

Summary

In a first aspect, the present disclosure refers to an Fc region, optionally isolated, comprising modification to increase the affinity of association between the Fc region and TRIM21.

5 In some examples, the modification comprises one or more substitutions to the amino acid sequence of a polypeptide of the Fc region. In some examples, the polypeptide comprises substitution at one or more positions corresponding to the following positions of IGHG1: 252, 253, 254, 256, 309, 310, 311, 314, 315, 345, 428, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439 or 440. In some examples, the polypeptide comprises substitution at one or more positions corresponding to the following positions of IGHG1: 253,
10 256, 433, 434, 435, 436 or 440. In some examples, the Fc region according to any one of claims 1 to 4, wherein the Fc region comprises a polypeptide comprising: I or L at the position corresponding to position 253; P, A, T, V, G, I, K, N or S at the position corresponding to position 256; S, V, H, A, D, P, Q, T or I at the position corresponding to position 433; H, R, N, I, L or M at the position corresponding to position 434; H or L at the position corresponding to position 435; Y, F, T, L, W or S at the position corresponding to
15 position 436; and S, G, I, R, Y, N, V, T, P, D, W, M, A, K, F, E or C at the position corresponding to position 440.

Unless stated otherwise, positions in polypeptides of Fc regions herein are numbered according to the EU numbering system as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed.
20 Public Health Service, National Institutes of Health, Bethesda, MD, 1991 (hereby incorporated by reference in its entirety).

In some examples, the Fc region comprises a polypeptide comprising an amino acid sequence having at least 60% sequence identity to one of SEQ ID NOs:167 to 175. In some examples, the Fc region
25 comprises a polypeptide comprising an amino acid sequence having at least 60% sequence identity to one of SEQ ID NOs:32 to 166. In another aspect, the present disclosure also refers to a polypeptide, optionally isolated, comprising: an amino acid sequence having at least 60% sequence identity to SEQ ID NO:10, wherein the polypeptide comprises the following amino acid residues at the specified positions numbered relative to SEQ ID NO:10: I or L at the position corresponding to position 26; P, A, T, V, G, I, K,
30 N or S at the position corresponding to position 29; S, V, H, A, D, P, Q, T or I at the position corresponding to position 206; H, R, N, I, L or M at the position corresponding to position 207; H or L at the position corresponding to position 208; Y, F, T, L, W or S at the position corresponding to position 209; and S, G, I, R, Y, N, V, T, P, D, W, M, A, K, F, E or C at the position corresponding to position 213; and wherein the amino acid sequence of the polypeptide is not identical to the amino acid sequence of a
35 constituent polypeptide of the Fc region of a wildtype immunoglobulin.

In some examples the polypeptide comprises an amino acid sequence having at least 60% sequence identity to one of SEQ ID NOs:167 to 175. In some examples the polypeptide comprises an amino acid sequence having at least 60% sequence identity to one of SEQ ID NOs:32 to 166.

40

In another aspect, the present disclosure also refers to a polypeptide, optionally isolated, comprising the amino acid sequence of one of SEQ ID NOs:32 to 166.

5 In another aspect, the present disclosure also refers to an Fc region, optionally isolated, comprising a polypeptide according to the present disclosure.

10 In another aspect, the present disclosure also refers to a polypeptide complex, optionally isolated, comprising an Fc region according to the present disclosure, or a polypeptide according to the present disclosure.

15 In another aspect, the present disclosure also refers to an antigen-binding molecule comprising an antigen-binding domain capable of specific binding to a target antigen and an Fc region according to the present disclosure, a polypeptide according to the present disclosure, or a polypeptide complex according to the present disclosure.

20 In some examples, the target antigen is an antigen of a pathogen, a cancer-associated antigen or an autoimmune disease-associated antigen. In some examples, the antigen-binding molecule comprises an antigen-binding domain capable of specific binding to an endocytosis receptor. In some examples, the antigen-binding molecule is a multispecific antigen-binding molecule. In some examples, the antigen-binding molecule further comprises an antigenic sequence of a target antigen.

25 In another aspect, the present disclosure also refers to an immunogen comprising an antigenic sequence of a target antigen and an Fc region according to the disclosure, a polypeptide according to the disclosure, or a polypeptide complex according to the disclosure.

30 In some examples, the target antigen is an antigen of a pathogen, a cancer-associated antigen or an autoimmune disease-associated antigen. In some examples, the immunogen additionally comprises an antigen-presenting cell (APC)-targeting region. In some examples, the APC-targeting region comprises or consists of a moiety capable of specific binding to an endocytosis receptor. In some examples the immunogen further comprises an antigen-binding domain capable of specific binding to a target antigen.

35 In another aspect, the present disclosure also refers to a nucleic acid, or a plurality of nucleic acids, optionally isolated, encoding an Fc region, a polypeptide, a polypeptide complex, an antigen-binding molecule or an immunogen according to the present disclosure.

In another aspect, the present disclosure also refers to an expression vector, or a plurality of expression vectors, comprising a nucleic acid or a plurality of nucleic acids according to the present disclosure.

40 In another aspect, the present disclosure also refers to a cell comprising an Fc region, a polypeptide, a polypeptide complex, an antigen-binding molecule, an immunogen, a nucleic acid or a plurality of nucleic acids or an expression vector or a plurality of expression vectors according to the present disclosure.

In another aspect, the present disclosure also refers to a method comprising culturing a cell comprising a nucleic acid or a plurality of nucleic acids or an expression vector or a plurality of expression vectors according to the disclosure under conditions suitable for expression of the Fc region, polypeptide,
5 antigen-binding molecule or immunogen from the nucleic acid(s) or expression vector(s).

In another aspect, the present disclosure also refers to a composition comprising an Fc region, a polypeptide, a polypeptide complex, an antigen-binding molecule, an immunogen, a nucleic acid or a plurality of nucleic acids, an expression vector or a plurality of expression vectors, or a cell according to
10 the present disclosure.

In another aspect, the present disclosure also refers to a polypeptide complex, optionally isolated, comprising an antigen-binding molecule or the immunogen according to the disclosure bound to the target antigen or a fragment thereof.
15

In another aspect, the present disclosure also refers to a pharmaceutical composition comprising an antigen-binding molecule, an immunogen or a polypeptide complex according to the present disclosure, and a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

20 In some examples, the present disclosure also refers to an antigen-binding molecule, an immunogen, a polypeptide complex, or a pharmaceutical composition according to the present disclosure, for use in a method of medical treatment or prophylaxis.

In some examples, the present disclosure also refers to an antigen-binding molecule, an immunogen, a polypeptide complex, or a pharmaceutical composition according to the present disclosure for use in a method of medical treatment or prevention of an infectious disease, a cancer or an autoimmune disease.
25

In some examples, the present disclosure also refers to the use of an antigen-binding molecule, an immunogen, a polypeptide complex, or a pharmaceutical composition according to the present disclosure in the manufacture of a medicament for use in a method of treatment or prevention of an infectious disease, a cancer or an autoimmune disease.
30

In some examples, the present disclosure also refers to a method of treating or preventing an infectious disease, a cancer or an autoimmune disease, comprising administering to a subject a therapeutically or prophylactically effective amount of an antigen-binding molecule, an immunogen, a polypeptide complex, or a pharmaceutical composition according to the present disclosure.
35

In some examples, the present disclosure also refers to an immunogen or a polypeptide complex according to the present disclosure, for use in a method for inducing or enhancing an immune response to a target antigen in a subject.
40

In some examples, the present disclosure also refers to the use of an immunogen or a polypeptide complex according to the present disclosure in the manufacture of a medicament for use in a method for inducing or enhancing an immune response to a target antigen in a subject.

5 In some examples, the present disclosure also refers to a method for inducing or enhancing an immune response to a target antigen in a subject, comprising administering an immunogen or a polypeptide complex according to the present disclosure to the subject.

The present disclosure also refers to a method comprising contacting a population of antigen-presenting
10 cells with an immunogen or a polypeptide complex according to the present disclosure.

The present disclosure also refers to a method for generating or expanding a population of immune cells specific for a target antigen, comprising contacting a population of immune cells with an antigen-presenting cell obtained by the method according to the disclosure.

15 In another aspect, the present disclosure also refers to an antigen-binding molecule according to the disclosure, an immunogen according to the disclosure, a polypeptide complex according to the disclosure, or a pharmaceutical composition according to the disclosure, for use in medical treatment or prophylaxis.

20 In another aspect, the present disclosure also refers to use of an antigen-binding molecule according to any one the disclosure, an immunogen according to the disclosure, a polypeptide complex according to the disclosure, or a pharmaceutical composition according to the disclosure in the manufacture of a medicament for use in treating or preventing an infectious disease, a cancer or an autoimmune disease.

25 In another aspect, the present disclosure also refers to an immunogen according to the disclosure, or a polypeptide complex according to the disclosure, for use in inducing or enhancing an immune response to a target antigen in a subject.

30 In another aspect, the present disclosure also refers to an *in vitro* method comprising contacting a population of antigen-presenting cells with an immunogen according to the disclosure, or a polypeptide complex according to the disclosure.

In another aspect, the present disclosure also refers to an *in vitro* method for generating or expanding a
35 population of immune cells specific for a target antigen, comprising contacting a population of immune cells with an antigen-presenting cell obtained by the method according to the disclosure.

Description

The present invention provides an Fc regions engineered for improved affinity to TRIM21, constituent
40 polypeptides thereof, and larger molecules and complexes comprising the engineered Fc regions.

Improved affinity for TRIM21 is demonstrated to provide advantageous properties relevant to therapeutic and prophylactic applications of molecules and complexes comprising the modified Fc regions. In particular, antigen-presenting cells stimulated with molecules/complexes comprising Fc regions modified for increased affinity to TRIM21 are shown to be capable of stimulating an improved cell-mediated immune response to target antigen.

TRIM21

Human tripartite motif containing-21 (TRIM21; also known as RNF81, RO52, SSA) is the protein identified by UniProt P19474. Alternative splicing of mRNA encoded by the human *TRIM21* gene yields two isoforms: isoform 1 (UniProt: P19474-1, v1; SEQ ID NO:1) and isoform 2 (UniProt: P19474-2; SEQ ID NO:2), which lacks the amino acid sequence corresponding to positions 169 to 245 of SEQ ID NO:1.

The structure and function of TRIM21 is reviewed e.g. in Foss et al., Immunol Rev. (2015) 268(1):328-39, which is hereby incorporated by reference in its entirety. TRIM21 is a cytosolic Fc receptor that is structurally unrelated to all other classes of Fc receptors (James et al., Proc Natl Acad Sci U S A. (2007) 104(15):6200-5). It is part of the TRIM family of proteins, which have cellular roles antiviral defence. Like other TRIM proteins, TRIM21 comprises an N-terminal RING domain (shown in SEQ ID NO:3) which has E3 ubiquitin ligase activity, a B-box (shown in SEQ ID NO:4), and a central coiled-coil domain (shown in SEQ ID NO:5). The C-terminal domain determines ligand specificity and function, and is referred to as the PRYSPRY domain (shown in SEQ ID NO:6). The PRYSPRY domain of TRIM21 contains the antibody binding site, and is a globular fold comprising a β -sandwich of two antiparallel β -sheets connected by flexible loops (James et al., Proc Natl Acad Sci U S A. (2007) 104(15):6200-5). TRIM21 exists as a dimer

Claims:

1. An Fc region, optionally isolated, comprising modification to increase the affinity of association between the Fc region and TRIM21.
- 5 2. The Fc region according to claim 1, wherein the modification comprises one or more substitutions to the amino acid sequence of a polypeptide of the Fc region.
3. The Fc region according to claim 2, wherein the polypeptide comprises substitution at one or more positions corresponding to the following positions of IGHG1 (EU numbering): 252, 253, 254, 256, 309,
10 310, 311, 314, 315, 345, 428, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439 or 440.
4. The Fc region according to any one of claims 1 to claim 3, wherein the Fc region comprises a polypeptide comprising substitution at one or more positions corresponding to the following positions of IGHG1 (EU numbering): 253, 256, 433, 434, 435, 436 or 440.
15
5. The Fc region according to any one of claims 1 to 4, wherein the Fc region comprises a polypeptide comprising: I or L at the position corresponding to position 253; P, A, T, V, G, I, K, N or S at the position corresponding to position 256; S, V, H, A, D, P, Q, T or I at the position corresponding to position 433; H, R, N, I, L or M at the position corresponding to position 434; H or L at the position corresponding to
20 position 435; Y, F, T, L, W or S at the position corresponding to position 436; and S, G, I, R, Y, N, V, T, P, D, W, M, A, K, F, E or C at the position corresponding to position 440.
6. The Fc region according to any one of claims 1 to 5, wherein the Fc region comprises a polypeptide comprising an amino acid sequence having at least 60% sequence identity to one of SEQ ID NOs:167 to
25 175.
7. The Fc region according to any one of claims 1 to 6, wherein the Fc region comprises a polypeptide comprising an amino acid sequence having at least 60% sequence identity to one of SEQ ID NOs:32 to
30 166.
8. A polypeptide, optionally isolated, comprising: an amino acid sequence having at least 60% sequence identity to SEQ ID NO:10, wherein the polypeptide comprises the following amino acid residues at the specified positions numbered relative to SEQ ID NO:10: I or L at the position corresponding to position 26; P, A, T, V, G, I, K, N or S at the position corresponding to position 29; S, V, H, A, D, P, Q, T or I at the
35 position corresponding to position 206; H, R, N, I, L or M at the position corresponding to position 207; H or L at the position corresponding to position 208; Y, F, T, L, W or S at the position corresponding to position 209; and S, G, I, R, Y, N, V, T, P, D, W, M, A, K, F, E or C at the position corresponding to position 213; and wherein the amino acid sequence of the polypeptide is not identical to the amino acid sequence of a constituent polypeptide of the Fc region of a wildtype immunoglobulin.
40

9. The polypeptide according to claim 8, wherein the polypeptide comprises an amino acid sequence having at least 60% sequence identity to one of SEQ ID NOs:167 to 175.
10. The polypeptide according to claim 8 or claim 9, wherein the polypeptide comprises an amino acid sequence having at least 60% sequence identity to one of SEQ ID NOs:32 to 166.
11. A polypeptide, optionally isolated, comprising the amino acid sequence of one of SEQ ID NOs:32 to 166.
12. An Fc region, optionally isolated, comprising a polypeptide according to any one of claims 8 to 11.
13. A polypeptide complex, optionally isolated, comprising an Fc region according to any one of claims 1 to 7 or claim 12, or a polypeptide according to any one of claims 8 to 11.
14. An antigen-binding molecule comprising an antigen-binding domain capable of specific binding to a target antigen and an Fc region according to any one of claims 1 to 7 or claim 12, a polypeptide according to any one of claims 8 to 11, or a polypeptide complex according to claim 13.
15. The antigen-binding molecule according to claim 14, wherein the target antigen is an antigen of a pathogen, a cancer-associated antigen or an autoimmune disease-associated antigen.
16. The antigen-binding molecule according to claim 14 or claim 15, wherein the antigen-binding molecule comprises an antigen-binding domain capable of specific binding to an endocytosis receptor.
17. The antigen-binding molecule according to any one of claims 14 to 16, wherein the antigen-binding molecule is a multispecific antigen-binding molecule.
18. The antigen-binding molecule according to any one of claims 14 to 17, wherein the antigen-binding molecule further comprises an antigenic sequence of a target antigen.
19. An immunogen comprising an antigenic sequence of a target antigen and an Fc region according to any one of claims 1 to 7 or claim 12, a polypeptide according to any one of claims 8 to 11, or a polypeptide complex according to claim 13.
20. The immunogen according to claim 19, wherein the target antigen is an antigen of a pathogen, a cancer-associated antigen or an autoimmune disease-associated antigen.
21. The immunogen according to claim 19 or claim 20, wherein the immunogen additionally comprises an antigen-presenting cell (APC)-targeting region.

22. The immunogen according to claim 21, wherein the APC-targeting region comprises or consists of a moiety capable of specific binding to an endocytosis receptor.
23. The immunogen according to any one of claims 19 to 22, wherein the immunogen further comprises
5 an antigen-binding domain capable of specific binding to a target antigen.
24. A nucleic acid, or a plurality of nucleic acids, optionally isolated, encoding an Fc region according to any one of claims 1 to 7 or claim 12, a polypeptide according to any one of claims 8 to 11, a polypeptide complex according to claim 13, an antigen-binding molecule according to any one of claims 14 to 18, or
10 an immunogen according to any one of claims 19 to 23.
25. An expression vector, or a plurality of expression vectors, comprising a nucleic acid or a plurality of nucleic acids according to claim 24.
- 15 26. A cell comprising an Fc region according to any one of claims 1 to 7 or claim 12, a polypeptide according to any one of claims 8 to 11, a polypeptide complex according to claim 13, an antigen-binding molecule according to any one of claims 14 to 18, an immunogen according to any one of claims 19 to 23, a nucleic acid or a plurality of nucleic acids according to claim 24, or an expression vector or a plurality of expression vectors according to claim 25.
20
27. A method comprising culturing a cell comprising a nucleic acid or a plurality of nucleic acids according to claim 24, or an expression vector or a plurality of expression vectors according to claim 25, under conditions suitable for expression of the Fc region, polypeptide, antigen-binding molecule or immunogen from the nucleic acid(s) or expression vector(s).
25
28. A composition comprising an Fc region according to any one of claims 1 to 7 or claim 12, a polypeptide according to any one of claims 8 to 11, a polypeptide complex according to claim 13, an antigen-binding molecule according to any one of claims 14 to 18, an immunogen according to any one of claims 19 to 23, a nucleic acid or a plurality of nucleic acids according to claim 24, an expression vector
30 or a plurality of expression vectors according to claim 25, or a cell according to claim 26.
29. A polypeptide complex, optionally isolated, comprising an antigen-binding molecule according to any one of claims 14 to 18, or the immunogen according to claim 23, bound to the target antigen or a fragment thereof.
35
30. A pharmaceutical composition comprising an antigen-binding molecule according to any one of claims 14 to 18, an immunogen according to any one of claims 19 to 23, or a polypeptide complex according to claim 29, and a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

31. An antigen-binding molecule according to any one of claims 14 to 18, an immunogen according to any one of claims 19 to 23, a polypeptide complex according to claim 29, or a pharmaceutical composition according to claim 30, for use in medical treatment or prophylaxis.
- 5 32. Use of an antigen-binding molecule according to any one of claims 14 to 18, an immunogen according to any one of claims 19 to 23, a polypeptide complex according to claim 29, or a pharmaceutical composition according to claim 30 in the manufacture of a medicament for use in treating or preventing an infectious disease, a cancer or an autoimmune disease.
- 10 33. An immunogen according to any one of claims 19 to 23, or a polypeptide complex according to claim 29, for use in inducing or enhancing an immune response to a target antigen in a subject.
34. An *in vitro* method comprising contacting a population of antigen-presenting cells with an immunogen according to any one of claims 19 to 23, or a polypeptide complex according to claim 29.
- 15 35. An *in vitro* method for generating or expanding a population of immune cells specific for a target antigen, comprising contacting a population of immune cells with an antigen-presenting cell obtained by the method according to claim 38.

20