PROTEINS SPECIFIC FOR BAFF AND B7RP1 AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of US Provisional Application Nos. 61/780,260, filed March 13, 2013, and 61/942,776, filed February 21, 2014, each of which are incorporated herein in their entirety.

FIELD

The bispecific molecules described herein are within the field of protein therapeutics.

BACKGROUND

Most therapeutic proteins bind to a single target protein with high specificity, thereby interfering with the activity of this single target protein. That protein may be a part of one or more biological pathways that mediate a human disease being treated, and the therapeutic protein may therefore inhibit disease progression. However, efficacy of therapeutic proteins is rarely complete for all patients. Incomplete efficacy of therapeutic proteins could be due in some cases to the complexity of a disease. For example, some diseases may be mediated by multiple biological pathways, or different biological pathways may play a predominant role in mediating disease activity in different patients having the same clinically-defined condition. Hence, in some diseases it may be advantageous to simultaneously inhibit at least two biological pathways.

SUMMARY

Herein is provided a bispecific protein that can bind to and inhibit the biological activity of both human B7-related protein 1 (B7RP1, also known as GL50 and T-cell co-stimulator ligand (ICOSLG)) and human B-cell activating factor (BAFF, also known as tumor necrosis factor superfamily, member 13b (TNFSF13B)). BAFF plays a role in B cell survival, and B7RP1 plays a role in T cell costimulation. Thus, a protein that inhibits the activity of both proteins interferes with the activity of both B and T cells.

In one aspect, there is provided a bispecific protein comprising:

a) antibody comprising a light chain complementarity determining region 1 (CDR1) comprising the amino acid sequence of SEQ ID NO:8, a light chain complementarity determining region 2 (CDR2) comprising the amino acid sequence of SEQ ID NO:9, a light chain complementarity determining region 3 (CDR3) comprising the amino acid sequence of SEQ ID NO:10, a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO:11, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO:12, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:13; and

b) a peptide comprising an amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3.

Such a protein can comprise an amino acid sequence comprising at least two copies of SEQ ID NO:1 separated by SEQ ID NO:6 or 7. In a further embodiment, such a protein can include an immunoglobulin light chain and an immunoglobulin heavy, and SEQ ID NO:6 or 7 can be downstream from the C-terminus of the heavy chain. In such embodiments, SEQ ID NO:6 or 7 can be flanked by peptides that bind to a protein other than that bound by the heavy and light chains.

In another aspect, there is provided a pharmaceutical composition comprising a therapeutically effective amount of the bispecific protein as described herein and a physiologically acceptable excipient.

Further, herein is described a pharmaceutical composition comprising any of the bispecific proteins herein described or the protein comprising the amino acid sequence of SEQ ID NO:6 or 7 and a physiologically acceptable excipient.

Also described herein is a nucleic acid encoding any polypeptide included in one of bispecific proteins or the proteins comprising SEQ ID NO:6 or SEQ ID NO:7 herein described. Exemplary nucleic acids encoding a polypeptide included in a bispecific protein include, for example, SEQ ID NOs: 55, 56, 60, 61, 62, and 63, among others. Vectors comprising such nucleic acids and host cells containing such vectors and/or nucleic acids are described. Further described herein is method for making a bispecific protein comprising culturing the host cell containing a nucleic acid encoding any of the bispecific proteins described herein under conditions such that the nucleic acid is expressed and recovering the protein from the cell mass or the culture medium. The host cell can be a mammalian cell, for example, a CHO cell, or a bacterial cell such as *Eschericha coli*.

In another aspect, there is provided a method for making a bispecific protein comprising culturing the host cell containing one or more nucleic acids encoding the bispecific protein as described herein; and/or a vector comprising said one or more nucleic acids; under conditions such that the nucleic acid is expressed and recovering the bispecific protein from the cell mass or the culture medium.

~~In another aspect,~~Also described herein is a method for treating systemic lupus erythematosus, including lupus nephritis, comprising administering to a patient a therapeutically effective amount of any of the bispecific proteins described herein or a pharmaceutical composition comprising such a bispecific protein.

Another therapeutic can be administered to the patient before, after, or concurrently with the bispecific protein. The other therapeutic can be a corticosteroid, an antimalarial, retinoic acid, an NSAID, cyclophosphamide, dehydroepiandrosterone, mycophenolate mofetil, azathioprine, chlorambucil, methotrexate, tacrolimus, dapsone, thalidomide, leflunomide, or cyclosporine.

~~In a further aspect, herein is described~~Also described herein is a method of treatment comprising administering to a patient a therapeutically effective amount of any of the bispecific proteins described herein or a pharmaceutical composition comprising a bispecific protein described herein, wherein the patient has a disease selected from the group

consisting of: ANCA-positive vasculitis, rheumatoid arthritis (RA), Crohn's disease, ulcerative colitis, celiac disease, pemphigus, pemphigoid, subacute cutaneous lupus erythematosus (SCLE), multiple sclerosis, chronic inflammatory demyelinating polyneuropathy (CIDP), myasthenia gravis, Goodpasture's syndrome, glomerulonephritis, autoimmune hemolytic anemia (AIHA), idiopathic thrombocytopenic purpura (ITP), chronic active hepatitis, primary billiary cirrhosis, Sjogren's syndrome, systemic sclerosis, Hashimoto's thyroiditis, Graves' disease, Addison's disease, and multiple endocrine neoplasia (MEN).

~~In another aspect, herein is described~~Also disclosed is a pharmaceutical composition comprising any of the bispecific proteins or the proteins comprising SEQ ID NO:6 or SEQ ID NO:7 herein described. The pharmaceutical composition can be, for example, for the treatment of systemic lupus erythematosus or lupus nephritis.

~~In another aspect,~~ Also disclosed is the use of any of the bispecific proteins provided herein as a medicament is described.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Diagrams of bispecific proteins that bind to BAFF and B7RP1. Across the top row are listed the identifier for each construct. Across the second row is a brief descriptive phrase relating to the structure of each construct. Across the bottom row is a diagram of the structure of each construct. The unfilled ovals represent constant regions of an immunoglobulin heavy or light chain. The ovals filled with horizontal lines represent immunoglobulin heavy or light chain variable (VH or VL) regions. The small, solidly filled squares and loops represent BAFF-binding peptides. The hinge regions are shown as heavy vertical lines, while the disulfide bridges are shown as heavy horizontal lines. The sequence of "G4S" in Figure 1 is disclosed in SEQ ID NO: 72.

Figure 2: Activity of bispecific proteins in a human B cell proliferation assay. The data shown in Figures 2A (top) and 2B (bottom) are from B cell proliferation assays performed as described in Example 1. In both panels, the x axis indicates the concentration (log[nM]) of the bispecific protein contained in the assay mixture, and the y axis indicates the amount of ^{3}H-thymidine uptake (counts per minute (cpm)). The meaning of each symbol is indicated by an identifier for each protein assayed. Meanings of the identifiers are shown in Figure 1 and explained in Example 1.

What is claimed is:

1. A bispecific protein,

wherein the protein can inhibit BAFF-mediated proliferation of human B cells and

wherein the protein can inhibit B7RP1-mediated proliferation of human T cells.

2. The bispecific protein of claim 1, wherein the bispecific protein comprises an IgG antibody comprising two immunoglobulin heavy chains having different amino acid sequences and two immunoglobulin light chains having different amino acid sequences.

3. The bispecific protein of claim 2, wherein the IgG antibody is an IgG1 antibody.

4. The bispecific protein of claim 2, wherein the IgG antibody is an IgG2 antibody.

5. The bispecific protein of claim 2, wherein the IgG antibody is an IgG4 antibody.

6. The bispecific protein of any one of claims 1 to 5, wherein the bispecific protein is a human or humanized IgG antibody.

7. The bispecific protein of any one of claims 1 to 6, which comprises a light chain complementarity determining region 1 (CDR1) comprising the amino acid sequence of SEQ ID NO:8, a light chain complementarity determining region 2 (CDR2) comprising the amino acid sequence of SEQ ID NO:9, a light chain complementarity determining region 3 (CDR3) comprising the amino acid sequence of SEQ ID NO:10, a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO:11, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO:12, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:13.

8. The bispecific protein of claim 7, which comprises a heavy chain variable region comprising SEQ ID NO:15 and a light chain variable region comprising SEQ ID NO:14.

9. The bispecific protein of claim 1, comprising:

(a) a polypeptide comprising an amino acid sequence having the following formula: A-L1-P-L2-P, wherein A is an immunoglobulin heavy chain of an

IgG antibody, L1 is a first linker of that is absent or is 3 to 40 amino acids long, P is a BAFF-binding peptide that is 10 to 40 amino acids long, and L2 is a peptide linker that is absent or is 5 to 50 amino acids long, and

(b) an immunoglobulin light chain.

10. The bispecific protein of claim 9, wherein the immunoglobulin heavy chain of (a) and the immunoglobulin light chain of (b) form an IgG antibody, comprising two molecules of the polypeptide of (a) and two molecules of the light chain of (b), that can bind B7RP1.

11. The bispecific protein of claim 9 or 10, wherein the immunoglobulin heavy chain is missing a lysine at its C-terminal end just upstream of L1.

12. The bispecific protein of any one of claims 9 to 11, wherein the IgG antibody is a human or humanized anti-B7RP1 IgG1 antibody.

13. The bispecific protein of any one of claims 9 to 11, wherein the anti-B7RP1 antibody is a human or humanized IgG2 antibody.

14. The bispecific protein of any one of claims 9 to 11, wherein the anti-B7RP1 antibody is a human or humanized IgG4 antibody.

15. The bispecific protein of any one of claims 9 to 14, wherein P has the amino acid sequence of SEQ ID NO:1 (LPGCKWDLLIKQWVCDPL).

16. The bispecific protein of any one of claims 9 to 15, wherein L1 has the amino acid sequence of SEQ ID NO:40 (GGGGG).

17. The bispecific protein of any one of claims 9 to 16, wherein the L2 has the amino acid sequence of SEQ ID NO:5.

18. The bispecific protein of claim 17, wherein L2 has the amino acid sequence of SEQ ID NO:7.

19. The bispecific protein of claim 17, wherein L2 has the amino acid sequence of SEQ ID NO:6.

20. The bispecific protein of any one of claims 9 to 19, comprising a light chain CDR1 comprising the amino acid sequence of SEQ ID NO:8 (RASQGISNWLA), a light chain CDR2 comprising the amino acid sequence of SEQ ID NO:9 (AASSLQS), a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:10 (QQYDSYPRT) , a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO:11 (SYWMS), a heavy chain CDR2 comprising the amino acid sequence of

SEQ ID NO:12 (YIKQDGNEKYYVDSVKG), and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:13 (EGILWFGDLPTF).

21. The bispecific protein of any one of claims 9 to 20, comprising an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO:14.

22. The bispecific protein of any one of claims 9 to 21, comprising an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:15.

23. The bispecific protein of any one of claims 15 to 22, wherein the immunoglobulin light chain of (b) comprises the amino acid sequence of SEQ ID NO:19.

24. The bispecific protein of any one of claims 15 to 23, wherein the polypeptide of (a) comprises the amino acid sequence of SEQ ID NO:17 or 18.

25. A bispecific protein comprising

(a) a polypeptide comprising the amino acid sequence of SEQ ID NO:17 or SEQ ID NO:18 and

(b) another polypeptide comprising the amino acid sequence of SEQ ID NO:19.

26. The bispecific protein of claim 25, which is a tetramer comprising two molecules of the polypeptide of (a) and two molecules of the polypeptide of (b).

27. The bispecific protein of claim 25 or 26, wherein the polypeptide of (a) comprises the amino acid sequence of SEQ ID NO:17.

28. The bispecific protein of claim 25 or 26, wherein the polypeptide of (b) comprises the amino acid sequence of SEQ ID NO:18.

29. A protein comprising a linker comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7.

30. The protein of claim 29, wherein the protein can inhibit BAFF-mediated proliferation of human B cells and wherein the protein can inhibit B7RP1-mediated proliferation of human T cells.

31. The protein of claim 30, which comprises the amino acid sequences of SEQ ID NO:1, SEQ ID NO:14, and/or SEQ ID NO:15.

32. A pharmaceutical composition comprising the bispecific protein of any one of claims 1 to 28 or the protein of claim 30 or 31 and a physiologically acceptable excipient.

33. A nucleic acid encoding the bispecific protein of any one of claims 1 to 28 or the protein of claim 30 or 31.

34. A vector comprising the nucleic acid of claim 33.

35. A host cell containing the nucleic acid of claim 33 and/or the vector of claim 34.

36. A method for making a bispecific protein comprising

culturing the host cell of claim 35 under conditions such that the nucleic acid is expressed and

recovering the protein from the cell mass or the culture medium.

37. The method of claim 36, wherein the host cell is a mammalian cell.

38. The method of claim 37, wherein the host cell is a CHO cell.

39. The method of claim 36, wherein the host cell is an *Eschericha coli* cell.

40. A method for treating systemic lupus erythematosus comprising administering to a patient a therapeutically effective amount of the bispecific protein of any one of claims 1 to 28, the protein of claims 30 or 31, or the pharmaceutical composition of claim 32.

41. The method of claim 40,

wherein another therapeutic is administered to the patient before, after, or concurrently with the bispecific protein, and

wherein the other therapeutic is a corticosteroid, an antimalarial, retinoic acid, an NSAID, cyclophosphamide, dehydroepiandrosterone, mycophenolate mofetil, azathioprine, chlorambucil, methotrexate, tacrolimus, dapsone, thalidomide, leflunomide, or cyclosporine.

42. The method of claim 41, wherein the other therapeutic is mycophenolate mofetil, a corticosteroid, an anti-malarial, methotrexate, or azathioprine.

43. A method of treatment comprising administering to a patient a therapeutically effective amount of the bispecific protein of any one of claims 1 to 28, the protein of claim 30 or 31, or the pharmaceutical composition of claim 32,

wherein the patient has a disease selected from the group consisting of: ANCA-positive vasculitis, rheumatoid arthritis (RA), Crohn's disease, ulcerative colitis, celiac disease, pemphigus, pemphigoid, subacute cutaneous lupus erythematosus (SCLE), multiple sclerosis, chronic inflammatory demyelinating polyneuropathy (CIDP), myasthenia gravis, Goodpasture's syndrome, glomerulonephritis, autoimmune hemolytic anemia (AIHA), idiopathic thrombocytopenic purpura (ITP), chronic active hepatitis, primary billiary cirrhosis, Sjogren's syndrome, systemic sclerosis, Hashimoto's thyroiditis, Graves' disease, Addison's disease, and multiple endocrine neoplasia (MEN).

44. The use of the bispecific protein of any one of claims 1 to 28 or the protein of any one of claims 29 to 31 as a medicament.

45. A pharmaceutical composition for the treatment of systemic lupus erythematosus comprising the bispecific protein of any of claims 1 to 28 or the protein of claim 30 or 31.

46. A pharmaceutical composition for the treatment of lupus nephritis comprising the bispecific protein of any of claims 1 to 28 or the protein of claim 30 or 31.

What is claimed is:

1. A bispecific protein comprising:

a) antibody comprising a light chain complementarity determining region 1 (CDR1) comprising the amino acid sequence of SEQ ID NO:8, a light chain complementarity determining region 2 (CDR2) comprising the amino acid sequence of SEQ ID NO:9, a light chain complementarity determining region 3 (CDR3) comprising the amino acid sequence of SEQ ID NO:10, a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO:11, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO:12, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:13; and

b) a peptide comprising an amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3.

2. The bispecific protein of claim 1, wherein the antibody is a human or humanized IgG antibody.

3. The bispecific protein of claim 1 or claim 2, which comprises a heavy chain variable region comprising SEQ ID NO:15 and a light chain variable region comprising SEQ ID NO:14.

4. The bispecific protein of claim 1, comprising:

(a) a polypeptide comprising an amino acid sequence having the following formula: A-L1-P-L2-P, wherein A is an immunoglobulin heavy chain of an IgG antibody, L1 is a first linker ~~of~~ that is absent or is 3 to 40 amino acids long, P is SEQ ID NO: 1, 2, or 3, and L2 is a peptide linker that is absent or is 5 to 50 amino acids long, and

(b) an immunoglobulin light chain.

5. The bispecific protein of claim 4, wherein the immunoglobulin heavy chain of (a) and the immunoglobulin light chain of (b) form an IgG antibody, comprising two molecules of the polypeptide of (a) and two molecules of the light chain of (b).

6. The bispecific protein of claim 4 or 5, wherein the immunoglobulin heavy chain is missing a lysine at its C-terminal end just upstream of L1.

7. The bispecific protein of any one of claims 4 to 6, wherein the IgG antibody is a human or humanized IgG1 antibody.

8. The bispecific protein of any one of claims 4 to 6, wherein the IgG antibody is a human or humanized IgG2 antibody.

9. The bispecific protein of any one of claims 4 to 6, wherein the IgG antibody is a human or humanized IgG4 antibody.

10. The bispecific protein of any one of claims 4 to 9, wherein P has the amino acid sequence of SEQ ID NO:1 (LPGCKWDLLIKQWVCDPL).

11. The bispecific protein of any one of claims 4 to 10, wherein L1 has the amino acid sequence of SEQ ID NO:40 (GGGGG).

12. The bispecific protein of any one of claims 4 to 11, wherein the L2 has the amino acid sequence of SEQ ID NO:5.

13. The bispecific protein of any of claims 4 to 11, wherein L2 has the amino acid sequence of SEQ ID NO:6.

14. The bispecific protein of any one of claims 4 to 13, comprising an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 14.

15. The bispecific protein of any one of claims 4 to 14, comprising an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:15.

16. The bispecific protein of any one of claims 10 to 15, wherein the immunoglobulin light chain of (b) comprises the amino acid sequence of SEQ ID NO:19.

17. The bispecific protein of any one of claims 10 to 16, wherein the polypeptide of (a) comprises the amino acid sequence of SEQ ID NO:17 or 18.

18. A bispecific protein comprising

(a) a polypeptide comprising the amino acid sequence of SEQ ID NO:17 or SEQ ID NO:18 and

(b) another polypeptide comprising the amino acid sequence of SEQ ID NO:19.

19. The bispecific protein of claim 18, which is a tetramer comprising two molecules of the polypeptide of (a) and two molecules of the polypeptide of (b).

20. The bispecific protein of claim 17 or 18, wherein the polypeptide of (a) comprises the amino acid sequence of SEQ ID NO:17.

21. The bispecific protein of claim 17 or 18, wherein the polypeptide of (a) comprises the amino acid sequence of SEQ ID NO:18.

22. The bispecific protein of claim 1 comprising a linker comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7.

23. The bispecific protein of claim 22, which comprises the amino acid sequences of SEQ ID NO:1, SEQ ID NO:14, and/or SEQ ID NO:15.

24. A pharmaceutical composition comprising a therapeutically effective amount of the bispecific protein of any one of claims 1 to 23 and a physiologically acceptable excipient.

25. A method for making a bispecific protein comprising

culturing the host cell containing one or more nucleic acids encoding the bispecific protein of any of claims 1 to 23; and/or a vector comprising said one or more nucleic acids; under conditions such that the nucleic acid is expressed and

recovering the bispecific protein from the cell mass or the culture medium.

26. The method of claim 25, wherein the host cell is a mammalian cell.

27. The method of claim 26, wherein the host cell is a CHO cell.

28. The method of claim 25, wherein the host cell is an *Eschericha coli* cell.