ANTI-FC EPSILON-R1 ALPHA (FCER1A) ANTIBODIES, BISPECIFIC ANTIGEN-BINDING MOLECULES THAT BIND FCER1A AND CD3, AND USES THEREOF

RELATED APPLICATIONS

[0001] This application is related to and claims priority of U.S. Provisional Application No. 62/721,921, filed on August 23, 2018. The entire contents of the foregoing application are expressly incorporated herein by reference.

REFERENCE TO SEQUENCE LISTING

[0002] This application incorporates by reference the Sequence Listing submitted in Computer Readable Form as file 10480WO01_118003-45320_SL.txt, created on August 21, 2019 and containing 67,307 bytes.

FIELD OF THE INVENTION

[0003] The present invention relates to antibodies, and antigen-binding fragments thereof, which are specific for FcεR1α, and methods of use thereof. The present invention also relates to bispecific antigen-binding molecules that bind FcεR1α and CD3, and methods of use thereof.

BACKGROUND

[0004] FcεR1 is a high affinity Fc receptor for Immunoglobulin E (IgE), and FcεR1 binds to IgE with an equilibrium dissociation constant (K_D) value of about 10^{-10} M. FcεR1 receptor crosslinking by allergen-bound IgE leads to cellular degranulation and subsequent allergic response, and the serum level of IgE is positively correlated with FcεR1.

[0005] Human FcεR1 is expressed in mast cells, basophils, monocytes, macrophages, mDCs, pDCs, Langerhans cells, eosinophils and platelets. Mast cells and basophils are innate effector cells that play a role in allergy and anaphylaxis via allergen mediated crosslinking of the IgE receptor, FcεR1α. Other roles include wound healing and mucosal immunity.

[0006] There are two types of human multimeric cell surface FcεR1 receptors, the tetrameric form and the trimeric form. The tetrameric human FcεR1 comprises an α chain, a β chain, and a homodimer of γ chains ($\alpha\beta\gamma_2$), and the trimeric human FcεR1 comprises an α chain and a homodimer of γ chains ($\alpha\gamma_2$). The α-chain of FcεR1 binds to a single IgE antibody molecule, while there is no reported role for β- and γ- chains in ligand binding.

[0007] Human FcεR1 binds to both human and murine IgE, and interleukin-4 (IL-4) enhances the expression of the α-chain in humans. In contrast, murine FcεR1 only has the tetrameric $\alpha\beta\gamma_2$ isoform and is expressed in mast cells and basophils. IL-4 does not enhance the expression of the α-chain of murine FcεR1.

[0008] CD3 is a homodimeric or heterodimeric antigen expressed on T cells in association with the T cell receptor complex (TCR) and is required for T cell activation. Functional CD3 is formed from the dimeric association of two of four different chains: epsilon, zeta, delta and gamma. The CD3 dimeric arrangements include gamma/epsilon, delta/epsilon, and zeta/zeta. Antibodies against CD3 have been shown to cluster CD3 on T cells, thereby causing T cell activation in a manner similar to the engagement of the TCR by peptide-loaded MHC molecules. Thus, anti-CD3 antibodies have been proposed for therapeutic purposes involving the activation of T cells.

[0009] Antigen-binding molecules that target FcεR1α, as well as bispecific antigen-binding molecules that bind both FcεR1α and CD3 would be useful in therapeutic settings in which specific targeting and T cell-mediated killing of cells that express FcεR1α is desired.

BRIEF SUMMARY OF THE INVENTION

[0009a] In one aspect, the present disclosure provides a bispecific antigen-binding molecule comprising a first antigen-binding domain that specifically binds human CD3, and a second antigen-binding domain that specifically binds human and/or cynomolgus FcεR1α, wherein:

the first antigen-binding domain comprises a heavy chain complementarity determining region (HCDR)1 having the amino acid sequence of SEQ ID NO:44, an HCDR2 having the amino acid sequence of SEQ ID NO:46, an HCDR3 having the amino acid sequence of SEQ ID NO:48, a light chain complementarity determining region (LCDR)1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32; and the second antigen-binding domain comprises: (i) an HCDR1 having the amino acid sequence of SEQ ID NO:4, an HCDR2 having the amino acid sequence of SEQ ID NO:6, an HCDR3 having the amino acid sequence of SEQ ID NO:8, an LCDR1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32; or (ii) an HCDR1 having the amino acid sequence of SEQ ID NO:12, an HCDR2 having the amino acid sequence of SEQ ID NO:14, an HCDR3 having the amino acid sequence of SEQ ID NO:16, an LCDR1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32; or (iii) an HCDR1 having the amino acid sequence of SEQ ID NO:20, an HCDR2 having the amino acid sequence of SEQ ID NO:22, an HCDR3 having the amino acid sequence of SEQ ID NO:24, an LCDR1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32.

[0009b] In another aspect, the present disclosure provides a bispecific antibody comprising a first binding arm that binds human CD3 and a second binding arm that binds human FcεR1α, wherein the first binding arm comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 56 and a light chain comprising the amino acid sequence of SEQ ID NO: 40, and wherein the second binding arm comprises a heavy chain comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 52, and 54, and a light chain comprising the amino acid sequence of SEQ ID NO: 40.

[0009c] In another aspect, the present disclosure provides a nucleic acid molecule encoding the bispecific antigen-binding molecule as disclosed herein.

[0009d] In another aspect, the present disclosure provides a vector comprising the nucleic acid molecule as disclosed herein.

[0009e] In another aspect, the present disclosure provides a host cell comprising the vector as disclosed herein.

[0009f] In another aspect, the present disclosure provides a pharmaceutical composition comprising the bispecific antigen-binding molecule as disclosed herein and a pharmaceutically acceptable carrier or diluent.

[0010] In a first example, the present disclosure provides antibodies and antigen-binding fragments thereof that bind to human FcεR1α. The antibodies according to this example are useful, *inter alia*, for targeting cells expressing FcεR1α. The present disclosure also provides bispecific antibodies and antigen-binding fragments thereof that bind human FcεR1α and human CD3. The bispecific antibodies according to this example are useful, *inter alia*, for targeting T cells expressing CD3, and for stimulating T cell activation, *e.g.*, under circumstances where T cell-mediated killing of cells expressing FcεR1α is beneficial or desirable. For example, the bispecific antibodies can direct CD3-mediated T cell activation to specific FcεR1α-expressing cells, such as mast cells or basophils.

[0011] Exemplary anti-FcεR1α antibodies of the present disclosure are listed in Tables 1 and 2 herein. Table 1 sets forth the amino acid sequence identifiers of the heavy chain variable regions (HCVRs) and light chain variable regions (LCVRs), as well as heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3), light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3), heavy chain (HC), and light chain (LC) of the exemplary anti-FcεR1α antibodies. Table 2 sets forth the sequence identifiers of the nucleic acid molecules encoding the HCVRs, LCVRs, HCDR1, HCDR2 HCDR3, LCDR1, LCDR2, LCDR3, HC and LC of the exemplary anti-FcεR1α antibodies.

[0012] The present disclosure provides antibodies, or antigen-binding fragments thereof, comprising an HCVR comprising an amino acid sequence selected from any of the HCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0013] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising an LCVR comprising an amino acid sequence selected from any of the LCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0014] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising an HCVR and an LCVR amino acid sequence pair (HCVR/LCVR) comprising any of the HCVR amino acid sequences listed in Table 1 paired with any of the LCVR amino acid sequences listed in Table 1. According to certain examples, the present disclosure provides antibodies, or antigen-binding fragments thereof, comprising an HCVR/LCVR amino acid sequence pair contained within any of the exemplary anti-FcεR1α antibodies listed in Table 1. In certain examples, the HCVR/LCVR amino acid sequence pair is of SEQ ID NOs: 2/26, 10/26, or 18/26 (*e.g.,* mAb17110, mAb17111, or mAb17112)

[0015] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR1 (HCDR1) comprising an amino acid sequence selected from any of the HCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0016] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR2 (HCDR2) comprising an amino acid sequence selected from any of the HCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0017] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR3 (HCDR3) comprising an amino acid sequence selected from any of the HCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0018] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR1 (LCDR1) comprising an amino acid sequence selected from any of the LCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0019] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR2 (LCDR2) comprising an amino acid sequence selected from any of the LCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0020] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR3 (LCDR3) comprising an amino acid sequence selected from any of the LCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0021] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising an HCDR3 and an LCDR3 amino acid sequence pair (HCDR3/LCDR3) comprising any of the HCDR3 amino acid sequences listed in Table 1 paired with any of the LCDR3 amino acid sequences listed in Table 1. According to certain examples, the present disclosure provides antibodies, or antigen-binding fragments thereof, comprising an HCDR3/LCDR3 amino

acid sequence pair contained within any of the exemplary anti-FcεR1α antibodies listed in Table 1. In certain examples, the HCDR3/LCDR3 amino acid sequence pair is of SEQ ID NOs: 8/32, 16/32, or 24/32 (*e.g.,* mAb17110, mAb17111, or mAb17112).

[0022] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within any of the exemplary anti-FcεR1α antibodies listed in Table 1. In certain examples, the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences set is selected from the group consisting of SEQ ID NOs: 4-6-8-28-30-32, 12-14-16-28-30-32, or 20-22-24-28-30-32 (*e.g.*, mAb17110, mAb17111, or mAb17112).

[0023] In a related example, the present disclosure provides antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within an HCVR/LCVR amino acid sequence pair as defined by any of the exemplary anti-FcεR1α antibodies listed in Table 1. For example, the present disclosure includes antibodies, or antigen-binding fragments thereof, comprising the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences set contained within an HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 2/26, 10/26, or 18/26 (*e.g.,* mAb17110, mAb17111, or mAb17112). Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. *See*, *e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, *J. Mol. Biol. 273*:927-948 (1997); and Martin *et al.*, *Proc. Natl. Acad. Sci. USA 86*:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antibody.

[0024] The present disclosure also provides nucleic acid molecules encoding anti-FcεR1α antibodies or portions thereof. For example, the present disclosure provides nucleic acid molecules encoding any of the HCVR amino acid sequences listed in Table 1; in certain examples the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0025] The present disclosure also provides nucleic acid molecules encoding any of the LCVR amino acid sequences listed in Table 1; in certain examples the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCVR nucleic acid sequences

listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0026] The present disclosure also provides nucleic acid molecules encoding any of the HCDR1 amino acid sequences listed in Table 1; in certain examples the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR1 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0027] The present disclosure also provides nucleic acid molecules encoding any of the HCDR2 amino acid sequences listed in Table 1; in certain examples the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR2 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0028] The present disclosure also provides nucleic acid molecules encoding any of the HCDR3 amino acid sequences listed in Table 1; in certain examples the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR3 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0029] The present disclosure also provides nucleic acid molecules encoding any of the LCDR1 amino acid sequences listed in Table 1; in certain examples the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR1 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0030] The present disclosure also provides nucleic acid molecules encoding any of the LCDR2 amino acid sequences listed in Table 1; in certain examples the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR2 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0031] The present disclosure also provides nucleic acid molecules encoding any of the LCDR3 amino acid sequences listed in Table 1; in certain examples the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR3 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0032] The present disclosure also provides nucleic acid molecules encoding an HCVR, wherein the HCVR comprises a set of three CDRs (*i.e.*, HCDR1-HCDR2-HCDR3), wherein the HCDR1-HCDR2-HCDR3 amino acid sequence set is as defined by any of the exemplary anti-FcεR1α antibodies listed in Table 1.

[0033] The present disclosure also provides nucleic acid molecules encoding an LCVR, wherein the LCVR comprises a set of three CDRs (*i.e.*, LCDR1-LCDR2-LCDR3), wherein the LCDR1-LCDR2-LCDR3 amino acid sequence set is as defined by any of the exemplary anti-FcεR1α antibodies listed in Table 1.

[0034] The present disclosure also provides nucleic acid molecules encoding both an HCVR and an LCVR, wherein the HCVR comprises an amino acid sequence of any of the HCVR amino acid sequences listed in Table 1, and wherein the LCVR comprises an amino acid sequence of any of the LCVR amino acid sequences listed in Table 1. In certain examples, the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto, and a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto. In certain examples according to this example of the disclosure, the nucleic acid molecule encodes an HCVR and LCVR, wherein the HCVR and LCVR are both derived from the same anti-FcεR1α antibody listed in Table 1.

[0035] The present disclosure also provides recombinant expression vectors capable of expressing a polypeptide comprising a heavy or light chain variable region of an anti-FcεR1α antibody. For example, the present disclosure includes recombinant expression vectors comprising any of the nucleic acid molecules mentioned above, *i.e.*, nucleic acid molecules encoding any of the HCVR, LCVR, and/or CDR sequences as set forth in Table 1. Also included within the scope of the present disclosure are host cells into which such vectors have been introduced, as well as methods of producing the antibodies or portions thereof by culturing the host cells under conditions permitting production of the antibodies or antibody fragments, and recovering the antibodies and antibody fragments so produced.

[0036] The present disclosure includes anti-FcεR1α antibodies having a modified glycosylation pattern. In some examples, modification to remove undesirable glycosylation sites may be useful, or an antibody lacking a fructose moiety present on the oligosaccharide chain, for example, to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield et al. (2002) JBC 277:26733). In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[0037] In another example, the disclosure provides a pharmaceutical composition comprising a recombinant human antibody or fragment thereof which specifically binds FcεR1α and a pharmaceutically acceptable carrier. In a related example, the disclosure features a composition which is a combination of an anti-FcεR1α antibody and a second therapeutic agent. In one example, the second therapeutic agent is any agent that is advantageously combined with

an anti-FcεR1α antibody. Additional combination therapies and co-formulations involving the anti-FcεR1α antibodies of the present disclosure are disclosed elsewhere herein.

[0038] In another example, the disclosure provides therapeutic methods for targeting/killing FcεR1α-expressing cells (*e.g.*, mast cells, or basophils) using an anti-FcεR1α antibody of the disclosure, wherein the therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an anti-FcεR1α antibody of the disclosure to a subject in need thereof. In some cases, the anti-FcεR1α antibodies (or antigen-binding fragments thereof) can be used for treating allergy, or may be modified to be more cytotoxic by methods, including but not limited to, modified Fc domains to increase ADCC (see *e.g.* Shield et al. (2002) JBC 277:26733), radioimmunotherapy, antibody-drug conjugates, or other methods for increasing the efficiency of FcεR1α expressing cells killing.

[0039] The present disclosure also includes the use of an anti-FcεR1α antibody of the disclosure in the manufacture of a medicament for the treatment of a disease or disorder (*e.g.*, allergy) related to or caused by FcεR1α-expressing cells.

[0040] In yet another example, the disclosure provides monospecific anti-FcεR1α antibodies for diagnostic applications, such as, *e.g.*, imaging reagents.

[0041] In yet another example, the disclosure provides therapeutic methods for stimulating T cell activation using an anti-CD3 antibody or antigen-binding portion of an antibody of the disclosure, wherein the therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an antibody of the present disclosure.

[0042] In another example, the present disclosure provides an isolated antibody or antigen-binding fragment thereof that binds human FcεR1α or binds cynomolgus (*Macaca fascicularis*) FcεR1α with a binding dissociation equilibrium constant (K_D) of less than about 250 nM as measured in a surface plasmon resonance assay at 25ºC. In yet another example, the present disclosure provides an isolated antibody or antigen-binding fragment thereof that binds human FcεR1α with a dissociative half-life (t½) of greater than about 0.54 minute or binds cynomolgus FcεR1α with a dissociative half-life (t½) of greater than about 0.6 minute as measured in a surface plasmon resonance assay at 25ºC.

[0043] The disclosure further provides an antibody or antigen-binding fragment that competes for binding to human FcεR1α with a reference antibody comprising an HCVR/LCVR amino acid sequence pair as set forth in Table 1. In another example, the disclosure provides an antibody or antigen-binding fragment that competes for binding to human FcεR1α with a reference antibody comprising an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/26; 10/26; and 18/26.

[0044] The disclosure furthermore provides an antibody or antigen-binding fragment, wherein the antibody or antigen-binding fragment thereof binds to the same epitope on human FcεR1α as a reference antibody comprising an HCVR/LCVR amino acid sequence pair as set forth in

Table 1. In another example, the antibody or antigen-binding fragment binds to the same epitope on human FcεR1α as a reference antibody comprising an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/26; 10/26, and 18/26.

[0045] The disclosure further provides an isolated antibody or antigen-binding fragment thereof that binds human FcεR1α, wherein the antibody or antigen-binding fragment comprises: the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having an amino acid sequence as set forth in Table 1; and the CDRs of a light chain variable region (LCVR) having an amino acid sequence as set forth in Table 1. In another example, the isolated antibody or antigen-binding fragment comprises the heavy and light chain CDRs of an HCVR/LCVR amino acid sequence pair selected from the group consisting of: 2/26; 10/26; and 18/26. In yet another example, the isolated antibody or antigen-binding fragment comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, selected from the group consisting of: SEQ ID NOs: 4-6-8-28-30-32; 12-14-16-28-30-32; and 20-22-24-28-30-32

[0046] In another example, the disclosure provides an isolated antibody or antigen-binding fragment thereof that binds human FcεR1α, wherein the antibody or antigen-binding fragment comprises: (a) a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 10, and 18; and (b) a light chain variable region (LCVR) having an amino acid sequence SEQ ID NO: 26. In a further example, the isolated antibody or antigen-binding fragment an HCVR/LCVR amino acid sequence pair selected from the group consisting of: SEQ ID NOs: 2/26; 10/26; and 18/26.

[0047] According to another example, the present disclosure provides bispecific antigen-binding molecules (*e.g.*, antibodies) that bind FcεR1α and CD3. Such bispecific antigen-binding molecules are also referred to herein as "anti-FcεR1α/anti-CD3 bispecific molecules," “anti-CD3/anti-FcεR1α bispecific molecules,” “anti-FcεR1α x CD3,” “anti-CD3 x FcεR1α,” or “FcεR1αxCD3 bsAbs.” The anti-FcεR1α portion of the anti-FcεR1α/anti-CD3 bispecific molecule is useful for targeting cells that express FcεR1α (*e.g.*, mast cells or basophils), and the anti-CD3 portion of the bispecific molecule is useful for activating T-cells. The simultaneous binding of FcεR1α on a mast cells or basophils and CD3 on a T-cell facilitates directed killing (cell lysis) of the targeted mast cells or basophils by the activated T-cell. The anti-FcεR1α/anti-CD3 bispecific molecules of the disclosure are therefore useful, *inter alia*, for treating diseases and disorders related to or caused by FcεR1α-expressing cells (*e.g.*, allergy).

[0048] The bispecific antigen-binding molecules according to this example of the present disclosure comprise a first antigen-binding domain that specifically binds human CD3, and a second antigen-binding domain that specifically binds FcεR1α. The present disclosure includes anti-FcεR1α/anti-CD3 bispecific molecules (*e.g.*, bispecific antibodies) wherein each antigen-binding domain comprises a heavy chain variable region (HCVR) paired with a light chain variable region (LCVR). In certain exemplary examples of the disclosure, the anti-CD3

antigen-binding domain and the anti-FcεR1α antigen binding domain each comprise different, distinct HCVRs paired with a common LCVR. For example, as illustrated in Example 1 herein, bispecific antibodies were constructed comprising a first antigen-binding domain that specifically binds CD3, wherein the first antigen-binding domain comprises an HCVR and an LCVR, each derived from an anti-CD3 antibody; and a second antigen-binding domain that specifically binds FcεR1α, wherein the second antigen-binding domain comprises an HCVR derived from an anti-FcεR1α antibody paired with the same LCVR. In such examples, the first and second antigen-binding domains comprise distinct anti-CD3 and anti-FcεR1α HCVRs but share a common LCVR. The amino acid sequence of this LCVR is shown, *e.g.*, in SEQ ID NO: 26, and the amino acid sequences of the corresponding CDRs (*i.e.*, LCDR1-LCDR2-LCDR3) are shown in SEQ ID NOs: 28, 30, and 32, respectively. Genetically modified mice can be used to produce fully human bispecific antigen-binding molecules comprising two different heavy chains that associate with an identical light chain that comprises a variable domain derived from one of two different human light chain variable region gene segments. Alternatively, variable heavy chains may be paired with one common light chain and expressed recombinantly in host cells. As such, the antibodies of the disclosure can comprise immunoglobulin heavy chains associated with a single rearranged light chain. In some examples, the light chain comprises a variable domain derived from a human Vκ1-39 gene segment or a Vκ3-20 gene segment. In other examples, the light chain comprises a variable domain derived from a human Vκ1-39 gene segment rearranged with a human Jκ5 or a human Jκ1 gene segment (WO 2017/053856, herein incorporated by reference).

[0049] The present disclosure provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises any of the HCVR amino acid sequences, any of the LCVR amino acid sequences, any of the HCVR/LCVR amino acid sequence pairs, any of the heavy chain CDR1-CDR2-CDR3 amino acid sequences, or any of the light chain CDR1-CDR2-CDR3 amino acid sequences as set forth in US publication 2014/0088295 published March 27, 2014 and WO 2018/067331 published April 12, 2018.

[0050] In addition, the present disclosure provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises any of the HCVR amino acid sequences as set forth in Table 3 herein. The first antigen-binding domain that specifically binds CD3 may also comprise any of the LCVR amino acid sequences as set forth in Tables 1, and 3 herein. The present disclosure also provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises any of the heavy chain CDR1-CDR2-CDR3 amino acid sequences as set forth in Table 3, and/or any of the light chain CDR1-CDR2-CDR3 amino acid sequences as set forth in Tables 1, and 3 herein.

[0051] According to certain examples, the present disclosure provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the first antigen-binding domain that specifically binds

CD3 comprises a heavy chain variable region (HCVR) having an amino acid sequence as set forth in Table 1 herein or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0052] The present disclosure also provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises a light chain variable region (LCVR) having an amino acid sequence as set forth in Tables 1, and 3 herein, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0053] The present disclosure also provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises an HCVR and LCVR (HCVR/LCVR) amino acid sequence pair as set forth in Table 3 herein.

[0054] The present disclosure also provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises a heavy chain CDR3 (HCDR3) domain having an amino acid sequence as set forth in Table 3 herein, or a substantially similar sequence thereto having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR3 (LCDR3) domain having an amino acid sequence as set forth in Tables 1, and 3 herein, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0055] In certain examples, the first antigen-binding domain that specifically binds CD3 comprises an HCDR3/LCDR3 amino acid sequence pair as set forth in Table 3 herein.

[0056] The present disclosure also provides anti-CD3/anti-FcεR1α bispecific antigen-binding molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises a heavy chain CDR1 (HCDR1) domain having an amino acid as set forth in Table 3 herein, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a heavy chain CDR2 (HCDR2) domain having an amino acid as set forth in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a heavy chain CDR3 (HCDR3) domain having an amino acid as set forth in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a light chain CDR1 (LCDR1) domain having an amino acid sequence as set forth in Tables 1, and 3 herein, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a light chain CDR2 (LCDR2) domain having an amino acid sequence as set forth in Tables 1, and 3 herein, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity, and a light chain CDR3 (LCDR3) domain having an amino acid sequence as set forth in Tables 1, and 3 herein , or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0057] Certain non-limiting, exemplary anti-CD3/anti-FcεR1α bispecific antigen-binding molecules of the disclosure include a first antigen-binding domain that specifically binds CD3 comprising HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, having the amino acid sequences as set forth in Table 3 herein.

[0058] The present disclosure further provides a bispecific antigen-binding molecule, wherein the first antigen-binding domain that specifically binds human CD3 comprises heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) from a heavy chain variable region (HCVR) comprising an amino acid sequence as set forth in Table 3 and light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) from a light chain variable region (LCVR) comprising an amino acid sequence as set forth in Tables 1, and 3.

[0059] In another example, the disclosure provides a bispecific antigen-binding molecule wherein the first antigen-binding domain that specifically binds human CD3 comprises heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) from a heavy chain variable region (HCVR) comprising an amino acid sequence of SEQ ID NO: 42., and light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) from a light chain variable region (LCVR) comprising an amino acid sequence of SEQ ID NO: 26.

[0060] The disclosure further provides a bispecific antigen-binding molecule, wherein the first antigen-binding domain that specifically binds human CD3 comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 comprising the amino acid sequences of SEQ ID Nos: 44-46-48-28-30-32.

[0061] In a further example, the disclosure provides a bispecific antigen-binding molecule, wherein the first antigen-binding domain that specifically binds human CD3 comprises the heavy and light chain CDRs of an HCVR/LCVR amino acid sequence pair of SEQ ID NO: 42/26.

[0062] In more examples, exemplary anti-CD3/anti-FcεR1α bispecific antigen-binding molecules of the disclosure include a bispecific antigen-binding molecule wherein the first antigen-binding domain that specifically binds human CD3 comprises an HCVR comprising HCDR1-HCDR2-HCDR3 having the amino acid sequences of SEQ ID NOs: 44-46-48**.**

[0063] The present disclosure also provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the second antigen-binding domain that specifically binds FcεR1α comprises a heavy chain variable region (HCVR) having the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 10, and 18, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0064] The present disclosure also provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the second antigen-binding domain that specifically binds FcεR1α comprises a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO: 26, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0065] The present disclosure also provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the second antigen-binding domain that specifically binds FcεR1α comprises an HCVR and LCVR (HCVR/LCVR) amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/26, 10/26, and 18/26.

[0066] The present disclosure also provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the second antigen-binding domain that specifically binds FcεR1α comprises a heavy chain CDR3 (HCDR3) domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 16, and 24, or a substantially similar sequence thereto having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR3 (LCDR3) domain having an amino acid sequence of SEQ ID NO: 32, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0067] In certain examples, the second antigen-binding domain that specifically binds FcεR1α comprises an HCDR3/LCDR3 amino acid sequence pair selected from the group consisting of SEQ ID NOs: 8/32, 16/32, and 24/32.

[0068] The present disclosure also provides anti-CD3/anti-FcεR1α bispecific antigen-binding molecules, wherein the second antigen-binding domain that specifically binds FcεR1α comprises a heavy chain CDR1 (HCDR1) domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 12, and 20, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a heavy chain CDR2 (HCDR2) domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 14, and 22, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a heavy chain CDR3 (HCDR3) domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 16, and 24, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a light chain CDR1 (LCDR1) domain having an amino acid sequence of SEQ ID NOs: 28, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR2 (LCDR2) domain having an amino acid sequence of SEQ ID NO: 30, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; ; and a light chain CDR3 (LCDR3) domain having an amino acid sequence of SEQ ID NOs: 32, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0069] Certain non-limiting, exemplary anti-CD3/anti-FcεR1α bispecific antigen-binding molecules of the disclosure include a second antigen-binding domain that specifically binds FcεR1α comprising HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively,

having the amino acid sequences selected from the group consisting of: SEQ ID NOs: 4-6-8-28-30-32, 12-14-16-28-30-32, and 20-22-24-28-30-32.

[0070] In a related example, the disclosure includes anti-CD3/anti-FcεR1α bispecific antigen-binding molecules wherein the second antigen-binding domain that specifically binds FcεR1α comprises the heavy and light chain CDR domains contained within heavy and light chain variable region (HCVR/LCVR) sequences selected from the group consisting of SEQ ID NOs: 2/26, 10/26, and 18/26.

[0071] In another example, the disclosure provides a bispecific antigen-binding molecule comprising: (a) a first antigen-binding domain that comprises three heavy chain complementarity determining regions HCDR1, HCDR2 and HCDR3, respectively, comprising the amino acid sequences of SEQ ID NOs: 44, 46 and 48, and three light chain complementarity determining regions LCDR1, LCDR2 and LCDR3, respectively, comprising the amino acid sequences of SEQ ID NOs: 28, 30 and 32, wherein the first antigen-binding domain specifically binds human CD3; and (b) a second antigen-binding domain that comprises three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) and three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3); wherein HCDR1 comprises an amino acid sequence selected from the group consisting of: SEQ ID NOs: 4, 12, and 20; HCDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 14, and 22; HCDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 16, and 24; LCDR1 comprises an amino acid sequence of SEQ ID NO: 28; LCDR2 comprises an amino acid sequence of SEQ ID NO: 30; and LCDR3 comprises an amino acid sequence of SEQ ID NO: 32, wherein the second antigen-binding arm specifically binds human FcεR1α.

[0072] In another example, the disclosure provides a bispecific antigen-binding molecule comprising a first antigen-binding domain that binds human CD3 and a second antigen-binding domain that binds human FcεR1α, wherein the second antigen-binding domain is derived from the antibody or antigen-binding fragment of any one of the anti-FcεR1α antibodies of the disclosure. In a further example, the disclosure provides a bispecific antigen-binding molecule comprising a first antigen-binding domain that specifically binds human CD3, and a second antigen-binding domain that specifically binds human FcεR1α.

[0073] The disclosure further provides a bispecific antigen-binding molecule which binds human cells expressing human CD3. In another example, the bispecific antigen-binding molecule binds human cells expressing human FcεR1α and/or cells expressing cynomolgus FcεR1α.

[0074] In another example the disclosure provides a bispecific antigen-binding molecule which inhibits allergic reaction in a subject (*e.g.*, mice) expressing human FcεR1α. The disclosure further provides bispecific antigen-binding molecules which deplete basophils or other FcεR1α-expressing cells in a subject (*e.g.*, mice) expressing human FcεR1α.

[0075] In another example the disclosure provides a bispecific antigen-binding molecule comprising a second antigen-binding domain that specifically binds a target cell expressing human FcεR1α with a binding ratio greater than 200 in the presence or absence of IgE or binds a target cell expressing cynomolgus FcεR1α with a binding ratio greater than 140 in the presence of absence of IgE, wherein such binding ratio is measured in an *in vitro* FACS binding assay.

[0076] In some examples, the antigen-binding molecule induces T cell-mediated killing of FcεR1α-expressing with an EC_{50} value of less than about 20 nM, as measured in an *in vitro* T cell-mediated cell killing assay, for example, where the FcεR1α expressing cells are basophils.

[0077] In some applications, the second antigen-binding domain binds human or cynomolgus FcεR1α with a K_D value of less than about 467 nM, as measured in an *in vitro* surface plasmon resonance binding assay at 25 ºC. In some instances, the second antigen-binding domain binds each of human FcεR1α and cynomolgus FcεR1α with an K_D value of less than about 450 nM, less than about 400 nM, less than about 350 nM, less than about 300 nM, less than about 250 nM, less than about 200 nM, less about 150 nM, less than about 100 nM, or less than about 50 nM.

[0078] In certain examples, anti-FcεR1α antibodies of the disclosure, antigen-binding fragments and bispecific antibodies thereof were made by replacing amino acid residues of a parental in a stepwise manner based on differences between the germline sequence and the parental antibody sequence.

[0079] In another example, the present disclosure provides an isolated bispecific antigen-binding molecule that competes for binding to FcεR1α, or binds to the same epitope on FcεR1α as a reference antibody, wherein the reference antibody comprises a first antigen-binding domain comprising an HCVR/LCVR pair comprising the amino acid sequences of SEQ ID NOs: 42/26, and a second antigen-binding domain comprising an HCVR/LCVR pair comprising the amino acid sequences of SEQ ID NOs: 2/26, 10/26 or 18/26.

[0080] In another example, the present disclosure provides an isolated bispecific antigen-binding molecule that competes for binding to human CD3, or binds to the same epitope on human CD3 as a reference antibody, wherein the reference antibody comprises a first antigen-binding domain comprising an HCVR/LCVR pair comprising the amino acid sequences of SEQ ID NOs: 42/26, and a second antigen-binding domain comprising an HCVR/LCVR pair comprising the amino acid sequences of SEQ ID NOs: 2/26, 10/26 or 18/26.

[0081] Any of the bispecific antigen-binding molecules discussed above or herein may be a bispecific antibody. In some examples, the bispecific antibody comprises a human IgG heavy chain constant region. In one example, the human IgG heavy chain constant region is isotype IgG1. In one example, the human IgG heavy chain constant region is isotype IgG4.

In various examples, the bispecific antibody comprises a chimeric hinge that reduces Fcγ receptor binding relative to a wild-type hinge of the same isotype.

[0082] The present disclosure also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain (HC) and a light chain (LC) amino acid sequence pair (HC/LC) comprising any of the HC amino acid sequences listed in Table 1 paired with any of the LC amino acid sequences listed in Table 1. According to certain examples, the present disclosure provides antibodies, or antigen-binding fragments thereof, comprising an HC/LC amino acid sequence pair contained within any of the exemplary anti-FcεR1α antibodies listed in Table 1. In certain examples, the HC/LC amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 34/40, 36/40, and 38/40.

[0083] The present disclosure also provides bispecific antibodies, or antigen-binding fragments thereof comprising a first heavy chain, a second heavy chain and a common light chain comprising any of the HC or LC amino acid sequences listed in Table 7. In certain examples, the bispecific antibodies comprise a first HC comprising an amino acid sequence of SEQ ID NO: 56; a second HC comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 52 and 54; and a common light chain comprising the amino acid sequence of SEQ ID NO: 40.

[0084] In one example, the disclosure provides a pharmaceutical composition comprising an anti-FcεR1α antigen-binding molecule or anti-FcεR1α/anti-CD3 bispecific antigen-binding molecule and a pharmaceutically acceptable carrier or diluent. The disclosure further provides a method for treating an FcεR1α -related disease, allergy or an IgE-related disease in a subject, the method comprising administering to the subject the pharmaceutical composition comprising an anti-FcεR1α antigen-binding molecule or anti-FcεR1α/anti-CD3 bispecific antigen-binding molecule and a pharmaceutically acceptable carrier or diluent. In some examples, the allergy or other IgE-related diseases are selected from the group consisting of allergic asthma, allergic rhinitis, hay fever, anaphylaxis, atopic dermatitis, chronic urticarial, food allergy, perennial allergy, drug allergy, and pollen allergy. In one example, the allery is severe allergy. In some cases, the allergy leads to anaphylaxis. In certain examples, the FcεR1α-related disease comprises severe allergy, mast cell activation disorder or mastocytosis. In certain examples, the method for treating allergy comprises administering to the subject the pharmaceutical composition comprising an anti-FcεR1α antigen-binding molecule or anti-FcεR1α/anti-CD3 bispecific antigen-binding molecule at a certain dose, as described elsewhere herein.

[0085] In another example, the present disclosure provides nucleic acid molecules encoding any of the HCVR, LCVR or CDR sequences of the anti-FcεR1α, and anti-CD3/anti-FcεR1α bispecific antigen-binding molecules disclosed herein, including nucleic acid molecules comprising the polynucleotide sequences as set forth in Tables 2, and 4 herein, as well as nucleic acid

molecules comprising two or more of the polynucleotide sequences as set forth in Tables 2, and 4 in any functional combination or arrangement thereof. Recombinant expression vectors carrying the nucleic acids of the disclosure, and host cells into which such vectors have been introduced, are also encompassed by the disclosure, as are methods of producing the antibodies by culturing the host cells under conditions permitting production of the antibodies, and recovering the antibodies produced.

[0086] The present disclosure provides nucleic acid molecules encoding any of the heavy chain amino acid sequences listed in Table 7. The present disclosure also provides nucleic acid molecules encoding any of the light chain amino acid sequences listed in Table 7.

[0087] The present disclosure includes anti-CD3/anti-FcεR1α bispecific antigen-binding molecules wherein any of the aforementioned antigen-binding domains that specifically bind CD3 are combined, connected or otherwise associated with any of the aforementioned antigen-binding domains that specifically bind FcεR1α to form a bispecific antigen-binding molecule that binds CD3 and FcεR1α.

[0088] The present disclosure includes anti-CD3/anti-FcεR1α bispecific antigen-binding molecules having a modified glycosylation pattern. In some applications, modification to remove undesirable glycosylation sites may be useful, or an antibody lacking a fructose moiety present on the oligosaccharide chain, for example, to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield et al. (2002) JBC 277:26733). In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[0089] In another example, the disclosure provides a pharmaceutical composition comprising an anti-CD3/anti-FcεR1α bispecific antigen-binding molecule as disclosed herein and a pharmaceutically acceptable carrier. In a related example, the disclosure features a composition which is a combination of an anti-CD3/anti-FcεR1α bispecific antigen-binding molecule and a second therapeutic agent. In one example, the second therapeutic agent is any agent that is advantageously combined with an anti-CD3/anti-FcεR1α bispecific antigen-binding molecule. Exemplary agents that may be advantageously combined with an anti-CD3/anti-FcεR1α bispecific antigen-binding molecule are discussed in detail elsewhere herein.

[0090] In yet another example, the disclosure provides therapeutic methods for targeting/ablating cells expressing FcεR1α using an anti-CD3/anti-FcεR1α bispecific antigen-binding molecule of the disclosure, wherein the therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an anti-CD3/anti-FcεR1α bispecific antigen-binding molecule of the disclosure to a subject in need thereof. The antibody or fragment thereof may be administered sub-cutaneously, intravenously, intradermally, intraperitoneally, orally or intramuscularly. In certain examples, an antibody of the disclosure is administered at a dose of about 0.001 mg/kg body weight to about 200 mg/kg body weight of

the subject. In certain examples, an antibody of the disclosure is administered at a dose comprising between 1 mg to 2500 mg of the antibody to a subject in need thereof.

[0091] The present disclosure also includes the use of an anti-CD3/anti-FcεR1α bispecific antigen-binding molecule of the disclosure in the manufacture of a medicament for the treatment of a disease or disorder related to or caused by FcεR1α-expressing cells.

[0092] Other examples will become apparent from a review of the ensuing detailed description.

DETAILED DESCRIPTION

[0093] Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0094] Unless defined otherwise, all 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. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

[0095] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All patents, applications and non-patent publications mentioned in this specification are incorporated herein by reference in their entireties.

Definitions

[0096] The expression "CD3," as used herein, refers to an antigen which is expressed on T cells as part of the multimolecular T cell receptor (TCR) and which consists of a homodimer or heterodimer formed from the association of two of four receptor chains: CD3-epsilon, CD3-delta, CD3-zeta, and CD3-gamma. All references to proteins, polypeptides and protein fragments herein are intended to refer to the human version of the respective protein, polypeptide or protein fragment unless explicitly specified as being from a non-human species. Thus, the expression "CD3" means human CD3 unless specified as being from a non-human species, *e.g.*, "mouse CD3," "monkey CD3," etc. Human CD3-epsilon comprises the amino acid sequence set forth as SEQ ID NO: 59; human CD3-delta comprises the amino acid sequence set forth as SEQ ID NO: 60; CD3-zeta comprises the amino acid sequence set forth as SEQ ID NO: 61; and CD3-gamma comprises the amino acid sequence set forth as SEQ ID NO: 62.

[0097] As used herein, "an antibody that binds CD3" or an "anti-CD3 antibody" includes antibodies and antigen-binding fragments thereof that specifically recognize a single CD3 subunit (*e.g.*, epsilon, delta, gamma or zeta), as well as antibodies and antigen-binding fragments thereof that specifically recognize a dimeric complex of two CD3 subunits (*e.g.*, gamma/epsilon, delta/epsilon, and zeta/zeta CD3 dimers). The antibodies and antigen-binding fragments of the present invention may bind soluble CD3 and/or cell surface expressed CD3. Soluble CD3 includes natural CD3 proteins as well as recombinant CD3 protein variants such as, *e.g.*, monomeric and dimeric CD3 constructs, that lack a transmembrane domain or are otherwise unassociated with a cell membrane.

[0098] As used herein, the expression "cell surface-expressed CD3" means one or more CD3 protein(s) that is/are expressed on the surface of a cell *in vitro* or *in vivo*, such that at least a portion of a CD3 protein is exposed to the extracellular side of the cell membrane and is accessible to an antigen-binding portion of an antibody. "Cell surface-expressed CD3" includes CD3 proteins contained within the context of a functional T cell receptor in the membrane of a cell. The expression "cell surface-expressed CD3" includes CD3 protein expressed as part of a homodimer or heterodimer on the surface of a cell (*e.g.*, gamma/epsilon, delta/epsilon, and zeta/zeta CD3 dimers). The expression, "cell surface-expressed CD3" also includes a CD3 chain (*e.g.*, CD3-epsilon, CD3-delta or CD3-gamma) that is expressed by itself, without other CD3 chain types, on the surface of a cell. A "cell surface-expressed CD3" can comprise or consist of a CD3 protein expressed on the surface of a cell which normally expresses CD3 protein. Alternatively, "cell surface-expressed CD3" can comprise or consist of CD3 protein expressed on the surface of a cell that normally does not express human CD3 on its surface but has been artificially engineered to express CD3 on its surface.

[0099] The expression “FcεR1α,” as used herein, refers to an α-chain of the high affinity Fc receptor (FcεR1) for IgE. FcεR1α is responsible for the binding of IgE to FcεR1. FcεR1α is expressed in mast cells, basophils, monocytes, macrophages, mDCs, pDCs, Langerhans cells, eosinophils and platelets. The amino acid sequence of human FcεR1α is set forth as SEQ ID NO: 63. The term “FcεR1α” includes recombinant FcεR1α protein or a fragment thereof. The term also encompasses FcεR1α protein or a fragment thereof coupled to, for example, histidine tag, mouse or human Fc, or a signal sequence such as ROR1 (for example, SEQ ID NOs: 57 or 58).

[00100] As used herein, "an antibody that binds FcεR1α" or an "anti-FcεR1α antibody" includes antibodies and antigen-binding fragments thereof that specifically recognize FcεR1α.

[00101] As used herein, the term “disease or disorder associated with expression of FcεR1α” includes any disease or disorder in which inhibition of expression and/or activity (*e.g.*, signaling) of FcεR1α and/or ablation of cells expressing FcεR1α is expected to alleviate symptoms and/or progression of the disorder. For example, such diseases and disorders include, but are not

limited to mast cell activation disorders, mastocytosis, and allergy, including but not limited to food allergy, pollen allergy, pet dander allergy, etc.

[00102] The term “allergy,” as used herein, refers to a condition caused by hypersensitivity of the immune system to a substance (allergen) in the environment. Allergies include, but are not limited to allergic asthma, hay fever, atopic dermatitis, chronic urticaria, food allergy, pet dander allergy, and pollen allergy. Symptoms of allergies may include, but are not limited to urticaria (*e.g.*, hives), angioedema, rhinitis, asthma, vomiting, sneezing, runny nose, shortness of breath, sinus inflammation, watery eyes, wheezing, bronchospasm, reduced peak expiratory flow (PEF), gastrointestinal distress, flushing, swollen lips, swollen tongue, reduced blood pressure, anaphylaxis, and organ dysfunction/failure. In one embodiment, the allergy is an anaphylactic allergy, which is a severe form of allergy that may cause death. Symptoms of anaphylaxis may include, but are not limited to rashes, throat or tongue swelling, airway swelling, shortness of breath, vomiting, lightheadedness, low blood pressure, etc.

[00103] The term "allergen," as used herein, includes any substance, chemical, particle or composition which is capable of stimulating an allergic response in a susceptible individual. Allergens may be contained within or derived from a food item such as, *e.g.*, dairy products (*e.g.*, cow's milk), egg, celery, sesame, wheat, soy, fish, shellfish, sugars (*e.g.*, sugars present on meat such as alpha-galactose), peanuts, other legumes (*e.g.*, beans, peas, soybeans, etc.), and tree nuts. Alternatively, an allergen may be contained within or derived from a non-food item such as, *e.g.*, dust (*e.g.*, containing dust mite), pollen, insect venom (*e.g.*, venom of bees, wasps, mosquitos, fire ants, etc.), mold, animal fur, animal dander, wool, latex, metals (*e.g.*, nickel), household cleaners, detergents, medication, cosmetics (*e.g.*, perfumes, etc.), drugs (*e.g.*, penicillin, sulfonamides, salicylate, etc.), therapeutic monoclonal antibodies (*e.g.*, cetuximab), ragweed, grass and birch. Exemplary pollen allergens include, *e.g.*, tree pollens such as birch pollen, cedar pollen, oak pollen, alder pollen, hornbeam pollen, aesculus pollen, willow pollen, poplar pollen, plantanus pollen, tilia pollen, olea pollen, Ashe juniper pollen, and *Alstonia scholaris* pollen.

[00104] The term "antigen-binding molecule" includes antibodies and antigen-binding fragments of antibodies, including, *e.g.*, bispecific antibodies.

[00105] The term "antibody", as used herein, means any antigen-binding molecule or molecular complex comprising at least one complementarity determining region (CDR) that specifically binds to or interacts with a particular antigen (*e.g.*, FcεR1α or CD3). The term "antibody" includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (*e.g.*, IgM). Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, C_H1, C_H2 and C_H3. Each light chain comprises a light chain variable region

(abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region comprises one domain (C_L1). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. In different embodiments of the invention, the FRs of the anti-FcεR1α antibody or anti-CD3 antibody (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[00106] The term "antibody", as used herein, also includes antigen-binding fragments of full antibody molecules. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[00107] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[00108] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the

V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H-V_H, V_H-V_L or V_L-V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[00109] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V_H-C_H1; (ii) V_H-C_H2; (iii) V_H-C_H3; (iv) V_H-C_H1-C_H2; (v) V_H-C_H1-C_H2-C_H3; (vi) V_H-C_H2-C_H3; (vii) V_H-C_L; (viii) V_L-C_H1; (ix) V_L-C_H2; (x) V_L-C_H3; (xi) V_L-C_H1-C_H2; (xii) V_L-C_H1-C_H2-C_H3; (xiii) V_L-C_H2-C_H3; and (xiv) V_L-C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[00110] As with full antibody molecules, antigen-binding fragments may be monospecific or multispecific (*e.g.*, bispecific). A multispecific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multispecific antibody format, including the exemplary bispecific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

[00111] The antibodies of the present invention may function through complement-dependent cytotoxicity (CDC) or antibody-dependent cell-mediated cytotoxicity (ADCC). "Complement-dependent cytotoxicity" (CDC) refers to lysis of antigen-expressing cells by an antibody of the invention in the presence of complement. "Antibody-dependent cell-mediated cytotoxicity" (ADCC) refers to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (*e.g.*, Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and thereby lead to lysis of the target cell. CDC and ADCC can be measured using assays that are well known and available in the art. (*See*, *e.g.*, U.S. Patent Nos 5,500,362 and 5,821,337, and Clynes *et al.* (1998) Proc. Natl. Acad. Sci. (USA) *95*:652-656). The constant region of an antibody is important in the ability of an antibody to fix complement

and mediate cell-dependent cytotoxicity. Thus, the isotype of an antibody may be selected on the basis of whether it is desirable for the antibody to mediate cytotoxicity.

[00112] In certain embodiments of the invention, the anti-FcεR1α monospecific antibodies or anti-FcεR1α/anti-CD3 bispecific antibodies of the invention are human antibodies. The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[00113] The antibodies of the invention may, in some embodiments, be recombinant human antibodies. The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic for human immunoglobulin genes (see *e.g.*, Taylor et al. (1992) Nucl. Acids Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[00114] Human antibodies can exist in two forms that are associated with hinge heterogeneity. In one form, an immunoglobulin molecule comprises a stable four chain construct of approximately 150-160 kDa in which the dimers are held together by an interchain heavy chain disulfide bond. In a second form, the dimers are not linked via inter-chain disulfide bonds and a molecule of about 75-80 kDa is formed composed of a covalently coupled light and heavy chain (half-antibody). These forms have been extremely difficult to separate, even after affinity purification.

[00115] The frequency of appearance of the second form in various intact IgG isotypes is due to, but not limited to, structural differences associated with the hinge region isotype of the

antibody. A single amino acid substitution in the hinge region of the human IgG4 hinge can significantly reduce the appearance of the second form (Angal et al. (1993) Molecular Immunology 30:105) to levels typically observed using a human IgG1 hinge. The instant invention encompasses antibodies having one or more mutations in the hinge, C_H2 or C_H3 region which may be desirable, for example, in production, to improve the yield of the desired antibody form.

[00116] The antibodies of the invention may be isolated antibodies. An "isolated antibody," as used herein, means an antibody that has been identified and separated and/or recovered from at least one component of its natural environment. For example, an antibody that has been separated or removed from at least one component of an organism, or from a tissue or cell in which the antibody naturally exists or is naturally produced, is an "isolated antibody" for purposes of the present invention. An isolated antibody also includes an antibody *in situ* within a recombinant cell. Isolated antibodies are antibodies that have been subjected to at least one purification or isolation step. According to certain embodiments, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[00117] The present invention also includes one-arm antibodies that bind FcεR1α. As used herein, a "one-arm antibody" means an antigen-binding molecule comprising a single antibody heavy chain and a single antibody light chain. The one-arm antibodies of the present invention may comprise any of the HCVR/LCVR or CDR amino acid sequences as set forth in Table 1.

[00118] The anti-FcεR1α or anti-FcεR1α/anti-CD3 antibodies disclosed herein may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences from which the antibodies were derived. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present invention includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back

to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies of the present invention may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present invention.

[00119] The present invention also includes anti-FcεR1α or anti-FcεR1α/anti-CD3 antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present invention includes anti-FcεR1α or anti-FcεR1α/anti-CD3 antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences set forth in Tables 1 and 3 herein.

[00120] The term "epitope" refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstance, an epitope may include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen.

[00121] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95%, and more preferably at least about 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain

instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[00122] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (*e.g.*, charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. *See, e.g.*, Pearson (1994) Methods Mol. Biol. 24: 307-331, herein incorporated by reference. Examples of groups of amino acids that have side chains with similar chemical properties include (1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; (2) aliphatic-hydroxyl side chains: serine and threonine; (3) amide-containing side chains: asparagine and glutamine; (4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; (5) basic side chains: lysine, arginine, and histidine; (6) acidic side chains: aspartate and glutamate, and (7) sulfur-containing side chains are cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443-1445, herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[00123] Sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as Gap and Bestfit which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. *See, e.g.*, GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (*e.g.*, FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000)

supra). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. *See, e.g.*, Altschul *et al.* (1990) J. Mol. Biol. 215:403-410 and Altschul *et al.* (1997) Nucleic Acids Res. 25:3389-402, each herein incorporated by reference.

Germline Mutations

[00124] The anti-FcεR1α and/or anti-CD3 antibodies disclosed herein comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy chain variable domains as compared to the corresponding germline sequences from which the antibodies were derived.

[00125] The present invention also includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"), and having desired binding properties to an FcεR1α or CD3 antigen, for example, weak or no detectable binding of anti-CD3 antibodies to CD3. Several such exemplary antibodies that recognize FcεR1α are described in Table 1. Several such exemplary antibodies that recognize CD3 are described in Table 3.

[00126] Furthermore, the antibodies of the present invention may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be tested for one or more desired properties such as, improved binding specificity, weak or reduced binding affinity, improved or enhanced pharmacokinetic properties, reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner given the guidance of the present disclosure are encompassed within the present invention.

[00127] The present invention also includes anti-FcεR1α antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present invention includes anti-CD3 antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences set forth in Table 1 herein. The antibodies and bispecific antigen-binding molecules of the present invention comprise one or more amino acid

substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences from which the individual antigen-binding domains were derived, while maintaining or improving the desired binding to FcεR1α or CD3, for example, weak or no detectable binding of anti-CD3 antibodies to CD3 antigen. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (*e.g.*, charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein, *i.e.* the amino acid substitution maintains or improves the desired binding affinity in the case of anti-FcεR1α and/or anti-CD3 binding molecules, for example, weak to no detectable binding or anti-CD3 antibodies to CD3 antigen. Examples of groups of amino acids that have side chains with similar chemical properties include (1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; (2) aliphatic-hydroxyl side chains: serine and threonine; (3) amide-containing side chains: asparagine and glutamine; (4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; (5) basic side chains: lysine, arginine, and histidine; (6) acidic side chains: aspartate and glutamate, and (7) sulfur-containing side chains are cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443-1445. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[00128] The present invention also includes antigen-binding molecules comprising an antigen-binding domain with an HCVR and/or CDR amino acid sequence that is substantially identical to any of the HCVR and/or CDR amino acid sequences disclosed herein, while maintaining or improving the desired property to FcεR1α and/or CD3 antigen. The term "substantial identity" or "substantially identical," when referring to an amino acid sequence means that two amino acid sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. *See, e.g.*, Pearson (1994) Methods Mol. Biol. 24: 307-331.

[00129] Sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and

other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as Gap and Bestfit which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. *See, e.g.*, GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (*e.g.*, FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) supra). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. *See, e.g.*, Altschul *et al.* (1990) J. Mol. Biol. 215:403-410 and Altschul *et al.* (1997) Nucleic Acids Res. 25:3389-402.

[00130] Once obtained, antigen-binding domains that contain one or more germline mutations are tested for decreased binding affinity utilizing one or more *in vitro* assays. Although antibodies that recognize a particular antigen are typically screened for their purpose by testing for high (*i.e.* strong) binding affinity to the antigen, the antibodies of the present invention exhibit weak binding or no detectable binding. Bispecific antigen-binding molecules comprising one or more antigen-binding domains obtained in this general manner are also encompassed within the present invention and are found to be advantageous as avidity-driven allergy therapies.

[00131] Unexpected benefits, for example, improved pharmacokinetic properties and low toxicity to the patient may be realized from the methods described herein.

Binding Properties of the Antibodies

[00132] As used herein, the term "binding" in the context of the binding of an antibody, immunoglobulin, antibody-binding fragment, or Fc-containing protein to either, *e.g.,* a predetermined antigen, such as a cell surface protein or fragment thereof, typically refers to an interaction or association between a minimum of two entities or molecular structures, such as an antibody-antigen interaction.

[00133] For instance, binding affinity typically corresponds to a K_D value of about 10^{-6} M or less, such as about 10^{-7} M or less, such as about 10^{-8} M or less, such as about 10^{-9} M or less when determined by, for instance, surface plasmon resonance (SPR) technology in a BIAcore 3000 instrument using the antigen as the ligand and the antibody, Ig, antibody-binding fragment, or Fc-containing protein as the analyte (or antiligand). Cell-based binding strategies, such as fluorescent-activated cell sorting (FACS) binding assays, are also routinely used, and FACS data correlates well with other methods such as radioligand competition binding and SPR (Benedict, CA, *J Immunol Methods*. 1997, 201(2):223-31; Geuijen, CA, et al. *J Immunol Methods*. 2005, 302(1-2):68-77).

[00134] Accordingly, the antibody or antigen-binding protein of the invention binds to the predetermined antigen or cell surface molecule (receptor) having an affinity corresponding to a K_D value that is at least ten-fold lower than its affinity for binding to a non-specific antigen (*e.g.*, BSA, casein). According to the present invention, the affinity of an antibody corresponding to a K_D value that is equal to or less than ten-fold lower than a non-specific antigen may be considered non-detectable binding, however such an antibody may be paired with a second antigen binding arm for the production of a bispecific antibody of the invention.

[00135] The term "K_D" (M) refers to the dissociation equilibrium constant of a particular antibody-antigen interaction, or the dissociation equilibrium constant of an antibody or antibody-binding fragment binding to an antigen. There is an inverse relationship between K_D and binding affinity, therefore the smaller the K_D value, the higher, *i.e.* stronger, the affinity. Thus, the terms “higher affinity” or “stronger affinity” relate to a greater ability to form an interaction and therefore a smaller K_D value, and conversely the terms “lower affinity” or “weaker affinity” relate to a lesser ability to form an interaction and therefore a larger K_D value. In some circumstances, a higher binding affinity (or K_D) of a particular molecule (*e.g.* antibody) to its interactive partner molecule (*e.g.* antigen X) compared to the binding affinity of the molecule (*e.g.* antibody) to another interactive partner molecule (*e.g.* antigen Y) may be expressed as a binding affinity ratio determined by dividing the larger K_D value (lower, or weaker, affinity) by the smaller K_D (higher, or stronger, affinity), for example expressed as 5-fold or 10-fold greater binding affinity, as the case may be.

[00136] The term "k_d" (sec -1 or 1/s) refers to the dissociation rate constant of a particular antibody-antigen interaction, or the dissociation rate constant of an antibody or antibody-binding fragment. Said value is also referred to as the k_{off} value.

[00137] The term "k_a" (M-1 x sec-1 or 1/M) refers to the association rate constant of a particular antibody-antigen interaction, or the association rate constant of an antibody or antibody-binding fragment.

[00138] The term "K_A" (M-1 or 1/M) refers to the association equilibrium constant of a particular antibody-antigen interaction, or the association equilibrium constant of an antibody or antibody-binding fragment. The association equilibrium constant is obtained by dividing the k_a by the k_d.

[00139] The term “EC50” or “EC_{50}” refers to the half maximal effective concentration, which includes the concentration of an antibody that induces a response halfway between the baseline and maximum after a specified exposure time. The EC_{50} essentially represents the concentration of an antibody where 50% of its maximal effect is observed. In certain embodiments, the EC_{50} value equals the concentration of an antibody of the invention that gives half-maximal binding to cells expressing CD3 or allergy related antigen, as determined by *e.g.* a FACS binding assay. Thus, reduced or weaker binding is observed with an increased EC_{50}, or half maximal effective concentration value.

[00140] In one embodiment, decreased binding can be defined as an increased EC_{50} antibody concentration which enables binding to the half-maximal amount of target cells.

[00141] In another embodiment, the EC_{50} value represents the concentration of an antibody of the invention that elicits half-maximal depletion of target cells by T cell cytotoxic activity. Thus, increased cytotoxic activity (*e.g.* T cell-mediated basophils killing) is observed with a decreased EC_{50}, or half maximal effective concentration value.

Bispecific Antigen-Binding Molecules

[00142] The antibodies of the present invention may be monospecific, bi-specific, or multispecific. Multispecific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, *e.g.*, Tutt et al., 1991, J. Immunol. 147:60-69; Kufer *et al.*, 2004, Trends Biotechnol. 22:238-244. The anti-FcεR1α monospecific antibodies or anti-FcεR1α/anti-CD3 bispecific antibodies of the present invention can be linked to or co-expressed with another functional molecule, *e.g.*, another peptide or protein. For example, an antibody or fragment thereof can be functionally linked (*e.g.*, by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment to produce a bi-specific or a multispecific antibody with a second or additional binding specificity.

[00143] Use of the expression "anti-CD3 antibody" or “anti-FcεR1α antibody” herein is intended to include both monospecific anti-CD3 or anti-FcεR1α antibodies as well as bispecific antibodies comprising a CD3-binding arm and an FcεR1α-binding arm. Thus, the present invention includes bispecific antibodies wherein one arm of an immunoglobulin binds human CD3, and the other arm of the immunoglobulin is specific for human FcεR1α. The CD3-binding arm can comprise any of the HCVR/LCVR or CDR amino acid sequences as set forth in Table 3 herein.

[00144] In certain embodiments, the CD3-binding arm binds to human CD3 and induces human T cell activation. In certain embodiments, the CD3-binding arm binds weakly to human CD3 and induces human T cell activation. In other embodiments, the CD3-binding arm binds weakly to human CD3 and induces ablation of mast cells and/or basophils in the context of a bispecific or multispecific antibody. In other embodiments, the CD3-binding arm binds or is associated weakly with human CD3, yet the binding interaction is not detectable by *in vitro* assays known in the art. The FcεR1α-binding arm can comprise any of the HCVR/LCVR or CDR amino acid sequences as set forth in Table 1 herein.

[00145] According to certain exemplary embodiments, the present invention includes bispecific antigen-binding molecules that specifically bind CD3 and FcεR1α. Such molecules may be referred to herein as, *e.g.*, "anti-CD3/anti-FcεR1α," or "anti-CD3xFcεR1α," or “anti-FcεR1α/anti-CD3,” or “anti-FcεR1αxCD3,” or "CD3xFcεR1α" bispecific molecules, or “FcεR1αxCD3” bispecific molecules, or other similar terminology (*e.g.*, anti-FcεR1α x anti-CD3).

[00146] The term "FcεR1α," as used herein, refers to the human FcεR1α protein unless specified as being from a non-human species (*e.g.*, "mouse FcεR1α," "monkey FcεR1α," etc.). The human FcεR1α protein has the amino acid sequence shown in SEQ ID NO: 63.

[00147] The aforementioned bispecific antigen-binding molecules that specifically bind CD3 and FcεR1α may comprise an anti-CD3 antigen-binding molecule which binds to CD3 with a weak binding affinity such as exhibiting a K_D of greater than about 40 nM, as measured by an *in vitro* affinity binding assay.

[00148] As used herein, the expression "antigen-binding molecule" means a protein, polypeptide or molecular complex comprising or consisting of at least one complementarity determining region (CDR) that alone, or in combination with one or more additional CDRs and/or framework regions (FRs), specifically binds to a particular antigen. In certain embodiments, an antigen-binding molecule is an antibody or a fragment of an antibody, as those terms are defined elsewhere herein.

[00149] As used herein, the expression "bispecific antigen-binding molecule" means a protein, polypeptide or molecular complex comprising at least a first antigen-binding domain and a second antigen-binding domain. Each antigen-binding domain within the bispecific antigen-binding molecule comprises at least one CDR that alone, or in combination with one or more additional CDRs and/or FRs, specifically binds to a particular antigen. In the context of the present invention, the first antigen-binding domain specifically binds a first antigen (*e.g.*, CD3), and the second antigen-binding domain specifically binds a second, distinct antigen (*e.g.*, FcεR1α).

[00150] In certain exemplary embodiments of the present invention, the bispecific antigen-binding molecule is a bispecific antibody. Each antigen-binding domain of a bispecific antibody comprises a heavy chain variable domain (HCVR) and a light chain variable domain (LCVR). In the context of a bispecific antigen-binding molecule comprising a first and a second antigen-binding domain (*e.g.*, a bispecific antibody), the CDRs of the first antigen-binding domain may be designated with the prefix "A1" and the CDRs of the second antigen-binding domain may be designated with the prefix "A2". Thus, the CDRs of the first antigen-binding domain may be referred to herein as A1-HCDR1, A1-HCDR2, and A1-HCDR3; and the CDRs of the second antigen-binding domain may be referred to herein as A2-HCDR1, A2-HCDR2, and A2-HCDR3.

[00151] The first antigen-binding domain and the second antigen-binding domain may be directly or indirectly connected to one another to form a bispecific antigen-binding molecule of the present invention. Alternatively, the first antigen-binding domain and the second antigen-binding domain may each be connected to a separate multimerizing domain. The association of one multimerizing domain with another multimerizing domain facilitates the association between the two antigen-binding domains, thereby forming a bispecific antigen-binding molecule. As used herein, a "multimerizing domain" is any macromolecule, protein, polypeptide, peptide, or

amino acid that has the ability to associate with a second multimerizing domain of the same or similar structure or constitution. For example, a multimerizing domain may be a polypeptide comprising an immunoglobulin C_H3 domain. A non-limiting example of a multimerizing component is an Fc portion of an immunoglobulin (comprising a C_H2-C_H3 domain), *e.g.*, an Fc domain of an IgG selected from the isotypes IgG1, IgG2, IgG3, and IgG4, as well as any allotype within each isotype group.

[00152] Bispecific antigen-binding molecules of the present invention will typically comprise two multimerizing domains, *e.g.*, two Fc domains that are each individually part of a separate antibody heavy chain. The first and second multimerizing domains may be of the same IgG isotype such as, *e.g.*, IgG1/IgG1, IgG2/IgG2, and IgG4/IgG4. Alternatively, the first and second multimerizing domains may be of different IgG isotypes such as, *e.g.*, IgG1/IgG2, IgG1/IgG4, IgG2/IgG4, etc.

[00153] In certain embodiments, the multimerizing domain is an Fc fragment or an amino acid sequence of from 1 to about 200 amino acids in length containing at least one cysteine residue. In other embodiments, the multimerizing domain is a cysteine residue, or a short cysteine-containing peptide. Other multimerizing domains include peptides or polypeptides comprising or consisting of a leucine zipper, a helix-loop motif, or a coiled-coil motif.

[00154] Any bispecific antibody format or technology may be used to make the bispecific antigen-binding molecules of the present invention. For example, an antibody or fragment thereof having a first antigen binding specificity can be functionally linked (*e.g.*, by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment having a second antigen-binding specificity to produce a bispecific antigen-binding molecule. Specific exemplary bispecific formats that can be used in the context of the present invention include, without limitation, *e.g.*, scFv-based or diabody bispecific formats, IgG-scFv fusions, dual variable domain (DVD)-Ig, Quadroma, knobs-into-holes, common light chain (*e.g.*, common light chain with knobs-into-holes, etc.), CrossMab, CrossFab, (SEED)body, leucine zipper, Duobody, IgG1/IgG2, dual acting Fab (DAF)-IgG, and Mab^2 bispecific formats (*see*, *e.g.*, Klein *et al.* 2012, mAbs 4:6, 1-11, and references cited therein, for a review of the foregoing formats).

[00155] In the context of bispecific antigen-binding molecules of the present invention, the multimerizing domains, *e.g.*, Fc domains, may comprise one or more amino acid changes (*e.g.*, insertions, deletions or substitutions) as compared to the wild-type, naturally occurring version of the Fc domain. For example, the invention includes bispecific antigen-binding molecules comprising one or more modifications in the Fc domain that results in a modified Fc domain having a modified binding interaction (*e.g.*, enhanced or diminished) between Fc and FcRn. In one embodiment, the bispecific antigen-binding molecule comprises a modification in a C_H2 or a C_H3 region, wherein the modification increases the affinity of the Fc domain to FcRn in an acidic

environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0). Non-limiting examples of such Fc modifications include, *e.g.*, a modification at position 250 (*e.g.,* E or Q); 250 and 428 (*e.g.,* L or F); 252 (*e.g.,* L/Y/F/W or T), 254 (*e.g.,* S or T), and 256 (*e.g.,* S/R/Q/E/D or T); or a modification at position 428 and/or 433 (*e.g.,* L/R/S/P/Q or K) and/or 434 (*e.g.,* H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.,* F or P), and 434. In one embodiment, the modification comprises a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.,* T250Q and M428L); and a 307 and/or 308 modification (*e.g.,* 308F or 308P).

[00156] The present invention also includes bispecific antigen-binding molecules comprising a first C_H3 domain and a second Ig C_H3 domain, wherein the first and second Ig C_H3 domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bispecific antibody to Protein A as compared to a bi-specific antibody lacking the amino acid difference. In one embodiment, the first Ig C_H3 domain binds Protein A and the second Ig C_H3 domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C_H3 may further comprise a Y96F modification (by IMGT; Y436F by EU). See, for example, US Patent No. 8,586,713. Further modifications that may be found within the second C_H3 include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 antibodies; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 antibodies; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 antibodies.

[00157] In certain embodiments, the Fc domain may be chimeric, combining Fc sequences derived from more than one immunoglobulin isotype. For example, a chimeric Fc domain can comprise part or all of a C_H2 sequence derived from a human IgG1, human IgG2 or human IgG4 C_H2 region, and part or all of a C_H3 sequence derived from a human IgG1, human IgG2 or human IgG4. A chimeric Fc domain can also contain a chimeric hinge region. For example, a chimeric hinge may comprise an "upper hinge" sequence, derived from a human IgG1, a human IgG2 or a human IgG4 hinge region, combined with a "lower hinge" sequence, derived from a human IgG1, a human IgG2 or a human IgG4 hinge region. A particular example of a chimeric Fc domain that can be included in any of the antigen-binding molecules set forth herein comprises, from N- to C-terminus: [IgG4 C_H1] - [IgG4 upper hinge] - [IgG2 lower hinge] - [IgG4 CH2] - [IgG4 CH3]. Another example of a chimeric Fc domain that can be included in any of the antigen-binding molecules set forth herein comprises, from N- to C-terminus: [IgG1 C_H1] - [IgG1 upper hinge] - [IgG2 lower hinge] - [IgG4 CH2] - [IgG1 CH3]. These and other examples of

chimeric Fc domains that can be included in any of the antigen-binding molecules of the present invention are described in US Publication 2014/0243504, published August 28, 2014, which is herein incorporated in its entirety. Chimeric Fc domains having these general structural arrangements, and variants thereof, can have altered Fc receptor binding, which in turn affects Fc effector function.

[00158] In certain embodiments, the invention provides an antibody heavy chain wherein the heavy chain constant region (CH) region comprises an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to any one of SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, or SEQ ID NO: 56. In some embodiments, the heavy chain constant region (CH) region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, and SEQ ID NO: 56.

Sequence Variants

[00159] The antibodies and bispecific antigen-binding molecules of the present invention may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences from which the individual antigen-binding domains were derived. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The antigen-binding molecules of the present invention may comprise antigen-binding domains which are derived from any of the exemplary amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antigen-binding domain was originally derived. In other embodiments, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antigen-binding domain was originally derived). Furthermore, the antigen-binding domains may contain any combination

of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antigen-binding domains that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. Bispecific antigen-binding molecules comprising one or more antigen-binding domains obtained in this general manner are encompassed within the present invention.

[00160] The present invention also includes antigen-binding molecules wherein one or both antigen-binding domains comprise variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present invention includes antigen-binding molecules comprising an antigen-binding domain having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (*e.g.*, charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. Examples of groups of amino acids that have side chains with similar chemical properties include (1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; (2) aliphatic-hydroxyl side chains: serine and threonine; (3) amide-containing side chains: asparagine and glutamine; (4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; (5) basic side chains: lysine, arginine, and histidine; (6) acidic side chains: aspartate and glutamate, and (7) sulfur-containing side chains are cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443-1445, herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[00161] The present invention also includes antigen-binding molecules comprising an antigen-binding domain with an HCVR, LCVR, and/or CDR amino acid sequence that is substantially identical to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein. The term "substantial identity" or "substantially identical," when referring to an amino acid sequence means that two amino acid sequences, when optimally aligned, such as by the programs GAP

or BESTFIT using default gap weights, share at least 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. *See, e.g.*, Pearson (1994) Methods Mol. Biol. 24: 307-331, herein incorporated by reference.

[00162] Sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as Gap and Bestfit which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. *See, e.g.*, GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (*e.g.*, FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) supra). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. *See, e.g.*, Altschul *et al.* (1990) J. Mol. Biol. 215:403-410 and Altschul *et al.* (1997) Nucleic Acids Res. 25:3389-402, each herein incorporated by reference.

pH-Dependent Binding

[00163] The present invention includes anti-FcεR1α antibodies, and anti-CD3/anti-FcεR1α bispecific antigen-binding molecules, with pH-dependent binding characteristics. For example, an anti-FcεR1α antibody of the present invention may exhibit reduced binding to FcεR1α at acidic pH as compared to neutral pH. Alternatively, anti-FcεR1α antibodies of the invention may exhibit enhanced binding to FcεR1α at acidic pH as compared to neutral pH. The expression "acidic pH" includes pH values less than about 6.2, *e.g.*, about 6.0, 5.95, 5,9, 5.85, 5.8, 5.75, 5.7, 5.65, 5.6, 5.55, 5.5, 5.45, 5.4, 5.35, 5.3, 5.25, 5.2, 5.15, 5.1, 5.05, 5.0, or less. As used herein, the expression "neutral pH" means a pH of about 7.0 to about 7.4. The expression "neutral pH" includes pH values of about 7.0, 7.05, 7.1, 7.15, 7.2, 7.25, 7.3, 7.35, and 7.4.

[00164] In certain instances, "reduced binding ... at acidic pH as compared to neutral pH" is expressed in terms of a ratio of the K_D value of the antibody binding to its antigen at acidic pH to the K_D value of the antibody binding to its antigen at neutral pH (or vice versa). For example, an antibody or antigen-binding fragment thereof may be regarded as exhibiting "reduced binding to

FcεR1α at acidic pH as compared to neutral pH" for purposes of the present invention if the antibody or antigen-binding fragment thereof exhibits an acidic/neutral K_D ratio of about 3.0 or greater. In certain exemplary embodiments, the acidic/neutral K_D ratio for an antibody or antigen-binding fragment of the present invention can be about 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, 20.0, 25.0, 30.0, 40.0, 50.0, 60.0, 70.0, 100.0 or greater.

[00165] Antibodies with pH-dependent binding characteristics may be obtained, *e.g.*, by screening a population of antibodies for reduced (or enhanced) binding to a particular antigen at acidic pH as compared to neutral pH. Additionally, modifications of the antigen-binding domain at the amino acid level may yield antibodies with pH-dependent characteristics. For example, by substituting one or more amino acids of an antigen-binding domain (*e.g.*, within a CDR) with a histidine residue, an antibody with reduced antigen-binding at acidic pH relative to neutral pH may be obtained.

Antibodies Comprising Fc Variants

[00166] According to certain embodiments of the present invention, anti-FcεR1α antibodies, and anti-CD3/anti-FcεR1α bispecific antigen-binding molecules, are provided comprising an Fc domain comprising one or more mutations which enhance or diminish antibody binding to the FcRn receptor, *e.g.*, at acidic pH as compared to neutral pH. For example, the present invention includes antibodies comprising a mutation in the C_H2 or a C_H3 region of the Fc domain, wherein the mutation(s) increases the affinity of the Fc domain to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0). Such mutations may result in an increase in serum half-life of the antibody when administered to an animal. Non-limiting examples of such Fc modifications include, *e.g.*, a modification at position 250 (*e.g.*, E or Q); 250 and 428 (*e.g.*, L or F); 252 (*e.g.*, L/Y/F/W or T), 254 (*e.g.*, S or T), and 256 (*e.g.*, S/R/Q/E/D or T); or a modification at position 428 and/or 433 (*e.g.*, H/L/R/S/P/Q or K) and/or 434 (*e.g.,* H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 and/or 308 (*e.g.,* F or P), and 434. In one embodiment, the modification comprises a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.,* T250Q and M428L); a 307 modification and/or a 308 modification (*e.g.,* 308F or 308P).

[00167] For example, the present invention includes anti-FcεR1α antibodies, and anti-CD3/anti-FcεR1α bispecific antigen-binding molecules, comprising an Fc domain comprising one or more pairs or groups of mutations selected from the group consisting of: 250Q and 248L (*e.g.*, T250Q and M248L); 252Y, 254T and 256E (*e.g.*, M252Y, S254T and T256E); 428L and 434S (*e.g.*, M428L and N434S); and 433K and 434F (*e.g.*, H433K and N434F). All possible combinations of

the foregoing Fc domain mutations, and other mutations within the antibody variable domains disclosed herein, are contemplated within the scope of the present invention.

Biological Characteristics of the Antibodies and Bispecific Antigen-Binding Molecules

[00168] According to certain embodiments, the present invention includes antibodies and antigen-binding fragments of antibodies that bind human FcεR1α (*e.g.*, at 25ºC) with a K_D of less than about 303 nM or bind cynomolgus FcεR1α (*e.g.*, at 25ºC) with a K_D of less than about 467 nM as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 3 herein. In certain embodiments, the antibodies or antigen-binding fragments of the present invention bind human or cynomolgus FcεR1α with a K_D of less than about 400 nM, less than about 500 nM, less than about 450 nM, less than about 400 nM, less than about 350 nM, less than about 300 nM, less than about 250 nM, less than about 200 nM, less than about 150 nM, or less than about 100 nM, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 3 herein (*e.g.*, mAb-capture or antigen-capture format), or a substantially similar assay. The present invention includes bispecific antigen-binding molecules (*e.g.*, bispecific antibodies which bind human or cynomolgus FcεR1α with a K_D of less than about 467 nM, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 3 herein (*e.g.*, mAb-capture or antigen-capture format), or a substantially similar assay.

[00169] The present invention also includes antibodies and antigen-binding fragments thereof that bind human FcεR1α with a dissociative half-life (t½) of greater than about 0.2 minute or greater than about 0.5 minutes or bind cynomolgus FcεR1α with a dissociative half-life (t½) of greater than about 0.3 minute or greater than about 0.6 minute as measured by surface plasmon resonance at 25ºC, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay. The present invention includes bispecific antigen-binding molecules (*e.g.*, bispecific antibodies) which bind human or cynomolgus FcεR1α with a K_D of greater than about 0.54 minutes or greater than about 1.1 minutes as measured by surface plasmon resonance at 25ºC, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay.

[00170] The present invention also includes antibodies and antigen-binding fragments thereof which bind specifically to human cell lines which express human or cynomolgus FcεR1α (*e.g.*, HEK293 cells engineered to express human or cynomolgus FcεR1α), as determined by a flow cytometry-based detection assay as set forth in Example 4 or a substantially similar assay.

[00171] The present invention also includes anti-CD3/anti-FcεR1α bispecific antigen-binding molecules which exhibit one or more characteristics selected from the group consisting of: (a) binding to FcεR1α expressed on cell surface in the absence or presence of IgE (*see*, *e.g.*, Example 4); (b) activating human CD3 signaling in the presence of FcεR1α expressing cells (*see*, *e.g.*, Example 5); (c) inducing T-cell mediated apoptosis of FcεR1α expressing cells *in vitro*

(*see*, *e.g.*, Example 6); (d) inducing T-cell mediated killing of basophils in a peripheral blood mononuclear cell (PBMC) population *in vitro* (*see*, *e.g.*, Example 6); (e) blocking allergen induced mast cell degranulation (*e.g.*, anaphylaxis) in mice expressing human FcεR1α (*see*, *e.g.*, Example 7); and (f) depleting splenic basophils in mice expressing human FcεR1α (*see*, *e.g.*, Example 7).

[00172] The present invention includes antibodies and antigen-binding fragments thereof that bind human CD3 with high affinity. The present invention also includes antibodies and antigen-binding fragments thereof that bind human CD3 with medium or low affinity, depending on the therapeutic context and particular targeting properties that are desired. The present invention also includes antibodies and antigen-binding fragments thereof that bind human CD3 with no measureable affinity. For example, in the context of a bispecific antigen-binding molecule, wherein one arm binds CD3 and another arm binds a target antigen (*e.g.*, FcεR1α), it may be desirable for the target antigen-binding arm to bind the target antigen with high affinity while the anti-CD3 arm binds CD3 with only moderate or low affinity or no affinity. In this manner, preferential targeting of the antigen-binding molecule to cells expressing the target antigen may be achieved while avoiding general/untargeted CD3 binding and the consequent adverse side effects associated therewith.

[00173] The present invention includes bispecific antigen-binding molecules (*e.g.*, bispecific antibodies) which are capable of simultaneously binding to human CD3 and a human FcεR1α. The binding arm that interacts with cells that express CD3 may have weak to no detectable binding as measured in a suitable *in vitro* binding assay. The extent to which a bispecific antigen-binding molecule binds cells that express CD3 and/or FcεR1α can be assessed by fluorescence activated cell sorting (FACS), as illustrated in Example 4 herein.

[00174] For example, the present invention includes antibodies, antigen-binding fragments, and bispecific antibodies thereof which specifically bind human T-cell lines which express CD3 but do not express FcεR1α, primate T-cells (*e.g.*, cynomolgus peripheral blood mononuclear cells [PBMCs]), and/or FcεR1α-expressing cells.

[00175] The present invention includes antibodies, antigen-binding fragments, and bispecific antibodies thereof that bind human CD3 with weak (*i.e.* low) or even no detectable affinity.

[00176] The present invention includes antibodies, antigen-binding fragments, and bispecific antibodies thereof that bind monkey (cynomolgus) CD3 with weak (*i.e.* low) or even no detectable affinity.

[00177] The present invention includes antibodies, antigen-binding fragments, and bispecific antibodies thereof that bind human CD3 and induce T cell activation.

[00178] The present invention includes anti-CD3/anti-FcεR1α bispecific antigen-binding molecules which are capable of inhibiting allergic response and/or depleting FcεR1α-expressing cells in a subject (*see*, *e.g.*, Example 7, in a passive cutaneous anaphylaxis (PCA) or a flow

cytometry-based assay, or substantially similar assays). For example, according to certain embodiments, anti-CD3/anti-FcεR1α bispecific antigen-binding molecules are provided, wherein a single administration of 25 mg/kg of the bispecific antigen-binding molecule to a subject causes a reduction in the number of FcεR1α-expressing cells in the subject (*e.g.*, the number of splenic basophils is significantly reduced).

Epitope Mapping and Related Technologies

[00179] The epitope on CD3 and/or FcεR1α to which the antigen-binding molecules of the present invention bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acids of a CD3 or FcεR1α protein. Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) of CD3 or FcεR1α. The antibodies of the invention may interact with amino acids contained within a single CD3 chain (*e.g.*, CD3-epsilon, CD3-delta or CD3-gamma), or may interact with amino acids on two or more different CD3 chains. The term "epitope," as used herein, refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstances, an epitope may include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen.

[00180] Various techniques known to persons of ordinary skill in the art can be used to determine whether an antigen-binding domain of an antibody "interacts with one or more amino acids" within a polypeptide or protein. Exemplary techniques include, *e.g.*, routine cross-blocking assay such as that described Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harbor, NY), alanine scanning mutational analysis, peptide blots analysis (Reineke, 2004, Methods Mol Biol 248:443-463), and peptide cleavage analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer, 2000, Protein Science 9:487-496). Another method that can be used to identify the amino acids within a polypeptide with which an antigen-binding domain of an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water to allow hydrogen-deuterium exchange to occur at all residues except for the residues protected by the antibody (which remain deuterium-labeled). After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues which

correspond to the specific amino acids with which the antibody interacts. *See, e.g.*, Ehring (1999) *Analytical Biochemistry* 267(2):252-259; Engen and Smith (2001) *Anal. Chem. 73*:256A-265A. X-ray crystallography of the antigen/antibody complex may also be used for epitope mapping purposes.

[00181] The present invention further includes anti-FcεR1α antibodies that bind to the same epitope as any of the specific exemplary antibodies described herein (*e.g.* antibodies comprising any of the amino acid sequences as set forth in Table 1 herein). Likewise, the present invention also includes anti-FcεR1α antibodies that compete for binding to FcεR1α with any of the specific exemplary antibodies described herein (*e.g.* antibodies comprising any of the amino acid sequences as set forth in Table 1 herein).

[00182] The present invention also includes bispecific antigen-binding molecules comprising a first antigen-binding domain that specifically binds human CD3 and/or cynomolgus CD3 with low or detectable binding affinity, and a second antigen binding domain that specifically binds human or cynomolgus FcεR1α, wherein the first antigen-binding domain binds to the same epitope on CD3 as any of the specific exemplary CD3-specific antigen-binding domains described herein, and/or wherein the second antigen-binding domain binds to the same epitope on FcεR1α as any of the specific exemplary FcεR1α-specific antigen-binding domains described herein.

[00183] Likewise, the present invention also includes bispecific antigen-binding molecules comprising a first antigen-binding domain that specifically binds human CD3, and a second antigen binding domain that specifically binds human FcεR1α, wherein the first antigen-binding domain competes for binding to CD3 with any of the specific exemplary CD3-specific antigen-binding domains described herein, and/or wherein the second antigen-binding domain competes for binding to FcεR1α with any of the specific exemplary FcεR1α-specific antigen-binding domains described herein.

[00184] One can easily determine whether a particular antigen-binding molecule (*e.g.*, antibody) or antigen-binding domain thereof binds to the same epitope as, or competes for binding with, a reference antigen-binding molecule of the present invention by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope on FcεR1α (or CD3) as a reference bispecific antigen-binding molecule of the present invention, the reference bispecific molecule is first allowed to bind to an FcεR1α protein (or CD3 protein). Next, the ability of a test antibody to bind to the FcεR1α (or CD3) molecule is assessed. If the test antibody is able to bind to FcεR1α (or CD3) following saturation binding with the reference bispecific antigen-binding molecule, it can be concluded that the test antibody binds to a different epitope of FcεR1α (or CD3) than the reference bispecific antigen-binding molecule. On the other hand, if the test antibody is not able to bind to the FcεR1α (or CD3) molecule following saturation binding with the reference bispecific antigen-binding molecule,

then the test antibody may bind to the same epitope of FcεR1α (or CD3) as the epitope bound by the reference bispecific antigen-binding molecule of the invention. Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference bispecific antigen-binding molecule or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, Biacore, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art. In accordance with certain embodiments of the present invention, two antigen-binding proteins bind to the same (or overlapping) epitope if, *e.g.*, a 1-, 5-, 10-, 20- or 100-fold excess of one antigen-binding protein inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, *e.g.*, Junghans et al., Cancer Res. 1990:50:1495-1502). Alternatively, two antigen-binding proteins are deemed to bind to the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antigen-binding protein reduce or eliminate binding of the other. Two antigen-binding proteins are deemed to have "overlapping epitopes" if only a subset of the amino acid mutations that reduce or eliminate binding of one antigen-binding protein reduce or eliminate binding of the other.

[00185] To determine if an antibody or antigen-binding domain thereof competes for binding with a reference antigen-binding molecule, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antigen-binding molecule is allowed to bind to an FcεR1α protein (or CD3 protein) under saturating conditions followed by assessment of binding of the test antibody to the FcεR1α (or CD3) molecule. In a second orientation, the test antibody is allowed to bind to an FcεR1α (or CD3) molecule under saturating conditions followed by assessment of binding of the reference antigen-binding molecule to the FcεR1α (or CD3) molecule. If, in both orientations, only the first (saturating) antigen-binding molecule is capable of binding to the FcεR1α (or CD3) molecule, then it is concluded that the test antibody and the reference antigen-binding molecule compete for binding to FcεR1α (or CD3). As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antigen-binding molecule may not necessarily bind to the same epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

Preparation of Antigen-Binding Domains and Construction of Bispecific Molecules

[00186] Antigen-binding domains specific for particular antigens can be prepared by any antibody generating technology known in the art. Once obtained, two different antigen-binding domains, specific for two different antigens (*e.g.*, CD3 and FcεR1α), can be appropriately arranged relative to one another to produce a bispecific antigen-binding molecule of the present invention using routine methods. (A discussion of exemplary bispecific antibody formats that

can be used to construct the bispecific antigen-binding molecules of the present invention is provided elsewhere herein). In certain embodiments, one or more of the individual components (*e.g.*, heavy and light chains) of the multispecific antigen-binding molecules of the invention are derived from chimeric, humanized or fully human antibodies. Methods for making such antibodies are well known in the art. For example, one or more of the heavy and/or light chains of the bispecific antigen-binding molecules of the present invention can be prepared using VELOCIMMUNE™ technology. Using VELOCIMMUNE™ technology (or any other human antibody generating technology), high affinity chimeric antibodies to a particular antigen (*e.g.*, CD3 or FcεR1α) are initially isolated having a human variable region and a mouse constant region. The antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc. The mouse constant regions are replaced with a desired human constant region to generate fully human heavy and/or light chains that can be incorporated into the bispecific antigen-binding molecules of the present invention.

[00187] Genetically engineered animals may be used to make human bispecific antigen-binding molecules. For example, a genetically modified mouse can be used which is incapable of rearranging and expressing an endogenous mouse immunoglobulin light chain variable sequence, wherein the mouse expresses only one or two human light chain variable domains encoded by human immunoglobulin sequences operably linked to the mouse kappa constant gene at the endogenous mouse kappa locus. Such genetically modified mice can be used to produce fully human bispecific antigen-binding molecules comprising two different heavy chains that associate with an identical light chain that comprises a variable domain derived from one of two different human light chain variable region gene segments. (*See*, *e.g.*, US 2011/0195454). Fully human refers to an antibody, or antigen-binding fragment or immunoglobulin domain thereof, comprising an amino acid sequence encoded by a DNA derived from a human sequence over the entire length of each polypeptide of the antibody or antigen-binding fragment or immunoglobulin domain thereof. In some instances, the fully human sequence is derived from a protein endogenous to a human. In other instances, the fully human protein or protein sequence comprises a chimeric sequence wherein each component sequence is derived from human sequence. While not being bound by any one theory, chimeric proteins or chimeric sequences are generally designed to minimize the creation of immunogenic epitopes in the junctions of component sequences, *e.g.* compared to any wild-type human immunoglobulin regions or domains.

Bioequivalents

[00188] The present invention encompasses antigen-binding molecules having amino acid sequences that vary from those of the exemplary molecules disclosed herein but that retain the ability to bind CD3 and/or FcεR1α. Such variant molecules may comprise one or more additions, deletions, or substitutions of amino acids when compared to parent sequence, but

exhibit biological activity that is essentially equivalent to that of the described bispecific antigen-binding molecules.

[00189] The present invention includes antigen-binding molecules that are bioequivalent to any of the exemplary antigen-binding molecules set forth herein. Two antigen-binding proteins, or antibodies, are considered bioequivalent if, for example, they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose under similar experimental conditions, either single does or multiple dose. Some antigen-binding proteins will be considered equivalents or pharmaceutical alternatives if they are equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on, *e.g.*, chronic use, and are considered medically insignificant for the particular drug product studied.

[00190] In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, and potency.

[00191] In one embodiment, two antigen-binding proteins are bioequivalent if a patient can be switched one or more times between the reference product and the biological product without an expected increase in the risk of adverse effects, including a clinically significant change in immunogenicity, or diminished effectiveness, as compared to continued therapy without such switching.

[00192] In one embodiment, two antigen-binding proteins are bioequivalent if they both act by a common mechanism or mechanisms of action for the condition or conditions of use, to the extent that such mechanisms are known.

[00193] Bioequivalence may be demonstrated by in vivo and in vitro methods. Bioequivalence measures include, *e.g.*, (a) an in vivo test in humans or other mammals, in which the concentration of the antibody or its metabolites is measured in blood, plasma, serum, or other biological fluid as a function of time; (b) an in vitro test that has been correlated with and is reasonably predictive of human in vivo bioavailability data; (c) an in vivo test in humans or other mammals in which the appropriate acute pharmacological effect of the antibody (or its target) is measured as a function of time; and (d) in a well-controlled clinical trial that establishes safety, efficacy, or bioavailability or bioequivalence of an antigen-binding protein.

[00194] Bioequivalent variants of the exemplary bispecific antigen-binding molecules set forth herein may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. In other contexts, bioequivalent antigen-binding proteins

may include variants of the exemplary bispecific antigen-binding molecules set forth herein comprising amino acid changes which modify the glycosylation characteristics of the molecules, *e.g.*, mutations which eliminate or remove glycosylation.

Species Selectivity and Species Cross-Reactivity

[00195] According to certain embodiments of the invention, antigen-binding molecules are provided which bind to human CD3. Also provided are antigen-binding molecules which bind to human FcεR1α. The present invention also includes antigen-binding molecules that bind to human CD3 to CD3 from one or more non-human species; and/or antigen-binding molecules that bind to human FcεR1α or and to FcεR1α from one or more non-human species, *e.g.*, cynomolgus.

[00196] According to certain exemplary embodiments of the invention, antigen-binding molecules are provided which bind to human CD3 and/or human FcεR1α and may bind or not bind, as the case may be, to one or more of mouse, rat, guinea pig, hamster, gerbil, pig, cat, dog, rabbit, goat, sheep, cow, horse, camel, cynomolgus, marmoset, rhesus, cynomolgus or chimpanzee CD3 and/or FcεR1α. For example, in a particular exemplary embodiment of the present invention bispecific antigen-binding molecules are provided comprising a first antigen-binding domain that binds human CD3, and a second antigen-binding domain that binds human or cynomolgus FcεR1α.

Therapeutic Formulation and Administration

[00197] The present invention provides pharmaceutical compositions comprising the antigen-binding molecules of the present invention. The pharmaceutical compositions of the invention are formulated with suitable carriers, excipients, and other agents that provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™, Life Technologies, Carlsbad, CA), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[00198] The dose of antigen-binding molecule administered to a patient may vary depending upon the age and the size of the patient, target disease, conditions, route of administration, and the like. The preferred dose is typically calculated according to body weight or body surface area. When a bispecific antigen-binding molecule of the present invention is used for

therapeutic purposes in an adult patient, it may be advantageous to intravenously administer the bispecific antigen-binding molecule of the present invention normally at a single dose of about 0.01 to about 50 mg/kg body weight, more preferably about 0.1 to about 25, about 1 to about 25, or about 5 to about 25 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. Effective dosages and schedules for administering a bispecific antigen-binding molecule may be determined empirically; for example, patient progress can be monitored by periodic assessment, and the dose adjusted accordingly. Moreover, interspecies scaling of dosages can be performed using well-known methods in the art (*e.g.*, Mordenti *et al.*, 1991, *Pharmaceut. Res. 8*:1351).

[00199] Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, *e.g.*, Wu et al., 1987, J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[00200] A pharmaceutical composition of the present invention can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[00201] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo

Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), and the HUMIRA™ Pen (Abbott Labs, Abbott Park IL), to name only a few.

[00202] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201). In another embodiment, polymeric materials can be used; see, Medical Applications of Controlled Release, Langer and Wise (eds.), 1974, CRC Pres., Boca Raton, Florida. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, 1984, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer, 1990, Science 249:1527-1533.

[00203] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (*e.g.*, ethanol), a polyalcohol (*e.g.*, propylene glycol, polyethylene glycol), a nonionic surfactant [*e.g.*, polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, *e.g.*, sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[00204] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the aforesaid antibody contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the aforesaid antibody is contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

Therapeutic Uses of the Antigen-Binding Molecules

[00205] The present invention includes methods comprising administering to a subject in need thereof a therapeutic composition comprising an anti-FcεR1α antibody or antigen-binding fragment thereof, or a bispecific antigen-binding molecule that specifically binds CD3 and FcεR1α. The therapeutic composition can comprise any of the antibodies or bispecific antigen-binding molecules as disclosed herein and a pharmaceutically acceptable carrier or diluent. As used herein, the expression "a subject in need thereof" means a human or non-human animal that exhibits one or more symptoms or indicia of an FcεR1α-related disease or disorder such as mast cell activation disorder, mastocytosis or an allergy (*e.g.*, a subject suffering from any type of allergies or exhibiting any allergic response), or who otherwise would benefit from an inhibition or reduction in FcεR1α activity or a depletion of FcεR1α+ cells (*e.g.*, anaphylaxis).

[00206] The antibodies and bispecific antigen-binding molecules of the invention (and therapeutic compositions comprising the same) are useful, *inter alia*, for treating any disease or disorder in which stimulation, activation and/or targeting of an immune response would be beneficial. In particular, the anti-FcεR1α antibodies or the anti-CD3/anti-FcεR1α bispecific antigen-binding molecules of the present invention may be used for the treatment, prevention and/or amelioration of any disease or disorder associated with or mediated by FcεR1α expression or activity or the proliferation of FcεR1α+ cells. The mechanism of action by which the therapeutic methods of the invention are achieved include killing of the cells expressing FcεR1α in the presence of effector cells, for example, by CDC, apoptosis, ADCC, phagocytosis, or by a combination of two or more of these mechanisms. Cells expressing FcεR1α which can be inhibited or killed using the bispecific antigen-binding molecules of the invention include, for example, mast cells and/or basophils.

[00207] The antigen-binding molecules of the present invention may be used to treat a disease or disorder associated with IgE or FcεR1α expression including, *e.g.*, mast cell activation disorder (such as mast cell activation syndrome), mastocytosis, or allergies including allergic asthma, hay fever, anaphylaxis, atopic dermatitis, chronic urticaria, food allergy, and pollen allergy. The allergies may be caused by exposure to one or more allergens, as listed elsewhere herein. According to certain embodiments of the present invention, the anti-FcεR1α antibodies or anti-FcεR1α/anti-CD3 bispecific antibodies are useful for treating a patient afflicted with severe allergy, including anaphylaxis. According to other related embodiments of the invention, methods are provided comprising administering an anti-FcεR1α antibody or an anti-CD3/anti-FcεR1α bispecific antigen-binding molecule as disclosed herein to a patient who is afflicted with anaphylaxis. Analytic/diagnostic methods known in the art, such as allergic reaction test, etc., may be used to ascertain whether a patient suffers anaphylaxis.

[00208] According to certain aspects, the present invention provides methods for treating a disease or disorder associated with FcεR1α expression (*e.g.*, anaphylaxis) comprising

administering one or more of the anti-FcεR1α or bispecific antigen-binding molecules described elsewhere herein to a subject after the subject has been determined to have allergy. For example, the present invention includes methods for treating allergy comprising administering an anti-FcεR1α antibody or an anti-CD3/anti-FcεR1α bispecific antigen-binding molecule to a patient 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks or 4 weeks, 2 months, 4 months, 6 months, 8 months, 1 year, or more after the subject has received other therapy *(e.g.*, anti-histamine therapy).

Combination Therapies and Formulations

[00209] The present invention provides methods which comprise administering a pharmaceutical composition comprising any of the exemplary antibodies and bispecific antigen-binding molecules described herein in combination with one or more additional therapeutic agents. Exemplary additional therapeutic agents that may be combined with or administered in combination with an antigen-binding molecule of the present invention include, *e.g.*, an IgE antagonist (*e.g.*, an anti-IgE antibody such as omalizumab) or small molecule inhibitor of IgE (*e.g.*,darpins such as darpin E2_76), an IL-25 inhibitor, an IL-4 inhibitor, an IL-4 receptor inhibitor (*e.g.*, an anti-IL-4R antibody such as dupilumab), an IL-33 inhibitor (*e.g.*, an anti-IL-33 antibody), a plasma cell ablating agent (*e.g.*, a BCMA x CD3 bispecific antibody) and a TSLP inhibitor. In certain embodiments, the plasma cell ablating agent is selected from the group consisting of a B-cell maturation antigen (BCMA) targeting agent, a proteasome inhibitor, a histone deacetylase inhibitor, a B-cell activating factor (BAFF) inhibitor, and an inhibitor of A proliferation inducing ligand (APRIL; CD256). In one embodiment, the BCMA targeting agent is selected from the group consisting of an anti-BCMA/anti-CD3 bispecific antibody, a chimeric antigen receptor against BCMA, and an anti-BCMA antibody conjugated to a cytotoxic drug.

[00210] Other agents that may be beneficially administered in combination with the antigen-binding molecules of the invention include allergy treatment medicines, including antihistamines, anti-inflammatory agents, corticoids, epinephrine, a bronchial dilator, a decongestant, leukotriene antagonists, or mast cell inhibitor (*e.g.*, cromolyn sodium).

[00211] The present invention also includes therapeutic combinations comprising any of the antigen-binding molecules mentioned herein and an inhibitor of IgE or FcεR1α, wherein the inhibitor is an aptamer, an antisense molecule, a ribozyme, an siRNA, a peptibody, a nanobody or an antibody fragment (*e.g.*, Fab fragment; $F(ab')_2$ fragment; Fd fragment; Fv fragment; scFv; dAb fragment; or other engineered molecules, such as diabodies, triabodies, tetrabodies, minibodies and minimal recognition units). The antigen-binding molecules of the invention may also be administered and/or co-formulated in combination with antivirals, antibiotics, analgesics, corticosteroids and/or NSAIDs.

[00212] The additional therapeutically active component(s) may be administered just prior to, concurrent with, or shortly after the administration of an antigen-binding molecule of the present

invention; (for purposes of the present disclosure, such administration regimens are considered the administration of an antigen-binding molecule "in combination with" an additional therapeutically active component).

[00213] The present invention includes pharmaceutical compositions in which an antigen-binding molecule of the present invention is co-formulated with one or more of the additional therapeutically active component(s) as described elsewhere herein.

Administration Regimens

[00214] According to certain embodiments of the present invention, multiple doses of an antigen-binding molecule (*e.g.*, an anti-FcεR1α antibody or a bispecific antigen-binding molecule that specifically binds FcεR1α and CD3) may be administered to a subject over a defined time course. The methods according to this aspect of the invention comprise sequentially administering to a subject in need thereof multiple doses of an antigen-binding molecule of the invention. As used herein, "sequentially administering" means that each dose of an antigen-binding molecule is administered to the subject at a different point in time, *e.g.*, on different days separated by a predetermined interval (*e.g.*, hours, days, weeks or months). The present invention includes methods which comprise sequentially administering to the patient a single initial dose of an antigen-binding molecule, followed by one or more secondary doses of the antigen-binding molecule, and optionally followed by one or more tertiary doses of the antigen-binding molecule.

[00215] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the antigen-binding molecule of the invention. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of the antigen-binding molecule, but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of an antigen-binding molecule contained in the initial, secondary and/or tertiary doses varies from one another (*e.g.*, adjusted up or down as appropriate) during the course of treatment. In certain embodiments, two or more (*e.g.*, 2, 3, 4, or 5) doses are administered at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (*e.g.*, "maintenance doses").

[00216] In one exemplary embodiment of the present invention, each secondary and/or tertiary dose is administered 1 to 26 (*e.g.*, 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 5½, 6, 6½, 7, 7½, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12, 12½, 13, 13½, 14, 14½, 15, 15½, 16, 16½, 17, 17½, 18, 18½, 19, 19½, 20, 20½, 21, 21½, 22, 22½, 23, 23½, 24, 24½, 25, 25½, 26, 26½, or more) weeks after the

immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of antigen-binding molecule which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[00217] The methods according to this aspect of the invention may comprise administering to a patient any number of secondary and/or tertiary doses of an antigen-binding molecule (*e.g.*, an anti-FcεR1α antibody or a bispecific antigen-binding molecule that specifically binds FcεR1α and CD3). For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[00218] In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 1 to 2 weeks after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 2 to 4 weeks after the immediately preceding dose. Alternatively, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

[00219] In one embodiment, the antigen-binding molecule (*e.g.*, an anti-FcεR1α antibody or a bispecific antigen-binding molecule that specifically binds FcεR1α and CD3) is administered to a subject as a weight-based dose. A "weight-based dose" *(e.g.,* a dose in mg/kg) is a dose of the antibody or the antigen-binding fragment thereof or the bispecific antigen-binding molecule that will change depending on the subject's weight.

[00220] In another embodiment, an antibody or the antigen-binding fragment thereof or a bispecific antigen-binding molecule is administered to a subject as a fixed dose. A "fixed dose" *(e.g.,* a dose in mg) means that one dose of the antibody or the antigen-binding fragment thereof or the bispecific antigen-binding molecule is used for all subjects regardless of any specific subject-related factors, such as weight. In one particular embodiment, a fixed dose of an antibody or the antigen-binding fragment thereof or a bispecific antigen-binding molecule of the invention is based on a predetermined weight or age.

[00221] In general, a suitable dose of the antigen binding molecule the invention can be in the range of about 0.001 to about 200.0 milligram per kilogram body weight of the recipient, generally in the range of about 1 to 50 mg per kilogram body weight. For example, the antibody

or the antigen-binding fragment thereof or the bispecific antigen-binding molecule can be administered at about 0.1 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 3 mg/kg, about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 40 mg/kg, about 50 mg/kg per single dose. Values and ranges intermediate to the recited values are also intended to be part of this invention.

[00222] In some embodiments, the antigen binding molecule of the invention is administered as a fixed dose of between about 1 mg to about 2500 mg. In some embodiments, the antigen binding molecule of the invention is administered as a fixed dose of about 1 mg, 2 mg, 3 mg, 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, about 30 mg, about 50 mg, about 75 mg, about 100 mg, about 125 mg, about 150 mg, about 175 mg, 200 mg, about 225 mg, about 250 mg, about 275 mg, about 300 mg, about 325 mg, about 350 mg, about 375 mg, about 400 mg, about 425 mg, about 450 mg, about 475 mg, about 500 mg, about 525 mg, about 550 mg, about 575 mg, about 600 mg, about 625 mg, about 650 mg, about 675 mg, about 700 mg, about 725 mg, about 750 mg, about 775 mg, about 800 mg, about 825 mg, about 850 mg, about 875 mg, about 900 mg, about 925 mg, about 950 mg, about 975 mg, about 1000 mg, about 1500 mg, about 2000 mg, or about 2500 mg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

Diagnostic Uses of the Antibodies

[00223] The anti-FcεR1α antibodies of the present invention may also be used to detect and/or measure FcεR1α, or FcεR1α-expressing cells in a sample, *e.g.*, for diagnostic purposes. For example, an anti-FcεR1α antibody, or fragment thereof, may be used to diagnose a condition or disease characterized by aberrant expression (*e.g.*, over-expression, under-expression, lack of expression, etc.) of FcεR1α. Exemplary diagnostic assays for FcεR1α may comprise, *e.g.*, contacting a sample, obtained from a patient, with an anti-FcεR1α antibody of the invention, wherein the anti-FcεR1α antibody is labeled with a detectable label or reporter molecule. Alternatively, an unlabeled anti-FcεR1α antibody can be used in diagnostic applications in combination with a secondary antibody which is itself detectably labeled. The detectable label or reporter molecule can be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I; a fluorescent or chemiluminescent moiety such as fluorescein isothiocyanate, or rhodamine; or an enzyme such as alkaline phosphatase, beta-galactosidase, horseradish peroxidase, or luciferase. Another exemplary diagnostic use of the anti-FcεR1α antibodies of the invention includes ^{89}Zr–labeled, such as ^{89}Zr-desferrioxamine–labeled, antibody for the purpose of noninvasive identification and tracking of mast cells, basophils, or other FcεR1α expressing cells in a subject (*e.g.* positron emission tomography (PET) imaging). (*See*, *e.g.,* Tavare, R. et al. Cancer Res. 2016 Jan 1;76(1):73-82; and Azad, BB. et al. Oncotarget. 2016 Mar 15;7(11):12344-58.) Specific exemplary assays that can be used to detect or measure FcεR1α in a sample include enzyme-

linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).

[00224] Samples that can be used in FcεR1α diagnostic assays according to the present invention include any tissue or fluid sample obtainable from a patient which contains detectable quantities of FcεR1α protein, or fragments thereof, under normal or pathological conditions. Generally, levels of FcεR1α in a particular sample obtained from a healthy patient (*e.g.*, a patient not afflicted with a disease or condition associated with abnormal FcεR1α levels or activity) will be measured to initially establish a baseline, or standard, level of FcεR1α. This baseline level of FcεR1α can then be compared against the levels of FcεR1α measured in samples obtained from individuals suspected of having an FcεR1α related disease (*e.g.*, a subject with allergy) or condition.

EXAMPLES

[00225] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1: Generation of Antibodies

Generation of Anti-FcεR1α Antibodies

[00226] Anti-FcεR1α antibodies were obtained by immunizing a genetically modified mouse with a human FcεR1α antigen (*e.g.*, hFcεR1α, SEQ ID NO: 63) or by immunizing an engineered mouse comprising DNA encoding human immunoglobulin heavy and kappa light chain variable regions with a human FcεR1α antigen.

[00227] Following immunization, splenocytes were harvested from each mouse and either (1) fused with mouse myeloma cells to preserve their viability and form hybridoma cells and screened for FcεR1α specificity, or (2) B-cell sorted (as described in US 2007/0280945A1) using a human FcεR1α fragment as the sorting reagent that binds and identifies reactive antibodies (antigen-positive B cells).

[00228] Chimeric antibodies to FcεR1α were initially isolated having a human variable region and a mouse constant region. The antibodies were characterized and selected for desirable characteristics, including affinity, selectivity, etc. If necessary, mouse constant regions were replaced with a desired human constant region, for example wild-type or modified IgG1 or IgG4 constant region, to generate a fully human anti-FcεR1α antibody. While the constant region

selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

[00229] Certain biological properties of the exemplary anti-FcεR1α antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

Generation of Anti-CD3 Antibodies

[00230] Anti-CD3 antibodies were generated as described in WO 2017/053856, which is herein incorporated by reference. An exemplary anti-CD3 antibody was selected for the production of bispecific anti-CD3/anti-FcεR1α antibodies in accordance with the present invention. Other anti-CD3 antibodies for use in preparing bispecific antibodies in accordance with the present invention can be found in, *e.g.*, WO 2014/047231.

[00231] Certain biological properties of the exemplary anti-CD3 antibodies generated in accordance with the methods of this Example are described in detail in the Examples herein.

Generation of Bispecific Antibodies that Bind FcεR1α and CD3

[00232] The present invention provides bispecific antigen-binding molecules that bind CD3 and FcεR1α; such bispecific antigen-binding molecules are also referred to herein as "anti-FcεR1α/anti-CD3 or anti-FcεR1αxCD3 bispecific molecules." The anti-FcεR1α portion of the anti-FcεR1α/anti-CD3 bispecific molecule is useful for targeting cells that express FcεR1α, and the anti-CD3 portion of the bispecific molecule is useful for activating T-cells. The simultaneous binding of FcεR1α on a cell and CD3 on a T-cell facilitates directed killing (cell lysis) of the targeted FcεR1α expressing cell by the activated T-cell.

[00233] Bispecific antibodies comprising an anti-FcεR1α-specific binding domain and an anti-CD3-specific binding domain were constructed using standard methodologies, wherein the anti-FcεR1α antigen binding domain and the anti-CD3 antigen binding domain each comprise different, distinct HCVRs paired with a common LCVR. In exemplified bispecific antibodies, the molecules were constructed utilizing a heavy chain from an anti-CD3 antibody, a heavy chain from an anti-FcεR1α antibody and a common light chain from the anti-CD3 antibody WO 2017/053856). In other instances, the bispecific antibodies may be constructed utilizing a heavy chain from an anti-CD3 antibody, a heavy chain from an anti-FcεR1α antibody and a light chain from an anti-CD3 antibody or a light chain from an anti-FcεR1α antibody light chain or any other light chain known to be promiscuous or pair effectively with a variety of heavy chain arms. The anti-FcεR1α antibodies and the anti-CD3 antibodies, from which any components of the bispecific antibodies are derived, are sometimes referred to as parental antibodies.

[00234] The bispecific antibodies described in the following examples comprise anti-CD3 binding arms; and anti-FcεR1α binding arm. Exemplified bispecific antibodies were manufactured having an IgG4 Fc domain (bsAb24919D, bsAb24920D, and bsAb24921D).

[00235] A summary of the component parts of the antigen-binding domains of the various anti-FcεR1αxCD3 bispecific antibodies constructed is set forth in Table 5.

Example 2: Heavy and Light Chain Variable Region Amino Acid and Nucleic Acid Sequences

[00236] Table 1 sets forth the amino acid sequence identifiers of the heavy and light chain variable regions (HCVR and LCVR), CDRs and heavy chains and light chains (HC and LC) of selected anti-FcεR1α antibodies of the invention. The corresponding nucleic acid sequence identifiers are set forth in Table 2.

Table 1: Amino Acid Sequence Identifiers

	SEQ ID NOs:									
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**	**HC**	**LC**
mAb17110	2	4	6	8	26	28	30	32	34	40
mAb17111	10	12	14	16	26	28	30	32	36	40
mAb17112	18	20	22	24	26	28	30	32	38	40

Table 2: Nucleic Acid Sequence Identifiers

	SEQ ID NOs:									
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**	**HC**	**LC**
mAb17110	1	3	5	7	25	27	29	31	33	39
mAb17111	9	11	13	15	25	27	29	31	35	39
mAb17112	17	19	21	23	25	27	29	31	37	39

[00237] Table 3 sets forth the amino acid sequence identifiers of the heavy and light chain variable regions (HCVR and LCVR), CDRs and heavy chain and light chain (HC and LC) of an exemplary anti-CD3 antibody of the invention. The corresponding nucleic acid sequence identifiers are set forth in Table 4.

Table 3: Amino Acid Sequence Identifiers

	SEQ ID NOs:									
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**	**HC**	**LC**
mAb7221G20	42	44	46	48	26	28	30	32	56	40

Table 4: Nucleic Acid Sequence Identifiers

	SEQ ID NOs:									
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**	**HC**	**LC**
mAb7221G20	41	43	45	47	25	27	29	31	55	39

[00238] A summary of the component parts of the various anti-FcεR1αxCD3 bispecific antibodies constructed is set forth in Table 5. Tables 6, 7 and 8 list the HCVR, LCVR, CDRs and heavy chain and light sequence identifiers of the bispecific antibodies.

Table 5: Summary of Component Parts of Anti-FcεR1α16xCD3 Bispecific Antibodies

Bispecific Antibody Identifier	**Anti-FcεR1α Antigen-Binding Domain**	**Anti-CD3 Antigen-Binding Domain**	**Common Light Chain Variable Region**
	Heavy Chain Variable Region	***Heavy Chain Variable Region***	
bsAb24919D	mAb17110	mAb7221G20	mAb7221G20
bsAb24920D	mAb17111	mAb7221G20	mAb7221G20
bsAb24921D	mAb17112	mAb7221G20	mAb7221G20

Table 6: Amino acid sequences of variable regions and CDRs of bispecific antibodies

Bispecific Antibody Identifier	Anti-FcεR1α antigen-binding domain SEQ ID NOs.				Anti-CD3 antigen-binding domain SEQ ID NOs.				Common Light chain variable region SEQ ID NOs			
	HCVR	HCDR1	HCDR2	HCDR3	HCVR	HCDR1	HCDR2	HCDR3	LCVR	LCDR1	LCDR2	LCDR3
bsAb24919D	2	4	6	8	42	44	46	48	26	28	30	32
bsAb24920D	10	12	14	16	42	44	46	48	26	28	30	32
bsAb24921D	18	20	22	24	42	44	46	48	26	28	30	32

Table 7: Heavy chain and light chain amino acid sequence identifiers of bispecific antibodies

Bispecific antibody Identifier	Anti-FcεR1α Heavy Chain	Anti-CD3 Heavy Chain	Common Light Chain
bsAb24919D	SEQ ID NO: 50	SEQ ID NO: 56	SEQ ID NO: 40
bsAb24920D	SEQ ID NO: 52	SEQ ID NO: 56	SEQ ID NO: 40
bsAb24921D	SEQ ID NO: 54	SEQ ID NO: 56	SEQ ID NO: 40

Table 8: Heavy chain and light chain nucleic acid sequence identifiers of bispecific antibodies

Bispecific antibody Identifier	Anti-FcεR1α Heavy Chain	Anti-CD3 Heavy Chain	Common Light Chain
bsAb24919D	SEQ ID NO: 49	SEQ ID NO: 55	SEQ ID NO: 39
bsAb24920D	SEQ ID NO: 51	SEQ ID NO: 55	SEQ ID NO: 39
bsAb24921D	SEQ ID NO: 53	SEQ ID NO: 55	SEQ ID NO: 39

Example 3: Surface Plasmon Resonance Derived Binding Affinities and Kinetic Constants of Human Monoclonal anti-FcεR1α Monospecific and anti-FcεR1α16xCD3 Bispecific antibodies

[00239] Equilibrium dissociation constants (K_D) for human or cynomolgus FcεR1α ectodomain binding to purified anti-FcεR1α monoclonal antibodies (mAbs) and CD3 x FcεR1α bispecific antibodies (bsAbs) were determined using a real-time surface plasmon resonance biosensor (SPR-Biacore), Biacore 8k. All binding studies were performed in 10mM HEPES, 150mM NaCl, 3mM EDTA, and 0.05% v/v surfactant Tween-20, pH 7.4 (HBS-ET) running buffer at 25°C and 37°C.

[00240] The Biacore CM4 sensor surface was first derivatized by amine coupling with a monoclonal mouse anti-human Fc antibody to capture approximately 500-900 RUs anti-FcεR1α monoclonal antibodies or anti-CD3 x FcεR1α bispecific monoclonal antibodies. 1 RU (response unit) represents 1 pg of protein per mm^2, as defined by the manufacturer. The ectodomain of human and cynomolgus FcεR1α reagents were expressed with a C-term myc-myc-6xHis tag – hFcεR1α.MMH (SEQ ID NO: 57) and mfFcεR1α.MMH (SEQ ID NO: 58). Different concentrations of FcεR1α reagents were prepared in HBS-ET running buffer (600nM – 7.4nM; serially diluted by 3-fold) and injected over anti-human Fc captured anti-FcεR1α monoclonal antibodies or anti-CD3 x FcεR1α bispecific monoclonal antibodies surfaces for 1 minute at a flow rate of 30µL/minute. The dissociation of bound FcεR1α reagents was monitored for 4 minutes in HBS-ET running buffer. Association (k_a) and dissociation (k_d) rate constants were determined by fitting the real-time binding sensorgrams to a 1:1 binding model with mass transport limitation using Biacore 8k evaluation software. Binding dissociation equilibrium constants (K_D) and dissociative half-lives (t½) were calculated from the kinetic rate constants as:

$$K_D\ (M) = \frac{kd}{ka}, \quad \text{and} \quad t½\ (min) = \frac{\ln(2)}{60 * kd}$$

[00241] Binding kinetics parameters for hFcεR1α.MMH and mfFcεR1α.MMH binding to different exemplary anti-FcεR1α monoclonal antibodies or anti-CD3 x FcεR1α bispecific monoclonal antibodies of the invention at 25°C and 37°C are shown in Table 9 through Table 12.

Table 9: Binding Kinetics Parameters of hFcεR1α.MMH Binding to Anti-FcεR1α Monoclonal Antibodies or Anti-CD3 X FcεR1α Bispecific Monoclonal Antibodies at 25°C.

mAb Captured	mAb Capture Level (RU)	600nM hFcεR1α. MMH Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
mAb17110	609 ± 1.2	130	7.19E+04	9.13E-03	1.27E-07	1.3
mAb17111	567 ± 0.8	91	7.84E+04	1.65E-02	2.11E-07	0.7
mAb17112	630 ± 0.9	123	8.80E+04	1.84E-02	2.09E-07	0.63
bsAb24919D	610 ± 0.7	72	6.82E+04	1.04E-02	1.52E-07	1.1
bsAb24920D	573 ± 1	46	6.59E+04	2.00E-02	3.03E-07	0.6
bsAb24921D	607 ± 0.6	69	7.74E+04	2.12E-02	2.74E-07	0.54
Isotype Control mAb	545 ± 1.7	-5	NB *	NB *	NB *	NB *

NB* indicates that no binding was observed under the current experimental conditions.

Table 10: Binding Kinetics Parameters of hFcεR1α.MMH Binding to Anti-FcεR1α Monoclonal Antibodies or Anti-CD3 X FcεR1α Bispecific Monoclonal Antibodies at 37°C.

mAb Captured	mAb Capture Level (RU)	600nM hFcεR1α. MMH Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
mAb17110	745 ± 1.5	93	8.88E+04	3.94E-02	4.44E-07	0.29
mAb17111	745 ± 2.2	52	8.21E+04	4.83E-02	5.88E-07	0.24
mAb17112	681 ± 0.6	87	1.13E+05	5.85E-02	5.17E-07	0.20
bsAb24919D	764 ± 1.5	43	7.35E+04	5.88E-02	8.01E-07	0.20
bsAb24920D	743 ± 3.5	19	6.39E+04	9.91E-02	1.55E-06	0.12
bsAb24921D	670 ± 0.6	40	9.56E+04	9.34E-02	9.77E-07	0.12
Isotype Control mAb	688 ± 3.5	-2	NB *	NB *	NB *	NB *

NB* indicates that no binding was observed under the current experimental conditions.

Table 11: Binding Kinetics Parameters of mfFcεR1α.MMH Binding to Anti-FcεR1α Monoclonal Antibodies or Anti-CD3 X FcεR1α Bispecific Monoclonal Antibodies at 25°C.

mAb Captured	mAb Capture Level (RU)	600nM mfFceR1a .MMH Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
mAb17110	596 ± 0.8	79	6.13E+04	1.36E-02	2.21E-07	0.8
mAb17111	620 ± 0.5	60	5.55E+04	1.37E-02	2.47E-07	0.8
mAb17112	594 ± 1.3	110	7.13E+04	1.39E-02	1.95E-07	0.8
bsAb24919D	597 ± 0.8	40	3.96E+04	1.44E-02	3.63E-07	0.8
bsAb24920D	625 ± 0.8	26	4.17E+04	1.95E-02	4.67E-07	0.6
bsAb24921D	563 ± 0.8	60	6.29E+04	1.61E-02	2.56E-07	0.7
Isotype Control mAb	610 ± 1.6	-9	NB *	NB *	NB *	NB *

NB* indicates that no binding was observed under the current experimental conditions.

Table 12: Binding Kinetics Parameters of mfFcεR1α.MMH Binding to Anti-FcεR1α Monoclonal Antibodies or Anti-CD3 X FcεR1α Bispecific Monoclonal Antibodies at 37°C.

mAb Captured	mAb Capture Level (RU)	600nM mfFceR1a .MMH Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
mAb17110	719 ± 3.5	53	5.94E+04	2.54E-02	4.28E-07	0.45
mAb17111	720 ± 3.4	38	5.17E+04	2.64E-02	5.10E-07	0.44
mAb17112	769 ± 0.7	81	9.94E+04	3.28E-02	3.30E-07	0.35
bsAb24919D	685 ± 1.7	20	3.86E+04	3.99E-02	1.03E-06	0.29
bsAb24920D	660 ± 2.7	10	1.33E+04	4.45E-02	3.35E-06	0.26
bsAb24921D	749 ± 1.2	37	8.56E+04	4.45E-02	5.20E-07	0.26
Isotype Control mAb	775 ± 2.8	-9	NB *	NB *	NB *	NB *

NB* indicates that no binding was observed under the current experimental conditions.

[00242] At 25°C, exemplary anti-FcεR1α monoclonal antibodies or anti-CD3 x FcεR1α bispecific monoclonal antibodies of the invention bound to hFcεR1α.MMH with K_D values ranging from 127nM to 303nM, as shown in Table 9. At 37°C, exemplary anti-FcεR1α

monoclonal antibodies or anti-CD3 x FcεR1α bispecific monoclonal antibodies of the invention bound to hFcεR1α.MMH with K_D values ranging from 444nM to 1.55uM, as shown in Table 10.

[00243] At 25°C, exemplary anti-FcεR1α monoclonal antibodies or anti-CD3 x FcεR1α bispecific monoclonal antibodies of the invention bound to mfFcεR1α.MMH with K_D values ranging from 195nM to 467nM, as shown in Table 11. At 37°C, exemplary anti-FcεR1α monoclonal antibodies or anti-CD3 x FcεR1α bispecific monoclonal antibodies of the invention bound to mfFcεR1α.MMH with K_D values ranging from 330nM to 3.35uM, as shown in Table 12.

Example 4: Anti-FcεR1α x CD3 Bispecific Antibodies Bind Specifically to Expressed FcεR1α on Jurkat and HEK293 Cells

[00244] In order to assess the binding to antigens expressed on cells by anti-FcεR1α monoclonal antibodies and anti-FcεR1α x CD3 bi-specific antibodies, flow cytometry experiment was performed with Jurkat/NFAT-Luc and HEK293/hFcεR1α /hFcεR1β / hFcεR1γ cells. Jurkat/NFAT-Luc cells are Jurkat cells engineered to stably express a luciferase reporter under the transcription control of Nuclear factor of activated T-cells (*NFAT*) response element. HEK293/hFcεR1α /hFcεR1β / hFcεR1γ cells are HEK293 cells engineered to stably express human FcεR1α, FcεR1β and FcεR1γ. To test the binding to monkey (cynomolgus, mf) FcεR1α (amino acids 4-260 of accession# XP_005541370.1 with alanine at position 81 changed to tryptophan), mfFcεR1α was stably expressed in HEK293 along with human FcεR1β and FcεR1γ. The resulting cell line, referred to hereafter as HEK293/mf FcεR1α /hFcεR1β / hFcεR1γ was isolated and maintained in DMEM medium supplemented with 10% FBS, 1X NEAA, 1X Penicillin/Streptomycin/L-Glutamine, 1μg/mL Puromycin, 100μg/mL of Hygromycin B and 500μg/ml of G418 sulfate. The reagents information is as follows: DMEM medium, Irvine Scientific, Cat# CRL-1573; Fetal bovine serum (FBS), Seradigm, Cat#1500-500; 100 X Penicillin / Streptomycin/L-Glutamine (Pen/Strep/Glut), Invitrogen, Cat# 10378-016; 100X Non-essential amino acids (NEAA), Irvine Scientific, Cat# 9034; Geneticin™ Selective Antibiotic (G418 Sulfate), Invitrogen, Cat# 11811-098; Hygromycin B, Calbiochem, Cat# 400049; Puromycin, Sigma, Cat# P-8833.

[00245] For flow cytometry analysis, HEK293, HEK293/hFcεR1α /hFcεR1β / hFcεR1γ and HEK293/mf FcεR1α /hFcεR1β / hFcεR1γ cells were collected after dissociation using Enzyme Free Dissociation Buffer (Millipore Cat# S-004), and the cells were pre-incubated with or without 70nM human IgE, for 30 minutes on ice in FACS buffer (PBS, without Ca^{++} and Mg^{++}, (Irvine Scientific, Cat# 9240) containing 2% FBS). Jurkat/NFAT-luc cells were also collected. The antibodies at the concentration of 70 nM were then added to 1 x 10^6 cells/well of each cell type at 4°C for 30 minutes. After incubation with primary antibodies, the cells were stained with 1.3 μg/ml of Allophycocyanin (APC) conjugated anti-human IgG secondary antibody (Jackson ImmunoResearch, Cat# 109-136-170) for 30 minutes on ice. Cells were fixed using BD

CytoFix™ (Becton Dickinson, Cat. # 554655) and analyzed on Accuri™ C6 (BD) or CytoFLEX Flow cytometer (Beckman Coulter). Unstained and secondary antibody alone controls were also tested for all cell lines and a sample was evaluated for viability using the Far Red Fluo viability dye (Thermo Fisher, Cat#L10120) according to the manufacturer's protocol. The results were analyzed using FlowJo software (version 10.0.8, FlowJo) to determine the geometric means of fluorescence for viable cells and the binding ratio was calculated with the mean fluorescence intensity (MFI) of the experimental condition normalized by the MFI of the unstained respective cells. The results were summarized in Tables 13 and 14.

Table 13: Binding of 70nM of Anti-FcεR1α Antibodies and Anti-FcεR1α x CD3 Antibodies to HEK293/hFcεR1α /hFcεR1β / hFcεR1γ and Jurkat/NFAT-luc Cells

		Binding Ratio (MFI of Treated / MFI of Unstained)			
		HEK293	HEK293/hFcεR1α /hFcεR1β / hFcεR1γ		Jurkat/NFAT-luc
Antibodies ID	Specificity	No IgE	No IgE	70nM IgE	No IgE
bsAb24919D	FcεR1α x hCD3	2	269	277	48
bsAb24920D	FcεR1α x hCD3	4	248	201	48
bsAb24921D	FcεR1α x hCD3	11	253	295	94
mAb17110	FcεR1α	2	171	396	1
mAb17111	FcεR1α	1	136	369	1
mAb17112	FcεR1α	8	158	367	1
Human IgG4 Stealth Control	Irrelevant protein	1	1	1	2
Human IgG4 Control	Irrelevant protein	1	1	1	1

Table 14: Binding of 70nM of Anti-FcεR1α Antibodies and Anti-FcεR1α x CD3ε Antibodies to HEK293/mf FcεR1α /hFcεR1β / hFcεR1γ Cells

		Binding Ratio (MFI of Treated / MFI of Unstained)		
		HEK293 Parental	HEK293/mfFcεR1α /hFcεR1β / hFcεR1γ	
Antibodies ID	Specificity	No IgE	No IgE	70nM IgE
bsAb24919D	FcεR1α x hCD3	1	166	236
bsAb24920D	FcεR1α x hCD3	2	142	169
bsAb24921D	FcεR1α x hCD3	2	169	284
mAb17110	FcεR1α	1	101	256
mAb17111	FcεR1α	1	102	190
mAb17112	FcεR1α	1	134	279
Human IgG4 Stealth Control	Irrelevant protein	1	1	1
Human IgG4 Control	Irrelevant protein	1	1	1

[00246] As shown in Table 13, exemplary anti-FcεR1α x CD3 bispecific antibodies bsAb24919D, bsAb24920D, and bsAb24921D, showed binding to human FcεR1α expressed in HEK293/hFcεR1α /hFcεR1β / hFcεR1γ cells without and with 70nM of human IgE with binding ratios of 201 – 295 and to and human CD3 expressed in Jurkat cells with binding ratios of 48 – 94. The exemplary bispecific antibodies of the invention showed minimal binding to HEK293 without FcεR1 receptors with binding ratios of 2 – 11. Anti-FcεR1α antibodies showed binding to HEK293/hFcεR1α /hFcεR1β / hFcεR1γ cells without and with 70nM of human IgE with binding ratios of 136 – 396 and to HEK293 or Jurkat cells with binding ratios 1 – 8. Isotype control antibodies showed no binding to any of the cells and secondary only controls showed binding ratios of 1.

[00247] As shown in Table 14, exemplary anti-FcεR1α x CD3ε bispecific antibodies bsAb24919D, bsAb24920D, and bsAb24921D, showed binding to monkey (cynomolgus) FcεR1α expressed in HEK293/mfFcεR1α /hFcεR1β / hFcεR1γ cells without and with 70nM of human IgE of 142 – 284. The exemplary bi-specific antibodies of the invention showed minimal binding to HEK293 cells without FcεR1 receptors with binding ratios of 1 – 2. Exemplary anti-FcεR1α antibodies of the invention showed binding to HEK293/mfFcεR1α /hFcεR1β / hFcεR1γ cells without and with 70nM of human IgE with binding ratios of 101 – 279 but not to HEK293. Isotype control antibodies showed no binding to any of the cells and secondary only controls showed binding ratios of 1.

Example 5: Activation of Human CD3 Signaling by Anti-FcεR1α x CD3 Bispecific Antibodies

[00248] In order to assess the activation of human CD3 signaling by anti-FcεR1α x CD3ε bi-specific antibodies in the presence of FcεR1α expressing cells, a bioassay with Jurkat/NFAT-luc and HEK293/hFcεR1α /hFcεR1β / hFcεR1γ cells was performed. Stable cell lines were developed. Jurkat cell line, a human T lymphocytic cell line, has been utilized to demonstrate CD3 mediated T cell receptor signaling (Abraham and Weiss, *Jurkat T cells and development of the T-cell receptor signaling paradigm*. Nat Rev Immunol. 2004 Apr;4(4):301-8). Jurkat cells were engineered to stably express a luciferase reporter under the transcription control of Nuclear factor of activated T-cells (NFAT) response element. The resulting cell line, referred to hereafter as Jurkat/NFAT-Luc was isolated and maintained in RPMI1640 medium (Irvine Scientific, Cat. #9160) supplemented with 10% FBS, 1X Penicillin/Streptomycin/L-Glutamine and 1μg/mL Puromycin. Additionally, HEK293 cells were transfected to stably express human FcεR1α (amino acids 1-257 of Uniprot # P12319-1), FcεR1β (amino acids 1-244 of Uniprot # Q01362-1) and FcεR1γ (amino acids 1-86 of Uniprot # P30273-1). The resulting cell line, referred to hereafter as HEK293/hFcεR1α /hFcεR1β / hFcεR1γ was isolated and maintained in DMEM medium (Irvine Science, Cat. #9033) supplemented with 10% FBS, 1X NEAA, 1X Penicillin/Streptomycin/L-Glutamine, 1μg/mL Puromycin, 100μg/mL of Hygromycin B and 500μg/ml of G418 sulfate.

[00249] A bioassay was performed to measure the CD3 signaling by exemplary anti-FcεR1α x CD3ε bi-specific antibodies of the invention. For the bioassay, HEK293 or HEK293/hFcεR1α /hFcεR1β / hFcεR1γ cells were plated at 10,000 cells per well in a 96-well plate in assay buffer with or without 10nM of human IgE in assay buffer (10% FBS in RPMI1640 (Irvine Scientific, Cat#9160) with pen/strep/glut) for 30 minutes at 37°C in 5% CO_2. Following the incubation, Jurkat/NFAT-luc cells were plated at 50,000 along with serially diluted exemplary anti-FcεR1α x CD3 bispecific antibodies of the invention, exemplary anti-FcεR1α of the invention or isotype control antibodies at concentrations ranging from 100nM to 2pM plus a sample containing buffer alone (no antibody). After 5.5 hours at 37°C in 5% CO_2, luciferase activity was measured with OneGlo™ reagent (Promega, # E6031) and Victor™ X multilabel plate reader (Perkin Elmer). The results were analyzed using nonlinear regression (4-parameter logistics) with Prism™6 software (GraphPad) to obtain EC_{50} values. The fold activation was calculated with the average RLU (relative light units) at the highest concentration of antibody normalized by the average RLU without antibody. The results were summarized in Table 15.

Table 15: Activation of Human CD3 by Anti-FcεR1α x CD3 Antibodies

		Jurkat/NFAT-luc		Jurkat/NFAT-luc			
		HEK293 cells		HEK293/hFcεR1α /hFcεR1β / hFcεR1γ cells			
		No IgE	10nM IgE	No IgE		10nM IgE	
Antibody ID	Specificity	EC_{50} [M]	EC_{50} [M]	EC_{50} [M]	Fold Activation	EC_{50} [M]	Fold Activation
bsAb24919D	FcεR1α x hCD3	No activation	No activation	3.4E-10	32	1.5E-09	32
bsAb24920D	FcεR1α x hCD3	No activation	No activation	6.8E-10	23	4.9E-09	25
bsAb24921D	FcεR1α x hCD3	No activation	No activation	2.3E-10	24	1.1E-09	27
mAb17110	FcεR1α	No activation	No activation	No activation	1	No activation	1
mAb17111	FcεR1α	No activation	No activation	No activation	1	No activation	1
mAb17112	FcεR1α	No activation	No activation	No activation	1	No activation	1
Human IgG4 Stealth Control	Irrelevant protein	No activation	No activation	No activation	1	No activation	1
Human IgG4 Control	Irrelevant protein	No activation	No activation	No activation	1	No activation	1

[00250] As shown in Table 15, exemplary anti-FcεR1α x CD3 bispecific antibodies of the invention, bsAb24919D, bsAb24920D, and bsAb24921D, showed activation of CD3 signaling in Jurkat/NFAT-luc cells with EC_{50} values ranging from 230pM to 680pM in the presence of HEK293/hFcεR1α /hFcεR1β / hFcεR1γ cells without human IgE and 1.1nM to 4.9nM with 10nM of human IgE. The highest activation was achieved by bsAb24919D with fold activation of 32 without and with 10nM of IgE. The exemplary bispecific antibodies of the invention showed minimal activation in the presence of HEK293 without FcεR1 receptors with fold activation ranging 1 – 3. Anti-FcεR1α and isotype control antibodies showed no activation with fold activation of 1 in any of the conditions tested.

Example 6: Effect of Anti-FcεR1α x Anti-CD3 Bispecific Antibodies in *in vitro* Killing Assays

[00251] To determine efficacy of exemplary anti-FcεR1α x anti-CD3 bispecific antibodies of the invention (bsAb24919D, bsAb24920D and bsAb24921D) in inducing T cell-mediated killing of FcεR1α -expressing cells in vitro, two separate experiments were used. In one experiment, engineered HEK293/hFcεR1α /hFcεR1β / hFcεR1γ cells were used as targets, while in the second experiment primary human basophils within a total peripheral blood mononuclear cell (PBMC) population were used as targets. In both instances similar protocols were used to activate T cells prior to the killing assay: CD8+ T cells were first isolated from human leukopacks (NY Blood Center) using a RossetteSep™ Human CD8+ T cell enrichment cocktail kit (STEMCELL Technologies, Cat. #15063) and placed in culture with CD3/CD28-coated Dynabeads® (Invitrogen, Cat. #11132D) to induce activation of the T cells. On day 2-3 of

culture, beads were removed using magnetic separation and the T cells were placed in culture. In one example (for use with HEK293/hFcεR1α /hFcεR1β / hFcεR1γ target cells), T cells were maintained in culture for 5 days, at which time IL-2 was added at 300U/ml to promote viability and growth, and the T cells were used 2 days after IL-2 addition. In the second example (for use with PBMC target cells), cells were maintained in culture for one day after removal of the beads and then used for the killing assay. In both instances, activated T cells were labeled with Carboxyfluorescein succinimidyl ester (CFSE, Thermo Fischer Scientific, Cat.# C34554) prior to setting up the killing assay to enable exclusion of the cells during analysis of the results.

[00252] To determine efficacy of exemplary anti-FcεR1α x anti-CD3 bispecific antibodies of the invention in inducing T cell-mediated killing HEK293/hFcεR1α /hFcεR1β / hFcεR1γ target cells, a killing assay that uses detection of two mediators of the apoptotic cascade as readout (cleaved caspase 3 and cleaved PARP) was used. To set up the killing assay, the activated T cells were mixed with the target cells at a ratio of 10 target cells per T cell and then plated in a 96-well plate. Serial five-fold antibody dilutions ranging in final concentration from 100nM to 10.24fM were added to the wells, and the cells were incubated overnight at 37 ºC to allow T cell-mediated killing to occur. Antibodies included exemplary anti-FcεR1α/CD3 bispecific antibodies of the invention and isotype control antibody. Following incubation, the cells were harvested and resuspended in pre-warmed BD cytofix (Cat. #554655) for 10 minutes at 37 ºC. Cells were then washed twice in MACS buffer (Miltenyi, Cat.#130-091-221) and made permeable by resuspending in ice-cold methanol and incubating at -20 ºC for at least 30 minutes or overnight. Following permeabilization, MACS buffer was added to the cells for 10 minutes to allow cell rehydration, followed by 2 washes with MACS buffer. Cells were then incubated with Fc-blocking antibody (Ebioscience, Cat. #14-9161-73), followed by staining with an antibody cocktail containing Alexa-647-conjugated anti-cleaved caspase 3 (Cell Signaling Technology, Cat. #9602S) and a PE-conjugated anti-cleaved PARP antibodies (BD Biosciences, Cat. #552933). Cleavage of caspase 3 and PARP are obligatory steps in the activation of the apoptotic cascade that is initiated after delivery of cytotoxic lytic granules from the CD8+ T cells to the targets. Thus, specific detection of these cleaved proteins serves as a readout of killing. After staining the cells were washed, resuspended in MACS buffer and acquired using an LSRFortessa instrument (BD Biosciences). Killed cells were identified as CFSE-, and apoptotic cells within this population were identified as cleaved caspase 3+ and cleaved PARP+. Data analysis was performed using Graphpad Prism software. The data points obtained were transformed using an X=Log(X) equation, and the transformed data were subjected to a linear regression analysis and fitted into a sigmoidal dose response curve. EC_{80} (eighty percent (80%) of maximal effective concentration, which includes the concentration of an antibody which induces a eighty percent (80%) response between the baseline and maximum after a specified exposure time) and top responses were derived from this analysis.

[00253] To determine efficacy of exemplary anti-FcεR1α x anti-CD3 bispecific antibodies of the invention in inducing T cell-mediated killing of primary basophils within a peripheral blood mononuclear cell (PBMC) population, an assay based on quantitation of these cells relative to the rest of the PBMC population was used. Fresh PBMCs were obtained from donor blood by Ficoll (GE Healthcare, Cat. #17-1440-03) purification and were mixed with activated T cells and antibody dilutions in a similar format as described above for the engineered HEK293/hFcεR1α /hFcεR1β / hFcεR1γ target cells. After overnight incubation, cells were harvested, incubated with a Live/Dead cell marker (Invitrogen Cat. # L34962), followed by incubation with an Fc-blocking antibody and staining with an antibody mix containing APC-conjugated anti-HLA-DR (BD Biosciences, Cat. # 559866) and BUV 395-conjugated anti-CD123 (BD Biosciences, Cat. # 564195) antibodies. The cells were then washed twice with MACS buffer and fixed in a solution containing BD Cytofix diluted 1:4 in PBS for 15 minutes. Cells were resuspended in MACS buffer and acquired in a LSRFortessa instrument. Dead cells were excluded from analysis using the Live/Dead cell marker, as were exogenous activated T cells that had previously been labeled with CFSE. Basophils within the remaining live PBMC population were identified as CD123+ HLA-DR-.

[00254] Data analysis was performed using Graphpad Prism software. The data points obtained were transformed using an X=Log(X) equation, and the transformed data were subjected to a linear regression analysis and fitted into a sigmoidal dose response curve. EC_{50}s were derived from this analysis. Maximum percent basophil decrease was calculated using the following formula:

100 – (100 x percent basophils in sample with highest antibody dose) / (Average percent basophils in all isotype control samples).

[00255] Table 16 summarizes dose-dependent increases in cleaved caspase 3 and cleaved PARP double positive HEK293/hFcεR1α /hFcεR1β / hFcεR1γ target cells in the presence of each exemplary bispecific antibodies of the invention (bsAb24919D, bsAb24920D and bsAb24921D), with EC_{80}s of 3.456 x 10^{-11} M, 1.264 x 10^{-10} M, and 6.128 x 10^{-11} M, respectively. Because this assay is based on capturing the early stages of apoptosis, the assay is stopped before the cells are fully killed, and the maximum percent of cells staining positive for the apoptotic markers was 56.04, 56.48 and 55.83 for cells incubated with bsAb24919D, bsAb24920D and bsAb24921D, respectively. Notably, no increases in the percentage of target cells positive for both apoptotic markers were observed when T cells were incubated together with the target cells in the absence of antibody relative to target cells incubated alone. In other words, induction of apoptosis is not observed when T cells were incubated together with the target cells in the presence of isotype control antibody only.

Table 16: EC80 and Maximum Percent of Apoptotic Cells From Dose Response Killing Curves of HEK293/hFcεR1α /hFcεR1β / hFcεR1γ Target Cells after Incubation with T Cells and FcεR1α x CD3 Bispecific Antibodies

	bsAb24919D	bsAb24920D	bsAb24921D
EC80	3.456e-11	1.264e-10	6.128e-11
Maximum percent apoptotic cells	56.04	56.48	55.83

[00256] Table 17 summarizes dose-dependent decreases in basophils within the total PBMC target population in the presence of exemplary bispecific antibodies of the invention (bsAb24919D, bsAb24920D and bsAb24921D), with EC_{50}s of 3.748 x 10^{-9} M, 2.003 x 10^{-8} M, and 4.003 x 10^{-9} M, respectively. Basophils were decreased by 90.57%, 80.1% and 90.92% with the highest dose of bsAb24919D, bsAb24920D and bsAb24921D, respectively, relative to the average percent of basophils within the PBMCs in all isotype-treated samples.

Table 17: EC50 and Maximum Percent Basophil Decrease from Dose Response Killing Curves of Basophils within PBMC Target Cells after Incubation with T Cells and Fcεr1α x CD3 Bispecific Antibodies

	bsAb24919D	bsAb24920D	bsAb24921D
EC50	3.748e-9	2.003e-8	4.003e-9
Maximum percent basophil decrease	90.57%	80.1%	90.92%

Example 7: *In vivo* Efficacy of Anti-FcεR1α x CD3 Bispecific Antibodies

[00257] Effect of anti-FcεR1α x anti-CD3 bispecific antibodies in the passive cutaneous anaphylaxis (PCA) *in vivo* model and in splenic basophil depletion was studied.

[00258] To determine efficacy of anti-FcεR1α x CD3 bispecific antibodies of the invention for blocking allergen induced mast cell degranulation, the passive cutaneous anaphylaxis (PCA) *in vivo* model was used. The PCA model assesses type 1 hypersensitivity and measures local mast cell activation-induced vascular permeability in ear tissue (Gilfillan, A. M. & Tkaczyk, C. Integrated signaling pathways for mast-cell activation. Nat. Rev. Immunol. 6, 218–230 (2006)). This model involves intradermal injection of an allergen-specific sera from allergic patients into a local area on the skin of mice that express the human high-affinity IgE receptor, FcεR1α, followed by intravenous injection of an allergen along with a dye. The allergic reaction causes capillary dilatation and increased vascular permeability at the site of sensitization, resulting in preferential accumulation of dye at this site. The dye can be extracted from the tissue and quantitated spectrophotometrically.

[00259] For the PCA assays, groups of mice humanized for FcεR1α and CD3 (n≥5 per experiment) were first injected subcutaneously with either an isotype control antibody or one of three exemplary FcεR1α x CD3 bispecific antibodies of the invention at a dose of 25mg/kg. Five days after antibody administration, mice were injected in the ear with serum from a cat allergic individual (IgE titer 585, diluted 1:5 in PBS). The following day the mice were administered intravenously (100µL per mouse) a solution of 1 µg/mL Fel D1 (Indoor biotech LTN-FD1-1) dissolved in 1X PBS containing 0.5% (w/v) Evan's blue dye (Sigma Aldrich, #E2129). One hour after antigen administration, mice were sacrificed, and the ears and spleens were excised and collected.

[00260] The ears were placed in 1 mL formamide and subsequently incubated for 3 days at 50°C to extract the Evan's blue dye. The ear tissue was then removed from the formamide, blotted to remove excess liquid and weighed. Two-hundred microliter aliquots of each formamide extract were transferred to 96 well plates in duplicate. Absorbance of the resulting supernatants was measured at 620nm. The optical density measured was converted to Evan's blue dye concentration using a standard curve and is represented as nanogram of Evan's blue dye per milligram ear tissue. Table 18 shows mean values ± the standard deviation for each group.

[00261] To assess basophils frequency a flow cytometry-based assay was used. Single cell suspensions were prepared from the collected spleens following red blood cell lysis (Sigma, Cat #R7757). The cells were then stained with a live/dead cell marker, followed by antibody staining with the antibody mixes containing BUV 395 conjugated anti-B220 (BD, Cat#563793), FITC conjugated anti-CD4 (BD, Cat#553031), FITC conjugated anti-CD8 (BD, Cat#557667) and PECy7 conjugated CD49b (EBIOSCIENCE, Cat#25-5971-82). After staining, the cells were washed twice with MACS buffer (Miltenyi Biotech Cat# 130-091-221), fixed with BD Cytofix (Cat# 554655) diluted 1:4 in PBS for 15 minutes, then resuspended in MACS buffer and stored at 4 degrees. On the day of acquisition, the cells were washed twice in BD Perm/wash buffer (Cat# 554723) and stained for intracellular FcεR1α with the eFluor450 conjugated anti-FcεR1α (EBIOSCIENCE, Cat#48-5899-42). The cells were then acquired in an LSRFortessa instrument and analyzed using FlowJo software. Basophils were identified as B220- CD4- CD8- CD49+ FcεR1α+. Percent reduction of basophils in individual antibody-administered mice was calculated with the following formula: 100 – (percent splenic basophils / mean percent splenic basophils in the isotype group), where percent splenic basophils are calculated relative to total live cells in the spleen. The results are shown in Table 19.

[00262] Evan's blue dye extravasation was observed in the ears of mice that were not administered antibody or in those administered an isotype control antibody, with a mean dye quantitation of 84.06 and 82.05 ng/mg, respectively (ng/mg refers to nanogram of Evan's blue dye per milligram of tissue). Table 18 demonstrates efficacy of the exemplary anti-FcεR1α x

CD3 bispecific antibodies of the invention (bsAb24919D and bsAb24921D) in the PCA model as indicated by a significant reduction of dye extravasation in the groups treated with these antibodies when compared to isotype control. A non-statistically significant trend towards reduced dye extravasation was observed in the group treated with bsAb24920D as compared to isotype control. As shown, bsAb24919D and bsAb24921D block mast cell degranulation in the passive cutaneous *in vivo* model against sensitization and subsequent challenge with Fel D1 as compared to isotype control demonstrating a significant reduction in dye extravasation of 74.64 ng/mg and 75.26 ng/mg respectively, while a more modest reduction of 48.56 ng/mg was observed with bsAb24920D. Statistical significance was determined as follows: Normality of all groups was first tested with the Shapiro-Wilk normality test. Because the data in all groups was normally distributed, a one-way ANOVA analysis was applied, with a Brown-Forsythe test to determine differences in standard deviations across the groups. Significantly different standard deviations were observed; thus, a non-parametric Kruskal-Wallis test was run instead with Dunn's multiple comparison test to determine statistical significance among the groups.

[00263] Spleens from mice that were not administered antibody were found to contain an average of 0.91% of basophils relative to total live cells, while those from mice administered isotype control antibody contained an average of 0.71% of basophils. Table 19 demonstrates efficacy of exemplary FcεR1α x CD3 bispecific antibodies of the invention (bsAb24919D, bsAb24920D and bsAb24921D) in depleting splenic basophils in the same mice from the PCA experiment described above. As shown, mice treated with any of the three antibodies showed a reduction in splenic basophils. While this reduction was significant for all three antibodies as compared to mice that were not administered antibody, the reduction was only statistically significant for mice treated with bsAb24919D when compared to the group administered an isotype control antibody. Mice treated with bsAb24919D, bsAb24920D and bsAb24921D showed 96, 93 and 92 percent reduction in basophils relative to the isotype group, respectively. Statistical significance was determined as follows: a Shapiro-Wilk normality test was first run, and all data groups we found to be normally distributed; thus, a one-way ANOVA analysis was applied, and a Brown-Forsythe test was used to test for differences in standard deviations across the groups. Significantly different standard deviations were observed in this test, so a non-parametric Kruskal-Wallis test was run instead with Dunn's multiple comparison test to determine statistical significance among the groups.

Table 18: Effect of Anti-FcεR1α x CD3 Bispecific Antibodies in the Passive Cutaneous Anaphylaxis (PCA) *in vivo* Model

Treatment	ng Evans Blue/mg tissue ±SD	Mean Difference compared to Isotype control
bsAb24919D (n=7)	7.41 ± 1.1	-74.64 (**)
bsAb24920D (n=7)	33.49 ± 16.54	-48.56 (ns)
bsAb24921D (n=7)	6.79 ± 2.77	-75.26(**)

25mg/kg total antibody concentration used for all groups administered antibody
*P≤.05, ***P≤.001, ***P≤.0001
n= number of mice per group

Table 19: Effect of Anti-FcεR1α x CD3 Bispecific Antibodies in Splenic Basophil Depletion

Treatment	Splenic Basophils (percent of live cells)	Mean Percent Decrease Relative to Average Basophils in Isotype Control group
bsAb24919D (n=7)	0.003 ± 0.0017	95.89 (**)
bsAb24920D (n=7)	0.005 ± 0.0018	92.96 (ns)
bsAb24921D (n=7)	0.003 ± 0.0026	92.43 (ns)

25mg/kg total antibody concentration used for all groups administered antibody
*P≤.05, ***P≤.001, ***P≤.0001
n= number of mice per group

[00264] For both PCA and basophil depletion assays, ablation of cells expressing FcεR1α was achieved using exemplary anti-FcεR1α x CD3 bispecific antibodies of the invention at lower dosage. bsAb24919D and bsAb24921D showed statistically significant inhibition of the PCA response and significant basophil loss in experiments repeated at lower doses of 1 mg/kg, 5 mg/kg, and 10 mg/kg (data not shown). Efficacy is similar at doses between 5mg/kg and 25mg/kg for both bsAb24919D and bsAb24921D in both PCA and basophil depletion assays (data not shown).

[00265] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Claims

What is claimed is:

1. A bispecific antigen-binding molecule comprising a first antigen-binding domain that specifically binds human CD3, and a second antigen-binding domain that specifically binds human and/or cynomolgus FcεR1α, wherein:
the first antigen-binding domain comprises a heavy chain complementarity determining region (HCDR)1 having the amino acid sequence of SEQ ID NO:44, an HCDR2 having the amino acid sequence of SEQ ID NO:46, an HCDR3 having the amino acid sequence of SEQ ID NO:48, a light chain complementarity determining region (LCDR)1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32; and
the second antigen-binding domain comprises:
(i) an HCDR1 having the amino acid sequence of SEQ ID NO:4, an HCDR2 having the amino acid sequence of SEQ ID NO:6, an HCDR3 having the amino acid sequence of SEQ ID NO:8, an LCDR1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32; or
(ii) an HCDR1 having the amino acid sequence of SEQ ID NO:12, an HCDR2 having the amino acid sequence of SEQ ID NO:14, an HCDR3 having the amino acid sequence of SEQ ID NO:16, an LCDR1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32; or
(iii) an HCDR1 having the amino acid sequence of SEQ ID NO:20, an HCDR2 having the amino acid sequence of SEQ ID NO:22, an HCDR3 having the amino acid sequence of SEQ ID NO:24, an LCDR1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32.

2. A bispecific antibody comprising a first binding arm that binds human CD3 and a second binding arm that binds human FcεR1α, wherein the first binding arm comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 56 and a light chain comprising the amino acid sequence of SEQ ID NO: 40, and wherein the second binding arm comprises a heavy chain comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 52, and 54, and a light chain comprising the amino acid sequence of SEQ ID NO: 40.

3. The bispecific antigen-binding molecule of claim 1, wherein the second antigen-binding domain comprises an HCDR1 having the amino acid sequence of SEQ ID NO:4, an HCDR2 having the amino acid sequence of SEQ ID NO:6, an HCDR3 having the amino acid sequence of SEQ ID NO:8, an LCDR1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32.

4. The bispecific antigen-binding molecule of claim 1, wherein the second antigen-binding domain comprises an HCDR1 having the amino acid sequence of SEQ ID NO:12, an HCDR2 having the amino acid sequence of SEQ ID NO:14, an HCDR3 having the amino acid sequence of SEQ ID NO:16, an LCDR1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32.

5. The bispecific antigen-binding molecule of claim 1, wherein the second antigen-binding domain comprises an HCDR1 having the amino acid sequence of SEQ ID NO:20, an HCDR2 having the amino acid sequence of SEQ ID NO:22, an HCDR3 having the amino acid sequence of SEQ ID NO:24, an LCDR1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32.

6. The bispecific antigen-binding molecule of claim 1, wherein the second antigen-binding domain comprises: (a) a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 10 or SEQ ID NO: 18; and (b) a light chain variable region (LCVR) having an amino acid sequence of SEQ ID NO: 26.

7. The bispecific antigen-binding molecule of claim 6, wherein the second antigen-binding domain comprises the HCVR/LCVR amino acid sequence pair of: SEQ ID NOs: 18/26.

8. The bispecific antigen-binding molecule of claim 1, wherein the antibody or antigen-binding fragment thereof or the bispecific antigen binding molecule binds to FcεR1α expressed on a cell surface in the presence of immunoglobulin E (IgE).

9. The bispecific antigen-binding molecule of claim 1 that is a bispecific antibody.

10. The bispecific antigen-binding molecule of claim 9, wherein the bispecific antibody comprises a human IgG heavy chain constant region.

11. The bispecific antigen-binding molecule of claim 10, wherein the human IgG heavy chain constant region is isotype IgG4.

12. The bispecific antigen-binding molecule of claim 10, wherein the human IgG heavy chain constant region is isotype IgG1.

13. The bispecific antigen-binding molecule of claim 9, wherein the bispecific antibody comprises a first heavy chain comprising an amino acid sequence of SEQ ID NO: 56, a second heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 52, and 54, and a light chain comprising an amino acid sequence of SEQ ID NO: 40.

14. A nucleic acid molecule encoding the bispecific antigen-binding molecule of claim 1.

15. A vector comprising the nucleic acid molecule of claim 14.

16. A host cell comprising the vector of claim 15.

17. A pharmaceutical composition comprising the bispecific antigen-binding molecule of claim 1 and a pharmaceutically acceptable carrier or diluent.