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 T-cell multimeric polypeptide comprising:

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

i) an epitope; and 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 T-cell 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 the first and the second immunomodulatory polypeptides bind different co-immunomodulatory polypeptides, and wherein one of the first and second immunomodulatory domains binds a first co-immunomodulatory polypeptide and a second co-immunomodulatory polypeptide and is a variant that binds to the first co-immunomodulatory polypeptide with a higher affinity than to the second co-immunomodulatory polypeptide.

[0003b] In one aspect, 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 recombinant polypeptide, wherein the recombinant polypeptide comprises, in order from N-terminus to C-terminus:

i) an epitope;

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

iii) a first immunomodulatory polypeptide;

iv) a proteolytically cleavable linker or a ribosome skipping signal;

v) a second immunomodulatory polypeptide;

vi) a second MHC polypeptide; and

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

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.

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

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

[0003f] In one aspect, 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.

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

[0003h] In one aspect, 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.

[0003i] In one aspect, 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.

[0003j] In one aspect, 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.

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

a) the multimeric polypeptide as described herein; and

b) a pharmaceutically acceptable excipient.

[0003l] In one aspect, 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.