T-CELL MODULATORY MULTIMERIC POLYPEPTIDES AND METHODS OF USE THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/338,410, filed May 18, 2016, which application is incorporated herein by reference in its entirety.

INTRODUCTION

[0002] An adaptive immune response involves the engagement of the T cell receptor (TCR), present on the surface of a T cell, with a small peptide antigen non-covalently presented on the surface of an antigen presenting cell (APC) by a major histocompatibility complex (MHC; also referred to in humans as a human leukocyte antigen (HLA) complex). This engagement represents the immune system's targeting mechanism and is a requisite molecular interaction for T cell modulation (activation or inhibition) and effector function. Following epitope-specific cell targeting, the targeted T cells are activated through engagement of costimulatory proteins found on the APC with counterpart costimulatory proteins the T cells. Both signals – epitope/TCR binding and engagement of APC costimulatory proteins with T cell costimulatory proteins – are required to drive T cell specificity and activation or inhibition. The TCR is specific for a given epitope; however, the costimulatory protein not epitope specific and instead is generally expressed on all T cells or on large T cell subsets.

SUMMARY

[0003] The present disclosure provides T-cell modulatory multimeric polypeptides comprising two different immunomodulatory polypeptides, at least one of which is a variant immunomodulatory polypeptide. The present disclosure provides nucleic acids comprising nucleotide sequences encoding the T-cell modulatory multimeric polypeptides, and host cells comprising the nucleic acids. The present disclosure provides methods of modulating the activity of a T cell; the methods comprise contacting the T cell with a T-cell modulatory multimeric polypeptide of the present disclosure.

[0003a] In one aspect, there is provided a multimeric polypeptide comprising:

a heterodimeric polypeptide comprising:

a) a first polypeptide comprising:

i) an epitope; and ii) a first major histocompatibility complex (MHC) polypeptide; and

b) a second polypeptide comprising:

i) a second MHC polypeptide; and ii) optionally an immunoglobulin (Ig) Fc polypeptide or a non-Ig scaffold; and

c) at least one first immunomodulatory polypeptide, wherein the at least one first immunomodulatory polypeptide is a CD80 polypeptide or variant thereof; and

d) at least one second immunomodulatory polypeptide, wherein the at least one second immunomodulatory is a 4-1BBL polypeptide or a variant thereof;

wherein the variant CD80 polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the CD80 amino acid sequence set forth in SEQ ID NO:1; and the variant 4-1BBL polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the CD4-1BBL amino acid sequence set forth in one of SEQ ID Nos: 2-4.

[0003b] In one example, there is provided a multimeric polypeptide comprising:

a) a first polypeptide comprising, in order from N-terminus to C-terminus:

i) an epitope;

ii) a first major histocompatibility complex (MHC) polypeptide; and

b) a second polypeptide comprising, in order from N-terminus to C-terminus:

i) a second MHC polypeptide; and

ii) optionally an immunoglobulin (Ig) Fc polypeptide or a non-Ig scaffold,

wherein the multimeric polypeptide comprises at least a first and a second immunomodulatory domain, wherein the first and the second immunomodulatory domains are each independently:

A) at the C-terminus of the first polypeptide;

B) at the N-terminus of the second polypeptide;

C) at the C-terminus of the second polypeptide; or

D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide,

wherein one of the first and second immunomodulatory domains is a variant CD80 polypeptide having at least one amino acid substitution relative to SEQ ID NO:1,

wherein one of the first and second immunomodulatory domains is a variant 4-1BBL polypeptide having at least one amino acid substitution relative to one of SEQ ID NOs:2-4.

[0003c] In one aspect, there is provided a nucleic acid comprising a nucleotide sequence encoding a first and/or second polypeptide as described herein.

[0003d] In one aspect, there is provided an expression vector comprising the nucleic acid as described herein.

[0003e] In one aspect, there is provided a host cell genetically modified with the expression vector as described herein.

[0003f] In one aspect, there is provided use of a multimeric polypeptide as described herein in the manufacture of a medicament for treating a cancer in an individual, wherein an effective amount of the multimeric polypeptide is to be administered to the individual, wherein the epitope is a cancer-associated peptide.

[0003g] In one aspect, there is provided use of a multimeric polypeptide as described herein in the manufacture of a medicament for treating an infection in an individual, wherein an effective amount of a multimeric polypeptide is to be administered to the individual, wherein the epitope is a pathogen-associated peptide, optionally wherein the infection is a virus-associated infection.

[0003h] In one example, there is provided a composition comprising:

a) a first nucleic acid comprising a nucleotide sequence encoding a first polypeptide comprising, in order from N-terminus to C-terminus:

i) an epitope;

ii) a first major histocompatibility complex (MHC) polypeptide; and

iii) a first immunomodulatory polypeptide; and

b) a second nucleic acid comprising a nucleotide sequence encoding a second polypeptide comprising, in order from N-terminus to C-terminus:

i) a second immunomodulatory polypeptide;

ii) a second MHC polypeptide; and

iii) an immunoglobulin (Ig) Fc polypeptide,

wherein one of the first and second immunomodulatory domains is a variant CD80 polypeptide having at least one amino acid substitution relative to SEQ ID NO:1, and

wherein one of the first and second immunomodulatory domains is a variant 4-1BBL polypeptide having at least one amino acid substitution relative to one of SEQ ID NOs:2-4.

[0003i] In one example, there is provided a host cell genetically modified with the composition as described herein.

[0003j] In one example, there is provided a method of producing the multimeric polypeptide as described herein, the method comprising:

a) culturing the host cell as described herein *in vitro* in a culture medium under conditions such that the host cell synthesizes the multimeric polypeptide; and

b) isolating the multimeric polypeptide from the host cell and/or from the culture medium.

[0003k] In one example, there is provided a method of selectively modulating the activity of an epitope-specific T cell, the method comprising contacting the T cell with the multimeric polypeptide as described herein, wherein said contacting selectively modulates the activity of the epitope-specific T cell.

[0003l] In one example, there is provided a method of selectively increasing the activity of an epitope-specific T cell in an individual, the method comprising administering to the individual an effective amount of the multimeric polypeptide as described herein effective to selectively increase the activity of an epitope-specific T cell in an individual.

[0003m] In one aspect, there is provided a composition comprising:

a) the multimeric polypeptide as described herein; and

b) a pharmaceutically acceptable excipient.

[0003n] In one example, there is provided a composition comprising:

a) the nucleic acid as described herein or the recombinant expression vector as described herein; and

b) a pharmaceutically acceptable excipient.

BRIEF DESCRIPTION OF THE DRAWINGS

[0004] **FIG. 1A-1C** schematically depict various embodiments of a T-cell modulatory multimeric polypeptide of the present disclosure. In these embodiments, disulfide bonds are formed between MHC (e.g., HLA) polypeptides present in separate polypeptides.

CLAIMS

What is claimed is:

1. A multimeric polypeptide comprising:

a heterodimeric polypeptide comprising:

a) a first polypeptide comprising:

i) an epitope; and

ii) a first major histocompatibility complex (MHC) polypeptide; and

b) a second polypeptide comprising:

i) a second MHC polypeptide; and

ii) optionally an immunoglobulin (Ig) Fc polypeptide or a non-Ig scaffold; and

c) at least one first immunomodulatory polypeptide, wherein the at least one first immunomodulatory polypeptide is a CD80 polypeptide or variant thereof; and

d) at least one second immunomodulatory polypeptide, wherein the at least one second immunomodulatory is a 4-1BBL polypeptide or a variant thereof;

wherein the variant CD80 polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the CD80 amino acid sequence set forth in SEQ ID NO:1; and the variant 4-1BBL polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the CD4-1BBL amino acid sequence set forth in one of SEQ ID Nos: 2-4.

2. The multimeric polypeptide of claim 1,

wherein the variant CD80 polypeptide binds a CD28 polypeptide with an affinity that is higher than the affinity of the variant CD80 polypeptide for a CTLA4 polypeptide, and

wherein the ratio of the binding affinity of the variant CD80 polypeptide for the CD28 polypeptide to the binding affinity of the variant CD80 polypeptide for the CTLA4 polypeptide is at least 1.5:1.

3. The multimeric polypeptide of claim 2, wherein the ratio of the binding affinity of the variant CD80 polypeptide for the CD28 polypeptide to the binding affinity of the variant CD80 polypeptide for the CTLA4 polypeptide is from 2:1 to 10:1.

4. The multimeric polypeptide according to any one of claims 1-3, wherein the variant 4-1BBL polypeptide exhibits reduced binding affinity to a 4-1BB polypeptide having an amino acid

sequence set forth in SEQ ID NO:51, compared to the binding affinity of a 4-1BBL polypeptide comprising the amino acid sequence set forth in one of SEQ ID NOs: 2-4 for the 4-1BB polypeptide.

5. The multimeric polypeptide according to any one of claims 1 to 4,

wherein the first immunomodulatory polypeptide is a variant CD80 polypeptide having at least one amino acid substitution relative to SEQ ID NO:1; and

wherein the second immunomodulatory polypeptide is a variant 4-1BBL polypeptide having at least one amino acid substitution relative to one of SEQ ID NOs:2-4.

6. The multimeric polypeptide of claim 5, wherein the variant CD80 polypeptide exhibits reduced binding affinity to a CD28 polypeptide having an amino acid sequence set forth in one of SEQ ID NOs:48-50, compared to the binding affinity of a CD80 polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:1, for the CD28 polypeptide.

7. The multimeric polypeptide of claim 1 to 6, wherein:

a1) the first polypeptide comprising, in order from N-terminus to C-terminus:

i) the epitope;

ii) the first MHC polypeptide; and

iii) a variant 4-1BBL polypeptide; and

b1) the second polypeptide comprising, in order from N-terminus to C-terminus:

i) a variant CD80 polypeptide;

ii) the second MHC polypeptide; or

a2) the first polypeptide comprising, in order from N-terminus to C-terminus:

i) the epitope;

ii) the first MHC polypeptide; and

iii) a variant CD80 polypeptide; and

b2) the second polypeptide comprising, in order from N-terminus to C-terminus:

i) a variant 4-1BBL polypeptide;

ii) the second MHC polypeptide.

8. A multimeric polypeptide of claim 1, wherein the at least one first immunomodulatory polypeptide is a CD80 polypeptide comprising a wild-type amino acid sequence.

9. A multimeric polypeptide of claim 8, wherein the CD80 polypeptide comprises the amino acid sequence set forth in SEQ ID NO:1.

10. A multimeric polypeptide of any one of claims 1, 8, and 9, wherein the at least one second immunomodulatory polypeptide is a 4-1BBL polypeptide comprising a wild-type amino acid sequence.

11. A multimeric polypeptide of claim 10, wherein the 4-1BBL polypeptide comprises the amino acid sequence set forth in one of SEQ ID NOs:2-4.

12. A multimeric polypeptide of any one of claims 1 and 8-11, wherein:

a1) the first polypeptide comprises, in order from N-terminus to C-terminus:

i) the epitope;

ii) the first MHC polypeptide; and

iii) at least one 4-1BBL polypeptide; and

b1) the second polypeptide comprises, in order from N-terminus to C-terminus:

i) a CD80 polypeptide; and

ii) the second MHC polypeptide; or

a2) the first polypeptide comprises, in order from N-terminus to C-terminus:

i) the epitope;

ii) the first MHC polypeptide; and

iii) a CD80 polypeptide; and

b2) the second polypeptide comprises, in order from N-terminus to C-terminus:

i) at least one 4-1BBL polypeptide;

ii) the second MHC polypeptide.

13. The multimeric polypeptide of any one of claims 1-12, wherein the first MHC polypeptide is a β2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide.

14. The multimeric polypeptide of any one of claims 1-12, wherein the first MHC polypeptide is an MHC Class II alpha chain polypeptide; and wherein the second MHC polypeptide is an MHC class II beta chain polypeptide.

15. The multimeric polypeptide of any one of claims 1-7, wherein the first immunomodulatory polypeptide is a variant CD80 immunomodulatory polypeptide that comprises a substitution of amino acid I67, K86, or D158.

16. The multimeric polypeptide of any one of claims 1-8, wherein the second immunomodulatory polypeptide is a variant 4-1BBL immunomodulatory polypeptide that comprises a substitution of K127.

17. The multimeric polypeptide of any one of claims 1-8, wherein the variant 4-1BBL polypeptide comprises an amino acid sequence having at least 90% amino acid sequence identity to the amino acid sequence PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL TGGLSY**X**EDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS PRSE (SEQ ID NO:11), wherein X is Ala, Gly, Val, Ile, Leu, Arg, His, Glu, or Asp.

18. The multimeric polypeptide of any one of claims 1-7, wherein the variant CD80 polypeptide comprises:

a) an amino acid sequence having at least 90% amino acid sequence identity to the amino acid sequence VIHVTK EVKEVATLSC GHNVSVEELA QTRIYWQKEK KMVLTMMSGD MNIWPEYKNR TIFDITNNLS **X**VILALRPSD EGTYECVVLK YEKDAFKREH LAEVTLSVKA DFPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS QDPETELYAV SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:5), where X is Ala, Gly, Val, Leu, Arg, His, Lys, Glu, or Asp; or

b) an amino acid sequence having at least 90% amino acid sequence identity to the amino acid sequence VIHVTK EVKEVATLSC GHNVSVEELA QTRIYWQKEK KMVLTMMSGD MNIWPEYKNR TIFDITNNLS IVILALRPSD EGTYECVVL**X** YEKDAFKREH LAEVTLSVKA DFPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS QDPETELYAV SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:7), where X is Ala, Gly, Val, Leu, Ile, Arg, His, Glu, or Asp; or

c) an amino acid sequence having at least 90% amino acid sequence identity to the amino acid sequence VIHVTK EVKEVATLSC GHNVSVEELA QTRIYWQKEK KMVLTMMSGD MNIWPEYKNR TIFDITNNLS IVILALRPSD EGTYECVVLK YEKDAFKREH LAEVTLSVKA DFPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS Q**X**PETELYAV SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:9), where X is Ala, Gly, Val, Leu, Ile, Arg, His, Lys, or Glu.

19. A nucleic acid comprising a nucleotide sequence encoding a first and/or second polypeptide according to any one of claims 1-18.

20. An expression vector comprising the nucleic acid of claim 19.

21. A host cell genetically modified with the expression vector of claim 20.

22. Use of a multimeric polypeptide of any one of claims 1 to 18 in the manufacture of a medicament for treating a cancer in an individual, wherein an effective amount of the multimeric polypeptide is to be administered to the individual, wherein the epitope is a cancer-associated peptide.

23. Use of a multimeric polypeptide of any one of claims 1 to 18 in the manufacture of a medicament for treating an infection in an individual, wherein an effective amount of a multimeric polypeptide is to be administered to the individual, wherein the epitope is a pathogen-associated peptide, optionally wherein the infection is a virus-associated infection.

24. A composition comprising:

a) the multimeric polypeptide of any one of claims 1-19; and

b) a pharmaceutically acceptable excipient.