sequence-based engineering efforts is the development of interleukin-2 (IL-2) variants dubbed “superkines” or super-2s [6].

[0009] Therefore, a solution is needed that takes advantage of the various approaches for inducing cytokine receptor signaling described above with respect to increased IL-2 receptor signaling without having to make sequence-based changes to the cytokines themselves. Moreover, such a solution should increase the affinity of cytokines to their signaling receptors while also increasing the overall number of cytokine-receptor signaling complexes per T cell. Furthermore, such a solution should result in increased activation and expansion of human immune cells.

SUMMARY

[0010] Disclosed are improved cell signaling complexes capable of facilitating the activation and expansion of immune cells. More specifically, disclosed are cell signaling complexes capable of facilitating the activation and expansion of immune cells (e.g., human donor peripheral blood T cells, human peripheral blood NK cells, etc.).

[0010a] In one aspect, provided herein is a chimeric cytokine complex, comprising:

a protein cage polypeptide, wherein the protein cage polypeptide comprises polypeptides that self-assemble into a tetrahedral pyramid structure;

a plurality of engineered Fc antibody domains non-covalently bound to the protein cage polypeptide; and

one or more cytokines covalently linked to each of the plurality of engineered Fc antibody domains.

[0010b] In another aspect, provided herein is a chimeric cytokine complex, comprising:

a protein cage polypeptide;

a plurality of engineered Fc antibody domains non-covalently bound to the protein cage polypeptide, wherein the plurality of engineered Fc antibody domains comprises between six and twelve engineered Fc antibody domains non-covalently bound to the protein cage polypeptide; and

one or more cytokines covalently linked to each of the plurality of engineered Fc antibody domains.

[0010c] In another aspect, provided herein is a chimeric cytokine complex, comprising:

a protein cage polypeptide;

a plurality of engineered Fc antibody domains non-covalently bound to the protein cage polypeptide; and

one or more cytokines covalently linked to each of the plurality of engineered Fc antibody domains, wherein each of the one or more cytokines is covalently linked to one of the engineered Fc antibody domains via an engineered metalloprotease-resistant linker sequence.

[0011] In some examples, a chimeric cytokine complex comprises a protein cage polypeptide, a plurality of engineered Fc antibody domains bound to the protein cage polypeptide, and one or more cytokines linked to each of the plurality of engineered Fc antibody domains.

[0012] Also disclosed is a method of activating and expanding immune cells. The method can comprise adding a chimeric cytokine complex to a population of immune cells. The chimeric cytokine complex can comprise a protein cage polypeptide, a plurality of engineered Fc antibody domains bound to the protein cage polypeptide, and one or more cytokines linked to each of the plurality of Fc antibody domains.

[0013] In some examples, the population of immune cells can be activated and expanded in vitro. In various examples, the population of immune cells can be human donor peripheral blood immune cells.

[0014] In various examples, the population of immune cells can be live T cells.

[0015] In various examples, the population of immune cells can be natural killer (NK) cells.

[0016] In some examples, the method can further comprise activating the population of T cells with an anti-CD3 and anti-CD28 T-cell activation reagent prior to adding the chimeric cytokine complex.

[0017] In various examples, the cytokines can be interleukins. The interleukins can be at least one of interleukin-2s (IL-2s), IL-7s, and IL-15s.

[0018] In various examples, the plurality of engineered Fc antibody domains can comprise between six and twelve engineered Fc antibody domains bound to the protein cage polypeptide. In some examples, between 12 and 24 cytokines can be linked to the plurality of engineered Fc antibody domains in total.

[0019] In various examples, each of the one or more cytokines can be linked to one of the engineered Fc antibody domains via an engineered metalloprotease-resistant linker sequence. In some examples, the engineered metalloprotease-resistant linker sequence can be between 7 to 13 amino acid residues in length.

[0020] In various examples, at least one of the engineered Fc antibody domains can be C-terminally linked to an N-terminus of at least one of the cytokines.

[0021] In some examples, one of the cytokines can be interleukin-2 (IL-2) and at least one of the engineered Fc antibody domains can be linked to the IL-2 via an engineered metalloprotease-resistant linker sequence comprising the amino acid sequence SLSPGKAPTS (SEQ ID NO:20).

[0022] In some examples, one of the cytokines is interleukin-7 (IL-7) and at least one of the engineered Fc antibody domains can be linked to the IL-7 via an engineered metalloprotease-resistant linker sequence comprising the amino acid sequence SLSPGKDCDIEGK (SEQ ID NO:21).

[0023] In some examples, one of the cytokines can be interleukin-15 (IL-15) and at least one of the engineered Fc antibody domains can be linked to the IL-15 via an engineered metalloprotease-resistant linker sequence comprising the amino acid sequence SLSPGKN (SEQ ID NO:22).

[0024] In some examples, at least one of the engineered Fc antibody domains can be N-terminally linked to a C-terminus of at least one of the cytokines.

[0025] In some examples, one of the cytokines can be interleukin-2 (IL-2) and at least one of the engineered Fc antibody domains can be linked to the IL-2 via an engineered metalloprotease-resistant linker sequence comprising the amino acid sequence TPKSCDKTHT (SEQ ID NO:23).

[0026] In some examples, one of the cytokines can be interleukin-7 (IL-7) and at least one of the engineered Fc antibody domains can be linked to the IL-7 via an engineered metalloprotease-resistant linker sequence comprising the amino acid sequence HPKSCDKTHT (SEQ ID NO:24).

[0027] In some examples, one of the cytokines can be interleukin-15 (IL-15) and at least one of the engineered Fc antibody domains can be linked to the IL-15 via an engineered metalloprotease-resistant linker sequence comprising the amino acid sequence TSPKSCDKTHT (SEQ ID NO:25).

[0028] In various examples, the engineered Fc antibody domains can be engineered human Fc antibody domains.

[0029] In some examples, the engineered human Fc antibody domains can be engineered human IgG1 Fc antibody domains.

[0030] In various examples, one of the engineered human IgG1 Fc antibody domains can comprise an amino acid sequence with at least about 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% amino acid identity to the amino acid sequence set forth in SEQ ID NO:1.

[0031] In some examples, the engineered human IgG1 Fc antibody domain can comprise at least one of the following point mutations relative to SEQ ID NO:1 to decrease affinity for certain Fcγ receptors and functionally reduce antibody-dependent cellular cytotoxicity (ADCC): P75L, R76W, Y80K, Y80P, Y80R, Y80G, and Y80A.

[0032] In some examples, the engineered human IgG1 Fc antibody domain can comprise the following point mutation relative to SEQ ID NO:1 to increase affinity for certain Fcγ receptors and functionally increase antibody-dependent cellular cytotoxicity (ADCC): Y80W.

[0033] In some examples, the engineered human IgG1 Fc antibody domain can comprise at least one of the following point mutations relative to SEQ ID NO:1 to decrease affinity for certain Fcγ receptors and have a neutral effect on other Fcγ receptors: S23A, E53A, E77A, Y80F, V87A, A111G, K122A, and D160A.

[0034] In some examples, the engineered human IgG1 Fc antibody domain can comprise at least one of the following point mutations relative to SEQ ID NO:1 to increase affinity for certain Fcγ receptors and have a neutral effect on other Fcγ receptors: E117, K118A, and A123T.

[0035] In some examples, the engineered human IgG1 Fc antibody domain can comprise at least one of the following point mutations relative to SEQ ID NO:1 to increase affinity for certain Fcγ receptors and decrease affinity for certain other Fcγ receptors: H52A, R85A, and K106A.

[0036] In some examples, the engineered human IgG1 Fc antibody domain can comprise at least one of the following point mutations relative to SEQ ID NO:1 to decrease affinity for certain Fcγ receptors: D54A, Q79A, and A111S.

[0037] In some examples, the engineered human IgG1 Fc antibody domain can comprise at least one of the following point mutations relative to SEQ ID NO:1 to increase affinity for certain Fcγ receptors: T40A and K74A.

[0038] In some examples, the Fc antibody domains can be engineered rabbit Fc antibody domains.

[0039] In various examples, the chimeric cytokine complex can further comprise a signal peptide linked to an N-terminus of at least one of the engineered Fc antibody domains or at least one of the cytokines.

[0040] In some examples, the signal peptide can be a murine Ig heavy signal peptide used for expression in Chinese hamster ovary (CHO) cells. In some examples, the signal peptide can comprise the amino acid sequence MGWSCIILFLVATATGVHS (SEQ ID NO:26).

[0041] In various examples, the protein cage polypeptide can comprise a polypeptide comprising an amino acid sequence with at least about 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% amino acid identity to the amino acid sequence set forth in any one of SEQ ID NOS: 8-15.

[0042] In some examples, the amino acid sequence making up the polypeptide of the protein cage polypeptide can comprise at least the following point mutation relative to the amino acid sequence set forth in any one of SEQ ID NOS: 8-15: Y294A.

[0043] In some examples, the protein cage polypeptide can comprise a polypeptide comprising a binding site for one of the engineered Fc antibody domains. The binding site can comprise the amino acid sequence RWGSGADCAWHLGELVWCTAGSGWE (SEQ ID NO:16).

[0044] In some examples, the protein cage polypeptide can comprise a polypeptide comprising a binding site for one of the engineered Fc antibody domains. The binding site can comprise the amino acid sequence GGRWGADCAWHLGELVWCTAGWEGG (SEQ ID NO:17).

[0045] In some examples, the protein cage polypeptide can comprise a polypeptide comprising a binding site for one of the engineered Fc antibody domains. The binding site can comprise the amino acid sequence GADCAWHLGELVWCTAG (SEQ ID NO:18).

[0046] In some examples, the protein cage polypeptide can comprise a polypeptide comprising a binding site for one of the engineered Fc antibody domains. The binding site can comprise the amino acid sequence RWGSGCDCAWHLGELVWCTCGSGWE (SEQ ID NO:19).

[0047] In some examples, the protein cage polypeptide can self-assemble into a tetrahedral pyramid structure.

[0048] In some examples, an Fc-cytokine complex is disclosed comprising an engineered Fc antibody domain and one or more cytokines linked to the engineered Fc antibody domain.

[0049] Also disclosed is a method of activating and expanding immune cells using the Fc-cytokine complex. The method can comprise adding an Fc-cytokine complex to a population of immune cells. The Fc-cytokine complex can comprise an Fc antibody domain and one or more cytokines linked to the Fc antibody domain.

[0050] In some examples, the population of immune cells can be activated and expanded in vitro. In various examples, the population of immune cells can be human donor peripheral blood immune cells. In various examples, the population of immune cells can be live T cells.

[0051] In some examples, the population of immune cells can be natural killer (NK) cells.

[0052] In some examples, the method can further comprise activating the population of T cells with an anti-CD3 and anti-CD28 T-cell activation reagent prior to adding the Fc-cytokine complex.

[0053] In various examples, the cytokines can be interleukins.

[0054] In some examples, the interleukins can be at least one of interleukin-2s (IL-2s), IL-7s, and IL-15s.

[0055] In various examples, each of the one or more cytokines can be linked to the engineered Fc antibody domain via an engineered metalloprotease-resistant linker sequence. In some examples, the engineered metalloprotease-resistant linker sequence can be between 7 to 13 amino acid residues in length.

[0056] In various examples, the engineered Fc antibody domain can be C-terminally linked to an N-terminus of at least one of the cytokines.

[0057] In some examples, one of the cytokines can be interleukin-2 (IL-2) and the engineered Fc antibody domain can be linked to the IL-2 via an engineered metalloprotease-resistant linker sequence comprising the amino acid sequence SLSPGKAPTS (SEQ ID NO:20).

[0058] In some examples, one of the cytokines can be interleukin-7 (IL-7) and the engineered Fc antibody domain can be linked to the IL-7 via an engineered

metalloprotease-resistant linker sequence comprising the amino acid sequence SLSPGKDCDIEGK (SEQ ID NO:21).

[0059] In some examples, one of the cytokines can be interleukin-15 (IL-15) and the engineered Fc antibody domain can be linked to the IL-15 via an engineered metalloprotease-resistant linker sequence comprising the amino acid sequence SLSPGKN (SEQ ID NO:22).

[0060] In various examples, the engineered Fc antibody domain can be N-terminally linked to a C-terminus of at least one of the cytokines.

[0061] In some examples, one of the cytokines can be interleukin-2 (IL-2) and the engineered Fc antibody domain can be linked to the IL-2 via an engineered metalloprotease-resistant linker sequence comprising the amino acid sequence TPKSCDKTHT (SEQ ID NO:23).

[0062] In some examples, one of the cytokines can be interleukin-7 (IL-7) and the engineered Fc antibody domain can be linked to the IL-7 via an engineered metalloprotease-resistant linker sequence comprising the amino acid sequence HPKSCDKTHT (SEQ ID NO:24).

[0063] In some examples, one of the cytokines can be interleukin-15 (IL-15) and the engineered Fc antibody domain can be linked to the IL-15 via an engineered metalloprotease-resistant linker sequence comprising the amino acid sequence TSPKSCDKTHT (SEQ ID NO:25).

[0064] In various examples, the engineered Fc antibody domain can be an engineered human Fc antibody domain. In some examples, the engineered human Fc antibody domain can be an engineered human IgG1 Fc antibody domain.

[0065] In some examples, the engineered human IgG1 Fc antibody domain can comprise an amino acid sequence with at least about 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% amino acid identity to the amino acid sequence set forth in SEQ ID NO:1.

[0066] In some examples, the engineered human IgG1 Fc antibody domain can comprise at least one of the following point mutations relative to SEQ ID NO:1 to decrease affinity for certain Fcγ receptors and functionally reduce antibody-dependent cellular cytotoxicity (ADCC): P75L, R76W, Y80K, Y80P, Y80R, Y80G, and Y80A.

[0067] In some examples, the engineered human IgG1 Fc antibody domain can comprise the following point mutation relative to SEQ ID NO:1 to increase affinity for certain Fcγ receptors and functionally increase antibody-dependent cellular cytotoxicity (ADCC): Y80W.

[0068] In some examples, the engineered human IgG1 Fc antibody domain can comprise at least one of the following point mutations relative to SEQ ID NO:1 to decrease affinity for certain Fcγ receptors and have a neutral effect on other Fcγ receptors: S23A, E53A, E77A, Y80F, V87A, A111G, K122A, and D160A.

[0069] In some examples, the engineered human IgG1 Fc antibody domain can comprise at least one of the following point mutations relative to SEQ ID NO:1 to increase affinity for certain Fcγ receptors and have a neutral effect on other Fcγ receptors: E117, K118A, and A123T.

[0070] In some examples, the engineered human IgG1 Fc antibody domain can comprise at least one of the following point mutations relative to SEQ ID NO:1 to increase affinity for certain Fcγ receptors and decrease affinity for certain other Fcγ receptors: H52A, R85A, and K106A.

[0071] In some examples, the engineered human IgG1 Fc antibody domain can comprise at least one of the following point mutations relative to SEQ ID NO:1 to decrease affinity for certain Fcγ receptors: D54A, Q79A, and A111S.

[0072] In some examples, the engineered human IgG1 Fc antibody domain can comprise at least one of the following point mutations relative to SEQ ID NO:1 to increase affinity for certain Fcγ receptors: T40A and K74A.

[0073] In some examples, the Fc antibody domain can be an engineered rabbit Fc antibody domain.

[0074] In various examples, the Fc-cytokine complex can further comprise a signal peptide linked to an N-terminus of the engineered Fc antibody domain or at least one of the cytokines. In some examples, the signal peptide can be a murine Ig heavy signal peptide used for expression in Chinese hamster ovary (CHO) cells. In some examples, the signal peptide can comprise the amino acid sequence MGWSCIILFLVATATGVHS (SEQ ID NO:26).

BRIEF DESCRIPTION OF THE DRAWINGS

[0075] Figs. 1A and 1B illustrate predicted structures that represent one embodiment of an engineered Fc antibody domain linked to a plurality of cytokines.

[0076] Fig. 1C illustrates a predicted structure of one embodiment of an engineered self-assembling protein cage polypeptide.

[0077] Fig. 1D illustrates a predicted structure of one embodiment of a cytokine (e.g., IL-2) and its receptor signaling complex.