

Durben et al. *Molecular Therapy* (2015); **23** 4, 648–655). Because of the resulting cytokine release syndrome, there have been significant blocks to the development of these antibodies for therapeutic purposes.

[0006] For example, Mack *et al.* (1995) PNAS 92:7021–7025 reported a bispecific single chain (bssc)-antibody with CD3-specificity, designated a bispecific T-cell engager (BiTE). However, as in earlier studies with different anti-CD3 containing bispecific antibodies, excessive T-cell activation and cytokine release induced upon *in vivo* application limits the safely applicable doses to less than 100 µg/day resulting in serum concentrations below 1 ng/ml.

[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 antibodies within the family comprise 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. Members of the antibody family may have an affinity (KD) for CD3 ranging from around about 10⁻⁶ to around about 10⁻¹¹ M. Certain members of the antibody family of the disclosure are cross-reactive with CD3 protein of Cynomolgus macaque, and a specific motif required for this cross-reactivity is identified, allowing the selection of antibodies for pre-clinical or clinical testing on this basis.

[0010] Anti-CD3 antibodies that have affinities (KD) of 50 nM or greater, 100 nM or greater, 500 nM or greater, or 1 µM 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.

[0011] In some examples, bispecific or multispecific antibodies are provided, which comprise at least a heavy chain variable region from the antibody family of the disclosure 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 specificity 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.

[0012] The family of CD3 specific antibodies of the disclosure comprise a VH domain, comprising CDR1, CDR2 and CDR3 sequences in a human VH framework. The CDR sequences may be situated, as an example, in the region of around amino acid residues 26-35; 53-59; and 98-117 for CDR1, CDR2 and CDR3, respectively, of the provided exemplary variable region sequences set forth in SEQ ID NO:1-68. 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.

[0013] The CDR sequences for an antibody of the disclosure may have the following sequence formulas. An X indicates a variable amino acid, which may be specific amino acids as indicated below.

CDR1

G₁ G₂ S₃ I₄ X₅ S₆ X₇ X₈ X₉ X₁₀

where:

X₅ may be any amino acid; in some examples X₅ is S or R;

X₇ and X₈ may be any amino acid; in some examples X₇ and X₈ are, independently, S or G. In some examples X₇X₈ are SS, or GG;

X₉ may be any amino acid; in some examples X₉ is H or Y; in some examples X₉ is H;

X₁₀ may be any amino acid, in some examples X₁₀ is Y or F; in some examples X₁₀ is Y.

In some examples, the CDR1 sequence has the formula: G G S I X₅ S H H G Y, where X₅ is as defined above. In some examples a CDR1 sequence of an anti-CD3 antibody of the disclosure comprises the sequence set forth in any of SEQ ID NO:1-68, residues 26-35.

CDR2

I₁' X₂' X₃' S₄' G₅' X₆' X₇'

where:

X₂' may be any amino acid; in some examples X₂' is S, Y or H;

X₃' may be any amino acid; in some examples X₃' is Y, H or R;

X₆' may be any amino acid; in some examples X₆' is S, N or I or R;

X₇' may be any amino acid; in some examples X₇' is T or P.

In some examples the CDR2 sequence has the formula: I X₂' X₃' S G S T; or I X₂' X₃' S G N P where X₂' and X₃' are as defined above. In some examples a CDR2 sequence of an anti-CD3 antibody of the disclosure comprises the sequence set forth in any of SEQ ID NO:1-68, residues 53-59.

CDR3

X₁" R₂" W₃" R₄" H₅" D₆" I₇" X₈" X₉" X₁₀" Y₁₁" P₁₂" Y₁₃" Y₁₄" Y₁₅" Y₁₆" G₁₇" M₁₈" D₁₉" V₂₀"

where:

X₁" may be any amino acid; in some examples X₁" is A or G.

X₈" may be any amino acid; in some examples X₈" is L or F.

X₉" may be any amino acid; in some examples X₉" is T or A

X₁₀" may be any amino acid; in some examples X₁₀" is G, A or R.

In some examples X₈" X₉" X₁₀" are F A A, which motif corresponds to antibodies that cross-react with the CD3 protein of Cynomolgus macaque. In other examples X₈" X₉" X₁₀" are L T A. In some examples a CDR3 sequence of an anti-CD3 antibody of the disclosure comprises the sequence set forth in any of SEQ ID NO:1-68, residues 98-117.

[0013a] In one aspect, there is provided an antigen-binding protein specific for CD3, comprising:
a heavy chain variable domain comprising:

(a) a CDR1 sequence of GGSIRSGGHY, a CDR2 sequence of ISYSGST, and a CDR3 sequence of ARWRHDILTAYPYYYYGMDV;
and a light chain variable domain.

[0014] In some examples the CD3-binding VH domain is paired with a light chain variable region domain. In some such examples the light chain is a fixed light chain. 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 contained in SEQ ID NO:69. In some examples, the CDR 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 any one of SEQ ID NO:1-69. 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: 1-69. 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 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:69, or a variable region comprising the set of CDR sequences in SEQ ID NO:69 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:70; or the tandem variable region sequence set forth in SEQ ID NO:71. 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:70. In some examples, the CDR sequence comprises amino acid residues 26-33; 51-58; 97-108 for CDR1, CDR2, CDR3, respectively.

[0017] 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. The composition may be lyophilized, suspended in solution, etc. and may be provided in a unit dose formulation.

[0017a] In another aspect, there is provided a pharmaceutical composition comprising an antigen-binding protein as described herein.

[0017b] In another aspect, there is provided a polynucleotide encoding an antigen-binding protein as described herein.

[0017c] In another aspect, there is provided a vector comprising a polynucleotide as described herein.

[0017d] In another aspect, there is provided a cell comprising a vector as described herein.

[0017e] In another aspect, there is provided a method of producing an antigen-binding protein as described herein, comprising growing a cell as described herein under conditions permissive for expression of the protein, and isolating the protein from the cells.

[0018] 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.

[0019] 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 use of an antigen-binding protein as described herein, in the preparation of a medicament for the treatment of an infection, and autoimmune disease, or cancer.

[0019b] In another aspect, there is provided the antigen-binding protein as described herein, or the pharmaceutical composition as described herein, for use in therapy in an individual.

[0020] 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 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 method of making an antigen-binding protein as described herein, comprising: immunizing OmniFlic or UniRat animals with an antigen other than CD3; and identifying antigen-specific heavy chain sequences that can be combined in a molecule containing the antigen-binding protein as described herein.

[0020b] In another aspect, there is provided a bispecific antibody comprising:

(i) a CD3-binding heavy chain variable domain comprising CDR1, CDR2 and CDR3 sequences of SEQ ID NO:18 in a human VH framework;

(ii) a light chain variable domain comprising CDR1, CDR2 and CDR3 sequences of SEQ ID NO:69 in a human VL framework; and

(iii) a single chain anti-BCMA variable domain comprising CDR1, CDR2 and CDR3 sequences at positions 26-33; 51-58; 97-108, respectively, of SEQ ID NO:70 in single or in tandem configuration.

[0020c] In another aspect, there is provided a bispecific antibody comprising:

(i) a CD3-binding heavy chain variable domain comprising CDR1, CDR2 and CDR3 sequences of SEQ ID NO:18 in a human VH framework;

(ii) a light chain variable domain comprising CDR1, CDR2 and CDR3 sequences of SEQ ID NO:69 in a human VL framework; and

(iii) a single chain variable domain that specifically binds to a pathogen antigen in single or in tandem configuration.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] 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 to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures.

[0022] **FIG. 1A-1B.** (FAM1_aCD3_CDR_seqalign). **FIG. 1A** shows an alignment of CDR1, 2 and 3 regions of all members of an antibody family recognizing human CD3. The CDR sequences correspond to amino acid residues 26-35; 53-59; and 98-117 for CDR1, CDR2 and CDR3, respectively, of the variable region sequences set forth in SEQ ID NO:1-68. **FIG. 1B** shows the CDR1, 2 and 3 regions of the fixed light chain (SEQ ID NO:69); and an exemplary anti-BCMA sequence (SEQ ID NO:70 and SEQ ID NO:71).

[0023] **FIG. 2A-2E.** Schematic models of bispecific human antibodies. **FIG. 2A** anti-CD3:anti-tumor-antigen bispecific antibody with common light chain (3 total unique chains). **FIG. 2B** anti-CD3:anti-tumor-antigen bispecific antibody with 2 unique light chains (4 total unique chains). **FIG. 2C** anti-CD3:anti-tumor-antigen bispecific antibody with heavy-chain only tumor antigen binding domain chain (3 unique chains). **FIG. 2D** anti-CD3:anti-tumor-antigen bispecific antibody

What is Claimed is:

1. An antigen-binding protein specific for CD3, comprising:
a heavy chain variable domain comprising:
(a) a CDR1 sequence of GGSIRSGGHY, a CDR2 sequence of ISYSGST, and a CDR3 sequence of ARWRHDILTAYPYYYYGMDV;
and a light chain variable domain.
2. The antigen-binding protein of claim 1, wherein said heavy chain CDR1, CDR2 and CDR3 sequences are present in a human VH framework.
3. The antigen-binding protein of any one of claims 1-2, wherein the heavy chain variable domain comprises an amino acid sequence set forth in SEQ ID NO: 18.
4. The antigen-binding protein of any one of claims 1-3, wherein the light chain variable domain comprises an amino acid sequence of SEQ ID NO: 69.
5. An antigen-binding protein of any one of claims 1-4, wherein when contacted with T cells in an activation assay the antigen binding protein induces release of reduced levels of one or both of IL-2 and IL-10 relative to a reference anti-CD3 antibody.
6. The antigen-binding protein of any one of claims 1-5, further comprising an Fc region.
7. The antigen-binding protein of claim 6, wherein the Fc region has been engineered to reduce effector functions.
8. The antigen-binding protein of any one of claims 1-7, wherein the affinity (KD) of the protein for human CD3 delta epsilon is around about 10^{-6} M to around about 10^{-11} M.
9. The antigen-binding protein of any one of claims 1-7, wherein the protein cross-reacts with human and *Cynomolgus* macaque CD3 protein.
10. The antigen-binding protein of any one of claims 1-9, wherein the protein comprises a single chain.

11. The antigen-binding protein of any one of claims 1-9, wherein the protein comprises two chains, or a multiple thereof.

12. The antigen-binding protein of any one of claims 1-9, wherein the protein comprises three chains.

13. The antigen-binding protein of any one of claims 1-9, wherein the protein comprises three chains and two antigen-binding arms, wherein the first antigen-binding arm comprises an antibody heavy chain and an antibody light chain, and the second antigen-binding arm comprises an antibody heavy chain that does not comprise a CH1 domain.

14. The antigen-binding protein of any one of claims 1-9, wherein the protein further comprises a heavy chain variable domain specific for a protein other than CD3.

15. The antigen-binding protein of any one of claims 1-14, wherein the protein further comprises a heavy chain variable domain specific for a protein other than CD3;

wherein when contacted with T cells in an activation assay the antigen binding protein induces release of reduced levels of one or both of IL-2 and IL-10 relative to a reference anti-CD3 antibody; and induces more than 30% tumor cytotoxicity in standard in vitro assays using tumor cells and human T cells.

16. The antigen-binding protein of claim 14, wherein the heavy chain variable domain specific for a protein other than CD3 is a heavy chain only variable domain.

17. The antigen-binding protein of claim 16, wherein the heavy chain variable domain specific for a protein other than CD3 further comprises a light chain variable domain.

18. The antigen-binding protein of claim 16, wherein the light chain variable domain is the same as the light chain variable domain of the CD3-binding region.

19. The antigen-binding protein of any one of claims 14-18, wherein the protein other than CD3 is a tumor associated antigen.

20. The antigen-binding protein of any one of claims 14-18, wherein the protein other than CD3 is a pathogen antigen.
21. The antigen-binding protein of any one of claims 14-18, wherein the protein other than CD3 is an immunoregulatory protein.
22. A pharmaceutical composition comprising an antigen-binding protein of any of claims 1-21.
23. The pharmaceutical formulation of claim 22, in a unit dose formula.
24. A polynucleotide encoding an antigen-binding protein of any of claims 1-21.
25. A vector comprising a polynucleotide of claim 24.
26. A cell comprising a vector of claim 25.
27. A method of producing an antigen-binding protein of any one of claims 1-21, comprising growing a cell according to claim 26 under conditions permissive for expression of the protein, and isolating the protein from the cells.
28. Use of an antigen-binding protein of any one of claims 1-21, in the preparation of a medicament for the treatment of an infection, and autoimmune disease, or cancer.
29. The antigen-binding protein of any one of claims 1-21, or the pharmaceutical composition of claim 22, for use in therapy in an individual.
30. The antigen-binding protein or the pharmaceutical composition according to claim 29 or the use of claim 28, wherein the individual is human.
31. A method of making an antigen-binding protein of claim 14, comprising:

immunizing OmniFlic or UniRat animals with an antigen other than CD3; and identifying antigen-specific heavy chain sequences that can be combined in a molecule containing the antigen-binding protein of any one of claims 1-13.

32. A bispecific antibody comprising:

(i) a CD3-binding heavy chain variable domain comprising CDR1, CDR2 and CDR3 sequences of SEQ ID NO:18 in a human VH framework;

(ii) a light chain variable domain comprising CDR1, CDR2 and CDR3 sequences of SEQ ID NO:69 in a human VL framework; and

(iii) a single chain anti-BCMA variable domain comprising CDR1, CDR2 and CDR3 sequences at positions 26-33; 51-58; 97-108, respectively, of SEQ ID NO:70 in single or in tandem configuration.

33. The bispecific antibody of claim 32, further comprising an Fc region.

34. The bispecific antibody of claim 33, wherein the CD3-binding heavy chain variable domain comprises the sequence of SEQ ID NO: 18, the light chain variable domain comprises SEQ ID NO: 69, and the BCMA-binding variable domain comprises SEQ ID NO: 70 or SEQ ID NO: 71.

35. A bispecific antibody comprising:

(i) a CD3-binding heavy chain variable domain comprising CDR1, CDR2 and CDR3 sequences of SEQ ID NO: 18 in a human VH framework;

(ii) a light chain variable domain comprising CDR1, CDR2 and CDR3 sequences of SEQ ID NO: 69 in a human VL framework; and

(iii) a single chain variable domain that specifically binds to a pathogen antigen in single or in tandem configuration.

36. The bispecific antibody of claim 35, further comprising an Fc region.

37. The bispecific antibody of claim 36, wherein the CD3-binding heavy chain variable domain comprises the sequence of SEQ ID NO: 18, the light chain variable domain comprises the sequence of SEQ ID NO: 69.