

receptor (FcR) interaction, or in an antigen-dependent manner when antigen is expressed on both normal and tumor cells. Both mechanisms may have been responsible for the toxicity observed in prior clinical studies. (See for example, Link et al. (1998) *Int. J. Cancer* 77(2):251-6; 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.

[0007] 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.

[0008] CD3 specific antibodies, and bispecific antibodies derived therefrom are provided by the invention.

Publications

[0009] 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. CD3 binding antibody sequences are provided in co-pending application PCT US2017/038377, herein specifically incorporated by reference.

SUMMARY

[0010] Compositions and methods of use thereof are provided for antibodies that bind to and activate signaling through CD3, e.g. activation of CD3⁺ T cells. The antibodies are characterized by binding to the CD3 epitope bound by the F2B antibody, which may be referred to herein as the F2B epitope. The F2B antibody comprises the set of CDR sequences of SEQ ID NO:1, and the fixed light chain sequences of SEQ ID NO:19. In some examples the antibody that binds to the F2B epitope comprises a heavy chain variable region sequence other than a sequence set forth in SEQ ID NO:1-18.

[0010a] In one aspect, the present disclosure refers to an isolated monoclonal antigen-binding protein that binds to CD3, wherein the antigen binding protein binds an epitope on CD3 comprising at least one residue selected from CD3 epsilon (SEQ ID NO:23): K73 and S83; and CD3 delta (SEQ ID NO:24) K82 and C93.

[0010b] In another aspect, the present disclosure refers to a pharmaceutical composition comprising an antigen-binding protein as described herein.

[0010c] In another aspect, the present disclosure refers to a polynucleotide encoding an antigen-binding protein as described herein.

[0010d] In another aspect, the present disclosure refers to a vector comprising the polynucleotide as described herein.

[0010e] In another aspect, the present disclosure refers to a cell comprising the vector as described herein.

[0010f] In another aspect, the present disclosure refers to 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 cell and/or a cell culture medium.

[0010g] In another aspect, the present disclosure refers to use of an antigen-binding protein as described herein in the manufacture of a medicament for the treatment of a disease.

[0010h] In yet another aspect, the present disclosure refers to an antigen-binding protein as described herein for use in the treatment of a disease.

- [0011]** Antibodies binding to the F2B epitope provide significant benefits in terms of biological activity. The antibodies minimize toxic cytokine release while maintaining effective tumor cell lysis. In some examples, anti-CD3 antibodies binding to the F2B epitope are characterized by a reduced propensity to induce cytokine release, upon binding to a competent T cell, e.g. for release of IL-2 and IFN γ . Without being bound by the theory, it is believed that binding to the specific epitope recognized by F2B provides for the unique and beneficial properties of antibodies described herein.
- [0012]** Antibodies binding to the F2B epitope may be selected for a binding affinity 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. Antibodies may be selected that induce a cytokine release that is not more than about 200% of the maximum cytokine release observed with the F2B antibody, and may be not more than about 150%, not more than 125%, not more than 100%, and may be less than the maximum observed for F2B in a comparative assay.
- [0013]** Antibodies may be selected that induce maximum IL-2 and IL-10 release in a comparative *in vitro* assay of not more than 20%, not more than 30%, and not more than 50% of the maximum release of a control anti-CD3 antibody, such as OKT-3 or TNB-383B.
- [0014]** The F2B epitope is characterized by binding to at least one residue selected from CD3 epsilon (SEQ ID NO:23): K73 and S83; and CD3 delta (SEQ ID NO:24) K82 and C93. In some examples the epitope comprises the region of CD3 epsilon defined by K73, N74, I75, G76, S77, D78, E79, D80, H81, L82, S83. In some examples the epitope comprises one or both of K73 and S83. In some examples the epitope comprises the region of CD3 delta defined by K82, E83, S84, T85, V86, Q87, V88, H89, Y90, R91, M92, C93. In some examples the epitope comprises one or both of K82 and C93. In some examples the F2B epitope comprises a conformational epitope involving residues of both CD3 delta and CD3 epsilon. In some examples the conformational epitope comprises each of residues CD3 $\square$ K73 and S83; CD3 $\square$ K82 and C93. In some examples antibodies that bind to the F2B epitope are not cross-reactive with cynomolgus CD3 protein.
- [0015]** In some examples, an antibody that binds to the F2B epitope is determined by competition assays between the antibodies disclosed herein and other antibodies. In some examples, the antibodies bind to a specific residue of CD3 that reduces cytokine release.
- [0016]** In some examples, bispecific or multispecific antibodies are provided, which comprise at least a heavy chain variable region from an antibody that binds to the F2B epitope. 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.

[0017] In some examples, an F2B epitope-binding antibody of the disclosure comprises a CD3-binding variable region, 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.

[0018] 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.

[0019] 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.

[0020] 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.

[0021] 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 a eukaryotic cell, such as a mammalian cell.

[0022] Examples of the disclosure include methods for producing an antigen-binding protein as described herein, comprising growing a host cell under conditions permissive for expression of the protein, and isolating the protein from the cell and/or a cell culture medium.

- [0023] Examples of the disclosure include methods of treatment, comprising administering to an individual an effective dose of an antigen-binding protein as described herein, or a pharmaceutical composition as described herein.
- [0024] Examples of the disclosure relate to use of an antigen-binding protein as described herein in the preparation of a medicament for the treatment of a disease.
- [0025] Examples of the disclosure include antigen-binding proteins as described herein for use in the treatment of a disease.
- [0026] In some examples, a method or use involves a human subject (e.g., a individual who is a human).

BRIEF DESCRIPTION OF THE DRAWINGS

- [0027] 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.
- [0028] **FIGS. 1A-1C.** **FIG. 1A** show an alignment of CDR1, 2 and 3 regions of members of antibody family 2 of SEQ ID NO:1-18, which specifically bind to human CD3, corresponding to residues 26-33; 51-58; and 97-112. **FIG. 1B** shows the CDR1, 2 and 3 regions of the fixed light chain (SEQ ID NO:19); and an exemplary anti-BCMA sequence (SEQ ID NO:20 and SEQ ID NO:21). **FIG. 1C** provides the CDR sequences of a reference anti-CD3 antibody (SEQ ID NO:22), ID 304704.
- [0029] **FIG. 2.** Schematic representation of molecule TNB-383B with the anti-CD3 arm (CD3_F2B or ID:312557) and a high affinity anti-BCMA arm.
- [0030] **FIG. 3.** Dose-response curves of cytokine release by PBMCs treated with TNB-383B and a positive control. Pre-cultured PBMCs were stimulated with a positive control (black crosses) or with TNB-383B (black square) at increasing concentrations. The positive control in this experiment was a bispecific anti-CD3/anti-BCMA antibody (TNB-384B). The anti-CD3 arm of this bispecific antibody recognizes a different epitope on human CD3 with high affinity ($K_D=30\text{nM}$, also named), but the anti-BCMA arm is identical to the anti-BCMA arm of TNB-383B. This positive control showed a similar cytokine secretion profile as OKT3 (data not shown).
- [0031] **FIG. 4.** Activation profile of T cell subsets after overnight stimulation with TNB-383B and a positive control (TNB-384B). Responses at plateau concentrations of positive control (132 ng/ml, black) and TNB-383B (1320 ng/ml, pattern) are shown. Cells were analyzed after a 24 hour incubation step as described in Example 1. CD69 expression was analyzed (percentage positive cells

WHAT IS CLAIMED IS:

1. An isolated monoclonal antigen-binding protein that binds to CD3, wherein the antigen binding protein binds an epitope on CD3 comprising at least one residue selected from CD3 epsilon (SEQ ID NO:23): K73 and S83; and CD3 delta (SEQ ID NO:24) K82 and C93.
2. The antigen-binding protein of claim 1, wherein the epitope on CD3 comprises a region of CD3 delta defined by K82, E83, S84, T85, V86, Q87, V88, H89, Y90, R91, M92, C93.
3. The antigen-binding protein of claim 1, wherein the epitope on CD3 comprises a region of CD3 epsilon defined by K73, N74, I75, G76, S77, D78, E79, D80, H81, L82, S83.
4. The antigen-binding protein of any one of claims 1-3, wherein the epitope comprises a conformational epitope comprising residues of both CD3 delta and CD3 epsilon.
5. The antigen-binding protein of any one of claims 1-4, wherein the conformational epitope comprises each of residues CD3 epsilon K73 and S83; and CD3 delta K82 and C93.
6. The antigen-binding protein of any one of claims 1-5, wherein the antibody does not cross-react with cynomolgus CD3 protein.
7. The antigen-binding protein of any one of claims 1-6, wherein the antigen-binding protein induces a cytokine release upon binding to a T cell that is not more than 200% of a maximum cytokine release observed with an F2B antibody.
8. The antigen-binding protein of any one of claims 1-7, wherein the binding affinity for CD3 is 50 nM or greater.
9. The antigen-binding protein of any one of claims 1-8, which is a human antibody.
10. The antigen-binding protein of any one of claims 1-8, which is a humanized antibody.
11. The antigen-binding protein of any one of claims 1-10, comprising a light chain variable region comprising a set of CDR sequences in SEQ ID NO:19.

12. The antigen-binding protein of any one of claims 1-11, comprising a light chain variable region comprising an amino acid sequence of SEQ ID NO:19.
13. The antigen-binding protein of any one of claims 1-12, further comprising an Fc region.
14. The antigen-binding protein of claim 13, wherein the Fc region has been engineered to reduce one or more effector functions.
15. The antigen-binding protein of any one of claims 1-14, further comprising an antigen binding domain that binds to a protein other than CD3.
16. The antigen-binding protein of claim 15, wherein the antigen binding domain that binds to a protein other than CD3 comprises a heavy chain only variable region.
17. The antigen-binding protein of claim 16, wherein the protein other than CD3 is a tumor associated antigen.
18. The antigen-binding protein of claim 16, wherein the protein other than CD3 is a pathogen antigen.
19. The antigen-binding protein of claim 16, wherein the protein other than CD3 is an immunoregulatory protein.
20. A pharmaceutical composition comprising an antigen-binding protein of any one of claims 1-19.
21. The pharmaceutical composition of claim 20, in a unit dose formula.
22. A polynucleotide encoding an antigen-binding protein of any one of claims 1-19.
23. A vector comprising the polynucleotide of claim 22.
24. A cell comprising the vector of claim 23.

25. A method of producing an antigen-binding protein of any one of claims 1-19, comprising growing a cell according to claim 24 under conditions permissive for expression of the protein, and isolating the protein from the cell and/or a cell culture medium.

26. Use of an antigen-binding protein of any one of claims 1-19 in the manufacture of a medicament for the treatment of a disease.

27. An antigen-binding protein of any one of claims 1-19 for use in the treatment of a disease.

28. The use of an antigen-binding protein of any one of claims 26-27, wherein the individual is a human.