the use of a relevant species, preferably a lower primate. The limitation now of the CD3 binding molecules suitable for therapeutic intervention in man described in the art is that the relevant species are higher primates, in particular cynomolgus monkeys. Accordingly, an anti-CD3 antibody capable of binding to both human and primate CD3 is highly desirable. Such antibodies have been described in United States Patent Publication No. 20100150918 and in PCT Publication WO2008/119567.

[0012] Despite such advances, a need remains for anti-human CD3 antibodies and their antigen-binding fragments that are capable of cross-reacting with CD3 of a non-human mammal (*e.g.*, a cynomolgous monkey). The present invention addresses this need and the need for improved therapeutics for cancer, autoimmunity and inflammatory diseases.

Summary

[0013] The present disclosure relates to CD3-binding molecules capable of binding to human and non-human CD3, and in particular to such molecules that are cross-reactive with CD3 of a non-human mammal (*e.g.*, a cynomolgus monkey). The disclosure also pertains to uses of such antibodies and antigen-binding fragments in the treatment of cancer, autoimmune and/or inflammatory diseases and other conditions.

[0014] In detail, the disclosure provides a CD3-binding molecule comprising an antigen-binding fragment of an antibody, wherein the antigen-binding fragment comprises an antibody CD3-specific VL domain and an antibody CD3-specific VH domain, wherein the CD3-specific VL domain and the CD3-specific VH domain form an antigen-binding domain capable of immunospecifically binding to both an epitope of human CD3 and to an epitope of the CD3 of a non-human mammal, wherein:

(I) the CD3-specific VL domain is selected from the group consisting of h-mab2 VL-1 (**SEQ ID NO:16**), h-mab2 VL-2 (**SEQ ID NO:18**), h-mab2 VL-3 (**SEQ ID NO:20**), h-mab2 VL-4 (**SEQ ID NO:22**), h-mab2 VL-5 (**SEQ ID NO:24**), h-mab2 VL-6 (**SEQ ID NO:26**), h-mab2 VL-7 (**SEQ ID NO:28**), h-mab2 VL-8 (**SEQ ID NO:30**), h-mab2 VL-9 (**SEQ ID NO:32**), and h-mab2 VL-10 (**SEQ ID NO:34**), and said CD3-specific VH domain is selected from the group consisting of h-mab2 VH-1 (**SEQ ID NO:36**), h-mab2 VH-2 (**SEQ ID**

NO:38), h-mab2 VH-3 (**SEQ ID NO:40**), h-mab2 VH-4 (**SEQ ID NO:42**), h-mab2 VH-5 (**SEQ ID NO:44**), h-mab2 VH-6 (**SEQ ID NO:46**), h-mab2 VH-6L (**SEQ ID NO:54**), h-mab2 VH-7 (**SEQ ID NO:48**), h-mab2 VH-8 (**SEQ ID NO:50**), h-mab2 VH-8L (**SEQ ID NO:55**), h-mab2 VH-8 di-1 (**SEQ ID NO:56**), h-mab2 VH-8 di-2 (**SEQ ID NO:57**), h-mab2 VH-6M (**SEQ ID NO:72**), h-mab2 VH-8M (**SEQ ID NO:74**), h-mab2 VH-2k (**SEQ ID NO:87**), and h-mab2 VH-5k (**SEQ ID NO:88**); or

(II) the CD3-specific VL domain is selected from the group consisting of h-mab1 VL-1 (**SEQ ID NO:10**) and h-mab1 VL-2 (**SEQ ID NO:12**), and the CD3-specific VH domain is h-mab1 VH (**SEQ ID NO:14**).

In one aspect, there is provided a CD3-binding molecule comprising an antigen-binding fragment of an antibody, wherein said antigen-binding fragment comprises an antibody CD3-specific VL domain and an antibody CD3-specific VH domain, wherein said CD3-specific VL domain and said CD3-specific VH domain form an antigen-binding domain capable of immunospecifically binding to both an epitope of human CD3 and to an epitope of the CD3 of a non-human mammal, wherein:

(I) said CD3-specific VL domain comprises the three complementarity determining regions (CDRs) of SEQ ID NO:5; and

(II) said CD3-specific VH domain comprises the three complementarity determining regions (CDRs) of SEQ ID NO:7, modified to comprise one or more amino acid substitutions selected from the group consisting of:

(i) I51T;

(ii) S52aN;

(iii) Y52cA;

(iv) N54S;

(v) A56T;

(vi) Y58E;

(vii) D61A; and

(viii) D65G;

wherein said numbering is according to the Kabat numbering scheme.

[0015] The disclosure particularly concerns the embodiment of the above-described CD3-binding molecule wherein the CD3-specific VL domain is h-mab2 VL-6 (**SEQ ID NO:26**).

[0016] The disclosure further concerns the embodiment of the above-described CD3-binding molecules wherein the CD3-specific VH domain is h-mab2 VH-8 (**SEQ ID NO:50**), h-mab2 VH-6 (**SEQ ID NO:46**), or h-mab2 VH-2k (**SEQ ID NO:87**).

[0017] The disclosure particularly concerns the embodiment of the above-described CD3-binding molecule wherein the molecule is an antibody, and particularly, wherein the antibody lacks an Fc region or comprises an Fc region that:

(A) lacks effector function or has reduced effector function; or

(B) impairs the ability of the Fc region of the antibody to bind to an Fc receptor;

wherein the reduction in effector function and the impairment of binding ability is relative to that of a wild-type Fc receptor.

[0018] The disclosure further concerns the embodiment of the above-described CD3-binding molecules wherein the molecule is a CD3-binding diabody that comprises a first polypeptide chain and a second polypeptide chain, the chains being covalently bonded to one another, wherein:

I. the first polypeptide chain comprises an amino terminus and a carboxy terminus and from N-terminus to C-terminus:

   (i) a domain (A) comprising the CD3-specific VL domain;

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2); and

(iii) a domain (C);

wherein the domains (A) and (B) do not associate with one another to form an epitope binding site;

and

(II) the second polypeptide chain comprises an amino terminus and a carboxy terminus and from N-terminus to C-terminus:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2);

(ii) a domain (E) comprising the CD3-specific VH domain; and

(iii) a domain (F);

wherein the domains (D) and (E) do not associate with one another to form an epitope binding site; and

wherein:

(1) the domains (A) and (E) associate to form the antigen-binding domain that is capable of immunospecifically binding to both human CD3 and to the CD3 of a non-human mammal;

(2) the domains (B) and (D) associate to form a binding site that immunospecifically binds to a second epitope, the second epitope being different from the CD3 epitope bound by the antigen-binding domain formed from the association of the domains (A) and (E); and

(3) the domains (C) and (F) are covalently associated together.

[0019] The disclosure further concerns the embodiment of the above-described CD3-binding molecules wherein the second epitope is not an epitope of CD3.

[0020] The disclosure further concerns the embodiment of the above-described CD3-binding molecules wherein the second epitope is an epitope of CD3 that is different from the CD3 epitope bound by the antigen-binding domain formed from the association of the domains (A) and (E).

[0021] The disclosure further concerns the embodiment of the above-described CD3-binding molecules or antibodies or diabodies in which such molecule humanized.

[0022] The disclosure further concerns the embodiment of the above-described CD3-binding molecules or antibodies or diabodies in which such molecule is capable of immunospecifically binding to CD3 and to fluorescein.

[0023] The disclosure further concerns the embodiment of the above-described CD3-binding molecules or diabodies in which such molecule is capable of immunospecifically binding to both: (i) CD3 and (ii)(a) a tumor antigen, or (ii)(b) a cell surface antigen, receptor or receptor ligand.

[0024] The disclosure further concerns the embodiment of the above-described CD3-binding molecules or diabodies in which the molecule or diabody is capable of immunospecifically binding to CD3 and to a tumor antigen expressed on a tumor cell, wherein the tumor cell is a tumor cell from a cancer selected from the group consisting of: breast cancer, prostate cancer, gastric cancer, lung cancer, stomach cancer, colon cancer, rectal cancer, pancreatic cancer, liver cancer, ovarian cancer, oral cavity cancer, pharyngeal cancer, esophageal cancer, laryngeal cancer, bone cancer, skin cancer, melanoma, uterine cancer, testicular cancer, bladder cancer, kidney cancer, brain cancer, glioblastoma, thyroid cancer, lymphoma, myeloma, and leukemia.

[0025] The disclosure further concerns the embodiment of the above-described CD3-binding molecules or diabodies in which the molecule or diabody is capable of immunospecifically binding to CD3 and to a cell surface antigen, receptor or receptor ligand, wherein the cell surface antigen, receptor or receptor ligand is HER2/neu, B7-H3, CD20, PSMA, IGF-1R., Ep-CAM, or is a molecule involved in a T cell – B cell association that leads to T cell or B cell activation in an adaptive immune response.

[0026] The disclosure further concerns the embodiment of the above-described CD3-binding molecules or diabodies in which the molecule or diabody is capable of immunospecifically binding to CD3 and to a molecule involved in the T cell – B cell association and the molecule involved in the T cell – B cell association is selected

from the group consisting of CD19, CD20, CD22, CD23, CD27, CD32B, CD38, CD40, CD79a, CD79b, CD80, CD86, LFA-I, LFA-3 and CFA-I.

[0027] The disclosure further concerns a pharmaceutical composition comprising any of the above-descibed CD3-binding molecules, antibodies or diabodies, and a pharmaceutically acceptable carrier, excipient or diluent. In another aspect, there is provided a pharmaceutical composition comprising the CD3-binding molecule as described herein, and a pharmaceutically acceptable carrier, excipient or diluent.

[0028] The disclosure further concerns the above-described pharmaceutical composition for use in the treatment of cancer or an autoimmune or inflammatory disease. In another aspect, there is provided use of the CD3-binding molecule as described herein, or of the pharmaceutical composition as described herein, in the manufacture of a medicament for the treatment of cancer or an autoimmune or inflammatory disease.

[0029] The disclosure further concerns the above-described pharmaceutical composition for use in the treatment of an autoimmune or inflammatory disease selected from the group consisting of: type I insulin-dependent diabetes, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, inflammatory bowel disease, myasthenia gravis, celiac's disease, Sjogren's syndrome, Grave's disease, Crohn's disease, autoimmune hepatitis, psoriasis, psoriatic arthritis, asthma, allergic rhinitis, effects from organ transplantation, or graft vs. host disease (GVHD). The disclosure particularly concerns the above-described pharmaceutical composition for use in the treatment of type I insulin-dependent diabetes.

Brief Description of the Drawings:

[0030] **Figures 1A-1B** show the results of a capture ELISA in which the ability of anti-CD3 antibody mAB1 (**Figure 1A**) or a chimeric derivative of antibody mAB1 (ch-mAb1) (**Figure 1B**) was assessed using human soluble CD3 ("shCD3").

[0031] **Figures 2A-2B** show the results of a capture ELISA in which the ability of anti-CD3 antibody mAB2 (**Figure 2A**) or a chimeric derivative of antibody mAB2 (ch-mAb2) (**Figure 2B**) was assessed using human soluble CD3 ("shCD3") or soluble cynomolgus monkey CD3 ("scCD3").

[0032] **Figure 3** show the results of analyses to determine the effect of variations in Kabat numbered framework residues 41-46 of the light chain of mAb2.

What Is Claimed Is:

Claim 1. A CD3-binding molecule comprising an antigen-binding fragment of an antibody, wherein said antigen-binding fragment comprises an antibody CD3-specific VL domain and an antibody CD3-specific VH domain, wherein said CD3-specific VL domain and said CD3-specific VH domain form an antigen-binding domain capable of immunospecifically binding to both an epitope of human CD3 and to an epitope of the CD3 of a non-human mammal, wherein:

(I) said CD3-specific VL domain comprises the three complementarity determining regions (CDRs) of SEQ ID NO:5; and

(II) said CD3-specific VH domain comprises the three complementarity determining regions (CDRs) of SEQ ID NO:7, modified to comprise one or more amino acid substitutions selected from the group consisting of:

(i) I51T;

(ii) S52aN;

(iii) Y52cA;

(iv) N54S;

(v) A56T;

(vi) Y58E;

(vii) D61A; and

(viii) D65G;

wherein said numbering is according to the Kabat numbering scheme.

Claim 2. The CD3-binding molecule of claim 2, wherein said CD3-specific VL domain is selected from the group consisting of h-mab2 VL-4 (SEQ ID NO:22), h-mab2 VL-6 (SEQ ID NO:26), h-mab2 VL-7 (SEQ ID NO:28), h-mab2 VL-8 (SEQ ID NO:30), h-mab2 VL-9 (SEQ ID NO:32), and h-mab2 VL-10 (SEQ ID NO:34).

Claim 3. The CD3-binding molecule of any one of claims 1-2, wherein said CD3-specific VH domain is selected from the group consisting of h-mab2 VH-6L (SEQ ID NO:54), h-mab2 VH-8L (SEQ ID NO:55), h-mab2 VH-8 di-1 (SEQ ID NO:56), h-mab2 VH-8 di-2 (SEQ ID NO:57), h-mab2 VH-6M (SEQ ID NO:72), h-mab2

VH-8M (SEQ ID NO:74), h-mab2 VH-2k (SEQ ID NO:87), or h-mab2 VH-5k (SEQ ID NO:88).

Claim 4. The CD3-binding molecule of any one of claims 1-2, wherein said CD3-specific VH domain comprises an amino acid sequence that differs, by comprising said amino acid substitution, from the amino acid sequence of h-mab2 VH-1 (SEQ ID NO:36), h-mab2 VH-2 (SEQ ID NO:38), h-mab2 VH-3 (SEQ ID NO:40), h-mab2 VH-4 (SEQ ID NO:42), h-mab2 VH-5 (SEQ ID NO:44), h-mab2 VH-6 (SEQ ID NO:46), h-mab2 VH-7 (SEQ ID NO:48), and h-mab2 VH-8 (SEQ ID NO:50).

Claim 5. The CD3-binding molecule of any one of claims 1-4, wherein said CD3-binding molecule is an antibody.

Claim 6. The CD3-binding antibody of claim 5, wherein said antibody:

(A) lacks an Fc region;

or

(B) comprises an Fc region that:

(i) lacks effector function; or

(ii) has reduced effector function; or

(iii) impairs the ability of the Fc region of said antibody to bind to an Fc receptor;

wherein said lack of effector function, said reduction in effector function, and said impairment of binding ability is relative to that of a wild-type Fc receptor.

Claim 7. The CD3-binding molecule of any one of claims 1-4, wherein said CD3-binding molecule is a CD3-binding diabody that comprises a first polypeptide chain and a second polypeptide chain, said chains being covalently bonded to one another, wherein:

(I) said first polypeptide chain comprises an amino terminus and a carboxy terminus and from N-terminus to C-terminus:

(i) a domain (A) comprising said CD3-specific VL domain;

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2); and

(iii) a domain (C);

wherein said domains (A) and (B) do not associate with one another to form an epitope binding site;

and

(II) said second polypeptide chain comprises an amino terminus and a carboxy terminus and from N-terminus to C-terminus:

(i) a domain (D) comprising a binding region of a light chain variable domain of said second immunoglobulin (VL2);

(ii) a domain (E) comprising said CD3-specific VH domain;

and

(iii) a domain (F);

wherein said domains (D) and (E) do not associate with one another to form an epitope binding site; and

wherein:

(1) said domains (A) and (E) associate to form said antigen-binding domain that is capable of immunospecifically binding to both human CD3 and to the CD3 of a non-human mammal;

(2) said domains (B) and (D) associate to form a binding site that immunospecifically binds to a second epitope, said second epitope being different from the CD3 epitope bound by the antigen-binding domain formed from said association of said domains (A) and (E); and

(3) said domains (C) and (F) are covalently associated together.

Claim 8. The CD3-binding diabody of claim 7, wherein said second epitope is not an epitope of CD3.

Claim 9. The CD3-binding diabody of claim 7, wherein said second epitope is an epitope of CD3 that is different from the CD3 epitope bound by the antigen-binding domain formed from said association of said domains (A) and (E).

Claim 10. The CD3-binding molecule of any one of claims 1-9, which is humanized.

Claim 11. The CD3-binding molecule of any one of claims 1-4 or 7-10, which is capable of immunospecifically binding to CD3 and to fluorescein.

Claim 12. The CD3-binding molecule of any one of claims 1-4 or 7-10, which is capable of immunospecifically binding to both: (i) CD3 and (ii)(a) a tumor antigen, or (ii)(b) a cell surface antigen, receptor or receptor ligand.

Claim 13. The CD3-binding molecule of claim 12, wherein said CD3-binding molecule is capable of immunospecifically binding to CD3 and to a tumor antigen expressed on a tumor cell, wherein said tumor cell is a tumor cell from a cancer selected from the group consisting of: breast cancer, prostate cancer, gastric cancer, lung cancer, stomach cancer, colon cancer, rectal cancer, pancreatic cancer, liver cancer, ovarian cancer, oral cavity cancer, pharyngeal cancer, esophageal cancer, laryngeal cancer, bone cancer, skin cancer, melanoma, uterine cancer, testicular cancer, bladder cancer, kidney cancer, brain cancer, glioblastoma, thyroid cancer, lymphoma, myeloma, and leukemia.

Claim 14. The CD3-binding molecule of claim 12, wherein said CD3-binding molecule is capable of immunospecifically binding to CD3 and to a cell surface antigen, receptor or receptor ligand, wherein said cell surface antigen, receptor or receptor ligand is HER2/neu, B7-H3, CD20, PSMA, IGF-1R., Ep-CAM, or is a molecule involved in a T cell – B cell association that leads to T cell or B cell activation in an adaptive immune response.

Claim 15. The CD3-binding molecule of claim 14, wherein said CD3-binding molecule is capable of immunospecifically binding to CD3 and to a molecule involved in said T cell – B cell association and said molecule involved in said T cell – B cell association is selected from the group consisting of CD19, CD20, CD22, CD23, CD27, CD32B, CD38, CD40, CD79a, CD79b, CD80, CD86, LFA-I, LFA-3 and CFA-I.

Claim 16. The CD3-binding molecule of any one of claims 7-10 and 12-14, wherein:

(A) said domain (B) comprises amino acid residues 119-238 of SEQ ID NO: 65; and

(B) said domain (D) comprises amino acid residues 1-107 of SEQ ID NO: 64.

Claim 17. The CD3-binding diabody of any one of claims 7-10 and 12-14, wherein:

(A) said domain (B) comprises amino acid residues 119-240 of SEQ ID NO: 67; and

(B) said domain (D) comprises amino acid residues 1-107 of SEQ ID NO: 66.

Claim 18. The CD3-binding diabody of any one of claims 7-17, wherein said CD3-specific VL domain is h-mab2 VL-6 (SEQ ID NO:26).

Claim 19. The CD3-binding molecule of any one of claims 1-4 or 7-18, wherein said CD3-binding diabody comprises an Fc domain or portion thereof.

Claim 20. The CD3-binding molecule of any one of claims 7-19, wherein:

(A) said polypeptide chain I additionally comprises an E coil sequence and said polypeptide chain II additionally comprises a K coil sequence; or

(B) said polypeptide chain I additionally comprises a K coil sequence and said polypeptide chain II additionally comprises an E coil sequence;

wherein said E coil sequence is amino acid residues 244-271 of SEQ ID NO: 62, and said K coil sequence is residues 247-274 of SEQ ID NO: 63.

Claim 21. A pharmaceutical composition comprising the CD3-binding molecule of any one of claims 1-20, and a pharmaceutically acceptable carrier, excipient or diluent.

Claim 22. Use of the CD3-binding molecule of any one of claims 1-20, or of the pharmaceutical composition of claim 21, in the manufacture of a medicament for the treatment of cancer or an autoimmune or inflammatory disease.

Claim 23. The use of claim 22, wherein said autoimmune or inflammatory disease is selected from the group consisting of: type I insulin-dependent diabetes, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, inflammatory bowel disease, myasthenia gravis, celiac's disease, Sjogren's syndrome, Grave's disease, Crohn's disease, autoimmune hepatitis, psoriasis, psoriatic arthritis, asthma, allergic rhinitis, effects from organ transplantation, or graft vs. host disease (GVHD).

Claim 24. The use of claim 23, wherein said autoimmune or inflammatory disease is type I insulin-dependent diabetes.