

[0006] The interaction of the T cell receptor (TCR) with its peptide-MHC ligand determines the activity of a T cell. The binding characteristics of this interaction has been studied in great detail and shown to control T cell function. The strength and nature of the TCR-peptide/MHC interaction determines whether T cells exert effector functions or are inactivated and deleted. Antibodies against CD3 activate T cells by changing the conformation of the CD3 ϵ chain and depending on the epitope may have either agonistic or antagonistic effects on T cells (Yoon et al., 1994 Immunity 1:563-569). In light of the significant side-effects of many T cell agonists it may be preferred to maintain potent anti-tumor effects while reducing the release of pro-inflammatory cytokines. However, partial agonistic anti-CD3 antibodies may alter the CD3 ϵ chain sub-optimally resulting in ineffective signaling, and most anti-CD3 antibodies are full agonists for both pathways. It is unclear whether these effector functions can be separated. Many existing anti-CD3 antibodies (for example SP-34, UCHT1, OKT3) have affinities in the range of 1-50 nM KD, however this may not be optimal for therapeutic use.

[0007] CD3 specific antibodies, and bispecific antibodies derived therefrom are provided by the invention.

Publications

[0008] CD3 antibodies are disclosed, for example, in U.S. Pat. Nos. 5,585,097; 5,929,212; 5,968,509; 6,706,265; 6,750,325; 7,381,803; 7,728,114. Bispecific antibodies with CD3 binding specificity are disclosed, for example, in U.S. Pat. Nos. 7,262,276; 7,635,472; 7,862,813; and 8,236,308, each herein specifically incorporated by reference.

SUMMARY

[0009] Compositions and methods of use thereof are provided for a family of closely related antibodies that bind to and activate signaling through CD3, e.g. activation of CD3⁺ T cells. The antibody family comprises a set of CDR sequences as defined herein. The family of antibodies provides a number of benefits that contribute to utility as clinically therapeutic agent(s). The antibodies within the family include members with a range of binding affinities, allowing the selection of a specific sequence with a desired affinity. The ability to fine tune affinity is of particular importance to manage the level of CD3 activation in an individual being treated, and thereby reduce toxicity.

[0010] In some examples, anti-CD3 antibodies have an affinity (KD) for CD3 ranging from around about 10⁻⁶ to around about 10⁻¹¹. Anti-CD3 antibodies that have affinities (KD) of 50 nM or greater, 100 nM or greater, 500 nM or greater, or 1 μ M or greater can be desirable to more closely mimic the TCR/MHC interaction and minimize toxic cytokine release while maintaining effective tumor cell lysis. In some examples, anti-CD3 antibodies are characterized or selected for reduced propensity to induce cytokine release, upon binding to a competent T cell,

e.g. for release of IL-2 and IFN γ . Antibodies may be selected for therapeutic use that optimize killing of tumor cells and reduced release of cytokines, e.g. an antibody that, within the family of antibody sequences described herein, induces a cytokine release that is less than about half the maximum observed for a family member in a comparative assay, and may be less, e.g. less and about 25% the maximum observed for a family member in a comparative assay. In some examples, bispecific or multispecific antibodies are provided, which comprise at least a heavy chain variable region from the antibody family and may comprise a heavy and light chain variable region provided herein. Bispecific antibodies comprise at least the heavy chain variable region of an antibody specific for a protein other than CD3, and may comprise a heavy and light chain variable region. In some such examples, the second antibody specifically binds to a tumor associated antigen, a targeting antigen, e.g. integrins, etc., a pathogen antigen, a checkpoint protein, and the like. Various formats of bispecific antibodies are within the ambit of the disclosure, including without limitation single chain polypeptides, two chain polypeptides, three chain polypeptides, four chain polypeptides, and multiples thereof.

[0011] Each of the CD3 specific antibodies comprises a VH domain, comprising CDR1, CDR2 and CDR3 sequences in a human VH framework. The family 2 CDR sequences may be situated, as an example, in the region of around amino acid residues 26-33; 51-58; and 97-112 for CDR1, CDR2 and CDR3, respectively, of the provided exemplary variable region sequences set forth in SEQ ID NO: 1-18. It will be understood by one of skill in the art that the CDR sequences may be in different position if a different framework sequence is selected, although generally the order of the sequences will remain the same.

[0012] The CDR sequences for a family 2 antibody may have the following sequence formulas. An X indicates a variable amino acid, which may be specific amino acids as indicated below.

CDR1

G₁ F₂ T₃ F₄ X₅ X₆ Y₇ A₈

where:

X₅ may be any amino acid; in some examples X₅ is D, A or H; in some examples X₅ is D.

X₆ may be any amino acid; in some examples X₆ is D or N; in some examples D₆ is D.

In some examples a CDR1 sequence of a family 2 anti-CD3 antibody comprises the sequence set forth in any of SEQ ID NO:1-18, residues 26-33.

CDR2

I₁' S₂' W₃' N₄' S₅' G₆' S₇' I₈'

In some examples a CDR2 sequence of a family 2 anti-CD3 antibody comprises the sequence set forth in any of SEQ ID NO: 1-18, residues 51-58.

CDR3

A₁^{''} K₂^{''} D₃^{''} S₄^{''} R₅^{''} G₆^{''} Y₇^{''} G₈^{''} X₉^{''} Y₁₀^{''} X₁₁^{''} X₁₂^{''} G₁₃^{''} G₁₂^{''} A₁₅^{''} Y₁₆^{''}

where:

X₉^{''} may be any amino acid, in some examples X₉^{''} is D or S; in some examples X₉^{''} is D;

X₁₁^{''} may be any amino acid, in some examples X₁₁^{''} is R or S;

X₁₂^{''} may be any amino acid, in some examples X₁₂^{''} is L or R.

[0013] In some examples a CD3 sequence of a family 2 anti-CD3 antibody has the formula A K D S R G Y G D Y X₁₁^{''} X₁₂^{''} G G A Y where X₁₁^{''} and X₁₂^{''} are as defined above. In some examples a CDR3 sequence of a family 2 anti-CD3 antibody comprises the sequence set forth in any of SEQ ID NO: 1-18, residues 97-112. In some examples the CD3-binding VH domain of a family 2 antibody is paired with a light chain variable region domain. In some such examples the light chain is a fixed light chain.

[0014] In some examples the light chain comprises a VL domain with CDR1, CDR2 and CDR3 sequences in a human VL framework. The CDR sequences may be those of SEQ ID NO:19. In some examples, the CDR1 sequence comprises amino acid residues 27-32; 50-52; 89-97 for CDR1, CDR2, CDR3, respectively.

[0015] In some examples the CDR sequences of an antibody of the disclosure are a sequence with at least 85% identity, at least 90% identity, at least 95% identity, at least 99% identity relative to a CDR sequence or set of CDR sequences in SEQ ID NO: 1-18. In some examples a CDR sequence of the disclosure comprises one, two, three or more amino acid substitutions relative to a CDR sequence or set of CDR sequences in any one of SEQ ID NO: 1-18. In some examples said amino acid substitution(s) are one or more of position 5 or 10 of CDR1, position 2, 6 or 7 of CDR2, position 1, 8, 9 or 10 of CDR3, relative to the family 2 formulas provided above.

[0016] In some examples, a bispecific antibody of the disclosure comprises a CD3-binding variable region described herein, paired with a light chain. In some examples the light chain comprises the variable region sequence set forth in SEQ ID NO:19, or a variable region comprising the set of CDR sequences in SEQ ID NO:19 and framework sequences. Various Fc sequences find use, including without limitation human IgG1, IgG2a, IgG2b, IgG3, IgG4, etc. In some examples, the second arm of the bispecific antibody comprises a variable region that specifically binds to a tumor-associated antigen. In some examples, the second arm of the bispecific antibody comprises a variable region that specifically binds to BCMA. In some examples the anti-BCMA arm is a single chain variable region, for example as shown in

Figure 2B. In some examples the anti-BCMA arm comprises the variable region sequence set forth in SEQ ID NO:20; or the tandem variable region sequence set forth in SEQ ID NO:21. The Fc sequence of the anti-BCMA arm may be, without limitation, human IgG1, IgG2a, IgG2b, IgG3, IgG4, etc. The CDR sequences may be those contained in SEQ ID NO:20. In some examples, the CDR sequence comprises amino acid residues 26-33; 51-58; 97-108 for CDR1, CDR2, CDR3, respectively.

[0016a] In one aspect, there is provided a multispecific antibody comprising:

(i) a first binding moiety that has binding specificity for human CD3 $\delta\epsilon$, comprising:

a heavy chain variable domain comprising (A) a CDR1 sequence comprising GFTFDDYA, a CDR2 sequence comprising ISWNSGSI, and a CDR3 sequence comprising AKDSRGYGDYRLGGAY, or

(B) a CDR1 sequence comprising GFTFHNYA, a CDR2 sequence comprising ISWNSGSI, and a CDR3 sequence comprising AKDSRGYGDYSLGGAY; and

a light chain variable domain comprising a CDR1 sequence comprising QSVSSN, a CDR2 sequence comprising GAS, and a CDR3 sequence comprising QQYNNWPWT; and (ii) a second binding moiety that has binding specificity for a tumor-associated antigen (TAA) or a tumor-specific antigen (TSA).

In other examples, pharmaceutical compositions are provided, comprising at least a CD3-binding VH domain of the disclosure, e.g. a monospecific, bispecific, etc. antibody or antibody-like protein comprising at least a CD3-binding VH domain of the disclosure; and a pharmaceutically acceptable excipient. In another aspect, there is provided a pharmaceutical composition comprising a multispecific antibody disclosed herein. In another example, there is provided a pharmaceutical composition comprising the bispecific three-chain antibody like molecule disclosed herein. In another example, there is provided a pharmaceutical composition comprising a bispecific antibody disclosed herein. The composition may be lyophilized, suspended in solution, etc. and may be provided in a unit dose formulation.

[0017] In some examples, a method is provided for treatment of cancer, the method comprising administering to an individual in need thereof an effective dose of a mono-specific, bi-specific, etc. antibody of the disclosure. Where the antibody is bispecific, a second antigen-binding site may specifically bind a tumor antigen, a checkpoint protein, etc. In various examples, the cancer is selected from the group consisting of ovarian cancer, breast cancer, gastrointestinal, brain cancer, head and neck cancer, prostate cancer, colon cancer, lung cancer, leukemia, lymphoma, sarcoma, carcinoma, neural cell tumors, squamous cell carcinomas, germ cell tumors, metastases, undifferentiated tumors, seminomas, melanomas, myelomas, neuroblastomas, mixed cell tumors, and neoplasias caused by infectious agents.

[0018] In some examples, a method is provided for treatment of infectious disease, the method comprising administering to an individual in need thereof an effective dose of a mono-specific,

bi-specific, etc. antibody of the disclosure. Where the antibody is bispecific, a second antigen-binding site may specifically bind a pathogen antigen, e.g. bacteria, viruses or parasites.

[0019a] In another aspect, there is provided a multispecific antibody disclosed herein, or the pharmaceutical composition disclosed herein, for use in therapy in an individual in need.

[0019b] In another aspect, there is provided use of an effective dose of the multispecific antibody disclosed herein, or the pharmaceutical composition disclosed herein, in the preparation of a medicament for treating a disease or condition in an individual in need, wherein the disease is an autoimmune disease or a cancer.

[0019] In other examples, a method is provided for the production of a bispecific antibody of the present disclosure comprising expressing the antibody sequences, e.g. one or more light chain encoding sequences, one or more heavy chain encoding sequences, in a single host cell. In another aspect, there is provided a method of producing a multispecific antibody disclosed herein, comprising growing a cell disclosed herein under conditions permissive for expression of the multispecific antibody, and isolating the multispecific antibody from the cell. In another example, there is provided a method of producing a bispecific antibody disclosed herein, comprising growing a cell disclosed herein under conditions permissive for expression of the bispecific antibody, and isolating the bispecific antibody from the cell. In various examples, the host cell may be a prokaryotic or an eukaryotic cell, such as a mammalian cell.

[0020a] In another aspect, there is provided a polynucleotide encoding a multispecific antibody disclosed herein. In another aspect, there is provided a polynucleotide encoding a bispecific antibody disclosed herein.

[0020b] In another aspect, there is provided a vector comprising a polynucleotide disclosed herein.

[0020c] In another aspect, there is provided a cell comprising a vector disclosed herein.

[0020d] In another aspect, there is provided a kit for treating a disease or disorder in an individual in need, comprising a multispecific antibody disclosed herein, or the pharmaceutical composition disclosed herein, and instructions for use, wherein the disease or disorder is an autoimmune disease or a cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is best understood from the following detailed description when read in conjunction with the accompanying drawings. The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee. It is emphasized that, according to common practice, the various features of the drawings are not

WHAT IS CLAIMED IS:

1. A multispecific antibody comprising:
 - (i) a first binding moiety that has binding specificity for human CD3 $\delta\epsilon$, comprising:
 - a heavy chain variable domain comprising
 - (A) a CDR1 sequence comprising GFTFDDYA, a CDR2 sequence comprising ISWNSGSI, and a CDR3 sequence comprising AKDSRGYGDYRLGGAY, or
 - (B) a CDR1 sequence comprising GFTFHNYA, a CDR2 sequence comprising ISWNSGSI, and a CDR3 sequence comprising AKDSRGYGDYSLGGAY; and
 - a light chain variable domain comprising a CDR1 sequence comprising QSVSSN, a CDR2 sequence comprising GAS, and a CDR3 sequence comprising QQYNNWPWT; and
 - (ii) a second binding moiety that has binding specificity for a tumor-associated antigen (TAA) or a tumor-specific antigen (TSA).
2. The multispecific antibody of claim 1, wherein the first binding moiety comprises a heavy chain variable domain comprising a CDR1 sequence comprising GFTFDDYA, a CDR2 sequence comprising ISWNSGSI, and a CDR3 sequence comprising AKDSRGYGDYRLGGAY.
3. The multispecific antibody of claim 1, wherein the first binding moiety comprises a heavy chain variable domain comprising a CDR1 sequence comprising GFTFHNYA, a CDR2 sequence comprising ISWNSGSI, and a CDR3 sequence comprising AKDSRGYGDYSLGGAY.
4. The multispecific antibody of any one of claims 1-3, wherein:
 - the CDR1, CDR2 and CDR3 sequences in the heavy chain variable domain of the first binding moiety are present in a human VH framework; and
 - the CDR1, CDR2 and CDR3 sequences in the light chain variable domain of the first binding moiety are present in a human Vkappa framework.
5. The multispecific antibody of claim 4, wherein:
 - the heavy chain variable domain of the first binding moiety comprises a sequence having at least 95% identity to SEQ ID NO: 1; and
 - the light chain variable domain of the first binding moiety comprises a sequence having at least 95% identity to SEQ ID NO: 19.
6. The multispecific antibody of claim 4, wherein:

the heavy chain variable domain of the first binding moiety comprises a sequence having at least 95% identity to SEQ ID NO: 13; and

the light chain variable domain of the first binding moiety comprises a sequence having at least 95% identity to SEQ ID NO: 19.

7. The multispecific antibody of claim 5, wherein:

the heavy chain variable domain of the first binding moiety comprises the sequence of SEQ ID NO: 1; and

the light chain variable domain of the first binding moiety comprises the sequence of SEQ ID NO: 19.

8. The multispecific antibody of claim 6, wherein:

the heavy chain variable domain of the first binding moiety comprises the sequence of SEQ ID NO: 13; and

the light chain variable domain of the first binding moiety comprises the sequence of SEQ ID NO: 19.

9. The multispecific antibody of claim 1, wherein the second binding moiety comprises a single heavy chain variable region, in a single or tandem configuration.

10. The multispecific antibody of claim 1, wherein the first binding moiety comprises a light chain polypeptide subunit and a heavy chain polypeptide subunit, and wherein the second binding moiety comprises a heavy chain polypeptide subunit.

11. The multispecific antibody of claim 10, wherein the light chain polypeptide subunit of the first binding moiety comprises a light chain constant domain.

12. The multispecific antibody of claim 10, wherein the heavy chain polypeptide subunit of the first binding moiety comprises at least one heavy chain constant domain.

13. The multispecific antibody of claim 12, wherein the heavy chain polypeptide subunit of the first binding moiety comprises a CH1 domain, a CH2 domain, and a CH3 domain.

14. The multispecific antibody of claim 13, wherein the heavy chain polypeptide subunit of the first binding moiety comprises a hinge region positioned between the CH1 domain and the CH2 domain.
15. The multispecific antibody of claim 10, wherein the heavy chain polypeptide subunit of the second binding moiety comprises at least one constant domain.
16. The multispecific antibody of claim 15, wherein the heavy chain polypeptide subunit of the second binding moiety comprises a CH2 domain and a CH3 domain, but no CH1 domain.
17. The multispecific antibody of claim 15 or 16, wherein the heavy chain polypeptide subunit of the second binding moiety comprises a hinge region positioned between the second binding moiety and the CH2 domain.
18. The multispecific antibody of any one of claims 13-17, wherein the CH2 and CH3 domains of the heavy chain polypeptide subunit of the first binding moiety and the CH2 and CH3 domains of the heavy chain polypeptide subunit of the second binding moiety together form an Fc region.
19. The multispecific antibody of claim 18, wherein the Fc region comprises a native-sequence human IgG1 Fc region.
20. The multispecific antibody of claim 18, wherein the Fc region comprises a variant human IgG1 Fc region.
21. The multispecific antibody of claim 20, wherein the variant human IgG1 Fc region has been engineered to reduce one or more effector functions.
22. The multispecific antibody of claim 18, wherein the Fc region comprises a native-sequence human IgG4 Fc region.
23. The multispecific antibody of claim 18, wherein the Fc region comprises a variant human IgG4 Fc region.
24. The multispecific antibody of claim 23, wherein the variant human IgG4 Fc region has been engineered to reduce one or more effector functions.

25. The multispecific antibody according to any one of claims 18-24, wherein the Fc region has been engineered to reduce homodimer formation.
26. A pharmaceutical composition comprising a multispecific antibody according to any one of claims 1-25.
27. A polynucleotide encoding a multispecific antibody according to any one of claims 1-25.
28. A vector comprising a polynucleotide according to claim 27.
29. A cell comprising a vector according to claim 28.
30. A method of producing a multispecific antibody according to any one of claims 1-25, comprising growing a cell according to claim 29 under conditions permissive for expression of the multispecific antibody, and isolating the multispecific antibody from the cell.
31. The multispecific antibody of any one of claims 1-25, or the pharmaceutical composition of claim 26, for use in therapy in an individual in need.
32. Use of an effective dose of the multispecific antibody according to any one of claims 1-25, or the pharmaceutical composition of claim 26, in the preparation of a medicament for treating a disease of condition in an individual in need, wherein the disease is an autoimmune disease or a cancer.
33. A kit for treating a disease or disorder in an individual in need, comprising a multispecific antibody of any one of claims 1-25, or the pharmaceutical composition of claim 26, and instructions for use, wherein the disease or disorder is an autoimmune disease or a cancer.
34. The kit of claim 33, further comprising at least one additional reagent.
35. The kit of claim 33, wherein the at least one additional reagent comprises a chemotherapeutic drug.
36. The multispecific antibody of claim 1, wherein the multispecific antibody is a bispecific antibody.