergen, B-cells bearing a surface-bound IgE antibody specific for the antigen are "activated" and developed into IgE-secreting plasma cells. The resulting allergen-specific IgE then circulates through the bloodstream and becomes bound to the surface of mast cells in tissues and basophils in the blood, through the high affinity receptor also known as FcεRI. The mast cells and basophils thereby become sensitized for the allergen. Subsequent exposure to the allergen causes a cross linking of the basophilic and mast cellular FcεRI which results in degranulation of these cells and a release of histamine, leukotrienes and platelet activating factors, eosinophil and neutrophil chemotactic factors and the cytokines IL-3, IL-4, IL-5 and GM-CSF which are responsible for clinical hypersensitivity and anaphylaxis.

[0004] Antagonists that block IgE-Receptor complex formation are useful as therapeutic agents to prevent allergic response. Several therapeutic anti-IgE antibodies have been developed. These anti-IgE antibodies block IgE from binding to the high-affinity receptor

FcεRI found on basophils and mast cells, and thereby prevent the release of histamine and other anaphylactic factors resulting in the pathological condition.

[0005] Anaphylaxis has been reported to occur in patients after receiving anti-IgE antibodies, such as omalizumab (e.g., Xolair®). Anaphylaxis is an acute systemic (multi-system) and very severe type I hypersensitivity allergic reaction. It is caused by degranulation of mast cells and basophils and mediated by IgE. Through 2006, 124 of 57,269 (about 0.2%) asthma patients had anaphylaxis after omalizumab administration. While there are no reports of fatal anaphylaxis as a result of omalizumab, some cases have been serious, and potentially life-threatening. For this reason, the FDA recommends that patients receiving omalizumab be monitored in the physician's office for a period of time after omalizumab administration, and health care providers administering omalizumab should be prepared to manage anaphylaxis that can be life-threatening. Sixty percent of the cases reported (124) has been after the first two doses of omalizumab. Therefore, it is possible that the reaction is from pre-existing antibodies in patients that recognize an epitope on omalizumab, as opposed to an anti-drug reaction that develops after drug administration. As anaphylaxis is associated with antibody of the IgE isotype, there is a need to develop an assay for detecting and quantitating the amount of IgE in a patient that is specific to the therapeutic anti-IgE antibody to assess the risk of anaphylaxis preferably before such anti-IgE antibody treatment and identify high risk patients.

[0006] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

[0007] In one aspect, the invention provides methods for detecting an anti-drug antibody of IgE isotype that bind to a therapeutic anti-IgE antibody in a sample, comprising the steps of: a) contacting a sample that may contain the anti-drug antibody with a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the relative binding affinity of the mutant therapeutic antibody to an IgE (such as human IgE) is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to the IgE; and b) detecting binding of the anti-drug antibody to the mutant therapeutic antibody.

[0008] In some embodiments, the relative binding affinity of the mutant therapeutic antibody is about 7.5% or less, about 5% or less, about 2.5% or less, about 2.0% or less,

about 1.5% or less, about 1% or less, about 0.9% or less, about 0.8% or less, about 0.7% or less, about 0.5% or less, about 0.25% or less, about 0.1% or less of the relative binding affinity of the therapeutic anti-IgE antibody.

[0009] In another aspect, the invention provides methods for detecting anti-drug antibodies of IgE isotype that bind to a therapeutic anti-IgE antibody in a sample, comprising the steps of: a) contacting a sample that may contain the anti-drug antibodies with a mutant therapeutic antibody having at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the potency of the mutant therapeutic antibody to an IgE (such as human IgE) is about 10% or less of the potency of the therapeutic anti-IgE antibody to the IgE; and b) detecting binding of the anti-drug antibodies to the mutant therapeutic antibody.

[0010] In some embodiments, the potency of the mutant therapeutic antibody is about 7.5% or less, about 5% or less, about 2.5% or less, about 2.0% or less, about 1.5% or less, about 1% or less, about 0.9% or less, about 0.8% or less, about 0.7% or less, about 0.5% or less, about 0.25% or less, about 0.1% or less of the potency of the therapeutic anti-IgE antibody.

[0011] Any of the mutant therapeutic antibodies provided herein may be used. In some embodiments, the mutant therapeutic antibody comprises one, two, three, four, five, or six amino acid mutations in CDR sequences of the heavy and/or light chain of the therapeutic anti-IgE antibody. In some embodiments, the therapeutic anti-IgE antibody is omalizumab, and the mutant therapeutic antibody comprises one, two, or three amino acid mutations in the first CDR of the light chain of omalizumab. In some embodiments, the therapeutic anti-IgE antibody is omalizumab, and the mutant therapeutic antibody comprises an amino acid substitution at position 34 (Asp) in the light chain (SEQ ID NO:1) of omalizumab. In some embodiments, the mutant therapeutic antibody comprises the heavy chain amino acid sequence of SEQ ID NO:2 and the light chain amino acid sequence of SEQ ID NO:1, wherein amino acids at positions 30 (Asp) and 34 (Asp) or positions 32 (Asp) and 34 (Asp) in the light chain are substituted. In some embodiments, the mutant therapeutic antibody comprises the heavy chain amino acid sequence of SEQ ID NO:2 and the light chain amino acid sequence of SEQ ID NO:1, wherein amino acid D (Asp) at positions 30, 32, and 34 are substituted in the light chain. In some embodiments, amino acid Asp is substituted with Ala. In some embodiments, the mutant therapeutic antibody comprises the heavy chain amino acid sequence of SEQ ID NO:2 and the light chain amino acid sequence of SEQ ID NO:1 with amino acid substitutions of Asp to Ala at positions 30, 32, and 34 in the light chain. In some embodiments, the therapeutic anti-IgE antibody is omalizumab, and the mutant therapeutic antibody comprises one, two, or three amino acid mutations in the third CDR of the heavy

chain of omalizumab. In some embodiments, the mutant therapeutic antibody comprises the heavy chain amino acid sequence of SEQ ID NO:2 and the light chain amino acid sequence of SEQ ID NO:1, wherein amino acids at positions 101 (His), 105 (His) and 107 (His) in the heavy chain (SEQ ID NO:2) are substituted. In some embodiments, amino acid His is substituted with Ala. In some embodiments, the mutant therapeutic antibody comprises the heavy chain amino acid sequence of SEQ ID NO:2 with amino acid substitutions of His to Ala at positions 101, 105, and 107 in the heavy chain and the light chain amino acid sequence of SEQ ID NO:1.

[0012] In some embodiments, the mutant therapeutic antibody is immobilized or captured to a surface. In some embodiments, the mutant therapeutic antibody is directly immobilized to a surface. In some embodiments, the mutant therapeutic antibody is conjugated to a label and is immobilized or captured to the surface through a capture agent that specifically binds to the label, wherein the capture agent is immobilized to the surface. In some embodiments, the label is biotin and the capture agent is streptavidin. In some embodiments, the label is digoxigenin and the capture agent is an anti-digoxigenin antibody.

[0013] In some embodiments, the sample is contacted with the mutant therapeutic antibody that is immobilized or captured to a surface. In some embodiments, the sample is contacted with the mutant therapeutic antibody before the mutant therapeutic antibody is captured to a surface. In some embodiments, the mutant therapeutic antibody is captured to a surface after the sample is contacted with the mutant therapeutic antibody and before detecting binding of the anti-drug antibody to the mutant therapeutic antibody.

[0014] In some embodiments, the binding of the anti-drug antibodies to the mutant therapeutic antibody is detected with a detecting agent. In some embodiments, the detecting agent is an FcεRIα polypeptide that binds to an Fc region of an IgE. Any of the FcεRIα polypeptides provided herein may be used. In some embodiments, the FcεRIα polypeptide comprises an extracellular domain of an FcεRIα subunit. In some embodiments, the FcεRIα polypeptide comprises an extracellular domain of an FcεRIα subunit fused to an IgG constant region. In some embodiments, the FcεRIα polypeptide is labeled. In some embodiments, the label is selected from the group consisting of biotin, digoxigenin, ruthenium, a radiologic label, a photoluminescent label, a chemiluminescent label, a fluorescent label, an electrochemiluminescent label, and an enzyme label. In some embodiments, the FcεRIα polypeptide is labeled with biotin, and the binding of the anti-drug antibody to the mutant therapeutic antibody is detected by streptavidin-HRP. In some embodiments, the FcεRIα polypeptide is labeled with digoxigenin, and the binding of the anti-drug antibody to the

mutant therapeutic antibody is detected by a HRP conjugated anti-digoxigenin antibody. In some embodiments, the FcεRIα polypeptide is labeled with ruthenium, and the binding of the anti-drug antibody to the mutant therapeutic antibody is detected by an electrochemiluminescence assay.

[0015] In some embodiments, the sample contains human serum or plasma. In some embodiments, the sample contains the therapeutic anti-IgE antibody. In some embodiments, the sample does not contain the therapeutic anti-IgE antibody. In some embodiments, the serum or plasma contains omalizumab. In other embodiments, the serum or plasma does not contain omalizumab.

[0016] In some embodiments, the methods further comprise a step of comparing the binding of the anti-drug antibodies to the mutant therapeutic antibody to a reference. In some embodiments, the reference is the detected binding between the mutant therapeutic antibody and a control antibody. In some embodiments, the control antibody is a positive control antibody that binds both the therapeutic anti-IgE antibody and the mutant therapeutic antibody with similar affinity. In some embodiments, the positive control antibody comprises a heavy chain variable region comprising the amino acid sequence shown in SEQ ID NO:7 and a light chain variable region comprising the amino acid sequence shown in SEQ ID NO:8. In some embodiments, the positive control antibody further comprises the heavy chain and light chain constant regions from a human IgE.

[0017] In another aspect, the invention also provides kits for detecting an anti-drug antibody of IgE isotype that binds to a therapeutic anti-IgE antibody in a sample comprising (a) a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the relative binding affinity of the mutant therapeutic antibody to an IgE (such as human IgE) is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to the IgE; and b) a detecting agent that binds to an Fc region of an IgE. Any of the mutant therapeutic antibodies provided herein may be used. In some embodiments, the detecting agent is an FcεRIα polypeptide. Any of the FcεRIα polypeptides provided herein may be included in the kit. In some embodiments, the FcεRIα polypeptide comprises an extracellular domain of an FcεRIα subunit. In some embodiments, the FcεRIα polypeptide comprises an extracellular domain of an FcεRIα subunit fused to an IgG constant region. In some embodiments, the FcεRIα polypeptide is labeled (such as labeled by biotin, digoxigenin, ruthenium, etc.). In some embodiments, the kit further comprises streptavidin-HRP or Amdex SA-HRP. In some embodiments, the kit further comprises HRP-conjugated anti-digoxigenin antibody for detecting digoxigenin labeled

FcεRIα polypeptide. In some embodiments, the kit further comprises a positive control antibody that binds both the therapeutic anti-IgE antibody and the mutant therapeutic antibody with similar affinity. In some embodiments, the positive control antibody comprises a heavy chain variable region comprising the amino acid sequence shown in SEQ ID NO:7 and a light chain variable region comprising the amino acid sequence shown in SEQ ID NO:8. In some embodiments, the positive control antibody further comprises the heavy chain and light chain constant regions from a human IgE.

[0018] In another aspect, the invention also provides kits for detecting anti-drug antibodies of IgE isotype that bind to a therapeutic anti-IgE antibody in a sample comprising a) a mutant therapeutic antibody having at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the potency of the mutant therapeutic antibody to an IgE (such as human IgE) is about 10% or less of the potency of the therapeutic anti-IgE antibody to the IgE; and b) a detecting agent that binds to an Fc region of an IgE. Any of the mutant therapeutic antibodies provided herein may be used. In some embodiments, the detecting agent is an FcεRIα polypeptide. Any of the FcεRIα polypeptides provided herein may be included in the kit. In some embodiments, the FcεRIα polypeptide comprises an extracellular domain of an FcεRIα subunit. In some embodiments, the FcεRIα polypeptide comprises an extracellular domain of an FcεRIα subunit fused to an IgG constant region. In some embodiments, the FcεRIα polypeptide is labeled (such as labeled by biotin, digoxigenin, ruthenium, etc.). In some embodiments, the kit further comprises streptavidin-HRP or Amdex SA-HRP. In some embodiments, the kit further comprises HRP-conjugated anti-digoxigenin antibody for detecting digoxigenin labeled FcεRIα polypeptide. In some embodiments, the kit further comprises a positive control antibody that binds both the therapeutic anti-IgE antibody and the mutant therapeutic antibody with similar affinity. In some embodiments, the positive control antibody comprises a heavy chain variable region comprising the amino acid sequence shown in SEQ ID NO:7 and a light chain variable region comprising the amino acid sequence shown in SEQ ID NO:8. In some embodiments, the positive control antibody further comprises the heavy chain and light chain constant regions from a human IgE.

[0019] In another aspect, the invention also provides methods for detecting an anti-drug antibody of IgE isotype that binds to a therapeutic anti-IgE antibody in a sample, comprising the steps of: (a) contacting a sample that may contain the anti-drug antibody with (i) a mutant therapeutic antibody and (ii) an FcεRIα polypeptide that binds to an Fc region of a human IgE, wherein the mutant therapeutic antibody comprises at least one amino acid mutation from the therapeutic anti-IgE antibody, and the relative binding affinity of the mutant

therapeutic antibody to human IgE is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE; (b) capturing the mutant therapeutic antibody to a surface; and (c) detecting binding of the anti-drug antibody to the mutant therapeutic antibody.

[0020] In some embodiments, excess amount of FcεRIα polypeptide is contacted with the sample in step (a). In some embodiments, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, or at least about 10-fold excess of FcεRIα polypeptide is contacted with the sample in step (a). Any of the FcεRIα polypeptides provided herein may be used. In some embodiments, the FcεRIα polypeptide comprises an extracellular domain of an FcεRIα subunit. The FcεRIα polypeptide may be labeled or not labeled.

[0021] Any of the mutant therapeutic antibodies provided herein may be used. In some embodiments, the mutant therapeutic antibody is labeled and is captured to the surface by a capture agent that specifically binds to the label. In some embodiments, the label is biotin and the surface is coated with streptavidin. In some embodiments, the binding of the anti-drug antibody to the mutant therapeutic antibody is detected by a labeled anti-human IgE antibody. In some embodiments, the FcεRIα polypeptide is labeled and the binding of the anti-drug antibody to the mutant therapeutic antibody is detected by a detecting agent that specifically binds to the label on the FcεRIα polypeptide. In some embodiments, the FcεRIα polypeptide is labeled with digoxigenin, and the binding of the anti-drug antibody to the mutant therapeutic antibody is detected by a HRP conjugated anti-digoxigenin antibody. In some embodiments, the FcεRIα polypeptide is labeled with ruthenium, and the binding of the anti-drug antibody to the mutant therapeutic antibody is detected by an electrochemiluminescence assay.

[0022] In another aspect, the invention also provides kits for detecting an anti-drug antibody of IgE isotype that binds to a therapeutic anti-IgE antibody in a sample comprising: (a) a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the relative binding affinity of the mutant therapeutic antibody to human IgE is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to human IgE; and (b) an FcεRIα polypeptide that binds to an Fc region of a human IgE. Any of the mutant therapeutic antibodies provided herein may be used. Any of the FcεRIα polypeptide described herein may be used. In some embodiments, excess amount of FcεRIα polypeptide is provided in the kit. In some embodiments, the FcεRIα polypeptide is labeled. In some embodiments, the kit further comprises a detecting agent that specifically

binds to the label on the FcεRIα polypeptide. In some embodiments, the kit further comprises an anti-human IgE antibody. In some embodiments, the anti-human IgE antibody is labeled.

[0023] In another aspect, the invention also provides methods for detecting an anti-drug antibody of IgE isotype that binds to a therapeutic anti-IgE antibody in a sample, comprising the steps of: (a) preincubating a sample that may contain the anti-drug antibody with excess amount of an FcεRIα polypeptide that binds to an Fc region of a human IgE; (b) incubating the preincubated sample from step (a) with the therapeutic anti-IgE antibody or a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, and the relative binding affinity of the mutant therapeutic antibody to a human IgE is reduced as compared to the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE; and (c) detecting binding of the anti-drug antibody to the therapeutic anti-IgE antibody or the mutant therapeutic antibody.

[0024] Any of the mutant therapeutic antibodies provided herein may be used. In some embodiments, the mutant therapeutic antibody comprises at least one amino acid mutation from the therapeutic anti-IgE antibody, and the relative binding affinity of the mutant therapeutic antibody to human IgE is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE.

[0025] In some embodiments, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, or at least about 10-fold excess of FcεRIα polypeptide is preincubated with the sample in step (a). Any of the FcεRIα polypeptides provided herein may be used.

[0026] In some embodiments, the therapeutic anti-IgE antibody or the mutant therapeutic antibody is captured to a surface before or after incubating with the sample in step (b). In some embodiments, the therapeutic anti-IgE antibody or the mutant therapeutic antibody is directly immobilized to a surface before incubating with the sample in step (b).

[0027] In some embodiments, the therapeutic anti-IgE antibody or the mutant therapeutic antibody is labeled and is captured to the surface through an immobilized capture agent that specifically binds to the label. In some embodiments, the therapeutic anti-IgE antibody or the mutant therapeutic antibody is labeled with biotin and is captured to a streptavidin coated surface.

[0028] In some embodiments, the binding of the anti-drug antibody to the therapeutic antibody or the mutant therapeutic antibody is detected by a HRP conjugated anti-human IgE antibody. In some embodiments, the FcεRIα polypeptide is labeled, and the binding of the

anti-drug antibody to the therapeutic anti-IgE antibody or the mutant therapeutic antibody is detected by detecting the label. In some embodiments, the FcεRIα polypeptide is labeled with digoxigenin, and the binding of the anti-drug antibody to the therapeutic antibody or the mutant therapeutic antibody is detected by a HRP conjugated anti-digoxigenin antibody. In some embodiments, the FcεRIα polypeptide is labeled with ruthenium, and the binding of the anti-drug antibody to the therapeutic anti-IgE antibody or the mutant therapeutic antibody is detected by an electrochemiluminescence assay.

[0029] In another aspect, the invention also provides kits for detecting an anti-drug antibody of IgE isotype that binds to a therapeutic anti-IgE antibody in a sample comprising: (a) the therapeutic anti-IgE antibody or a mutant therapeutic antibody thereof, wherein the mutant therapeutic antibody comprises at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the relative binding affinity of the mutant therapeutic antibody to human IgE is reduced as compared to the relative binding affinity of the therapeutic anti-IgE antibody to human IgE; and (b) an FcεRIα polypeptide that binds to an Fc region of a human IgE. Any of the mutant therapeutic antibodies provided herein may be used. In some embodiments, the kit further comprises an anti-human IgE antibody. In some embodiments, the anti-human IgE antibody is labeled. Any of the FcεRIα polypeptide provided herein may be used. In some embodiments, the FcεRIα polypeptide is labeled. In some embodiments, the kit further comprises a detecting agent that specifically binds to the label on the FcεRIα polypeptide.

[0030] In another aspect, the invention provides methods of identifying a patient having a risk of anaphylactic reaction to a therapeutic anti-IgE antibody, comprising the steps of: (a) contacting a sample from the patient with a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the relative binding affinity of the mutant therapeutic antibody to human IgE is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE; and (b) detecting binding of an anti-drug antibody of IgE isotype to the mutant therapeutic antibody, wherein the presence and/or the level of the anti-drug antibody in the sample indicates that the patient has a risk of anaphylactic reaction to the therapeutic anti-IgE antibody.

[0031] In another aspect, the invention provides methods of identifying a patient having a risk of anaphylactic reaction to a therapeutic anti-IgE antibody, comprising the steps of: (a) contacting a sample from the patient with a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the potency of the mutant therapeutic antibody to human IgE is about 10% or less of the potency of the

therapeutic anti-IgE antibody to said human IgE; and (b) detecting binding of an anti-drug antibody of IgE isotype to the mutant therapeutic antibody, wherein the presence and/or the level of the anti-drug antibody in the sample indicates that the patient has a risk of anaphylactic reaction to the therapeutic anti-IgE antibody.

[0032] In another aspect, the invention provides methods of identifying a patient having a risk of anaphylactic reaction to a therapeutic anti-IgE antibody, comprising the steps of: (a) contacting a sample from a patient with (i) a mutant therapeutic antibody and (ii) an FcεRIα polypeptide that binds to an Fc region of a human IgE, wherein the mutant therapeutic antibody comprises at least one amino acid mutation from the therapeutic anti-IgE antibody, and the relative binding affinity of the mutant therapeutic antibody to human IgE is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE; (b) capturing the mutant therapeutic antibody to a surface; and (c) detecting binding of an anti-drug antibody of IgE isotype to the mutant therapeutic antibody, wherein the presence and/or the level of the anti-drug antibody in the sample indicates that the patient has a risk of anaphylactic reaction to the therapeutic anti-IgE antibody.

[0033] In another aspect, the invention provides methods of identifying a patient having a risk of anaphylactic reaction to a therapeutic anti-IgE antibody, comprising the steps of: (a) preincubating a sample from a patient with excess amount of an FcεRIα polypeptide that binds to an Fc region of a human IgE; (b) incubating the preincubated sample from step (a) with the therapeutic anti-IgE antibody or a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, and the relative binding affinity of the mutant therapeutic antibody to human IgE is reduced as compared to the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE; and (c) detecting binding of an anti-drug antibody of IgE isotype to the therapeutic anti-IgE antibody or the mutant therapeutic antibody, wherein the presence and/or the level of the anti-drug antibody in the sample indicates that the patient has a risk of anaphylactic reaction to the therapeutic anti-IgE antibody.

[0034] In another aspect, the invention provides methods of treating a patient having an IgE-mediated disorder, comprising the steps of: (a) determining the level of an anti-drug antibody of IgE isotype to a therapeutic anti-IgE antibody in a sample from the patient; (b) administering an effective amount of the therapeutic anti-IgE antibody to the patient if the level of the anti-drug antibody in the sample do not indicate that the patient has a risk of anaphylactic reaction to the therapeutic anti-IgE antibody. The level of the anti-drug antibody may be determined by any of the methods provided herein.

[0035] In another aspect, the invention provides uses of a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody in the preparation of a kit for detecting an anti-drug antibody of IgE isotype that binds to a therapeutic anti-IgE antibody in a sample, wherein the detection comprises the steps of: (a) contacting a sample that may contain the anti-drug antibody with the mutant therapeutic antibody, wherein the relative binding affinity of the mutant therapeutic antibody to human IgE is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE; and (b) detecting binding of the anti-drug antibody to the mutant therapeutic antibody.

[0036] In another aspect, the invention provides uses of a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody in the preparation of a kit for detecting an anti-drug antibody of IgE isotype that binds to a therapeutic anti-IgE antibody in a sample, wherein the detection comprises the steps of: (a) contacting a sample that may contain the anti-drug antibody with the mutant therapeutic antibody, wherein the potency of the mutant therapeutic antibody to human IgE is about 10% or less of the potency of the therapeutic anti-IgE antibody to said human IgE; and (b) detecting binding of the anti-drug antibody to the mutant therapeutic antibody.

[0037] In another aspect, the invention provides uses of (i) a mutant therapeutic antibody and (ii) an FcεRIα polypeptide that binds to an Fc region of a human IgE in the preparation of a kit for detecting an anti-drug antibody of IgE isotype that binds to a therapeutic anti-IgE antibody in a sample, wherein the detection comprises the steps of: (a) contacting a sample that may contain the anti-drug antibody with (i) the mutant therapeutic antibody and (ii) the FcεRIα polypeptide, wherein the mutant therapeutic antibody comprises at least one amino acid mutation from the therapeutic anti-IgE antibody, and the relative binding affinity of the mutant therapeutic antibody to human IgE is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE; (b) capturing the mutant therapeutic antibody to a surface; and (c) detecting binding of the anti-drug antibody to the mutant therapeutic antibody.

[0038] In another aspect, the invention provides uses of (i) a therapeutic anti-IgE antibody or a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody and (ii) an FcεRIα polypeptide that binds to an Fc region of a human IgE in the preparation of a kit for detecting an anti-drug antibody of IgE isotype that binds to the therapeutic anti-IgE antibody in a sample, wherein the detection comprises the steps of: (a) preincubating a sample that may contain the anti-drug antibody with excess

amount of the FcεRIα polypeptide; (b) incubating the preincubated sample from step (a) with the therapeutic anti-IgE antibody or the mutant therapeutic antibody, and the relative binding affinity of the mutant therapeutic antibody to human IgE is reduced as compared to the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE; and (c) detecting binding of the anti-drug antibody to the therapeutic anti-IgE antibody or the mutant therapeutic antibody.

[0039] In another aspect, the invention provides uses of a mutant therapeutic antibody comprising at least one amino acid mutation from a therapeutic anti-IgE antibody in the preparation of a kit for identifying a patient having a risk of anaphylactic reaction to the therapeutic anti-IgE antibody, wherein the identifying comprises the steps of: (a) contacting a sample from the patient with the mutant therapeutic antibody, wherein the relative binding affinity of the mutant therapeutic antibody to human IgE is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE; and (b) detecting binding of an anti-drug antibody of IgE isotype to the mutant therapeutic antibody, wherein the presence and/or the level of the anti-drug antibody indicate the patient has a risk of anaphylactic reaction to the therapeutic anti-IgE antibody.

[0040] In another aspect, the invention provides uses of a mutant therapeutic antibody comprising at least one amino acid mutation from a therapeutic anti-IgE antibody in the preparation of a kit for identifying a patient having a risk of anaphylactic reaction to the therapeutic anti-IgE antibody, wherein the identifying comprises the steps of: (a) contacting a sample from the patient with the mutant therapeutic antibody, wherein the potency of the mutant therapeutic antibody to human IgE is about 10% or less of the potency of the therapeutic anti-IgE antibody to said human IgE; and (b) detecting binding of an anti-drug antibody of IgE isotype to the mutant therapeutic antibody, wherein the presence and/or the level of the anti-drug antibody in the sample indicates that the patient has a risk of anaphylactic reaction to the therapeutic anti-IgE antibody.

[0041] In another aspect, the invention provides uses of (i) a mutant therapeutic antibody and (ii) an FcεRIα polypeptide that binds to an Fc region of a human IgE in the preparation of a kit for identifying a patient having a risk of anaphylactic reaction to a therapeutic anti-IgE antibody, wherein the identifying comprises the steps of: (a) contacting a sample from a patient with (i) the mutant therapeutic antibody and (ii) the FcεRIα polypeptide, wherein the mutant therapeutic antibody comprises at least one amino acid mutation from the therapeutic anti-IgE antibody, and the relative binding affinity of the mutant therapeutic antibody to human IgE is about 10% or less of the relative binding affinity of the therapeutic anti-IgE

antibody to said human IgE; (b) capturing the mutant therapeutic antibody to a surface; and (c) detecting binding of an anti-drug antibody of IgE isotype to the mutant therapeutic antibody, wherein the presence and/or the level of the anti-drug antibody in the sample indicates that the patient has a risk of anaphylactic reaction to the therapeutic anti-IgE antibody.

[0042] In another aspect, the invention provides uses of (i) an FcεRIα polypeptide that binds to an Fc region of a human IgE and (ii) a therapeutic anti-IgE antibody or a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody in the preparation of a kit for identifying a patient having a risk of anaphylactic reaction to a therapeutic anti-IgE antibody, wherein the identifying comprises the steps of: (a) preincubating a sample from a patient with excess amount of the FcεRIα polypeptide; (b) incubating the preincubated sample from step (a) with the therapeutic anti-IgE antibody or the mutant therapeutic antibody, and the relative binding affinity of the mutant therapeutic antibody to human IgE is reduced as compared to the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE; and (c) detecting binding of an anti-drug antibody of IgE isotype to the therapeutic anti-IgE antibody or the mutant therapeutic antibody, wherein the presence and/or the level of the anti-drug antibody in the sample indicates that the patient has a risk of anaphylactic reaction to the therapeutic anti-IgE antibody.

[0043] In another aspect, the invention also provides compositions comprising a therapeutic anti-IgE antibody for treating a patient having an IgE-mediated disorder, wherein the level of an anti-drug antibody of IgE isotype to the therapeutic anti-IgE antibody in a sample from the patient has been determined and the level of the anti-drug antibody in the sample does not indicate that the patient has a risk of anaphylactic reaction to the therapeutic anti-IgE antibody. In another aspect, the invention provides a therapeutic anti-IgE antibody for treating a patient having an IgE-mediated disorder, wherein the level of an anti-drug antibody of IgE isotype to the therapeutic anti-IgE antibody in a sample from the patient has been determined and the level of the anti-drug antibody in the sample does not indicate that the patient has a risk of anaphylactic reaction to the therapeutic anti-IgE antibody.

[0044] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art.

BRIEF DESCRIPTION OF THE FIGURES

[0045] Figure 1A shows the light chain amino acid sequence of antibody E25 (SEQ ID NO:1). Figure 1B shows the heavy chain amino acid sequence of antibody E25 (SEQ ID NO:2). The CDR regions as defined by Chothia are shown in boldface, while the CDR regions as defined by Kabat are delineated with brackets.

[0046] Figure 2A is a diagrammatic representation of an ELISA assay to compare the binding affinity of E25 and E25-AAA mutant to purified human IgE. Figure 2B is a graph showing binding of E25-AAA mutant to human IgE as compared to binding of E25 to human IgE. E25-AAA mutant had about 100x less affinity for IgE than E25.

[0047] Figure 3 is a diagrammatic representation of a potency assay for therapeutic anti-IgE antibodies.

[0048] Figure 4A is a graph showing binding of AME2 to E25 as compared to binding of AME2 to E25-AAA mutant. Figure 4B is a graph showing binding of AME10 to E25 as compared to binding of AME10 to E25-AAA mutant. Figure 4C is a graph showing binding of AME1 to E25 as compared to binding of AME1 to E25-AAA mutant. Figure 4D is a graph showing binding of AME7 to E25 as compared to binding of AME7 to E25-AAA mutant. Figure 4E is a graph showing binding of AME9 to E25 as compared to binding of AME9 to E25-AAA mutant. Figure 4F is a graph showing binding of AME13 to E25 as compared to binding of AME13 to E25-AAA mutant. Figure 4G is a graph showing binding of AME4 to E25 as compared to binding of AME4 to E25-AAA mutant. Figure 4H is a graph showing binding of AME5 to E25 as compared to binding of AME5 to E25-AAA mutant.

[0049] Figure 5 shows an E25-specific IgE chimeric antibody engineered as a positive control antibody for the assay system. The variable regions of the chimeric antibody are from antibody AME2 which specifically binds to Fab fragment of E25, and the constant regions of the chimeric antibody are from a human IgE antibody.

[0050] Figure 6A is diagrammatic representation of an assay system for testing binding of the chimeric E25-specific IgE positive control antibody to E25 antibody or E25-AAA mutant antibody. Figure 6B is a graph showing that the chimeric E25-specific IgE positive control antibody binds to E25 and 25-AAA mutant with similar affinity.

[0051] Figure 7 is a diagrammatic representation of an assay for detecting E25-specific antibodies of IgE isotype using E25-AAA mutant antibody.

[0052] Figure 8 shows a E25-specific IgE standard curve to determine the sensitivity of an assay for detecting E25-specific antibodies of IgE isotype using E25-AAA mutant antibody. This figure shows the results using the assay format described in Figure 7.

[0053] Figure 9 shows the drug tolerance of the assay for detecting E25-specific antibodies of IgE isotype using E25-AAA mutant antibody in the presence of increasing concentrations of E25.

[0054] Figure 10 is a diagrammatic representation of an assay for detecting E25-specific antibodies of IgE isotype using a semi-homogenous ELISA format.

[0055] Figure 11 is a diagrammatic representation of an assay for detecting E25-specific antibodies of IgE isotype using a semi-homogenous MSD-ECLA format.

[0056] Figure 12 is a diagrammatic representation of an assay for detecting E25-specific antibodies of IgE isotype using a "blocking" homogenous ELISA format.

[0057] Figure 13 is a diagrammatic representation of an assay for detecting E25-specific antibodies of IgE isotype using a "blocking" homogenous ELISA format.

[0058] Figure 14 is a diagrammatic representation of an assay for detecting E25-specific antibodies of IgE isotype using a homogeneous MSD-ECLA format.

[0059] Figure 15 is a diagrammatic representation of an assay for detecting E25-specific antibodies of IgE isotype using a semi-homogeneous ELISA format. Figure 15 left panel shows the assay using biotin-labeled E25 (or biotin-labeled E25 mutant). Figure 15 right panel shows the assay using E25 (or E25 mutant).

[0060] Figure 16 is a diagrammatic representation of an assay for detecting E25-specific antibodies of IgE isotype using a semi-homogeneous ELISA format. Figure 16 left panel shows the assay using biotin-labeled E25 (or biotin-labeled E25 mutant). Figure 16 right panel shows the assay using E25 (or E25 mutant).

[0061] Figure 17 is a diagrammatic representation of an assay for detecting E25-specific antibodies of IgE isotype using a semi-homogeneous MSD-ECLA format. Figure 17 left panel shows the assay using biotin-labeled E25 (or biotin-labeled E25 mutant). Figure 17 right panel shows the assay using E25 (or E25 mutant).

DETAILED DESCRIPTION

[0062] The present invention provides methods and reagents that are useful to detect IgE isotype anti-drug antibodies that are specific to a therapeutic anti-IgE antibody (such as omalizumab, XOLAIR®). The challenges with development of such an assay include the

difficulty of distinguishing between endogenous IgE (IgE with the Fc region available for binding by an anti-IgE therapeutic antibody) versus IgE specific to the therapeutic anti-IgE antibody because the endogenous IgE interferes with detection of the IgE specific to the therapeutic anti-IgE antibody. The invention provides a method and reagents that can differentiate between the endogenous IgE and the IgE specific to the therapeutic anti-IgE antibody, and specifically detect the IgE specific to the therapeutic anti-IgE antibody.

A. General Techniques

[0063] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, "Molecular Cloning: A Laboratory Manual", second edition (Sambrook et al., 1989); "Oligonucleotide Synthesis" (M. J. Gait, ed., 1984); "Animal Cell Culture" (R. I. Freshney, ed., 1987); "Methods in Enzymology" (Academic Press, Inc.); "Current Protocols in Molecular Biology" (F. M. Ausubel et al., eds., 1987, and periodic updates); "PCR: The Polymerase Chain Reaction", (Mullis et al., eds., 1994).

[0064] 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.

B. Definitions

[0065] As used herein, an "anti-drug antibody" is an antibody wherein the variable regions of the antibody bind to a therapeutic anti-IgE antibody. For example, antibodies with variable regions that bind to therapeutic antibody omalizumab (E25) described herein are anti-drug antibodies.

[0066] The term "antibody" is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.,* bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity or function.

[0067] "Antibody fragments" comprise a portion of a full length antibody, generally the antigen binding or variable region thereof . Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[0068] A "Fab" fragment contains a variable and constant domain of the light chain and a variable domain and the first constant domain (CH1) of the heavy chain. $F(ab)'_2$ antibody fragments comprise a pair of Fab fragments that are generally covalently linked near their carboxy termini by hinge cysteines. Other chemical couplings of antibody fragments are also known.

[0069] "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0070] The term "monoclonal antibody" as used herein refers to an antibody from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope(s), except for possible variants that may arise during production of the monoclonal antibody, such variants generally being present in minor amounts. Such monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones or recombinant DNA clones. It should be understood that the selected target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity *in vivo*, to create a multispecific antibody, *etc.*, and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this invention. 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. In addition to their specificity, the monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins. 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, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including, for example, the hybridoma method (*e.g.,* Kohler *et al., Nature*, 256:495 (1975); Harlow *et al., Antibodies: A Laboratory Manual,* (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681, (Elsevier, N.Y., 1981)), recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567), phage display technologies (see, *e.g.,* Clackson *et al., Nature,* 352:624-628 (1991); Marks *et al., J. Mol. Biol.*, 222:581-597 (1991); Sidhu *et al., J. Mol. Biol.* 338(2):299-310 (2004); Lee *et al., J.Mol.Biol.*340(5):1073-1093 (2004); Fellouse, *Proc. Nat. Acad. Sci. USA* 101(34):12467-12472 (2004); and Lee *et al. J. Immunol. Methods* 284(1-2):119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, e.g., WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits *et al., Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits *et al., Nature*, 362:255-258 (1993); Bruggemann *et al., Year in Immuno.*, 7:33 (1993); U.S. Patent Nos. 5,545,806; 5,569,825; 5,591,669 (all of GenPharm); 5,545,807; WO 1997/17852; U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks *et al., Bio/Technology*, 10: 779-783 (1992); Lonberg *et al., Nature*, 368: 856-859 (1994); Morrison, *Nature*, 368: 812-813 (1994); Fishwild *et al., Nature Biotechnology*, 14: 845-851 (1996); Neuberger, *Nature Biotechnology*, 14: 826 (1996); and Lonberg and Huszar, *Intern. Rev. Immunol.*, 13: 65-93 (1995).

[0071] The monoclonal antibodies herein specifically include "chimeric" antibodies. "Chimeric" antibodies (immunoglobulins) have a portion of the heavy and/or light chain identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; and Morrison *et al., Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)). Humanized antibody as used herein is a subset of chimeric antibodies.

[0072] "Humanized" forms of non-human (*e.g.,* murine) antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient or acceptor antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance such as binding affinity. Generally, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence although the FR regions may include one or more amino acid substitutions that improve binding affinity. The number of these amino acid substitutions in the FR are typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al., Nature* 321:522-525 (1986); Reichmann *et al., Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

[0073] A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the known techniques for making human antibodies. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

[0074] The term "hypervariable region," "HVR," or "HV," when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six HVRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. See, e.g., Xu et al., Immunity 13:37-45 (2000); Johnson and Wu, in Methods in Molecular Biology 248:1-25 (Lo, ed., Human Press, Totowa, NJ, 2003). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional

and stable in the absence of light chain. See, e.g., Hamers-Casterman et al., Nature 363:446-448 (1993); Sheriff et al., Nature Struct. Biol. 3:733-736 (1996).

[0075] A number of HVR delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat HVRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

Loop	Kabat	AbM	Chothia	Contact
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35B (Kabat Numbering)	H26-H35B	H26-H32	H30-H35B
H1	H31-H35 (Chothia Numbering)	H26-H35	H26-H32	H30-H35
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

[0076] HVRs may comprise "extended HVRs" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the VL and 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the VH. The variable domain residues are numbered according to Kabat et al., supra, for each of these definitions.

[0077] "Framework" or "FR" residues are those variable domain residues other than the HVR residues as herein defined.

[0078] The term "variable domain residue numbering as in Kabat" or "amino acid position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat et al., supra. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue

52 of H2 and inserted residues (e.g. residues 82a, 82b, and 82c, etc. according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

[0079] The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) (e.g, Kabat et al., *Sequences of Immunological Interest.* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The "EU numbering system" or "EU index" is generally used when referring to a residue in an immunoglobulin heavy chain constant region (e.g., the EU index reported in Kabat et al., supra). The "EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody. Unless stated otherwise herein, references to residue numbers in the variable domain of antibodies means residue numbering by the Kabat numbering system. Unless stated otherwise herein, references to residue numbers in the constant domain of antibodies means residue numbering by the EU numbering system (e.g., see United States Provisional Application No. 60/640,323, Figures for EU numbering).

[0080] The term "Fc region" is used to define the C-terminal region of an immunoglobulin heavy chain which may be generated by papain digestion of an intact antibody. The Fc region may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at about position Cys226, or from about position Pro230, to the carboxyl-terminus of the Fc region. The Fc region of an immunoglobulin generally comprises two constant domains, a CH2 domain and a CH3 domain, and optionally comprises a CH4 domain. By "Fc region chain" herein is meant one of the two polypeptide chains of an Fc region.

[0081] "Binding" or "specific binding" generally refers to binding between two molecules (such as between an antibody and one or more targets, an anti-IgE antibody and an IgE, and an anti-drug antibody and the drug) with sufficient affinity. Preferably, the extent of binding of an antibody to an unrelated molecule is less than about 10% of the binding of the antibody to a target as measured, e.g., by a radioimmunoassay (RIA). In some embodiments, the antibody that binds to its target has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, or ≤ 0.1 nM.

[0082] "Binding affinity" generally refers to the strength of the sum total of monovalent 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. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present invention. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

[0083] In one embodiment, the "Kd" or "Kd value" according to this invention is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. 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 ug/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% 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.

[0084] According to another embodiment, the Kd or Kd value may be measured by using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE®-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at -10 response

units (RU). Briefly, 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 ul/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% 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 sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio koff/kon. See, e.g., Chen et al., J. Mol. Biol. 293:865-881 (1999). If the on-rate exceeds 106 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.

[0085] An "on-rate," "rate of association," "association rate," or "kon" according to this invention can also be determined as described above using a BIACORE ®-2000 or a BIACORE ®-3000 system (BlAcore, Inc., Piscataway, NJ).

[0086] The term "substantially similar" or "substantially the same," as used herein, denotes a sufficiently high degree of similarity between two numeric values (for example, one associated with an antibody of the invention and the other associated with a reference/comparator antibody), such that one of skill in the art would consider the difference between the two values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said values (e.g., relative binding affinity values). The difference between said two values is, for example, less than about 50%, less than about 40%, less than about 30%, less than about 20%, and/or less than about 10% as a function of the reference/comparator value

[0087] The term "sample", as used herein, refers to a composition that is obtained or derived from a subject of interest. Samples include, but are not limited to, whole blood, serum, or plasma from an individual.

[0088] "Total IgE" refers to a total amount of IgE present in a sample, including free, unbound IgE and IgE complexed with a binding partner. "Free IgE" refers to IgE not bound to a binding partner.

[0089] A "subject", an "individual", or a "patient" used herein is a mammal, more preferably a human. Mammals include, but are not limited to, humans, primates, farm animal, sport animals (e.g., horses), rodents, and pets (*e.g.*, dogs and cats).

[0090] As used herein, method for "aiding assessment" refers to methods that assist in making a clinical determination (*e.g.*, risk of anaphylaxis), and may or may not be conclusive with respect to the definitive assessment.

[0091] As used herein, a "reference value" can be an absolute value; a relative value; a value that has an upper and/or lower limit; a range of values; an average value; a median value; a mean value; or a value as compared to a particular control or baseline value.

[0092] The term "detecting" or "detection" is used in the broadest sense to include both qualitative and quantitative measurements of a specific molecule, herein measurements of a specific analyte molecule such as an IgE or an anti-drug antibody. In one aspect, a detection method described herein is used to identify the mere presence of an analyte molecule of interest in a sample. In another aspect, a detection method can be used to quantify an amount of analyte molecule in a sample. In still another aspect, the method can be used to determine the relative binding affinity of an analyte molecule of interest for a target molecule.

[0093] The term "detecting agent", "detection agent", "detecting reagent", and "detection reagent" are used interchangeably to refer to an agent that detects an analyte molecule, either directly via a label, such as a fluorescent, enzymatic, radioactive, or chemiluminescent label, that can be linked to the detecting agent, or indirectly via a labeled binding partner, such as an antibody or receptor that specifically binds the detecting agent. Examples of detecting agents include, but are not limited to, an antibody, antibody fragment, soluble receptor, receptor fragment, and the like.

[0094] By "correlate" or "correlating" is meant comparing, in any way, the performance and/or results of a first analysis or protocol with the performance and/or results of a second analysis or protocol. For example, one may use the results of a first analysis or protocol in carrying out a second protocols and/or one may use the results of a first analysis or protocol to determine whether a second analysis or protocol should be performed.

[0095] The term "assay surface" or "surface" means a substrate on which a capture agent may be immobilized for use in an immunoassay. Suitable assay surfaces include polymeric

assay plate, chips, fluidity cards, magnetic beads, resins, cellulose polymer sponge, and the like.

[0096] The term "binding domain" refers to the region of a polypeptide that binds to another molecule. In the case of an Fc receptor polypeptide or FcR, the binding domain can comprise a portion of a polypeptide chain thereof (e.g. the α-chain thereof) that is responsible for binding an Fc region of an immunoglobulin or other Fc region containing molecule. One useful binding domain is the extracellular domain (ECD) of an Fc receptor α-chain polypeptide. As described herein, the extracellular domain of the FcεRIα-chain contains a binding domain that binds the Fc region of an Ig, for example IgE.

[0097] The term "capture agent" or "capture reagent" refers to a agent capable of binding and capturing a target molecule or analyte molecule in a sample. Typically, a capture agent or reagent is immobilized, for example, on a solid substrate, such as a microparticle or bead, microtiter plate, column resin, chip, fluidity card, magnetic bead, cellulose polymer sponge, and the like. The capture agent can be an antigen, soluble receptor, antibody, a mixture of different antibodies, and the like.

[0098] "Chimeric" polypeptides are polypeptides in which a portion of the polypeptide sequence is derived from one species, while at least one other portion corresponds to a sequence derived from a different species.

[0099] The term "label" when used herein refers to a compound or composition which is conjugated or fused directly or indirectly to a reagent such as a nucleic acid probe, a polypeptide or an antibody and facilitates detection or capture of the reagent to which it is conjugated or fused. The label may itself be detectable (*e.g.,* radioisotope, fluorescent, photoluminescent, chemiluminescent, or electrochemiluminescent labels), detectable after binding to another molecule, or in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

[0100] The term "target molecule" refers to a specific binding target of an analyte molecule. A target molecule can be, for example, an antigen if the analyte molecule is an antibody. The target molecule can be, for example, a polypeptide or antibody having therapeutic activity. In one embodiment, the target molecule is a therapeutic antibody and the analyte molecule is an anti-drug antibody that binds the therapeutic antibody.

[0101] "Analyte" and "analyte molecule," as used herein, refer to a molecule that is analyzed by the methods of the invention, and includes, but is not limited to, anti-drug antibodies.

[0102] "Treating" or "treatment" refers to clinical intervention in an attempt to alter the natural course of the individual or cell being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, the therapeutic antibodies described herein are used to delay development of a disease or disorder or to slow the progression of a disease or disorder.

[0103] An "effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. A "therapeutically effective amount" of a therapeutic agent may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the therapeutic agent are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0104] A "conservative substitution" as used herein, replaces a selected amino acid with another that is not substantially different in character. Amino acids grouped according to character include positively charged amino acids: Lys, Arg, His; negatively charged amino acids: Asp, Glu; amide amino acids: Asn, Gln; aromatic amino acids: Phe, Tyr, Trp; hydrophobic amino acids: Pro, Gly, Ala, Val, Leu, Ile, Met; and uncharged hydrophilic amino acids: Ser, Thr. Preferred conservative amino acid substitutions are shown below:

Conservative Amino Acid Substitutions

Target AA	**Replacement Selected From**	**Preferred Substitution**
Ala	Pro, Gly, Ala, Val, Leu, Ile, Met, Ser, Thr	Ser
Arg	Lys, Arg, His, Ser, Ala Ser, Ala	Lys
Asn	Lys, Arg, His, Asn, Gln, Ser, Ala Ser, Ala	Gln, Ser, Ala
Asp	Asp, Glu, Asn, Gln, Ser, Ala	Glu, Ser, Ala
Cys	Pro, Gly, Ala, Val, Leu, Ile, Met, Ser, Thr	Ala, Ser
Gln	Lys, Arg, His, Asn, Gln, Ser, Ala	Asn, Ser, Ala
Glu	Asp, Glu, Asn, Gln Ser, Ala	Asp,Ser, Ala

Gly	Pro, Gly, Ala, Val, Leu, Ile, Met, Ser, Thr	Pro, Ala
His	Lys, Arg, His, Ser, Ala	Ser, Ala
Ile	Pro, Gly, Ala, Val, Leu, Met	Ala, Val, Leu
Leu	Pro, Gly, Ala, Val, Ile, Met	Ala, Val, Ile
Lys	Arg, His, Ser, Ala	Arg, Ser, Ala
Met	Pro, Gly, Ala, Val, Leu, Ile	Ala, Val, Leu, Ile
Phe	Lys, Arg, His, Tyr, Trp Ala, Val, Leu, Ile	Tyr, Ala, Val, Leu, Ile
Pro	Lys, Arg, His, Phe, Tyr, Trp, Gly, Ala	Phe, Gly, Ala
Ser	Lys, Arg, His, Thr, Ala	Thr, Ala
Thr	Lys, Arg, His, Ser, Ala	Ser, Ala
Trp	Phe, Tyr, Trp, Ala	Phe, Ala
Tyr	Phe, Tyr, Trp, Ala, Val, Leu, Ile	Phe, Ala, Val, Leu, Ile
Val	Pro, Gly, Ala, Val, Leu, Ile, Met, Ser, Ala	Leu, Ile, Ser, Ala

[0105] The terms, "protein," "peptide," and "polypeptide" are used interchangeably to denote an amino acid polymer or a set of two or more interacting or bound amino acid polymers.

[0106] "Polypeptide" refers to a peptide or protein containing two or more amino acids linked by peptide bonds, and includes peptides, oligomers, proteins, and the like. Polypeptides can contain natural, modified, or synthetic amino acids. Polypeptides can also be modified naturally, such as by post-translational processing, or chemically, such as amidation acylation, cross-linking, and the like.

[0107] As used herein, "a", "an", and "the" can mean singular or plural (i.e., can mean one or more) unless indicated otherwise.

[0108] Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter *per se*. For example, description referring to "about X" includes description of "X."

[0109] It is understood that aspect and variations of the invention described herein include "consisting" and/or "consisting essentially of" aspects and variations.

C. Methods of the Invention

[0110] The invention provides methods and reagents that are useful to detected IgE isotype anti-drug antibodies that specifically bind to a therapeutic anti-IgE antibody. The invention also provides methods for identifying an individual having a risk of anaphylaxis to a therapeutic anti-IgE antibody treatment by measuring the presence and/or the level of IgE isotype anti-drug antibodies that bind to the therapeutic antibody in a sample from the

individual, and assessing the risk of anaphylaxis based on the presence and/or the level of the IgE isotype anti-drug antibodies in the sample. The invention further provides methods for treating an individual having IgE-mediated disorders comprising determining the presence and/or level of anti-drug antibodies to a therapeutic anti-IgE antibody in a sample from the individual, and administering an effective amount of the therapeutic anti-IgE antibody to the individual if the level of the anti-drug antibodies in the sample indicates that the individual does not have a risk of analphylactic reaction to the therapeutic anti-IgE antibody.

Therapeutic anti-IgE antibodies and mutant therapeutic antibodies

[0111] The methods of the invention is useful to detect anti-drug antibodies of IgE isotype that specifically bind to an anti-IgE therapeutic antibody. The difficulties of developing such an assay include the ability to distinguish binding to an endogenous IgE to which the anti-IgE antibody targets and to an IgE that specifically binds to the anti-IgE antibody (i.e., anti-drug antibody of IgE isotype). In some embodiments, the IgE is a human IgE.

[0112] As used herein, an "anti-IgE antibody" or a "therapeutic anti-IgE antibody" is an antibody that binds to an IgE in such a manner so as to inhibit or substantially reduce the binding of such IgE to the high affinity receptor (FcεRI). Exemplary anti-IgE antibodies, include, for example, E25 (omalizumab), E26, E27, as well as CGP-5101 (Hu-901) and the HA antibody. The amino acid sequences of the heavy and light chain variable domains and the full length heavy and light chain of the humanized anti-IgE antibodies E25, E26, and E27 are disclosed, for example in U.S. Pat. No. 6,172,213 (Figures 2 and 12) and WO 99/01556. The CGP-5101 (Hu-901) antibody is described in Corne et al., 1997, *J. Clin. Invest.* 99(5): 879-887, WO 92/17207, and ATTC Deposit Nos. BRL-10706, 11130, 11131, 11132, and 11133. Figure 1 shows the full-length amino acid sequences of anti-IgE antibody E25 (omalizumab). The HA antibody is antibody MAb2 (CL-2C) shown in Table 2, Example 10 in WO2004/070011, and WO2004/070010. The cell line that produces the HA antibody was deposited at American Type Culture Collection (ATCC) on December 3, 2003 with ATCC No. PTA-5678.

[0113] In some embodiments, the methods of the invention use a mutant anti-IgE antibody that has a significant lower binding affinity (including relative binding affinity) and/or potency to an IgE (such as a human IgE) than the unmodified therapeutic anti-IgE antibody. The mutant therapeutic anti-IgE antibody may be designed to have one or more of the following characteristics: a) the binding affinity (including relative binding affinity) of the mutant antibody to an IgE is about 10% or less of the binding affinity (including relative

binding affinity) of the therapeutic anti-IgE antibody to the IgE; b) the potency of the mutant antibody to an IgE is about 10% or less of the potency of the therapeutic anti-IgE antibody; c) the mutant antibody has the same or similar tertiary structure as the therapeutic anti-IgE antibody; d) the mutant antibody has the same or similar glycan levels as the therapeutic anti-IgE antibody; and e) the mutant antibody has the same or similar binding affinity to one or more control anti-drug antibodies as compared to the therapeutic anti-IgE antibody. A mutant therapeutic antibody having the minimum number of amino acid mutations in the variable regions effective to reduce relative binding affinity and/or potency to an IgE may be selected for use in the assays described herein. In some embodiments, the mutant antibody comprises one, two, three, four, five, or six amino acid mutations (e.g., substitutions, deletions, or additions) in one or more CDRs (such as one, two, or three of CDR1, CDR2, and CDR3) of the heavy and/or light chain of the therapeutic anti-IgE antibody.

[0114] In some embodiments, the potency of the mutant antibody to an IgE is about 10% or less, about 7.5% or less, about 5% or less, about 2.5% or less, about 2.0% or less, about 1.5% or less, about 1% or less, about 0.9% or less, about 0.8% or less, about 0.7% or less, about 0.5% or less, about 0.25% or less, or about 0.1% or less of the potency of the therapeutic anti-IgE antibody to the IgE.

[0115] In some embodiments, the relative binding affinity of the mutant therapeutic antibody to an IgE is about 10% or less, about 7.5% or less, about 5% or less, about 2.5% or less, about 2.0% or less, about 1.5% or less, about 1% or less, about 0.9% or less, about 0.8% or less, about 0.7% or less, about 0.5% or less, about 0.25% or less, or about 0.1% or less of the relative binding affinity of the therapeutic anti-IgE antibody to the IgE.

[0116] In some embodiments, the therapeutic anti-IgE antibody is omalizumab, and the mutant antibody comprises one, two, or three amino acid mutations in the first CDR of the light chain and/or one, two, or three amino acid mutations in the third CDR of the heavy chain. In some embodiments, the therapeutic anti-IgE antibody is omalizumab, and the mutant antibody comprises the heavy chain variable region amino acid sequence from SEQ ID NO:2 and the light chain variable region amino acid sequence from SEQ ID NO:1 wherein amino acid Asp at position 34, positions 30 and 34, positions 32 and 34, or positions 30, 32, and 34 of SEQ ID NO:1 are substituted. In some embodiments, amino acid Asp at position 30, 32, and/or 34 of SEQ IN NO:1 are substituted by Ala. In some embodiments, the mutant antibody comprises the heavy chain amino acid sequence of SEQ ID NO:2 and the light chain amino acid sequence of SEQ ID NO:1 with amino acid substitutions from Asp to Ala at positions 30, 32, and 34 of SEQ ID NO:1. Any of the anti-IgE antibodies described in

Presta et al. (*J. Immunol.* 151:2623-2632, 1993) having the relative binding affinity and/or the potency to IgE of about 10% or less of the relative binding affinity or the potency of therapeutic antibody E25 may be used as mutant therapeutic antibody in the methods described herein.

[0117] Anti-drug antibodies may be generated and used as a control to screen for mutant antibodies. These control antibodies may bind with similar affinity or equally well to the mutant antibody and unmodified therapeutic anti-IgE antibody. In some embodiments, the control anti-drug antibody binds to Fab fragment of the anti-IgE antibody. In some embodiments, the control anti-drug antibody binds to one or more CDRs of the anti-IgE antibody. A binding assay described in Example 2 may be used to test and screen mutant antibodies using a control anti-drug antibody (such as a control antibody shown in Figure 5). See Figure 6A for assay methods.

[0118] The potency of a therapeutic anti-IgE antibody or a mutant therapeutic antibody is determined by measuring the ability of the therapeutic anti-IgE antibody or the mutant therapeutic antibody to bind to IgE in competition with the high affinity receptor (FcεRI) as compared to a reference control. Typical assay methods include immunoassays, such as ELISA, ECLA, and the like that include a capture agent bound to an assay surface to capture and immobilize the desired target molecule. Captured target molecules are detected with a detection agent that binds the target molecule and provides a detection label for quantification.

[0119] In some embodiments, the potency of a therapeutic anti-IgE antibody or a mutant therapeutic antibody is determined by an inhibition ELISA as shown in Figure 3. Increasing concentrations of an anti-IgE antibody or a mutant antibody is incubated with labeled IgE. The mixture is added to a plate containing an immobilized FcεRIα polypeptide as a capture agent. The anti-IgE antibody or the mutant antibody that binds labeled IgE effectively inhibits the binding of the labeled IgE to the capture agent, reducing the detectable signal. Thus, an anti-IgE potency of the sample is inversely correlated with the signal detected.

[0120] FcεRIα polypeptides described herein can be used in such assays as capture agents that bind IgE. The amount of captured IgE can be compared with a control, for example a standard lot or other standard having a known amount of an anti-IgE antibody; and/or with a control lacking an anti-IgE antibody. A reduced signal detected from the labeled IgE is compared with the control and the amount of inhibition is correlated to the potency of the anti-IgE antibody or the mutant antibody.

[0121] Binding affinity (including relative binding affinity) of a mutant therapeutic antibody or a therapeutic anti-IgE antibody to an IgE may be measured using ELISA or

BIAcore™ surface plasmon resonance (SPR) system (BIAcore, INC, Piscaway NJ). Relative binding affinity is a comparison of the binding of the drug to its target compared with another drug. For example, using an ELISA assay, a therapeutic anti-IgE antibody or a mutant antibody, or a fragment thereof (such as a Fab) is immobilized to a surface, and purified IgE (such as human IgE) with increased concentration (such as from 0.1 ng/ml to 10,000 ng/ml) is then incubated with the immobilized therapeutic anti-IgE antibody or the mutant antibody. A detecting agent (such as a goat anti-human IgE antibody) labeled with HRP is allowed to bind to any IgE bound to the immobilized therapeutic anti-IgE antibody or mutant antibody. The signal generated by the HRP is measured. See, e.g., Figures 2A and 2B. The relative reduction in binding affinity of the mutant therapeutic antibody as compared to the therapeutic anti-IgE antibody is determined. Additionally, the relative binding affinity may be measured by immobilizing IgE (such as human IgE) directly to a surface (ELISA plate), incubating with varying concentrations of an anti-IgE therapeutic antibody or mutant antibody, and then detecting the bound anti-IgE therapeutic antibody or mutant antibody using an HRP-labeled anti-human IgG antibody. Alternatively, BIAcore assays may be used to measure the binding affinity of human IgE to the immobilized therapeutic anti-IgE antibody or the mutant antibody (such as Fab fragments).

[0122] Other properties of the therapeutic anti-IgE antibody and the mutant antibody, such as primary and tertiary structures and glycan levels, are tested using known method.

FcεRIα polypeptides

[0123] An FcεRIα polypeptide can be used as a capture agent, a detecting agent and/or a blocking agent in the assays described herein. The term "FcεRI polypeptide" is used to describe a polypeptide that binds to the Fc region of an IgE or IgE Fc-region containing molecule, and a polypeptide that forms a receptor that binds to the Fc region of an IgE or IgE Fc-region containing molecule. FcεRI receptor may include an Fc receptor polypeptide α-chain and an Fc receptor polypeptide homo- or heterodimer of the ε-chain. FcεRI α-chains contain an extracellular domain ("ECD") that binds to the Fc domain-containing agent, for example an immunoglobulin (Ig). FcRs are reviewed in Ravetch and Kinet, 1991, *Annu. Rev. Immunol.* 9: 457–492; Capel et al., 1994, *Immunomethods* 4: 25–34; and de Haas et al., 1995 *J. Lab. Clin. Med.* 126: 330–341. The physiology and pathology of the high affinity IgE receptor (FcεRI) are reviewed in Kinet, 1999, *Annu. Rev. Immunol.* 17: 931–972.

[0124] In some embodiments, the FcεRI polypeptide may comprise Fc binding domain sequences (such as extracellular domain sequences) from a human or a non-human primate

(such as cynomolgus monkey, rhesus monkey, chimpanzee) FcεRIα polypeptide. FcεRIα polypeptide may also include synthetic FcεRIα polypeptide, variants of FcεRIα polypeptide, fusion proteins comprising FcεRIα polypeptide, and chimeric proteins comprising FcεRIα polypeptide. In some embodiments, the FcεRIα polypeptide binds human IgE with similar affinity as wild type human or non-human primate FcεRIα and does not block the CDR epitopes of human IgE from binding to the therapeutic anti-IgE antibody.

[0125] The immature FcεRIα polypeptides contain native signal sequence, and mature polypeptides lack signal sequence. The FcεRIα polypeptides include immature FcεRIα polypeptides containing native signal sequence and mature polypeptides lacking signal sequence. In some embodiments, the FcεRIα polypeptides may include those having the amino acid sequence of SEQ ID NO: 3 (cynomolgus), SEQ ID NO: 4 (rhesus), SEQ ID NO: 5 (chimpanzee), or SEQ ID NO: 6 (human) as well as variants thereof having at least 90% (for example, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) sequence identity with the sequence of SEQ ID NO: 3, 4, 5, or 6.

FcεRIα IgE-binding Fragment Polypeptides

[0126] In some embodiments, the FcεRIα polypeptide comprises an IgE binding fragment of FcεRIα. IgE-binding fragments of FcεRIα preferably retain high affinity for IgE. In one example, the IgE-binding fragment comprises an extracellular domain ("ECD") of a human or a non-human primate FcεRIα, and can be the ECD of SEQ ID NO: 3, 4, 5, or 6, or of a variant thereof having at least 90% sequence identity to SEQ ID NO: 3, 4, 5 or 6.

[0127] The amino acid sequences of cynomolgus, rhesus, chimpanzee, and human FcεRIα are shown in Table 1 below. Any of the non-human primate FcεRIα polypeptides described in WO 08/028068 may be used.

Table 1. Primate FcεRIα Mature Sequences

```
       +1         10          20          30          40          50
Cyno     VPQKPTVSLN PPWNRIFKGE NVTLTCNGSN FFEVSSMKWF HNGSLSEVAN
Rhesus   VPQKPTVSLN PPWNRIFKGE NVTLTCNGSN FFEVSSMKWF HNGSLSEVAN
Chimp    VPQKPKVSLN PPWNRIFKGE NVTLTCNGNN FFEVSSTKWF HNGSLSEETN
Human    VPQKPKVSLN PPWNRIFKGE NVTLTCNGNN FFEVSSTKWF HNGSLSEETN

                60          70          80          90         100
Cyno     SSLNIVNADF EDSGEYKCQH QQFDDSEPVH LEVFSDWLLL QASAEVVMEG
Rhesus   SSLNIVNADF EDSGEYKCQH QQFDDSEPVH LEVFSDWLLL QASAEVVMEG
Chimp    SSLNIVNAKF EDSGEYKCQH QQVNESEPVY LEVFSDWLLL QASAEVVMEG
Human    SSLNIVNAKF EDSGEYKCQH QQVNESEPVY LEVFSDWLLL QASAEVVMEG

               110         120         130         140         150
Cyno     QPLFLRCHSW RNWDVYKVIY YKDGEALKYW YENHNISITN TTVEDSGTYY
Rhesus   QPLFLRCHSW RNWDVYKVIY YKDGEALKYW YENHNISITN ATVEDSGTYY
Chimp    QPLFLRCHGW RNWDVYKVIY YKDGEALKYW YENHNISITN ATVEDSGTYY
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Human     QPLFLRCHGW RNWDVYKVIY YKDGEALKYW YENHNISITN ATVEDSGTYY

                  160         170      *  180         190          200
Cyno      CTGKLWQLDC ESEPLNITVI KAQHDKYWLQ FLIPLLVAIL FAVDTGLFIS
Rhesus    CTGKLWQLDC ESEPLNITVI KAQHDKYWLQ FLIPLLVAIL FAVDTGLFIS
Chimp     CTGKVWQLDY ESEPLNITVI KAPREKYWLQ FFIPLLVAIL FAVDTGLFIS
Human     CTGKVWQLDY ESEPLNITVI KAPREKYWLQ FFIPLLVVIL FAVDTGLFIS

                 210         220          230 232
Cyno      TQQQVTFLLK IKRTRKGFKL LNPHPKPNPK SN    (SEQ ID NO: 3)
Rhesus    TQQQVTFLLK IKRTRKGFKL LNPHPKPNPK SN    (SEQ ID NO: 4)
Chimp     TQQQVTFLLK IKRTRKGFRL LTPHPKPNPK NN    (SEQ ID NO: 5)
Human     TQQQVTFLLK IKRTRKGFRL LNPHPKPNPK NN**  (SEQ ID NO: 6)
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*ECD - residues V1-K176
**US Patent No. 6,602,983

[0128] The FcεRIα ECD can extend, for example, from residue V1 to K171, A172, P173, H/R174, D/E175, or K176 of the FcεRIα polypeptides, numbered as shown in Table 1. In some embodiments, the FcεRI polypeptide comprises any of the following FcεRIα ECD fragments: V1-K171, V1-A172, V1-Q/P173, V1-H/R174, V1-D/E 175, or V1-K176. Exemplary FcεRIα ECD polypeptides thus include those polypeptides comprising residues V1 to K171, V1 to A172, V1 to P173, V1 to H/R174, V1 to D/E175, or V1 to K176 of SEQ ID NO: 3, 4, 5, or 6, and of variants thereof having at least 90% (for example, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) identity with SEQ ID NO: 3, 4, 5, or 6.

[0129] Additional fragments include truncations and deletion mutants of the ECDs that retain high affinity binding to IgE.

FcεRIα variant polypeptides

[0130] In some embodiments, the FcεRIα polypeptide comprises a variant FcεRIα polypeptide. Variant FcεRIα polypeptides are those having at least one amino acid substitution, deletion, or insertion as compared to a native polypeptide. FcεRIα variants can have one or more conservative amino acid substitution (as defined herein), replacing a target residue with a corresponding residue of the same general character, for example, a Lys for an Arg. Such amino acid substitutions can be made without altering the general function of the polypeptide. The FcεRIα variant polypeptide can also include non-conservative substitutions.

[0131] A variant FcεRIα polypeptide may have one or more substitution replacing an amino acid of a first species FcεRIα with a corresponding amino acid of a second species FcεRIα. For example, the encoded polypeptide can contain one or more (but no more than 14) amino acid substitutions at positions 29, 37, 48, 49, 59, 73, 74, 75, 80, 141, 155, 160, 173, 174, or 175, as shown in Table 1. The one or more substitutions can include, for example, one or more (and fewer than 14) of the following amino acid substitutions:

S29N M37T V48E A49T D59K

F73V D74N D75E H80V T141A

L155V C160Y Q173P H174R D175E

[0132] Structural information derived from the crystal structure of human FcεRI complexed with the Fc domain of human IgE indicates that Tyr 160 is located near the receptor:ligand interface. Because a Cys at this interface may impede binding, the FcεRIα polypeptides may be mutated to replace Cys160 with tyrosine to improve binding of cynomolgus and rhesus FcεRIα to human IgE. In some embodiments, the FcεRIα polypeptide comprises an FcεRIα polypeptide that has been mutated to include the Cys160 to Tyrosine mutation. For example, the mutated cyno sequence is shown below.

pRKgD cynoFcεRI.6xHisTyr160

-55
MGGAA ARLGAVILFV VIVGLHGVRG KYALADASLK MADPNRFRGK DLPVLDQLLE
+1 VPQKPTVSLN PPWNRIFKGE NVTLTCNGSN FFEVSSMKWF HNGSLSEVAN
SSLNIVNADF EDSGEYKCQH QQFDDSEPVH LEVFSDWLLL QASAEVVMEG
QPLFLRCHSW RNWDVYKVIY YKDGEALKYW YENHNISITN TTVDSGTYYC
TGKLWQLD**Y**E SEPLNITVIK AQHDK HHHHHH (SEQ ID NO:11)

Chimeric FcεRIα polypeptides

[0133] In some embodiments, the FcεRIα polypeptide is a chimeric polypeptide, for example, a chimeric polypeptide formed of two or more portions of different FcεRIα polypeptides. For example, a chimeric FcεRIα polypeptide can be formed of two or more portions derived from two or more of SEQ ID NO: 3, 4, 5, and 6. An exemplary chimeric polypeptide is the cynomolgus/rhesus chimeric polypeptide comprising residues 1-141 of the rhesus FcεRIα ECD and residues 142-171 of the cyno FcεRIα ECD, and having the amino acid sequence of SEQ ID NO: 12 (see right below). Additional chimeric polypeptides contemplated include human/cyno, human/rhesus, human/chimpanzee, cyno/chimpanzee, rhesus/chimpanzee, and the like chimeras, each comprising a portion of the named species FcεRIα ECD.

rhesusSScynoFceRI.6xhis tyr160

-25 +1
MAPAM ESPTLLCVAL LFFAPDGVLA VPQKPTVSLN PPWNRIFKGE NVTLTCNGSN
FFEVSSMKWF HNGSLSEVAN SSLNIVNADF EDSGEYKCQH QQFDDSEPVH LEVFSDWLLL
QASAEVVMEG QPLFLRCHSW RNWDVYKVIY YKDGEALKYW YENHNISITN TTVEDSGTYY
CTGKLWQLD**Y** ESEPLNITVI KAQHDK HHHHHH (SEQ ID NO:12)

Fusion Proteins

[0134] In some embodiments, the FcεRIα polypeptide is a fusion protein, for example, an FcεRIα polypeptide fused to one or more heterologous polypeptide. Such fusion proteins can comprise at least an FcεRIα IgE binding fragment, for example at least an FcεRIα ECD, fused at the carboxy or amino terminus, to a heterologous polypeptide. The heterologous polypeptide can be any polypeptide, and generally is a polypeptide that confers a specific property to the fusion protein.

[0135] Heterologous polypeptides can provide for secretion, improved stability, or facilitate purification of the FcεRIα polypeptides. Non-limiting examples of such peptide tags include the 6-His tag, Gly/His6/GST tag, thioredoxin tag, hemaglutinin tag, Glylh156 tag, and OmpA signal sequence tag. For example, an extracellular domain of an FcεRIα polypeptide can be fused to a His tag, for example $(His)_6$, including a $Gly(His)_6$-gst tag. The $Gly(His)_6$-gst tag provides for ease of purification of polypeptides encoded by the nucleic acid.

[0136] Using the ECD of each species as described above, different forms of FcεRIα polypeptide may be constructed and expressed in mammalian cells, for example, monomeric forms containing an extracellular domain (residues 1-176) of the receptor, six C-terminal histidine residues, and a signal sequence. For example, FcεRIα polypeptide may comprise a monomeric form containing a native signal sequence at the N-terminus for the ECD, and a HIS6 tag:

cyno FcεR1 (1-176) his monomer

MAPAM ESPTLLCVAL LFFAPDGVLA VPQKPTVSLN PPWNRIFKGE NVTLTCNGSN FFEVSSMKWF HNGSLSEVAN SSLNIVNADF EDSGEYKCQH QQFDDSEPVH LEVFSDWLLL QASAEVVMEG QPLFLRCHSW RNWDVYKVIY YKDGEALKYW YENHNISITN TTVEDSGTYY CTGKLWQLDY ESEPLNITVI KAQHDK(176)HHHHHH (SEQ ID NO: 13)

[0137] FcεRIα polypeptide may also comprise the ECD fused to the signal sequence and first 27 amino acids of the herpes simplex virus (HSV) gD protein shown below.

MGGAAARLGAVILFVVIVGLHGVRGKYALADASLKMADPNRFRGKDLPVLDQLLE (SEQ ID NO: 14)

[0138] In some embodiments, an FcεRIα polypeptide can be any of the three specific fusion proteins, each containing an HSV gD signal sequence (underlined below) fused to an FcεRIα ECD and a 6XHis tag:

gD**cyno** FcεRIα 1-176 6Xhis (SEQ ID NO: 15),

gD**rhesus** FcεRIα 1-176 6Xhis (SEQ ID NO: 16), and

gD**chimp** FcεRIα 1-176 6Xhis (SEQ ID NO: 17).

gDcyno FcεRIα 1-176 6XHis

MGGAA ARLGAVILFV VIVGLHGVRG KYALADASLK MADPNRFRGK DLPVLDQLLE
+1 VPQKPTVSLN PPWNRIFKGE NVTLTCNGSN FFEVSSMKWF HNGSLSEVAN
SSLNIVNADF EDSGEYKCQH QQFDDSEPVH LEVFSDWLLL QASAEVVMEG
QPLFLRCHSW RNWDVYKVIY YKDGEALKYW YENHNISITN TTVEDSGTYY
CTGKLWQLDC ESEPLNITVI KAQHDK HHHHHH (SEQ ID NO: 15)

gDrhesus FcεRIα 1-176 6XHis

MGGAA ARLGAVILFV VIVGLHGVRG KYALADASLK MADPNRFRGK DLPVLDQLLE
+1 VPQKPTVSLN PPWNRIFKGE NVTLTCNGSN FFEVSSMKWF HNGSLSEVAN
SSLNIVNADF EDSGEYKCQH QQFDDSEPVH LEVFSDWLLL QASAEVVMEG
QPLFLRCHSW RNWDVYKVIY YKDGEALKYW YENHNISITN ATVEDSGTYY
CTGKLWQLDC ESEPLNITVI KAQHDKYWLQ FLIPLLVAIL FAVDTGLFIS
TQQQVTFLLK IKRTRKGFKL LNPHPKPNPK SN HHHHHH (SEQ ID NO: 16)

gDchimp FcεRIα 1-176 6XHis

MGGAA ARLGAVILFV VIVGLHGVRG KYALADASLK MADPNRFRGK DLPVLDQLLE
+1 VPQKPKVSLN PPWNRIFKGE NVTLTCNGNN FFEVSSTKWF HNGSLSEETN
SSLNIVNAKF EDSGEYKCQH QQVNESEPVY LEVFSDWLLL QASAEVVMEG
QPLFLRCHGW RNWDVYKVIY YKDGEALKYW YENHNISITN
ATVEDSGTYY CTGKVWQLDY ESEPLNITVI KAPREKYWLQ
FFIPLLVAIL FAVDTGLFIS TQQQVTFLLK IKRTRKGFRL
LTPHPKPNPK NN HHHHHH (SEQ ID NO: 17)

[0139] The FcεRIα polypeptides can also be fused to the immunoglobulin constant domain of an antibody to form immunoadhesin molecules. For example, a fusion polypeptide comprises an extracellular domain of an FcεRIα polypeptide and an Fc portion of an IgG, which may be used in any of the methods provided herein. In some embodiments, the fusion polypeptide FcεRIα-IgG comprises the following sequence:

[1]VPQKPKVSLN PPWNRIFKGE NVTLTCNGNN FFEVSSTKWF HNGSLSEETN SSLNIVNAKF[60]
[61]EDSGEYKCQH QQVNESEPVY LEVFSDWLLL QASAEVVMEG QPLFLRCHGW RNWDVYKVIY[120]
[121]YKDGEALKYW YENHNISITN ATVEDSGTYY CTGKLWQLDY ESEPLNITVI KAPREKYWLD[180]
[181]KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG[240]
[241]VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG[300]
[301]QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD[360]
[361]GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK[406] (SEQ ID NO:18)

[0140] In some embodiments, the FcεRIα polypeptide is a fusion protein comprising an FcεRIα polypeptide fused to an Fc domain of IgG which forms a dimeric form of FcεRIα. Cysteine residues present in the IgG Fc domain permit dimerization of the fusion polypeptide. For example, the FcεRIα-encoding nucleic acid fragment may be fused into the Fc domain of IgG shown below:

Fc domain of IgG

VTDKTHTCPP CPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS
HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK
EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC
LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW
QQGNVFSCSV MHEALHNHYT QKSLSLSPGK (SEQ ID NO: 19)

[0141] The FcεRIα polypeptide may contain a native rhesus signal sequence (SS), a portion of the rhesus FcεRIα ECD (residues V1-A141) and a portion of the cynomolgus FcεRIα ECD (residues T142-K171), fused to the Fc domain of immunoglobulin G protein. The cysteine residues of the IgG domain permit disulfide bonds to form an FcεRIα polypeptide dimer. In some embodiments, the FcεRI polypeptide comprises the FcεRIα-IgG fusion protein with the sequence shown below:

rhesus (1-141)/cyno (142-171) FcεRIα-IgG fusion protein (1-171)

-25 +1
MAPAM ESPTLLCVAL LFFAPDGVLA VPQKPTVSLN PPWNRIFKGE NVTLTCNGSN
FFEVSSMKWF HNGSLSEVAN SSLNIVNADF EDSGEYKCQH QQFDDSEPVH LEVFSDWLLL
QASAEVVMEG QPLFLRCHSW RNWDVYKVIY YKDGEALKYW YENHNISITN **A**TVEDSGTYY
CTGKLWQLDY ESEPLNITVI KVTDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP
EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK
EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI
AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
QKSLSLSPGK (SEQ ID NO: 20)

[0142] Additional chimeric rhesus/cyno FcεRIα-IgG fusion proteins include the fusion proteins made by varying the length of the chimeric FcεRIα polypeptide from 1-171 to 1-178 with increasing lengths of the sequence 171KAQHDKYW178. These include:

rhesus/cyno FcεRIα-IgG fusion protein (1-172) (SEQ ID NO: 21)

rhesus/cyno FcεRIα-IgG fusion protein (1-173) (SEQ ID NO: 22)

rhesus/cyno FcεRIα-IgG fusion protein (1-174) (SEQ ID NO: 23)

rhesus/cyno FcεRIα-IgG fusion protein (1-175) (SEQ ID NO: 24)

rhesus/cyno FcεRIα-IgG fusion protein (1-176) (SEQ ID NO: 25)

rhesus/cyno FcεRIα-IgG fusion protein (1-177) (SEQ ID NO: 26)

rhesus/cyno FcεRIα-IgG fusion protein (1-178) (SEQ ID NO: 27)

[0143] For example, an FcεRIα polypeptide may be rhesus/cyno FcεRIα-IgG fusion protein (1-178) with the sequence shown below:

rhesus/cyno FcεRIα-IgG fusion protein (1-178)

-25 MAPAM ESPTLLCVAL LFFAPDGVLA VPQKPTVSLN PPWNRIFKGE NVTLTCNGSN
FFEVSSMKWF HNGSLSEVAN SSLNIVNADF EDSGEYKCQH QQFDDSEPVH LEVFSDWLLL
QASAEVVMEG QPLFLRCHSW RNWDVYKVIY YKDGEALKYW YENHNISITN ATVEDSGTYY

CTGKLWQLDY ESEPLNITVI **KAQHDKYW**VT DKTHTCPPCP APELLGGPSV FLFPPKPKDT
LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH
QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK
GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
ALHNHYTQKS LSLSPGK (SEQ ID NO: 27)

[0144] FcεRIα polypeptides include polypeptides made by various combinations of cyno, rhesus, chimp, and human FcεRIα polypeptides which produces a variety of chimeric FcεRIα polypeptides. For example, an FcεRIα polypeptide may comprise cyno/human FcεRIα-IgG (1-178) shown below:

cyno/Human FcεRIα-IgG (1-178)

MAPAM ESPTLLCVAL LFFAPDGVLA VPQKPTVSLN PPWNRIFKGE NVTLTCNGSN
FFEVSSMKWF HNGSLSEVAN SSLNIVNADF EDSGEYKCQH QQFDDSEPVH LEVFSDWLLL
QASAEVVMEG QPLFLRCHSW RNWDVYKVIY YKDGEALKYW YENHNISITN ATVEDSGTYY
CTGKVWQLDY ESEPLNITVI KAPREKYWVT DKTHTCPPCP APELLGGPSV FLFPPKPKDT
LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH
QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK
GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
ALHNHYTQKS LSLSPGK (SEQ ID NO: 28)

[0145] In some embodiments, the FcεRIα polypeptide is labeled (such as a biotin, a digoxigenin, a ruthenium, a radiologic, a photoluminescent, a chemiluminescent, a fluorescent, or an electrochemiluminescent label).

[0146] The inventions also provide polynucleotides encoding any of FcεRIα polypeptides described herein. The inventions further provide variant polynucleotide sequences that can be at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical, to a nucleic acid sequence encoding a full length native sequence, a mature sequence lacking a signal sequence, or an extracellular domain of the polypeptide of SEQ ID NOs: 3, 4, 5, or 6, and are less than 100% identical to a nucleic acid sequence encoding a full length native sequence, mature sequence lacking a signal sequence, or an extracellular domain of a native sequence.

[0147] Alterations of the FcεRIα nucleic acid and amino acid sequences can be accomplished by a number of known techniques. For example, mutations can be introduced at particular locations by procedures known to the skilled artisan, such as oligonucleotide-directed mutagenesis, for example, described by Walder et al., 1986, Gene, 42:133; Bauer et al., 1985, Gene 37:73; Craik, 1985, BioTechniques, 12-19; Smith et al., 1981, Genetic

Engineering: Principles and Methods, Plenum Press; U.S. Patent No. 4,518,584, and U.S. Patent No. 4,737,462.

[0148] Methods of making nucleotides encoding FcεRI polypeptides and expression of FcεRI polypeptides in mammalian cells are known to one of ordinary skill in the art. For example, plasmids encoding the constructed forms of FcεRI polypeptides described above can be transfected into 293S human embryonic kidney cells using either calcium phosphate precipitation or Fugene® (Roche, Indianapolis, IN) transfection methods. Supernatants from transfected cell cultures are collected after several days of growth and FcεRI polypeptide can be purified by affinity chromatography using column matrix immobilized antibodies directed against the HSV gD tag (MAb5B6 coupled to controlled pore glass), or using metal chelating resins directed against the 6X histidine fusion tag (Ni-NTA-Agarose, Qiagen, Valencia, CA).

[0149] Polypeptides and proteins (such as, anti-IgE antibodies, mutant antibodies, control anti-drug antibodies, FcεRI polypeptides, etc.) described herein may be produced and isolated or purified using methods known in the art. "Purified" means that a molecule is present in a sample at a concentration of at least 95% by weight, or at least 96%, 97%, 98%, or 99% by weight of the sample in which it is contained. Any recombinant DNA or RNA method can be used to create the host cell that expresses the target polypeptides of the invention, including, but not limited to, transfection, transformation or transduction. Methods and vectors for genetically engineering host cells with the polynucleotides of the present invention, including fragments and variants thereof, are well known in the art, and can be found, for example, in Current Protocols in Molecular Biology, Ausubel et al., eds. (Wiley & Sons, New York, 1988, and updates). Exemplary vectors and host cells are described in the Examples below.

[0150] Host cells are genetically engineered to express the polypeptides described herein. The vectors include DNA encoding any of the polypeptides described herein, operably linked to suitable transcriptional or translational regulatory sequences, such as those derived from a mammalian, microbial, viral, or insect gene. Examples of regulatory sequences include transcriptional promoters, operators, or enhancers, mRNA ribosomal binding sites, and appropriate sequences that control transcription and translation. Nucleotide sequences are operably linked when the regulatory sequence functionally relates to the DNA encoding the target protein.

[0151] Such polypeptides may be included to allow, for example, secretion, improved stability, or facilitated purification of the polypeptide. A polynucleotide sequence encoding an appropriate signal peptide can be incorporated into expression vectors. A DNA sequence

for a signal peptide (secretory leader) may be fused in-frame to the target sequence so that target protein is translated as a fusion protein comprising the signal peptide. The DNA sequence for a signal peptide can replace the native nucleic acid encoding a signal peptide or in addition to the nucleic acid sequence encoding the native sequence signal peptide. A signal peptide that is functional in the intended host cell promotes extracellular secretion of the polypeptide. Preferably, the signal sequence will be cleaved from the target polypeptide upon secretion from the cell. Non-limiting examples of signal sequences that can be used in practicing the invention include the yeast I-factor and the honeybee melatin leader in Sf9 insect cells.

[0152] Selection of suitable vectors to be used for the cloning of polynucleotide molecules encoding the polypeptides will depend upon the host cell in which the vector will be transformed, and, where applicable, the host cell from which the target polypeptide is to be expressed. Suitable host cells for expression of the polypeptides include prokaryotes, yeast, and higher eukaryotic cells.

[0153] Expression vectors for use in prokaryotic hosts generally comprise one or more phenotypic selectable marker genes. Such genes generally encode, *e.g.*, a protein that confers antibiotic resistance or that supplies an auxotrophic requirement. A wide variety of such vectors are readily available from commercial sources. Examples include pSPORT vectors, pGEM vectors (Promega), pPROEX vectors (LTI, Bethesda, MD), Bluescript vectors (Stratagene), and pQE vectors (Qiagen).

[0154] The polypeptides or proteins produced from the host cells may be further purified using known methods.

Methods for detecting anti-drug antibodies of IgE isotype that bind to a therapeutic anti-IgE antibody

[0155] In one aspect, the invention provides methods for detecting anti-drug antibodies of IgE isotype that bind to a therapeutic anti-IgE antibody in a sample from an individual, comprising the steps of: (a) contacting a sample that may contain the anti-drug antibodies with a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the relative binding affinity of the mutant therapeutic antibody to an IgE (such as a human IgE) is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to the IgE; and (b) detecting binding of the anti-drug antibodies to the mutant therapeutic antibody.

[0156] In another aspect, the invention provides methods for detecting anti-drug antibodies of IgE isotype that bind to a therapeutic anti-IgE antibody in a sample from an individual, comprising the steps of: (a) contacting a sample that may contain the anti-drug antibodies with a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the potency of the mutant therapeutic antibody is about 10% or less of the potency of the therapeutic anti-IgE antibody; and (b) detecting binding of the anti-drug antibodies to the mutant therapeutic antibody.

[0157] Methods known in the art may be used to detect binding between the anti-drug antibodies and the mutant therapeutic antibody. ELISA, BIAcore®, Immunocap®, RIA (RadioImmunoAssay) assays may be used. The assays may be homogeneous, semi-homogeneous, or non-homogeneous. For example, most ELISAs utilize antibodies and/or ligands for capture and detection of a target protein. These ELISAs can utilize either homogeneous, semi-homogeneous, or non-homogeneous assay formats to maximize sensitivity or reduce matrix interference.

[0158] Homogeneous assays utilize a format where both the capture agent and detection agent (or ligands) are pre-incubated simultaneously with the matrix sample containing the target protein in a liquid-phase reaction. The capture agent-target protein-detection agent complex is then captured on a solid-phase (such as a streptavidin-coated ELISA plate), washed, and quantitated by detecting the amount of the detection agent captured to the surface (e.g., by the addition of an appropriate substrate solution if the detection agent is labeled with an enzyme). Semi-homogeneous assays utilize a format where the capture agent alone is pre-incubated with the matrix sample in a liquid-phase reaction. This capture agent-target protein complex is then captured on a solid phase, washed, then incubated with a detection agent, washed, and quantitated. Non-homogeneous assays do not utilize any liquid-phase pre-incubation step, but instead utilize sequential steps. The capture agent is captured to the solid-phase, washed, the matrix sample containing the target protein is then added and bound by the capture agent, washed, bound by the detection reagent, washed, and finally quantitated.

[0159] For example, in a non-homogeneous assay, a mutant therapeutic antibody described herein is immobilized to a surface and used as a capture agent for binding to the anti-drug IgE antibodies. The mutant therapeutic antibody may be directly or indirectly immobilized to the surface. In some embodiments, the mutant therapeutic antibody is conjugated to a label and is captured to the surface through a capture agent that specifically binds to the label, wherein the capture agent is immobilized to the surface. The directly or indirectly immobilized

mutant therapeutic antibody is incubated with a sample from an individual that may contain anti-drug antibodies of IgE isotype. Since the mutant antibody is designed to have reduced binding affinity or potency to an IgE, the amount of the IgE antibodies bound to the mutant therapeutic antibody correlates with the anti-drug antibodies in the sample. Binding of the anti-drug IgE antibodies to the immobilized mutant antibody is detected using a detection agent (such as an FcεRI polypeptide that binds to the Fc region of an IgE). An example of such assays is shown in Figures 7.

[0160] Semi-homogenous assays may also be used for detecting anti-drug antibodies of IgE isotype in a sample. In some embodiments, the detection comprises the steps: 1) preincubating a sample from an individual that may contain anti-drug antibodies of IgE isotype with a labeled mutant therapeutic antibody; 2) incubating the preincubated sample with an immobilized molecule (such as streptavidin) that binds to the label on the mutant therapeutic antibody; and 3) detecting binding of the anti-drug antibodies of IgE isotype to the mutant therapeutic antibody using a detection agent (such as an FcεRI polypeptide that binds to the Fc region of an IgE). Washing steps may be included between the incubation steps to remove molecules unbound to the solid phase. Examples of such assays are shown in Figures 11 and 12.

[0161] "Blocking" homogenous assays may also be used for detecting anti-drug antibodies of IgE isotype in a sample. For example, the invention provides methods for detecting an anti-drug antibody of IgE isotype that binds to a therapeutic anti-IgE antibody in a sample, comprising the steps of: (a) preincubating a sample that may contain the anti-drug antibody with (i) the a mutant therapeutic anti-IgE antibody, and (ii) an FcεRIα polypeptide that binds to an Fc region of an IgE (such as an FcεRIα polypeptide comprising an extracellular domain of an FcεRIα subunit); (b) capturing the mutant therapeutic antibody in step (a) to a surface; and (c) detecting binding of the anti-drug antibody to the mutant therapeutic antibody.

[0162] "Blocking" semi-homogenous assays may also be used for detecting anti-drug antibodies of IgE isotype in a sample from an individual. For example, the invention provides methods for detecting an anti-drug antibody of IgE isotype that binds to a therapeutic anti-IgE antibody in a sample, comprising the steps of: (a) preincubating a sample that may contain the anti-drug antibody with an FcεRIα polypeptide that binds to an Fc region of an IgE, (b) incubating the preincubated sample from step (a) with the therapeutic anti-IgE antibody or a mutant thereof; and (c) detecting binding of the anti-drug antibody to the therapeutic anti-IgE antibody or the mutant antibody. The mutant therapeutic antibody may be captured to a surface before or after incubating with the preincubated sample.

[0163] In some embodiments, the sample is preincubated with excess amount of the FcεRIα polypeptide in the blocking assays. As used therein, "excess" amount of FcεRIα polypeptide means that the amount of the FcεRIα polypeptide added is higher than the highest level of baseline total IgE expected in a sample. For example, the baseline total IgE may be from 30 IU/mL to 700 IU/mL for patients with 30 – 150 kg body weight. In some embodiments, the amount of the FcεRIα polypeptide added is at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, or at least 10-fold of the amount of the total IgE in the sample. In some embodiments, the mutant therapeutic antibody comprises at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the relative binding affinity of the mutant therapeutic antibody to an IgE is reduced as compared to the relative binding affinity of the therapeutic anti-IgE antibody to the IgE. In some embodiments, the relative binding affinity of the mutant therapeutic antibody to an IgE is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to the IgE. Any of the mutant therapeutic antibodies described herein may be used.

[0164] In some embodiments, the method comprises the steps: 1) preincubating a sample from an individual that may contain anti-drug antibodies of IgE isotype with a labeled mutant therapeutic antibody in the presence of at least about 10-fold excess of either an unlabeled or labeled FcεRIα polypeptide; 2) incubating the preincubated sample to an immobilized molecule (such as streptavidin) that binds to the label (such as biotin) on the mutant therapeutic antibody; and 3) detecting binding of the anti-drug antibodies of IgE isotype to the mutant therapeutic antibody using a detection agent (such as a labeled anti-human IgE antibody or a labeled antibody specific for the label on the FcεRI polypeptide). Washing steps are included between the incubation steps to remove molecules unbound to the solid phase (such as endogenous non-drug specific IgE). Examples of such assays are shown in Figures 12, 13 and 14.

[0165] In some embodiments, the method comprises the steps: 1) preincubating a sample from an individual that may contain anti-drug antibodies of IgE isotype with at least about 10-fold excess of either an unlabeled or labeled FcεRIα polypeptide; 2) incubating the preincubated sample from step 1) with an immobilized anti-IgE therapeutic antibody or an immobilized mutant therapeutic antibody; 3) detecting binding of the anti-drug antibodies of IgE isotype to the therapeutic antibody or the mutant therapeutic antibody using a detection agent (such as a labeled anti-human IgE antibody or a labeled antibody specific for the label on the FcεRIα polypeptide that binds to the Fc region of an IgE). Washing steps are included

between the incubation steps to remove molecules unbound to the solid phase (such as endogenous non-drug specific IgE). Examples of such assays are shown in Figures 15, 16, and 17.

[0166] In any of the methods described herein, the therapeutic anti-IgE antibody or a mutant antibody may comprise a label or may be conjugated to a label. In some embodiments, the methods comprising a step of capturing the labeled therapeutic anti-IgE antibody or the mutant antibody to a surface before detecting binding of the anti-drug antibody to the therapeutic anti-IgE antibody or the mutant antibody, wherein a capture agent that specifically binds to the label is immobilized to the surface. Any of the solid phase or surface (such as small sheets, Sephadex, polyvinyl chloride, plastic beads, microparticles, assay plates, or test tubes manufactured from polyethylene, and polystyrene) described herein may be used. In some embodiments, the surface is a cellulose polymer sponge (ImmunoCAP®, Phadia). In some embodiments, the surface is not a cellulose polymer sponge (ImmunoCAP®, Phadia). In some embodiments, the therapeutic anti-IgE antibody or the mutant antibody is labeled with biotin, and capture agent is streptavidin. In some embodiments, the FcεRIα polypeptide comprises a label, and the binding of the anti-drug antibody to the therapeutic anti-IgE antibody or the mutant antibody is detected by detecting binding of the FcεRIα polypeptide to the anti-drug antibody.

[0167] In some embodiments of the methods described herein, an FcεRIα polypeptide is used a detecting agent to detect binding of the anti-drug antibodies to the therapeutic anti-IgE antibody or the mutant therapeutic antibody. In some embodiments, the FcεRIα polypeptide comprises a label or is conjugated to a label. In some embodiments, the label on the FcεRIα polypeptide is digoxigenin, and the binding of the FcεRIα polypeptide to the anti-drug antibody is detected using a HRP conjugated anti-digoxigenin antibody. In some embodiments, the label on the FcεRIα polypeptide is ruthenium, and the binding of the FcεRIα polypeptide to the anti-drug antibody is detected using electrochemiluminescence.

[0168] In some embodiments of the methods described herein, an FcεRIα polypeptide is used as a blocking agent to block binding of the non-drug specific IgE in the sample to the therapeutic anti-IgE antibody or the mutant therapeutic antibody. In some embodiments, the binding of the anti-drug antibody to the therapeutic anti-IgE antibody or the mutant antibody is detected by detecting using a HRP conjugated anti-human IgE antibody.

[0169] The samples that may be used in the methods described herein include blood samples from individuals before treatment with an anti-IgE therapeutic antibody or after treatment with an anti-IgE therapeutic antibody. In some embodiments, blood samples are

collected from individuals who have discontinued the anti-IgE antibody treatment for at least 16 weeks. In some embodiments, blood samples are collected from individuals who have discontinued the anti-IgE antibody treatment for at least 16 weeks but not more than 18 months since the last does of the anti-IgE therapeutic antibody. In some embodiments, the samples are serum or plasma samples. The serum or plasma samples can be prepared using standard technology known in the art.

[0170] A positive control may be used to develop the assay, to evaluate assay sensitivity and drug tolerance, and/or used a control for the assay. A positive control may be used in any of the methods described herein. In some embodiments, the assay includes testing a positive control anti-drug antibody. A positive antibody that binds to both the therapeutic anti-IgE antibody and the mutant therapeutic antibody may be used. In some embodiments, the positive control anti-drug antibody binds to the Fab fragment of the anti-IgE antibody. In some embodiments, the positive control anti-drug antibody binds to one or more CDRs of the anti-IgE antibody. In some embodiments, the positive control antibody binds both the therapeutic anti-IgE antibody and the mutant therapeutic antibody with similar affinity. In some embodiments, the relative binding affinity of the positive control antibody to the therapeutic anti-IgE antibody is within about 10-fold, within about 9-fold, within about 8-fold, within about 7-fold, within about 6-fold, within about 5-fold, within about 4-fold, within about 3-fold, or within about 2-fold difference compared to relative binding affinity of the positive control antibody to the mutant therapeutic antibody. In some embodiments, the difference between the relative binding affinities of the positive control antibody to the therapeutic anti-IgE antibody and to the mutant therapeutic antibody is less than about 50%, less than about 40%, less than about 30%, less than about 20%, and/or less than about 10%. For example, a positive control antibody is a chimeric antibody comprising the variable regions from an anti-drug antibody (including a CDR-specific anti-drug antibody) and constant regions from an IgE (such as human IgE). In some embodiments, the control anti-drug antibody is a murine antibody against omalizumab (E25). Examples of anti-E25 antibodies that may be used as a positive control (such as AME2) are described in Example 2. In some embodiments, binding the anti-drug antibodies in a sample to the immobilized mutant antibody and binding of the positive control antibody to the immobilized mutant antibody are detected and compared.

[0171] The heavy and light chain variable region amino acid and nucleic acid sequences of antibody AME2 are shown below.

AME2 heavy chain variable region amino acid sequence (SEQ ID NO:7)

QVQLQQSGAELMKPGASVKISCKATGYTFSSHWIEWVKQRSGHGLEWIGEILPGSGS
INYNEKFKGKATFTADTSSNTAYMQLSSLASEDSAVYYCGREGADYGYDVAMDYW
GQGASVTVSS

AME2 light chain variable region amino acid sequence (SEQ ID NO:8)

QIVITQSPAIMSASPGEKVTITCSATSSVNYMHWFQQKPGTSPKLWIYGTSHLASGVP
ARFSGSGSGTSYSLTISRMEAEDAATYYCQQRSRYPFTFGSGTKLEIKR

AME2 heavy chain variable region nucleic acid sequence (SEQ ID NO:9)

CAAGTTCAACTGCAGCAGTCTGGCGCTGAGCTGATGAAGCCTGGGGCCTCAGTG
AAGATATCCTGCAAGGCTACTGGCTACACATTCAGTAGCCACTGGATAGAGTGG
GTGAAACAGAGGTCTGGACATGGCCTTGAGTGGATTGGAGAGATTCTACCTGGA
AGTGGTAGTATTAATTACAATGAGAAGTTCAAGGGCAAGGCCACATTCACAGCA
GACACATCCTCCAACACAGCCTACATGCAACTCAGCAGCCTGGCATCTGAGGACT
CTGCCGTCTATTATTGTGGAAGAGAGGGGGCCGACTATGGTTACGACGTTGCTAT
GGACTACTGGGGTCAAGGAGCCTCGGTCACCGTCTCCTCG

AME2 light chain variable region nucleic acid sequence (SEQ ID NO:10)

CAAATTGTTATCACCCAGTCTCCAGCAATCATGTCTGCATCTCCAGGGGAGAAGG
TCACCATAACCTGTAGTGCCACCTCAAGTGTAAATTACATGCACTGGTTCCAGCA
GAAGCCAGGCACTTCTCCCAAACTCTGGATTTATGGCACATCCCACCTGGCTTCT
GGAGTCCCTGCTCGCTTCAGTGGCAGTGGATCTGGGACCTCTTACTCTCTCACAA
TCAGCCGAATGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGCAAAGGAGTC
GTTACCCATTCACGTTCGGCTCGGGGACAAAGCTCGAGATCAAACGG

[0172] In some embodiments, the heavy chain variable region of antibody AME2 is fused to a heavy chain constant region of a human IgE and the light chain variable region of antibody AME2 is fused to a light chain constant region of a human IgE to form a chimeric antibody. For the example, the following heavy and light chain constant regions of a human IgE may be used in a chimeric antibody.

A human IgE heavy chain constant region amino acid sequence (SEQ ID NO:29)

ASTQSPSVFPLTRCCKNIPSNATSVTLGCLATGYFPEPVMVTWDTGSLNGTTMTLPAT
TLTLSGHYATISLLTVSGAWAKQMFTCRVAHTPSSTDWVDNKTFSVCSRDFTPPTVK
ILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDXSTASTTQEGELAS
TQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSRPSPFDLFI
RKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGT
RDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLA
CLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAEWEQKD
EFICRAVHEAASPSQTVQRAVSVNPGK

A human IgE light chain constant region amino acid sequence (SEQ ID NO:30)
ADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTD
QDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

[0173] In some embodiments, a capture reagent (e.g., a mutant antibody, an anti-IgE antibody, an FcεRIα polypeptide, or streptavidin) is immobilized to a solid phase by insolubilizing the capture reagent either before the assay procedure, as by adsorption to a water-insoluble matrix or surface (U.S. Pat. No. 3,720,760) or non-covalent or covalent coupling, for example, using glutaraldehyde or carbodiimide cross-linking, with or without prior activation of the support with, for example, nitric acid and a reducing agent as described in U.S. Pat. No. 3,645,852 or in Rotmans et al., 1983, J. Immunol. Methods, 57:87-98, or after the assay procedure. In some embodiments, the capture reagent (e.g., the mutant antibody) after immobilization is available to bind a target molecule (e.g., the anti-drug antibodies) from a sample.

[0174] The solid phase or surface used for immobilization can be any inert support or carrier that is essentially water insoluble and useful in immunoassays, including supports in the form of, for example, surfaces, particles, porous matrices, cellulose polymer sponge (ImmunoCAP®, Phadia), and the like. Examples of commonly used supports include small sheets, Sephadex, polyvinyl chloride, plastic beads, microparticles, assay plates, or test tubes manufactured from polyethylene, polypropylene, polystyrene, and the like. In some embodiments, the solid phase or surface is a cellulose polymer sponge (ImmunoCAP®, Phadia). In some embodiments, the solid phase or surface is not a cellulose polymer sponge (ImmunoCAP®, Phadia). Such supports include 96-well microtiter plates, as well as particulate materials such as filter paper, agarose, cross-linked dextran, and other polysaccharides. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates described in U.S. Pat. Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are suitably employed for capture reagent immobilization. In an embodiment the immobilized capture reagent is coated on a microtiter plate. The preferred solid phase is a multi-well microtiter plate that can be used to analyze several samples at one time.

[0175] The solid phase is coated with the capture reagent (such as a mutant therapeutic antibody described herein) that can be linked by a non-covalent or covalent interaction or physical linkage, as desired. Techniques for attachment include those described in U.S. Pat.

No. 4,376,110 and the references cited therein. If covalent attachment of the capture reagent to the plate is utilized, the plate or other solid phase can be incubated with a cross-linking agent together with the capture reagent. Commonly used cross-linking agents for attaching the capture reagent to the solid phase substrate include, for example, 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate yield photoactivatable intermediates capable of forming cross-links in the presence of light.

[0176] If polystyrene plates are utilized, the wells in the plate are preferably coated with the capture reagent (typically diluted in a buffer such as 0.05 M sodium carbonate or 0.15 M phosphate buffered saline (PBS), pH 7.2 or 7.4) by incubation for at least about 10 hours, more preferably at least overnight, at temperatures of about 4-20°C., more preferably about 4-8°C., and at a pH of about 8-12, more preferably about 9-10, and most preferably about 9.6. If shorter coating times (1-2 hours) are desired, the plate is coated at 37°C or plates with nitrocellulose filter bottoms such, as for example, Millipore MULTISCREEN^{TM}. The plates can be stacked and coated in advance of the assay, allowing for an immunoassay to be carried out simultaneously on several samples in a manual, semi-automatic, or automatic fashion, such as by using robotics. Polystyrene plates can be coated with streptavidin using the method described above.

[0177] The coated plates are typically treated with a blocking agent that binds non-specifically to, and saturates, the binding sites to prevent unwanted binding of free analyte molecules to excess binding sites on the wells of the plate. Examples of appropriate blocking agents include, for example, gelatin, bovine serum albumin, egg albumin, casein, and non-fat milk. The blocking treatment typically takes place under conditions of ambient temperatures for about 1-4 hours, preferably about 1.5 to 3 hours.

[0178] After coating and blocking, the sample to be analyzed is diluted as necessary and added to the immobilized phase. The preferred dilution rate is about 5-15%, preferably about 10%, by volume. Buffers that can be used for dilution include for example (a) phosphate buffered saline (PBS) containing 0.5% BSA, 0.05% TWEEN 20^{TM}, detergent (P20), 5 mM EDTA, 0.25% Chaps surfactant, 0.2% beta-gamma globulin, and 0.35M NaCl, pH 7.0; (b) PBS containing 0.5% BSA and 0.05% P20; (c) PBS containing 0.5% BSA, 0.05% P20, 5 mM EDTA, and 0.35 M NaCl, pH 6.35; (d) PBS containing 0.5% BSA, 0.05% P20, 5 mM EDTA,

0.2% beta-gamma globulin, and 0.35 M NaCl; (e) PBS containing 0.5% BSA, 0.05% P20, 5 mM EDTA, 0.25% Chaps, and 0.35 M NaCl; and (f) PBS containing 0.5% P20.

[0179] For sufficient sensitivity, it is preferred that the immobilized capture reagent is in molar excess of the maximum molar concentration of the analyte (such as anti-drug antibodies of IgE isotype) anticipated in the sample after appropriate dilution. Depending on the analyte, the capture reagent can compete for binding sites with the detecting antibody yielding inaccurate results. Therefore, the final concentration of the capture reagent will normally be determined empirically to maximize the sensitivity of the assay over the range of interest.

[0180] In some embodiments, the assay system has a sensitivity for anti-drug IgE of about 0.1 IU/ml to about 0.5 IU/ml (such as about 0.1 IU/ml, about 0.2 IU/ml, about 0.3 IU/ml, about 0.4 IU/ml, or about 0.5 IU/ml). In some embodiments, the assay system has total IgE tolerance of 700 IU/ml or higher. In some embodiments, the assay system has total IgE tolerance of 800 IU/ml or higher. In some embodiments, the assay system has drug tolerance (such as omalizumab tolerance) of at least about 50 ng/ml (such as about 50 ng/ml to about 200 ng/ml). In some embodiments, the drug tolerance for the assay system is at least about 50 ng/ml, at least about 75 ng/ml, at least about 100 ng/ml, at least about 125 ng/ml, or at least about 150 ng/ml.

[0181] Conditions for incubation of sample and capture reagent are selected to maximize sensitivity of the assay and to minimize dissociation. Incubation time depends primarily on the temperature. For example, the incubation time is from about 0.5 to 3 hours (including1.5-3 hours) at 20-38°C (including 36-38°C), or overnight at room temperature. To maximize the anti-drug IgE sensitivity and the anti-IgE drug tolerance of the assay, incubation times greater than about 10 hours are used if possible. If the sample is a biological fluid (such as blood or serum) incubation times can be lengthened by adding a protease inhibitor to the sample to prevent proteases in the biological fluid from degrading the analyte.

[0182] The pH of the incubation buffer is chosen to maintain a significant level of specific binding of the capture reagent to the analyte being captured. In some embodiments, the pH of the incubation buffer is about 6-9.5 (including pH about 6-7). In some embodiments, the pH of the incubation buffer is about 7.2. Various buffers can be employed to achieve and maintain the desired pH during this step, including borate, phosphate, carbonate, Tris-HCl or Tns-phosphate, acetate, barbital, and the like. The particular buffer employed is usually not critical, however, and in individual assays one buffer may be preferred over another.

[0183] The sample is separated from the immobilized capture reagent with a wash solution to remove uncaptured analyte (such as anti-drug antibodies) from the system. The wash solution is generally a buffer. The incubation buffers described above are suitable wash solutions. The pH of the wash solution is determined as described above for the incubation buffer. In an embodiment, the pH of the wash solution is about 6-9, more preferably about 6-7. Washes can be done one or more times. Minimizing the number of washes, however, to retain molecules that bind the target molecule with low affinity increases the background noise of the assay. In some embodiments, the system is washed three times. The temperature of the wash solution is typically from about 0-40°C, more preferably about 4-30°C. An automated plate washer can be utilized. A cross-linking agent or other suitable agent can be added to the wash solution to covalently attach the captured analyte to the capture reagent.

[0184] Following removal of uncaptured analyte molecules from the system, the captured analyte molecules are contacted with a detecting agent, such as an antibody or an FcεRIα polypeptide, such as at a temperature of about 20-40°C, about 36-38°C, or room temperature. In some embodiments, the analyte is an anti-drug antibody of the IgE isotype, the detecting agent is a labeled FcεRIα-IgG chimeric receptor.

[0185] The temperature and time for contacting the analyte molecule with the detecting agent is dependent primarily on the detection means employed. For example, when horseradish peroxidase (HRP) conjugated to streptavidin (SA-HRP) is used as the means for detection, the detecting agent is preferably incubated with the captured analyte for about 0.5-2 hours, more preferably about 1 hour. The system is washed as described above to remove unbound detecting agent from the system and developed by adding peroxidase substrate and incubating the plate for about 15 minutes at room temperature or until good color is visible. In an embodiment, a molar excess of the detecting agent is added to the system after the unbound analyte has been washed from the system.

[0186] The affinity of the detecting agent must be sufficiently high such that small amounts of analyte can be detected. A fluorimetric or chemilimunescent label moiety has greater sensitivity in immunoassays compared to a conventional colorimetric label. The binding affinity of the selected detecting agent must be considered in view of the binding affinity of the capture agent, such that the detecting agent does not strip the analyte from the capture reagent.

[0187] The label moiety is any detectable functionality that does not interfere with the binding of the captured analyte to the detecting agent. Examples of suitable label moieties include moieties that can be detected directly, such as fluorochrome, chemiluminescent, and

radioactive labels, as well as moieties, such as enzymes, that must be reacted or derivatized to be detected. Examples of such labels include 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. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, beta-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 HPP, lactoperoxidase, or microperoxidase, biotin/avidin, biotin/streptavidin, biotin/Streptavidin-beta-galactosidase with MUG, digoxigenin, ruthenium, spin labels, bacteriophage labels, stable free radicals, and the like.

[0188] Conjugation of the label moiety to the detecting agent, such as for example an antibody or an FcεRIα polypeptide, is a standard manipulative procedure in immunoassay techniques. See, for example, O'Sullivan et al. "Methods for the Preparation of Enzyme-antibody Conjugates for Use in Enzyme Immunoassay," in Methods in Enzymology, ed. J. J. Langone and H. Van Vunakis, Vol. 73 (Academic Press, New York, N.Y., 1981), pp. 147-166. Conventional methods are available to bind the label moiety covalently to proteins or polypeptides. For example, coupling agents such as dialdehydes, carbodiimides, dimaleimides, bis-imidates, bis-diazotized benzidine, and the like can be used to label antibodies with the above-described fluorescent, chemiluminescent, and enzyme labels. See, for example, U.S. Pat. No. 3,940,475 (fluorimetry) and U.S. Pat. No. 3,645,090 (enzymes); Hunter et al., 1962, Nature, 144:945; David et al., 1974, Biochemistry, 13:1014-1021; Pain et al., 1981, J. Immunol Methods, 40:219-230; and Nygren J., 1982, Histochem. and Cytochem., 30:407-412. Preferred labels herein are fluorescent or chemiluminescent to increase amplification and sensitivity to about 5-10 pg/ml. In an embodiment, the label moiety is HRP.

[0189] The amount of analyte bound to the capture reagent is determined by washing away unbound detecting agent from the immobilized phase and measuring the amount of detecting agent bound to the analyte using a detection method appropriate to the label. In an embodiment, the label moiety is an enzyme. In the case of enzyme moieties, the amount of developed color is a direct measurement of the amount of captured analyte. For example, when HRP is the label moiety, color is detected by quantifying the optical density (O.D.) absorbance (e.g., at 450 nm). In another embodiment, the quantity of analyte bound to the

capture reagent is determined in-directly. The signal of an unlabeled detecting agent can be amplified for detection with an anti-detecting agent antibody conjugated to a label moiety. For example, the signal of an unlabeled mouse antibody that binds the target molecule can be amplified with a sheep anti-mouse IgG antibody labeled with HRP. The label moiety is detected using a detection method appropriate to the label. For example, HRP can be detected by reacting HRP with a calorimetric substrate and measuring the optical density of the reacted substrate at 450 nm absorbance.

[0190] The pH and/or temperature of the system can be varied to identify molecules that bind the target molecule.

Methods for assessing or aiding assessment of risk for anaphylaxis to a therapeutic anti-IgE antibody treatment

[0191] The methods described herein may be used to assess or aid assessment of risk for an anaphylactic reaction to the administration of a therapeutic anti-IgE antibody. The methods described herein may also be used for identifying patients having a risk for anaphylactic reaction to the administration of a therapeutic anti-IgE antibody.

[0192] Blood samples from patients treated with a therapeutic anti-IgE antibody (such as E25, omalizumab) with anaphylaxis and patients without hypersensitivity reactions are collected. Data including clinical histories, allergy skin test results and immunogenicity evaluations are collected. The amount of anti-drug antibodies of IgE isotype in the samples are tested using the assays described herein. A correlation between the allergy skin test, anaphylaxis and the level of anti-drug antibodies of IgE isotype is established. Samples will be collected after anaphylaxis or after all participants including controls have discontinued anti-IgE treatment for at least 16 weeks but no more than 18 months. The established correlation can be used to establish a reference level, and can be used to assess or aid assessment of risk of anaphylaxis to a therapeutic anti-IgE antibody before a patient is treated with the therapeutic anti-IgE antibody.

[0193] In one aspect, the invention provides a method for assessing or aiding assessment of risk in a patient for an anaphylactic reaction to the administration of a therapeutic anti-IgE antibody, comprising the steps of: a) detecting the level of anti-drug antibodies of IgE isotype that bind to the therapeutic anti-IgE antibody in a sample from the patient before anti-IgE antibody treatment; and b) comparing the level detected in step a) to a reference level. In some embodiments, patients having the level of anti-drug antibodies of IgE isotype higher than a reference level is excluded from the anti-IgE antibody treatment.

[0194] In another aspect, the invention provides methods of identifying a patient having a risk of anaphylactic reaction to a therapeutic anti-IgE antibody, comprising detecting the presence and/or the level of anti-drug antibodies of IgE isotype in a sample from the patient using any of the methods described herein, wherein the presence and/or the level of the anti-drug antibody in the sample indicates that the patient has a risk of anaphylactic reaction to the therapeutic anti-IgE antibody.

Methods for treating IgE-mediated disorders

[0195] The anti-drug antibodies of IgE isotype in a patient may be assessed using the assay methods described herein before or after the patient is treated with an anti-IgE antibody. The invention provides a method for treating an IgE-mediated disorder in an individual with an anti-IgE antibody comprising comparing the level of anti-drug antibodies of IgE isotype in a sample from the individual to a reference level; and administering an effective amount of the anti-IgE antibody to the individual if the level of anti-drug antibodies in the sample is lower than a reference level. In one aspect, the invention provides a method for identifying patient having high-risk of anaphylaxis comprising comparing the level of anti-drug antibodies of IgE isotype in a sample from an individual to a reference level, wherein the individual is identified as having high-risk of anaphylaxis if the level of anti-drug antibodies in the sample is higher than a reference level. In another aspect, the invention provides methods of treating a patient having an IgE-mediated disorder, comprising the steps of: (a) determining the level of an anti-drug antibody of IgE isotype to a therapeutic anti-IgE antibody in a sample from the patient using any of the methods described herein; (b) administering an effective amount of the therapeutic anti-IgE antibody to the patient if the level of the anti-drug antibody in the sample do not indicate that the patient has a risk of anaphylactic reaction to the therapeutic anti-IgE antibody.

[0196] For the prevention or treatment of IgE-mediated disorders, the appropriate dosage of an anti-IgE antibody, will depend on the type of disease to be treated, the severity and course of the disease, whether the anti-IgE antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the agent, and the discretion of the attending physician. The anti-IgE antibody is suitably administered to the patient at one time or over a series of treatments. In some embodiments, the anti-IgE antibody is omalizumab. The anti-IgE antibody may be in liquid formulations or is reconstituted from lyophilized formulations. Formulations suitable for anti-IgE antibodies

are described in U.S. Pat. No. 6,875,432; and U.S. Pub. Nos. 2004/0197324 and 2005/0158303.

[0197] The anti-IgE antibody is administered to an individual in need of treatment, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes.

[0198] The IgE-mediated disorders include allergic rhinitis, asthma (e.g., allergic asthma and non-allergic asthma), atopic dermatitis, allergic gastroenteropathy, hypersensitivity (e.g., anaphylaxis, urticaria, food allergies etc.), allergic bronchopulmonary aspergillosis, parasitic diseases, interstitial cystitis, hyper-IgE syndrome, ataxia-telangiectasia, Wiskott-Aldrich syndrome, thymic alymphoplasia, IgE myeloma and graft-versus-host reaction. In yet a further specific aspect, the IgE-mediated disorder is food allergy, anaphylaxis, contact dermatitis and allergic purpura.

[0199] The IgE-mediated disorders treatable by the method of the invention also include ataxia-telangiectasia, Churg-Strauss Syndrome, eczema, enteritis, gastroenteropathy, graft-versus-host reaction, hyper-IgE (Job's) syndrome, hypersensitivity (e.g., anaphylactic hypersensitivity, candidiasis, vasculitis), IgE myeloma, inflammatory bowel disease (e.g., Crohn's disease, ulcerative colitis, indeterminate colitis and infectious colitis), mucositis (e.g., oral mucositis, gastrointestinal mucositis, nasal mucositis and proctitis), necrotizing enterocolitis and esophagitis, parasitic diseases (e.g., trypanosomiasis), hypersensitivity vasculitis, urticaria and Wiskott-Aldrich syndrome.

[0200] The IgE-mediated disorders treatable by the method of the invention also include Addison's disease (chronic adrenocortical insufficiency), alopecia, hereditary angioedema, anigioedema (Bannister's disease, angioneurotic edema), ankylosing spondylitis, aplastic anemia, arteritis, amyloidosis, immune disorders, such as autoimmune hemolytic anemia, autoimmune oophoritis, autoimmune orchitis, autoimmune polyendocrine failure, autoimmune hemolytic anemia, autoimmunocytopenia, autoimmune glomerulonephritis, Behcet's disease, bronchitis, Buerger's disease, bullous pemphigoid, Caplan's syndrome (rheumatoid pneumoconiosis), carditis, celiac sprue, Chediak-Higashi syndrome, chronic obstructive lung Disease (COPD), Cogan-Reese syndrome (iridocorneal endothelial syndrome), CREST syndrome, dermatitis herpetiformis (Duhring's disease), diabetes mellitus, eosinophilic fasciitis, eosinophilic nephritis, episcleritis, extrinsic allergic alveolitis, familial paroxysmal polyserositis, Felty's syndrome, fibrosing alveolitis, glomerulonephritis,

Goodpasture's syndrome, granulocytopenia, granuloma, granulomatosis, granuloma myositis, Graves' disease, Guillain-Barre syndrome (polyneuritis), Hashimoto's thyroiditis (lymphadenoid goiter), hemochromatosis, histocytosis, hypereosinophilic syndrome, irritable bowel syndrome, juvenile arthritis, keratitis, leprosy, lupus erythematosus, Lyell's disease, Lyme disease, mixed connective tissue disease, mononeuritis, mononeuritis multiplex, Muckle-Wells syndrome, mucocutaneous lymphoid syndrome (Kawasaki's disease), multicentric reticulohistiocystosis, multiple sclerosis, myasthenia gravis, mycosis fungoides, panninculitis, pemphigoid, pemphigus, pericarditis, polyneuritis, polyarteritis nodoas, psoriasis, psoriatic arthritis, pulmonary arthritis, pulmonary adenomatosis, pulmonary fibrosis, relapsing polychondritis, rheumatic fever, rheumatoid arthritis, rhinosinusitis (sinusitis), sarcoidosis, scleritis, sclerosing cholangitis, serum sickness, Sezary syndrome, Sjogren's syndrome, Stevens-Johnson syndrome, systemic mastocytosis, transplant rejection, thrombocytopenic purpura, thymic alymphoplasia, uveitis, vitiligo, Wegener's granulomatosis.

[0201] The IgE-mediated disorders may be treated by an anti-IgE antibody in combination with known methods of treatments for IgE-mediated disorders, either as combined or additional treatment steps or as additional components of a therapeutic formulation. For example, the treatment includes an anti-IgE antibody in combination with an antihistamine, a sympathomimetic, a bronchodilator, a glucocorticoid, a non-steroidal anti-inflammatory drug, a decongestant, a cough suppressant or an analgesic. In another specific aspect, the anti-IgE antibody is administered in combination with a treatment regimen of allergen desensitization.

D. Kits

[0202] The invention also provide kits for use in the methods described herein.

[0203] In one aspect, the invention provides kits for detecting anti-drug antibodies of IgE isotype that bind to a therapeutic anti-IgE antibody in a sample, comprising a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the relative binding affinity of the mutant therapeutic antibody to an IgE (such as human IgE) is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to the IgE. In some embodiments, the kits further comprise a detecting agent that binds to an Fc region of a human IgE (such as an FcεRIα polypeptide described herein).

[0204] In another aspect, the invention provides kits for detecting anti-drug antibodies of IgE isotype that bind to a therapeutic anti-IgE antibody in a sample, comprising a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the potency of the mutant therapeutic antibody to an IgE is about 10% or less of the potency of the therapeutic anti-IgE antibody to the IgE. In some embodiments, the kits further comprise a detecting agent that binds to an Fc region of a human IgE (such as an FcεRIα polypeptide described herein).

[0205] In another aspect, the invention provides kits for detecting anti-drug antibodies of IgE isotype that bind to a therapeutic anti-IgE antibody in a sample, comprising (a) a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the relative binding affinity of the mutant therapeutic antibody to an IgE (such as human IgE) is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to the IgE; and (b) an FcεRIα polypeptide that binds to an Fc region of a human IgE.

[0206] In another aspect, the invention provides kits for detecting anti-drug antibodies of IgE isotype that bind to a therapeutic anti-IgE antibody in a sample, comprising (a) the therapeutic anti-IgE antibody or a mutant therapeutic antibody thereof, wherein the mutant therapeutic antibody comprises at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the relative binding affinity of the mutant therapeutic antibody to an IgE (such as human IgE) is reduced as compared to the relative binding affinity of the therapeutic anti-IgE antibody to the IgE; and (b) an FcεRIα polypeptide that binds to an Fc region of a human IgE.

[0207] In some embodiments, the kits further comprise a positive control antibody that binds both the therapeutic anti-IgE antibody and the mutant therapeutic antibody. In some embodiments, the positive control antibody binds both the therapeutic anti-IgE antibody and the mutant therapeutic antibody with similar affinity. In some embodiments, the positive control antibody comprises the heavy and light variable regions from an antibody that specific binds to Fab fragment of the anti-IgE antibody and constant regions from a human IgE. In some embodiments, the positive control antibody comprises a heavy chain variable region comprising the amino acid sequence shown in SEQ ID NO:7, and a light chain variable region comprising the amino acid sequence shown in SEQ ID NO:8. In some embodiments, the positive control antibody binds to the complex of Fab fragment of the anti-IgE antibody and IgE.

[0208] The reagents of the kits (such as therapeutic anti-IgE antibody, the mutant therapeutic antibody, the positive control antibody, and/or the FcεRIα polypeptide) may be in a container. In some embodiments, the therapeutic anti-IgE antibody, the mutant therapeutic antibody, positive control antibody, and/or the FcεRIα polypeptide comprise a label.

[0209] In some embodiments, the therapeutic anti-IgE antibody or the mutant therapeutic antibody is immobilized directly or indirectly to a surface. In some embodiments, the therapeutic anti-IgE antibody or the mutant therapeutic antibody is conjugated to a label (such as a biotin). In some embodiments, the therapeutic anti-IgE antibody or the mutant therapeutic antibody is conjugated to a label is captured to a surface through an immobilized capture agent that specifically binds to the label. In some embodiments, the label is biotin and the capture agent is streptavidin.

[0210] In some embodiments, the detecting agent or FcεRIα polypeptide is conjugated to a label (such as a biotin, digoxigenin, or ruthenium). In some embodiments, the detecting agent is a labeled FcεRI polypeptide. In some embodiments, the kit further comprises streptavidin-HRP or Amdex SA-HRP. In some embodiments, the kit further comprises HRP-conjugated anti-digoxigenin antibody for detecting digoxigenin labeled FcεRI polypeptide. In some embodiments, the kit further comprises labeled anti-human IgE antibody (such as a polyclonal antibody or a monoclonal antibody). In some embodiments, the labeled anti-human IgE antibody is a HRP-conjugated anti-human IgE antibody.

[0211] The kits of the invention may further comprise any instructions for use in accordance with any of the methods described herein. In some embodiments, these instructions comprise a description of testing the amount of anti-drug antibodies of IgE isotype in a patient sample according to any methods described herein. The kits may further comprise a description of assessing risk of anaphylaxis of a patient before treatment with the anti-IgE antibody. The instructions may be provided on a label or package insert. Kits may optionally comprise additional components such as buffers and reagents for carrying out the methods described herein.

[0212] The kits of the invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. Also contemplated are packages for use in combination with a specific device, such as a device for signal detection in an ELISA assay.

[0213] The following are examples of the methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

EXAMPLES

Example 1. Preparation of a mutant antibody from anti-IgE antibody omalizumab

[0214] The antibody omalizumab (E25 or rhuMAbE25) is a humanized anti-human IgE antibody described in U.S. Pub. No. 2005/0158303 and U.S. Pat. No. 6,172,213. The amino acid sequences of the heavy and light chain variable region of E25 are provided in Figure 2 in US 6,172,213, and the amino acid sequences of the full length heavy and light chain of E25 are provided in Figure 12 in US 6,172,213. The heavy chain and light chain amino acid sequences of antibody E25 are shown in Figures 1A and 1B. A mutant E25, referred to as E25-AAA mutant, containing three amino acid substitutions in the light chain CDR1 was generated. The mutations are substitutions from D to A at positions 30, 32, 34 shown in SEQ ID NO:1. This mutant antibody is described in Presta et al., J. Immunol. 151:2623-2632, 1993.

[0215] The binding affinity of this mutant antibody to human IgE relative to E25 was tested as shown in Figure 2A. E25 or E25-AAA mutant was immobilized on an ELISA plate, increasing concentration of purified human IgE was added to the plate. Binding of human IgE to E25 or to E25-AAA mutant was detected by a goat anti-human IgE conjugated with a HRP. The OD at 450 nm was measured. These experiments were carried out using known methods. See, e.g., Engvall et al., *Immunochemistry* 8:871-4, 1971; Presta et al., *J. Immunol.* 151:2623-2632, 1993. As shown in Figure 2B, E25-AAA mutant has about 100x less binding affinity to human IgE than E25.

[0216] To compare the primary structure of E25 with E25-AAA mutant, Lys-C peptide mapping was used. Lys-C enzyme was used for digestion so that the 3 amino acid substitutions were all in the same peptide (light chain 1-43). The peptide map profiles showed only two peak differences in the mutant, the parent peptide LC 1-43 that disappeared, and a new peak not present in E25. LC-MS was run and the mass of the new peak in the mutant was confirmed as LC 1-43 with the 3 Ala replacing the 3 Asp. Therefore this analysis confirmed the mutant had the same primary structure of E25, with the exception of the 3 Ala substituting the 3 Asp in the LC.

[0217] The charge distribution of E25-AAA mutant was also studied. The charge distribution of monoclonal antibodies is usually specific to the molecule. In this case the amino acid substitutions in the mutant changed the pI of the molecule significantly (from 7.6 to ~9), therefore the migration time of the mutant was very different. Additionally, differences in the profiles are expected due to the substitution of Asp32 which contributes to

the heterogeneity (it isomerizes) of the charge distribution of E25. The iCIEF (imaged capillary isoelectric focusing) profiles for E25 and E25-AAA mutant were similar, though not identical, with similar amounts of acidic and basic variants.

Example 2. Comparison of binding of anti-drug antibodies to E25 and E25-AAA mutant

[0218] Murine monoclonal antibodies specific to E25 were generated. As shown in Table 2 below, AME1, AME7, AME9, AME2, AME10, AME13, AME4, and AME5 are antibodies that bind to E25. AME1, AME7, AME9, AME2, AME10, AME4, and AME5 are mouse IgG1, and AME13 is a mouse IgG2 antibody. E25 is a humanized antibody derived from MAE11 as described in Example 1 and in Presta et al., *J. Immunol.* 151:2623-2632, 1993. MAE1 is a control anti-human IgE monoclonal antibody, and has different CDRs from MAE11. MAE 1 and MAE11 are mouse IgG antibodies. Control antibody (full length) is an IgG antibody with framework residues similar to E25, but binds a different antigen. Negative binding to MAE1 demonstrated that AMEs were specific to E25 sequences only. To test whether these anti-drug antibodies bind equally well to E25 and E25-AAA mutant, binding assays were carried out using methods known in the art. See, e.g., Engvall et al., *Immunochemistry* 8:871-4, 1971. E25 or E25-AAA mutant was immobilized on a ELISA plate, increasing concentration of a purified anti-drug antibody (AME1, AME7, AME9, AME2, AME10, AME14, AME4, or AME5) was added to the plate. Binding of the anti-drug antibody to E25 or E25-AAA mutant was detected by an anti-mouse IgG antibody conjugated with a HRP. The OD at 450 nm was measured. As shown in Figures 4A-4H and Table 2 below, AME2, AME10, AME4 and AME5 are specific for E25 Fab and are specific for E25 framework region; AME1, AME7, and AME 9 are specific for E25 CDRs; AME13 is specific for E25 framework region. Since AME2, AME10, AME13, AME4, AME5, and AME7 bind to both E25 and E25-AAA mutant, these antibodies may be used to test and screen mutant anti-IgE antibodies that may be used in assay described herein.

Table 2. Mouse antibodies that bind to E25 and E25-AAA mutant

Ab	E25 Full-Length	E25 Fab	Control Ab Full-Length	MAE11[c]	MAE1	E25 Fab/IgE Complex	E25 Full-Length	E25-AAA Mutant Full-Length
AME1	+	+	-	+	-	-	+	-

AME7	+	+	-	+	-	-	+	+/-
AME9	+	+	-	+	-	-	+	-
AME2	+	+	-	-	-	+	+	+
AME10	+	+	-	-	-	-	+	+
AME13	+	-	+	-	-	-	+	+
AME4	+	+	+	-	-	-	+	+
AME5	+	+	+	-	-	+	+	+

Example 3. Preparation of an E25-specific IgE positive control antibody

[0219] Figure 5 shows a positive control antibody that may be used in the assay system described herein. This antibody has the heavy and light chain variable regions from AME2 and constant regions from a human IgE.

[0220] The positive control antibody was tested using an assay shown in Figure 6A. The surface of an ELISA plate was coated with human FcεRIα IgG chimeric receptor. E25-specific IgE positive control antibody was added to the plate and incubated to allow binding to the immobilized receptor. Either E25 or E25-AAA mutant with increasing concentration was added to the plate and incubated to allow binding of E25 or E25-AAA mutant. Binding of E25 or E25-AAA mutant to the plate was measured using HRP-anti-human IgG antibody. The results are shown in Figure 6B. The experiment indicates that the positive control antibody shown in Figure 3 binds equally well to E25 and E25-AAA mutant and may be used in the assay system described herein as a positive control for the assay or for screening additional mutant antibodies.

Example 4. Detection of anti-drug antibodies of IgE isotype in a sample using direct ELISA format

[0221] Figure 7 shows an assay system for detecting E25-specific IgE. E25-AAA mutant antibody is used to coat the surface of an ELISA plate. Alternatively, the mutant antibody is immobilized on the surface of a cellulose polymer sponge (ImmunoCAP® design, Phadia). A patient serum sample is added to the surface and incubated under a condition to allow binding of any E25-specific IgE to E25-AAA mutant. A biotin labeled human FcεRIα-IgG chimeric receptor (e.g., as described in WO 08/028068) is added to the ELISA plate (or ImmunoCAP®) to detect any E25-specific IgE bound to E25-AAA mutant. SA-HRP (streptavidin-horseradish peroxidase conjugate) is added to detect biotin- FcεRI-IgG.

[0222] Alternatively, another detecting system is used for detecting binding E25-specific IgE to E25-AAA mutant. The following steps are used for a direct ELISA assay: a) coating a plate overnight at 2-8°C with E25-AAA mutant; b) adding assay diluent (PBS, 0.5% BSA, 0.05% polysorbate 20, and 0.05% ProClin 300) to the plate and incubating it for 2 hours at room temperature with agitation; c) adding 1:2 diluted serum samples containing E25-specific IgE and non-E25-specific IgE to the plate and incubating it overnight at room temperature with agitation; d) adding biotin-labeled FcεR1-IgG to the plate and incubating it for 1 hour at room temperature with agitation; e) adding Amdex-streptavidin-HRP to the plate and incubating it 1 hour at room temperature with agitation; f) adding tetramethylbenzidine (TMB) substrate to the plate and incubating it for about 15 minutes; and g) adding 1M phosphoric acid to the plate and reading the absorbance at A_{450}-A_{650}. The plate is also washed three times between each of the steps before step f).

Example 5. Detection of anti-drug antibodies of IgE isotype in a sample using semi-homogeneous and homogeneous assays

[0223] Figure 10 shows a semi-homogeneous ELISA format to detect E25-specific IgE in a sample. The following steps are used: a) preincubating serum samples containing E25-specific IgE and non-E25-specific IgE with biotin-labeled E25-AAA mutant for overnight at room temperature with agitation; b) adding assay diluent (PBS, 0.5% BSA, 0.05% Polysorbate 20, and 0.05% ProClin 300) to a streptavidin-coated plate and incubating it for 1-2 hours at room temperature with agitation or using a pre-blocked streptavidin-coated plate (such as Reacti-Bind Streptavidin Coated High Binding Capacity (HBC) Clear 96-well Plate(s) with Super Blocker BSA, Pierce cat. #15500); c) adding the preincubated serum samples to the plate and incubating them for 0.5-2 hours (e.g., 1 hour) at room temperature with agitation; d) adding digoxigenin-labeled FcεR1-IgG to the plate and incubating it for 1 hour at room temperature with agitation; e) adding HRP-labeled anti-digoxigenin antibody to the plate and incubating it for 1 hour at room temperature with agitation; f) adding TMB substrate to the plate and incubating it for about 15 minutes; and g) adding 1M phosphoric acid to the plate and reading the absorbance at A_{450}-A_{650}. The plate is washed three times between steps, for example, after each of the steps of b) to e).

[0224] For example, 1 ug/mL of biotin-labeled E25-AAA Mutant in Assay Diluent (PBS, 0.5% BSA, 0.05% Polysorbate 20, 0.05% ProClin 300) is diluted 1:1 with human serum and pre-incubated together overnight at room temperature with agitation. The 1:2 pre-incubated serum sample is then added to a streptavidin-coated microtiter plate (Pierce cat. #15500),

incubated for 1 hour at room temperature with agitation, then washed. Bound E25-specific IgE is detected by incubation with ~ 250 ng/mL of DIG-labeled FceR1-IgG in Assay Diluent for 1 hour at room temperature with agitation. The plate is washed and incubated with ~ 1:6000 HRP-labeled mouse anti-DIG MAb (Jackson ImmunoResearch cat. #200-032-156) in Assay Diluent for 1 hour at room temperature with agitation. The plate is washed a final time and incubated with TMB substrate for 15-30 minutes for color development and measurement.

[0225] Figure 11 shows a semi-homogeneous MSD-ECLA format to detect E25-specific IgE in a sample. The following steps are used: a) preincubating serum samples containing E25-specific IgE and non-E25-specific IgE with biotin-labeled mutant E25 (such as E25-AAA mutant) for overnight at room temperature with agitation; b) adding assay diluent (PBS, 0.5% BSA, 0.05% Polysorbate 20, and 0.05% ProClin 300) to a MSD streptavidin-coated plate (Meso Scale Discovery (MSD), Gaithersburg, MD, USA) and incubating it for 1-2 hours at room temperature with agitation; c) adding the preincubated serum samples to the streptavidin-coated plate and incubating it for 1-2 hours at room temperature with agitation; d) adding ruthenium-labeled FcεR1-IgG and incubating for 1-2 hours at room temperature with agitation; and e) adding MSD TPA read buffer and immediately reading the signal. The plate is washed between steps, for example, after each of the steps of b) to d).

[0226] Figure 12 shows a "blocking" homogeneous ELISA format to detect E25-specific IgE in a sample. The following steps are used: a) preincubating serum samples containing E25-specific IgE and non-E25 specific IgE with a biotin-labeled mutant E25 (such as E25-AAA mutant) and a greater than 10 fold excess FcεR1-IgG for overnight at room temperature with agitation; b) adding assay diluent (PBS, 0.5% BSA, 0.05% Polysorbate 20, and 0.05% ProClin 300) to a streptavidin-coated plate and incubating it for 1-2 hours at room temperature with agitation; c) adding the preincubated serum samples to the plate and incubating it for 1-2 hours at room temperature with agitation; d) adding HRP-labeled anti-Human IgE antibody to the plate and incubating it for 1-2 hours at room temperature with agitation; e) adding TMB substrate to the plate and incubating it for about 15 minutes; and f) adding 1M phosphoric acid to the plate and reading the absorbance at A_{450}-A_{650}. The plate is washed between steps, for example, after each of the steps of b) to d).

[0227] Figure 13 shows a "blocking" homogeneous ELISA format to detect E25-specific IgE in a sample. The following steps are used: a) preincubating serum samples containing E25-specific IgE and non-E25 specific IgE with a biotin-labeled mutant-E25 (such as E25-AAA mutant) and greater than 10-fold excess of digoxigenin-labeled FcεR1-IgG for

overnight at room temperature with agitation; b) adding assay diluent (PBS, 0.5% BSA, 0.05% Polysorbate 20, and 0.05% ProClin 300) to a streptavidin-coated plate and incubating it for 1-2 hours at room temperature with agitation; c) adding the preincubated serum samples to the plate and incubating it for 1-2 hours at room temperature with agitation; d) adding HRP-labeled anti-digoxigenin antibody to the plate and incubating it for 1-2 hours at room temperature with agitation; e) adding TMB substrate to the plate and incubating for about 15 minutes; and f) adding 1M phosphoric acid to the plate and reading the absorbance at A_{450}-A_{650}. The plate is washed between steps, for example, after each of the steps of b) to d).

[0228] Figure 14 shows a homogeneous "blocking" MSD-ECLA format to detect E25-specific IgE in a sample. The following steps are used: a) preincubating serum samples containing E25-specific IgE and non-E25 specific IgE with a biotin-labeled mutant E25 (such as E25-AAA mutant) and a greater than 10 fold excess of ruthenium-labeled FcεR1-IgG for overnight at room temperature with agitation; b) adding assay diluent (PBS, 0.5% BSA, 0.05% Polysorbate 20, and 0.05% ProClin 300) to a streptavidin-coated plate and incubating it for 1-2 hours at room temperature with agitation; c) adding the preincubated serum samples to the plate and incubating it for 1-2 hours at room temperature with agitation; d) adding MSD TPA read buffer and immediately reading the signal. The plate is washed between steps, for example, after each of the steps of b) to c).

[0229] Figure 15 shows a semi-homogeneous "blocking" ELISA format to detect E25-specific IgE in a sample. The following steps are used: a) coating a plate overnight at 2-8°C with E25 (or an E25 mutant (such as E25-AAA mutant)) (Fig. 15, right panel) or adding biotin-labeled E25 (or a biotin-labeled E25 mutant (such as E25-AAA mutant)) (Fig. 15, left panel) to a pre-coated streptavidin plate and incubating it for 1-2 hours at room temperature with agitation; b) adding assay diluent (PBS, 0.5% BSA, 0.05% polysorbate 20, and 0.05% ProClin 300) to the plate and incubating it for 2 hours at room temperature with agitation; c) preincubating serum samples containing E25-specific IgE and non-E25 specific IgE with greater than 10-fold excess of unlabeled FcεR1-IgG and incubating them overnight at room temperature with agitation; d) adding the preincubated serum samples to the plate and incubating it for 1-2 hours at room temperature with agitation; e) adding HRP-labeled anti-human IgE antibody to the plate and incubating it for 1-2 hours at room temperature with agitation; f) adding TMB substrate to the plate and incubating for about 15 minutes; and g) adding 1M phosphoric acid to the plate and reading the absorbance at A_{450}-A_{650}. The plate is washed between steps, for example, after each of the steps of a), b), d), and e).

[0230] Figure 16 shows a semi-homogeneous "blocking" ELISA format to detect E25-specific IgE in a sample. The following steps are used: a) coating a plate overnight at 2-8°C with E25 (or an E25 mutant (such as E25-AAA mutant)) (Fig. 16, right panel) or adding biotin-labeled E25 (or a biotin-labeled E25 mutant (such as E25-AAA mutant)) (Fig. 16, left panel) to a pre-coated streptavidin plate and incubating it for 1-2 hours at room temperature with agitation; b) adding assay diluent (PBS, 0.5% BSA, 0.05% polysorbate 20, and 0.05% ProClin 300) to the plate and incubating it for 2 hours at room temperature with agitation; c) preincubating serum samples containing E25-specific IgE and non-E25 specific IgE with greater than 10-fold excess of digoxigenin-labeled FcεR1-IgG for overnight at room temperature with agitation; d) adding the preincubated serum samples to the plate and incubating it for 1-2 hours at room temperature with agitation; e) adding HRP-labeled anti-digoxigenin antibody to the plate and incubating it for 1-2 hours at room temperature with agitation; f) adding TMB substrate to the plate and incubating for about 15 minutes; and g) adding 1M phosphoric acid to the plate and reading the absorbance at A_{450}-A_{650}. The plate is washed between steps, for example, after each of the steps of a), b), d) and e).

[0231] Figure 17 shows a semi-homogeneous "blocking" MSD-ECLA format to detect E25-specific IgE in a sample. The following steps are used: a) coating a plate overnight at 2-8°C with E25 (or an E25 mutant (such as E25-AAA mutant)) (Fig. 17, right panel) or adding biotin-labeled E25 (or a biotin-labeled E25 mutant) (Fig. 17, left panel) to a pre-coated streptavidin plate and incubating it for 1-2 hours at room temperature with agitation; b) adding assay diluent (PBS, 0.5% BSA, 0.05% polysorbate 20, and 0.05% ProClin 300) to the plate and incubating it for 2 hours at room temperature with agitation; c) preincubating serum samples containing E25-specific IgE and non-E25 specific IgE with greater than 10-fold excess of ruthenium-labeled FcεR1-IgG for overnight at room temperature with agitation; d) adding the preincubated serum samples to the plate and incubating it for 1-2 hours at room temperature with agitation; and e) adding MSD TPA read buffer and immediately reading the signal. The plate is washed between steps, for example, after each of the steps of a), b) and d).

Example 6. Assay Sensitivity for Anti-drug-specific Antibody of IgE Isotype

[0232] The sensitivity for E25 specific IgE antibodies of the assay system described in Example 4 (Figure 7) was determined. A microtiter plate was coated overnight at 4°C with E25-AAA Mutant in 0.05M Na Carbonate Buffer, pH 9.6, washed 3X with Wash Buffer (PBS, 0.05% Polysorbate 20, pH 7.2) and then blocked with Assay Diluent (PBS, 0.05%

Polysorbate 20, 0.5% BSA, 0.05% ProClin 300, pH 7.2) for two hours at room temperature. A E25-specific IgE (Positive Control shown in Figure 5) Standard Curve was prepared by adding 0.4 - 1000 ng/mL of Positive Control (PC) to neat normal human serum pool (NHS Pool) and then diluting each standard sample 1:2 in Assay Diluent. The 1:2 Positive Control Standard Curve Samples were added to the E25-AAA Mutant coated microtiter plate and allowed to incubate overnight at room temperature with agitation. The microtiter plate was then washed 6X with Wash Buffer.

[0233] Biotin-labeled rhuFcεR1-IgG diluted in Assay Diluent (PBS, 0.5% BSA, 0.05% Polysorbate 20, and 0.05% ProClin 300) was added to the microtiter plate and allowed to incubate for 1 hour at room temperature with agitation. The microtiter plate was then washed 6X with Wash Buffer. Amdex Streptavidin-Horseradish Peroxidase (Amdex SA-HRP) diluted in Assay Diluent was added to the microtiter plate and allowed to incubate for 1 hour at room temperature with agitation. The microtiter plate was then washed 6X with Wash Buffer. TMB Substrate was then added to the microtiter plate and allowed to incubate for 15 minutes at room temperature. Phosphoric Acid was then added to the microtiter plate to stop the color development and the absorbance signal of each well read using a plate reader at 450 nm with a 650 nm reference.

[0234] The E25-specific IgE (PC) Standard Curve is shown in Figure 8. The minimum quantifiable concentration (MQC) of E25-specific IgE antibodies was 0.2 IU/ml (0.48 ng/ml) for this assay system.

[0235] The sensitivity for E25 specific IgE antibodies of the assay system described in Figure 10 was determined. A E25-specific IgE (Positive Control shown in Figure 5) Standard Curve was prepared by adding 0.1 - 100 IU/mL of Positive Control (PC) to neat normal human serum pool (NHS Pool) and then diluting each standard sample 1:2 in Assay Diluent (PBS, 0.05% Polysorbate 20, 0.5% BSA, 0.05% ProClin 300, pH 7.2) containing 1 µg/mL of biotin-labeled E25-AAA Mutant. The 1:2 Positive Control Standard Curve Samples were allowed to pre-incubate overnight at room temperature with agitation. A microtiter plate pre-coated with streptavidin (Pierce cat. #15125) was washed 3X with Wash Buffer (PBS, 0.05% Polysorbate 20, pH 7.2). The pre-incubated 1:2 Positive Control Standard Curve Samples were added to the streptavidin coated microtiter plate and allowed to incubate for 1 hour at room temperature with agitation. The microtiter plate was then washed 3X with Wash Buffer. Digoxigenin-labeled rhuFcεR1-IgG diluted in Assay Diluent was added to the microtiter plate and allowed to incubate for 1 hour at room temperature with agitation. The microtiter plate was then washed 3X with Wash Buffer. Anti-Digoxigenin

monoclonal antibody-Horseradish Peroxidase (HRP-Anti-DIG MAb, Jackson ImmunoResearch Laboratories Inc. cat. #200-032-156) diluted in Assay Diluent was added to the microtiter plate and allowed to incubate for 1 hour at room temperature with agitation. The microtiter plate was then washed 3X with Wash Buffer. TMB Substrate was then added to the microtiter plate and allowed to incubate for 15 minutes at room temperature. Phosphoric Acid was then added to the microtiter plate to stop the color development and the absorbance signal of each well read using a plate reader at 450 nm with a 650 nm reference. The E25-specific IgE (PC) Standard Curve is shown in Table 3 below. The minimum quantifiable concentration (MQC) of E25-specific IgE antibodies was 0.1 IU/ml (0.24 ng/ml) for this assay system. The method for Table 4 below is the same as described above for Table 3 with the following changes: 1) A E25-specific IgE (Positive Control shown in Figure 5) Standard Curve was prepared by adding 0.1 – 6.4 IU/mL instead of 0.1 – 100 IU/mL of Positive Control (PC) to neat normal human serum pool (NHS Pool).; and 2) The pre-coated streptavidin plate was Pierce cat. #15500 instead of Pierce cat. #15125.

Table 3. Semi-homogeneous ELISA format sensitivity

Xolair-Specific IgE, IU/mL	Pierce SA-Plate	
	OD	Signal/ Noise Ratio
100	3.905	92.8
30	3.899	92.6
10	3.379	80.3
3	1.365	32.4
1	0.472	11.2
0.3	0.182	4.3
0.1	**0.087**	**2.1**
0	0.042	1.00

Standard Curve in NHS Pool with about 159 IU/ml total IgE.

Table 4. Semi-homogeneous ELISA format sensitivity

Xolair-Specific IgE, IU/mL	Pierce SA-Plate	
	OD	Signal/ Noise Ratio
6.4	1.708	33.7
3.2	1.029	20.3
1.6	0.575	11.4
0.8	0.287	5.7
0.4	0.167	3.3
0.2	0.109	2.2
0.1	**0.078**	**1.5**
0	0.051	1.00

Example 7. Drug-Tolerance of Assay System for Detection of Anti-drug-Specific Antibody of IgE Isotype

[0236] The drug tolerance of the assay system described in Example 4 (Figure 7) was tested in the presence of 0.8 IU/ml (2 ng/ml) of Positive Control (E25-specific IgE shown in Figure 5) and increasing concentrations of E25. Microtiter plates were coated with E25-AAA mutant as described in Example 6. E25 Drug-Tolerance Test Samples were prepared by adding 1 - 1000 ng/mL E25 to neat NHS Pool containing 2 ng/mL of PC and then diluting each drug-tolerance sample 1:2 in Assay Diluent. The 1:2 E25 Drug-Tolerance Samples were then added to the E25-AAA Mutant coated microtiter plate and further processed to detect E25 specific antibodies as described in Example 6. The results of this assay are shown in Figure 9. In the presence of 0.8 IU (2 ng/ml) of E25-specific antibodies, the E25 tolerance of the assay was ~130ng/ml E25.

[0237] The drug tolerance of the assay system described in Figure 10 was tested in the presence of 0.2, 1, and 5 IU/ml (0.48, 2.4, and 12 ng/ml) of Positive Control (E25-specific IgE shown in Figure 5) and 0, 10, 50, and 150 ng/mL concentrations of E25. A E25-specific IgE (Positive Control) Standard Curve was prepared by adding 0.1 – 6.4 IU/mL of Positive Control (PC) to neat normal human serum pool (NHS Pool). E25 Drug-Tolerance Test Samples were prepared by adding 0, 10, 50, and 150 ng/mL E25 to neat NHS Pool or 3 individual Allergic Asthma human sera with up to 812 IU/ml of non-specific IgE containing 0.2, 1, and 5 IU/ml of PC. Both the Standard Curve and Drug Tolerance samples were then diluted 1:2 in Assay Diluent (PBS, 0.05% Polysorbate 20, 0.5% BSA, 0.05% ProClin 300, pH 7.2) containing 1 ug/mL of biotin-labeled E25-AAA Mutant. The 1:2 Samples were allowed to pre-incubate overnight at room temperature with agitation. A microtiter plate pre-coated with streptavidin (Pierce cat. #15500) was washed 3X with Wash Buffer (PBS, 0.05%

Polysorbate 20, pH 7.2). The pre-incubated 1:2 Samples were added to the streptavidin coated microtiter plate and allowed to incubate for 1 hour at room temperature with agitation. The microtiter plate was then washed 3X with Wash Buffer. Digoxigenin-labeled rhuFcεR1-IgG diluted in Assay Diluent was added to the microtiter plate and allowed to incubate for 1 hour at room temperature with agitation. The microtiter plate was then washed 3X with Wash Buffer. Anti-Digoxigenin monoclonal antibody-Horseradish Peroxidase (HRP-Anti-DIG MAb, Jackson ImmunoResearch Laboratories Inc. cat. #200-032-156) diluted in Assay Diluent was added to the microtiter plate and allowed to incubate for 1 hour at room temperature with agitation. The microtiter plate was then washed 3X with Wash Buffer. TMB Substrate was then added to the microtiter plate and allowed to incubate for 15 minutes at room temperature. Phosphoric Acid was then added to the microtiter plate to stop the color development and the absorbance signal of each well read using a plate reader at 450 nm with a 650 nm reference. The results of this assay are shown in Table 5 below. In the presence of 0.2 IU (0.48 ng/ml) of E25-specific antibodies, the E25 tolerance of the assay was ~50 ng/ml E25.

Table 5. Semi-homogeneous ELISA format drug tolerance

Serum Total IgE, IU/Ml	E25-Specific IgE Added, IU/mL	E25 Added, ng/mL	Drug-specific IgE Detected, IU/mL
1 = 107 IU/mL	0.2	0	0.26
		16.7	0.17
		50	**0.13**
		150	QNS
Pool = 159 IU/mL	0.2	0	0.32
		16.7	0.24
		50	**0.18**
		150	< 0.1
5 = 419 IU/mL	0.2	0	0.37
		16.7	0.29
		50	**0.17**
		150	< 0.1
7 = 812 IU/mL	0.2	0	0.37
		16.7	0.33
		50	0.26
		150	**0.14**

[0238] The total IgE interference for the assay system described in Figure 10 was also tested. A E25-specific IgE (Positive Control) Standard Curve was prepared by adding 0.1 –

6.4 IU/mL of Positive Control (PC) to neat normal human serum pool (NHS Pool). Ten Total IgE Interference Samples consisting of 9 human serum samples from individuals diagnosed with Allergic Asthma (Sera provided by the company Bioreclamation, Westbury, NY) and a normal human serum pool with varying Total IgE levels of 107 – 2446 IU/mL were chosen for analysis. Both the Standard Curve and the ten Total IgE Interference samples were then diluted 1:2 in Assay Diluent (PBS, 0.05% Polysorbate 20, 0.5% BSA, 0.05% ProClin 300, pH 7.2) containing 1 ug/mL of biotin-labeled E25-AAA Mutant. The 1:2 Samples were allowed to pre-incubate overnight at room temperature with agitation. A microtiter plate pre-coated with streptavidin (Pierce cat. #15500) was washed 3X with Wash Buffer (PBS, 0.05% Polysorbate 20, pH 7.2). The pre-incubated 1:2 Samples were added to the streptavidin coated microtiter plate and allowed to incubate for 1 hour at room temperature with agitation. The microtiter plate was then washed 3X with Wash Buffer. Digoxigenin-labeled rhuFcεR1-IgG diluted in Assay Diluent was added to the microtiter plate and allowed to incubate for 1 hour at room temperature with agitation. The microtiter plate was then washed 3X with Wash Buffer. Anti-Digoxigenin monoclonal antibody-Horseradish Peroxidase (HRP-Anti-DIG MAb, Jackson ImmunoResearch Laboratories Inc. cat. #200-032-156) diluted in Assay Diluent was added to the microtiter plate and allowed to incubate for 1 hour at room temperature with agitation. The microtiter plate was then washed 3X with Wash Buffer. TMB Substrate was then added to the microtiter plate and allowed to incubate for 15 minutes at room temperature. Phosphoric Acid was then added to the microtiter plate to stop the color development and the absorbance signal of each well read using a plate reader at 450 nm with a 650 nm reference.

[0239] Table 6 below shows that there was no total IgE interference if total IgE in the sample was at ≤ 800 IU/ml.

Table 6. Semi-homogeneous ELISA format total IgE interference

Serum Total IgE, IU/mL	OD	Non-Specific IgE Detected, IU/mL
1 = 107 IU/mL	0.034	< 0.1
2 = 145 IU/mL	0.042	< 0.1
Pool = 159 IU/mL	0.051	< 0.1
3 = 213 IU/mL	0.041	< 0.1
4 = 286 IU/mL	0.050	< 0.1
5 = 419 IU/mL	0.050	< 0.1
6 = 664 IU/mL	0.048	< 0.1

7 = 812 IU/mL	0.052	< 0.1
8 = 1767 IU/mL	0.084	0.12
9 = 1855 IU/mL	0.115	0.23
10 = 2446 IU/mL	0.128	0.28

[0240] The accuracy of the assay system described in Figure 10 was also tested. The accuracy of the assay system described in Figure 10 was tested in the presence of 0, 0.2, 1, and 5 IU/ml (0.48, 2.4, and 12 ng/ml) of Positive Control (E25-specific IgE shown in Figure 5). A E25-specific IgE (Positive Control) Standard Curve was prepared by adding 0.1 – 6.4 IU/mL of Positive Control (PC) to neat normal human serum pool (NHS Pool). Accuracy Test Samples were prepared by adding 0, 0.2, 1, and 5 IU/ml of PC to neat NHS Pool or 3 individual Allergic Asthma human sera with up to 812 IU/ml of non-specific IgE. Both the Standard Curve and Accuracy samples were then diluted 1:2 in Assay Diluent (PBS, 0.05% Polysorbate 20, 0.5% BSA, 0.05% ProClin 300, pH 7.2) containing 1 ug/mL of biotin-labeled E25-AAA Mutant. The 1:2 Samples were allowed to pre-incubate overnight at room temperature with agitation. A microtiter plate pre-coated with streptavidin (Pierce cat. #15500) was washed 3X with Wash Buffer (PBS, 0.05% Polysorbate 20, pH 7.2). The pre-incubated 1:2 Samples were added to the streptavidin coated microtiter plate and allowed to incubate for 1 hour at room temperature with agitation. The microtiter plate was then washed 3X with Wash Buffer. Digoxigenin-labeled rhuFcεR1-IgG diluted in Assay Diluent was added to the microtiter plate and allowed to incubate for 1 hour at room temperature with agitation. The microtiter plate was then washed 3X with Wash Buffer. Anti-Digoxigenin monoclonal antibody-Horseradish Peroxidase (HRP-Anti-DIG MAb, Jackson ImmunoResearch Laboratories Inc. cat. #200-032-156) diluted in Assay Diluent was added to the microtiter plate and allowed to incubate for 1 hour at room temperature with agitation. The microtiter plate was then washed 3X with Wash Buffer. TMB Substrate was then added to the microtiter plate and allowed to incubate for 15 minutes at room temperature. Phosphoric Acid was then added to the microtiter plate to stop the color development and the absorbance signal of each well read using a plate reader at 450 nm with a 650 nm reference. The results are shown in Table 7 below. There seems to be a trend toward over-recovery of IgE with increasing levels of total IgE.

Table 7. Semi-homogeneous ELISA format accuracy

Serum Total IgE, IU/mL	E25-Specific IgE Added, IU/mL	Drug-specific IgE Detected, IU/mL	% Recovery of Expected IgE
1 = 107 IU/mL	0	< 0.1	
	0.2	0.26	130
	1	1.20	120
	5	4.00	80
Pool = 159 IU/mL	0	< 0.1	
	0.2	0.32	160
	1	1.19	119
	5	4.35	87
5 = 419 IU/mL	0	< 0.1	
	0.2	0.37	185
	1	1.44	144
	5	5.87	117
7 = 812 IU/mL	0	< 0.1	
	0.2	0.37	185
	1	1.33	133
	5	5.68	114

[0241] The Total IgE (non-specific IgE) levels of the individual Allergic Asthma sera were determined by the sera vendor (Bioreclamation) using the commercial Total IgE assay from Phadia.

[0242] The Total IgE level of the NHS Pool was determined using a method for detecting total free IgE in a human serum sample. Samples drawn prior to administration of E25 were incubated with a plate coated with rhuFcεRI-IgG. Binding between IgE in the sample and the rhuFcεRI-IgG was detected by adding biotin-conjugated anti-human IgE antibodies to the plate, and followed by adding streptavidin-conjugated-beta-galactosidase reagent. The plate was washed and incubated with 0.34 mg/mL MUG (4-methylumbelliferyl b-D-galactoside) in 0.1 M Sodium Phosphate, 1 mM $MgCl_2$, pH 7.5. This reaction was then stopped with the addition of 0.3M Glycine, pH 10.5 and the fluorescent signal read. The signal correlates with the level of IgE in the serum sample.

[0243] 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.

CLAIMS

What is claimed is:

1. A method for detecting an anti-drug antibody of IgE isotype that binds to a therapeutic anti-IgE antibody in a sample, comprising the steps of:

(a) contacting a sample that may contain the anti-drug antibody with a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the relative binding affinity of the mutant therapeutic antibody to human IgE is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE; and

(b) detecting binding of the anti-drug antibody to the mutant therapeutic antibody.

2. The method of claim 1, wherein the relative binding affinity of the mutant therapeutic antibody to the human IgE is about 5% or less of the relative binding affinity of the therapeutic anti-IgE antibody to the human IgE.

3. The method of claim 1 or 2, wherein the relative binding affinity of the mutant therapeutic antibody to the human IgE is about 2.5% or less of the relative binding affinity of the therapeutic anti-IgE antibody to the human IgE.

4. The method of any one of claims 1-3, wherein the relative binding affinity of the mutant therapeutic antibody to the human IgE is about 1% or less of the relative binding affinity of the therapeutic anti-IgE antibody to the human IgE.

5. The method of any one of claims 1-4, wherein the relative binding affinity is measured by comparing the binding to the human IgE in an ELISA assay.

6. A method for detecting an anti-drug antibody of IgE isotype that binds to a therapeutic anti-IgE antibody in a sample, comprising the steps of:

(a) contacting a sample that may contain the anti-drug antibody with a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the potency of the mutant therapeutic antibody to human IgE is about 10% or less of the potency of the therapeutic anti-IgE antibody to said human IgE; and

(b) detecting binding of the anti-drug antibody to the mutant therapeutic antibody.

7. The method of any one of claims 1-6, wherein the mutant therapeutic antibody comprises one, two, three, four, five, or six amino acid mutations in the CDR sequences of the heavy and/or light chain of the therapeutic anti-IgE antibody.

8. The method of any one of claims 1-7, wherein the therapeutic anti-IgE antibody is omalizumab, and the mutant therapeutic antibody comprises one, two, or three amino acid mutations in the first CDR of the light chain.

9. The method of claim 8, wherein the mutant therapeutic antibody comprises the heavy chain amino acid sequence of SEQ ID NO:2 and the light chain amino acid sequence of SEQ ID NO:1, wherein amino acid Asp at positions 30, 32, and 34 are substituted in the light chain.

10. The method of claim 9, wherein the mutant therapeutic antibody comprises the heavy chain amino acid sequence of SEQ ID NO:2 and the light chain amino acid sequence of SEQ ID NO:1 with amino acid substitutions of Asp to Ala at positions 30, 32, and 34.

11. The method of any one of claims 1-10, wherein the mutant therapeutic antibody is captured to a surface.

12. The method of claim 11, wherein the mutant therapeutic antibody is directly immobilized to the surface.

13. The method of claim 11, wherein the mutant therapeutic antibody is labeled and is captured to the surface through a capture agent that specifically binds to the label, wherein the capture agent is immobilized to the surface.

14. The method of claim 13, wherein the label is biotin and the capture agent is streptavidin.

15. The method of claim 13, wherein the label is digoxigenin and the capture agent is an anti-digoxigenin antibody.

16. The method of any one of claims 1-15, wherein the sample is contacted with the mutant therapeutic antibody that is captured to a surface.

17. The method of any one of claims 1-15, wherein the sample is contacted with the mutant therapeutic antibody before the mutant therapeutic antibody is captured to a surface.

18. The method of any one of claims 1-17, wherein the binding of the anti-drug antibodies to the mutant therapeutic antibody is detected with a detecting agent.

19. The method of claim 18, wherein the detecting agent is an FcεRIα polypeptide that binds to an Fc region of a human IgE.

20. The method of claim 19, wherein the FcεRIα polypeptide comprises an extracellular domain of an FcεRIα subunit.

21. The method of claim 20, wherein the FcεRIα polypeptide comprises an extracellular domain of an FcεRIα subunit fused to an IgG constant region.

22. The method of any one of claims 19-21, wherein the FcεRIα polypeptide is labeled.

23. The method of claim 22, wherein the label on the FcεRIα polypeptide is selected from the group consisting of biotin, digoxigenin, ruthenium, a radiologic label, a photoluminescent label, a chemiluminescent label, a fluorescent label, an electrochemiluminescent label, and an enzyme label.

24. The method of claim 22 or 23, wherein the label on the FcεRIα polypeptide is detected by a second detecting agent that specifically binds to the label on the FcεRIα polypeptide.

25. The method of any one of claims 1-7 and 11-24, wherein the therapeutic anti-IgE antibody is omalizumab.

26. The method of any one of claims 1-25, wherein the sample contains human serum or plasma.

27. The method of claim 26, wherein the serum or plasma contains the therapeutic antibody.

28. The method of claim 26, wherein the serum or plasma does not contain the therapeutic antibody.

29. The method of any one of claims 1-28, further comprising a step of comparing the binding of the anti-drug antibodies to the mutant therapeutic antibody detected in step b) to a reference.

30. The method of claim 29, wherein the reference is the detected binding between the mutant therapeutic antibody and a control antibody.

31. The method of claim 30, wherein the control antibody is a positive control antibody that binds both the therapeutic anti-IgE antibody and the mutant therapeutic antibody with similar affinity.

32. The method of claim 31, wherein the positive control antibody comprises a heavy chain variable region comprising the amino acid sequence shown in SEQ ID NO:7 and a light chain variable region comprising the amino acid sequence shown in SEQ ID NO:8.

33. A kit for detecting an anti-drug antibody of IgE isotype that binds to a therapeutic anti-IgE antibody in a sample comprising:

(a) a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the relative binding affinity of the mutant therapeutic antibody to human IgE is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to human IgE; and

(b) a detecting agent that binds to an Fc region of a human IgE.

34. The kit of claim 33, wherein the detecting agent is an FcεRIα polypeptide comprising an extracellular domain of an FcεRIα subunit.

35. The kit of claim 34, wherein the FcεRIα polypeptide comprises an extracellular domain of an FcεRIα subunit fused to an IgG constant region.

36. The kit of any one of claims 33-35, further comprising a positive control antibody that binds both the therapeutic anti-IgE antibody and the mutant therapeutic antibody with similar affinity.

37. The kit of claim 36, wherein the positive control antibody comprises a heavy chain variable region comprising the amino acid sequence shown in SEQ ID NO:7 and a light chain variable region comprising the amino acid sequence shown in SEQ ID NO:8.

38. A kit for detecting an anti-drug antibody of IgE isotype that binds to a therapeutic anti-IgE antibody in a sample comprising:

(a) a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the potency of the mutant therapeutic antibody to human IgE is about 10% or less of the potency of the therapeutic anti-IgE antibody to human IgE; and

(b) a detecting agent that binds to an Fc region of a human IgE.

39. The kit of claim 38, wherein the detecting agent is an FcεRIα polypeptide comprising an extracellular domain of an FcεRIα subunit.

40. The kit of claim 39, wherein the FcεRIα polypeptide comprises an extracellular domain of an FcεRIα subunit fused to an IgG constant region.

41. The kit of any one of claims 38-40, further comprising a positive control antibody that binds both the therapeutic anti-IgE antibody and the mutant therapeutic antibody with similar affinity.

42. The kit of claim 41, wherein the positive control antibody comprises a heavy chain variable region comprising the amino acid sequence shown in SEQ ID NO:7 and a light chain variable region comprising the amino acid sequence shown in SEQ ID NO:8.

43. A method for detecting an anti-drug antibody of IgE isotype that binds to a therapeutic anti-IgE antibody in a sample, comprising the steps of:

(a) contacting a sample that may contain the anti-drug antibody with (i) a mutant therapeutic antibody and (ii) an FcεRIα polypeptide that binds to an Fc region of a human IgE, wherein the mutant therapeutic antibody comprises at least one amino acid mutation from the therapeutic anti-IgE antibody, and the relative binding affinity of the mutant therapeutic antibody to human IgE is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE;

(b) capturing the mutant therapeutic antibody to a surface; and

(c) detecting binding of the anti-drug antibody to the mutant therapeutic antibody.

44. The method of claim 43, wherein excess amount of FcεRIα polypeptide is contacted with the sample in step (a).

45. The method of claim 44, wherein at least about 10-fold excess of FcεRIα polypeptide is contacted with the sample in step (a).

46. The method of any one of claims 43-45, wherein the FcεRIα polypeptide comprises an extracellular domain of an FcεRIα subunit.

47. The method of any one of claims 43-46, wherein the mutant therapeutic antibody is labeled and is captured to the surface by a capture agent that specifically binds to the label.

48. The method of claim 47, wherein the label is biotin and the surface is coated with streptavidin.

49. The method of any one of claims 43-48, wherein the binding of the anti-drug antibody to the mutant therapeutic antibody is detected by a labeled anti-human IgE antibody.

50. The method of any one of claims 43-48, wherein the FcεRIα polypeptide is labeled and the binding of the anti-drug antibody to the mutant therapeutic antibody is detected by a detecting agent that specifically binds to the label on the FcεRIα polypeptide.

51. A kit for detecting an anti-drug antibody of IgE isotype that binds to a therapeutic anti-IgE antibody in a sample comprising:

(a) a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the relative binding affinity of the mutant therapeutic antibody to human IgE is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to human IgE; and

(b) an FcεRIα polypeptide that binds to an Fc region of a human IgE.

52. The kit of claim 51, further comprising an anti-human IgE antibody.

53. The kit of claim 52, wherein the anti-human IgE antibody is labeled.

54. The kit of any one of claims 51-53, wherein the FcεRIα polypeptide is labeled.

55. The kit of any one of claims 51-54, further comprising a detecting agent that specifically binds to the label on the FcεRIα polypeptide.

56. A method for detecting an anti-drug antibody of IgE isotype that binds to a therapeutic anti-IgE antibody in a sample, comprising the steps of:

(a) preincubating a sample that may contain the anti-drug antibody with excess amount of an FcεRIα polypeptide that binds to an Fc region of a human IgE;

(b) incubating the preincubated sample from step (a) with the therapeutic anti-IgE antibody or a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, and the relative binding affinity of the mutant therapeutic antibody to human IgE is reduced as compared to the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE; and

(c) detecting binding of the anti-drug antibody to the therapeutic anti-IgE antibody or the mutant therapeutic antibody.

57. The method of claim 56, wherein the mutant therapeutic antibody comprises at least one amino acid mutation from the therapeutic anti-IgE antibody, and the relative binding affinity of the mutant therapeutic antibody to human IgE is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE.

58. The method of claim 56 or 57, wherein at least about 10-fold excess of FcεRIα polypeptide is preincubated with the sample in step (a).

59. The method of any one of claims 56-58, wherein the therapeutic anti-IgE antibody or the mutant therapeutic antibody is captured to a surface before or after incubating with the sample in step (b).

60. The method of any one of claims 56-59, wherein the therapeutic anti-IgE antibody or the mutant therapeutic antibody is directly immobilized to a surface before incubating with the sample in step (b).

61. The method of any one of claims 56-59, wherein the therapeutic anti-IgE antibody or the mutant therapeutic antibody is labeled and is captured to the surface through an immobilized capture agent that specifically binds to the label.

62. The method of claim 61, wherein the therapeutic anti-IgE antibody or the mutant therapeutic antibody is labeled with biotin and is captured to a streptavidin coated surface.

63. The method of any one of claims 56-62, wherein the binding of the anti-drug antibody to the therapeutic anti-IgE antibody or the mutant therapeutic antibody is detected by a HRP conjugated anti-human IgE antibody.

64. The method of any one of claims 56-62, wherein the FcεRIα polypeptide is labeled, and the binding of the anti-drug antibody to the therapeutic anti-IgE antibody or the mutant therapeutic antibody is detected by detecting the label.

65. The method of claim 64, wherein the FcεRIα polypeptide is labeled with digoxigenin, and the binding of the anti-drug antibody to the therapeutic anti-IgE antibody or the mutant therapeutic antibody is detected by a HRP conjugated anti-digoxigenin antibody.

66. The method of claim 64, wherein the FcεRIα polypeptide is labeled with ruthenium, and the binding of the anti-drug antibody to the therapeutic anti-IgE antibody or the mutant therapeutic antibody is detected via electrochemiluminescence.

67. A kit for detecting an anti-drug antibody of IgE isotype that binds to a therapeutic anti-IgE antibody in a sample comprising:

(a) the therapeutic anti-IgE antibody or a mutant therapeutic antibody thereof, wherein the mutant therapeutic antibody comprises at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the relative binding affinity of the mutant therapeutic antibody to human IgE is reduced as compared to the relative binding affinity of the therapeutic anti-IgE antibody to human IgE; and

(b) an FcεRIα polypeptide that binds to an Fc region of a human IgE.

68. The kit of claim 67, further comprising an anti-human IgE antibody.

69. The kit of claim 68, wherein the anti-human IgE antibody is labeled.

70. The kit of any one of claims 67-69, wherein the FcεRIα polypeptide is labeled.

71. The kit of claim 70, further comprising a detecting agent that specifically binds to the label on the FcεRIα polypeptide.

72. A method of identifying a patient having a risk of anaphylactic reaction to a therapeutic anti-IgE antibody, comprising the steps of:

(a) contacting a sample from the patient with a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the relative binding affinity of the mutant therapeutic antibody to human IgE is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE; and

(b) detecting binding of an anti-drug antibody of IgE isotype to the mutant therapeutic antibody, wherein the presence and/or the level of the anti-drug antibody indicate the patient has a risk of anaphylactic reaction to the therapeutic anti-IgE antibody.

73. A method of identifying a patient having a risk of anaphylactic reaction to a therapeutic anti-IgE antibody, comprising the steps of:

(a) contacting a sample from the patient with a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein

the potency of the mutant therapeutic antibody to human IgE is about 10% or less of the potency of the therapeutic anti-IgE antibody to said human IgE; and

(b) detecting binding of an anti-drug antibody of IgE isotype to the mutant therapeutic antibody, wherein the presence and/or the level of the anti-drug antibody in the sample indicates that the patient has a risk of anaphylactic reaction to the therapeutic anti-IgE antibody.

74. A method of identifying a patient having a risk of anaphylactic reaction to a therapeutic anti-IgE antibody, comprising the steps of:

(a) contacting a sample from a patient with (i) a mutant therapeutic antibody and (ii) an FcεRIα polypeptide that binds to an Fc region of a human IgE, wherein the mutant therapeutic antibody comprises at least one amino acid mutation from the therapeutic anti-IgE antibody, and the relative binding affinity of the mutant therapeutic antibody to human IgE is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE;

(b) capturing the mutant therapeutic antibody to a surface; and

(c) detecting binding of an anti-drug antibody of IgE isotype to the mutant therapeutic antibody, wherein the presence and/or the level of the anti-drug antibody in the sample indicates that the patient has a risk of anaphylactic reaction to the therapeutic anti-IgE antibody.

75. A method of identifying a patient having a risk of anaphylactic reaction to a therapeutic anti-IgE antibody, comprising the steps of:

(a) preincubating a sample from a patient with excess amount of an FcεRIα polypeptide that binds to an Fc region of a human IgE;

(b) incubating the preincubated sample from step (a) with the therapeutic anti-IgE antibody or a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, and the relative binding affinity of the mutant therapeutic antibody to human IgE is reduced as compared to the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE; and

(c) detecting binding of an anti-drug antibody of IgE isotype to the therapeutic anti-IgE antibody or the mutant therapeutic antibody, wherein the presence and/or the level of the anti-drug antibody in the sample indicates that the patient has a risk of anaphylactic reaction to the therapeutic anti-IgE antibody.

76. A method of treating a patient having an IgE-mediated disorder, comprising the steps of:

(a) determining the level of an anti-drug antibody of IgE isotype to a therapeutic anti-IgE antibody in a sample from the patient;

(b) administering an effective amount of the therapeutic anti-IgE antibody to the patient for treating the IgE-mediated disorder if the level of the anti-drug antibody in the sample does not indicate that the patient has a risk of anaphylactic reaction to the therapeutic anti-IgE antibody.

77. The method of claim 76, wherein the level of the anti-drug antibody in the sample is determined by a method comprising the steps:

(a) contacting a sample from the patient with a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the relative binding affinity of the mutant therapeutic antibody to human IgE is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE; and

(b) detecting binding of an anti-drug antibody of IgE isotype to the mutant therapeutic antibody.

78. The method of claim 76, wherein the level of the anti-drug antibody in the sample is determined by a method comprising the steps:

(a) contacting a sample from the patient with a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, wherein the potency of the mutant therapeutic antibody to human IgE is about 10% or less of the potency of the therapeutic anti-IgE antibody to said human IgE; and

(b) detecting binding of an anti-drug antibody of IgE isotype to the mutant therapeutic antibody.

79. The method of claim 76, wherein the level of the anti-drug antibody in the sample is determined by a method comprising the steps:

(a) contacting a sample from a patient with (i) a mutant therapeutic antibody and (ii) an FcεRIα polypeptide that binds to an Fc region of a human IgE, wherein the mutant therapeutic antibody comprises at least one amino acid mutation from the therapeutic anti-IgE

antibody, and the relative binding affinity of the mutant therapeutic antibody to human IgE is about 10% or less of the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE;

(b) capturing the mutant therapeutic antibody to a surface; and

(c) detecting binding of an anti-drug antibody of IgE isotype to the mutant therapeutic antibody.

80. The method of claim 76, wherein the level of the anti-drug antibody in the sample is determined by a method comprising the steps:

(a) preincubating a sample from a patient with excess amount of an FcεRIα polypeptide that binds to an Fc region of a human IgE;

(b) incubating the preincubated sample from step (a) with the therapeutic anti-IgE antibody or a mutant therapeutic antibody comprising at least one amino acid mutation from the therapeutic anti-IgE antibody, and the relative binding affinity of the mutant therapeutic antibody to human IgE is reduced as compared to the relative binding affinity of the therapeutic anti-IgE antibody to said human IgE; and

(c) detecting binding of an anti-drug antibody of IgE isotype to the therapeutic anti-IgE antibody or the mutant therapeutic antibody.