

265:10506-10513 (1989). There remains a need in the art to identify and characterize modified human IgM antibodies with modified CDC activity.

SUMMARY

[0005] In one aspect, this disclosure provides a modified human IgM constant region that includes one or more amino acid substitutions relative to a wild-type human IgM constant region, where at least one amino acid substitution is at a position in the C μ 3 domain ranging from T302 of SEQ ID NO: 1 to K322 of SEQ ID NO: 1, and where a modified IgM antibody that includes the modified IgM constant region and a heavy chain variable region specific for a target antigen exhibits reduced complement-dependent cytotoxicity (CDC) of cells expressing the target antigen relative to a corresponding wild-type human IgM antibody.

[0005a] In another aspect, the present disclosure provides modified human IgM antibody comprising the modified human IgM constant region as described herein, and a heavy chain variable region (VH) situated amino terminal to the modified human IgM constant region, wherein the modified human IgM antibody specifically binds to a target antigen and exhibits reduced complement dependent cytotoxicity (CDC) of cells expressing the target antigen relative to a corresponding wild-type human IgM antibody.

[0006] In certain examples, the modified human IgM constant region includes at least one amino acid substitution is at amino acid T302, C303, T304, V305, T306, H307, T308, D309, L310, P311, S312, P313, L314, K315, Q316, T317, I318, S319, R320, P321, and/or K322 of SEQ ID NO: 1. For example, the modified human IgM constant region can include at least one amino acid substitution is at position L310 of SEQ ID NO: 1, position P311 of SEQ ID NO: 1, position P313 of SEQ ID NO: 1, position K315 of SEQ ID NO: 1, or any combination thereof.

[0007] In certain examples at least one amino acid substitution can be at position L310 of SEQ ID NO: 1, e.g., L310 of SEQ ID NO: 1 can be substituted with alanine (L310A), serine (L310S), aspartic acid (L310D) or glycine (L310G). In certain examples L310 of SEQ ID NO: 1 can be substituted with alanine (L310A), for example, the modified IgM constant region can include SEQ ID NO: 15. In certain examples L310 of SEQ ID NO: 1 can be substituted with aspartic acid (L310D), for example, the modified IgM constant region can include SEQ ID NO: 23.

[0008] In certain examples at least one amino acid substitution can be at position P311 of SEQ ID NO: 1, e.g., P311 of SEQ ID NO: 1 can be substituted with alanine (P311A), serine (P311S), or glycine (P311G). In certain examples P311 of SEQ ID NO: 1 can be substituted with alanine (P311A), for example, the modified IgM constant region can include SEQ ID NO: 2.

[0009] In certain examples at least one amino acid substitution can be at position P313 of SEQ ID NO: 1, e.g., P313 of SEQ ID NO: 1 can be substituted with alanine (P313A), serine (P313S), or glycine (P313G). In certain examples P313 of SEQ ID NO: 1 can be substituted with serine (P313S), for example the modified IgM constant region can include SEQ ID NO: 3.

[0010] In certain examples at least one amino acid substitution can be at position K315 of SEQ ID NO: 1, e.g., K315 of SEQ ID NO: 1 can be substituted with alanine (K315A), serine (K315S), aspartic acid (K315D), glutamine (K315Q), or glycine (P313G). In certain examples K315 of SEQ ID NO: 1 can be substituted with alanine (K315A), for example the modified IgM constant region can include SEQ ID NO: 16. In certain examples K315 of SEQ ID NO: 1 can be substituted with aspartic acid (K315D), for example the modified IgM constant region can include SEQ ID NO: 24. In certain examples K315 of SEQ ID NO: 1 can be substituted with glutamine (K315Q), for example the modified IgM constant region can include SEQ ID NO: 25.

[0011] In certain examples the modified human IgM constant region as provided herein can include two or more amino acid substitutions. For example, the modified human IgM constant region as provided herein can include amino acid substitutions at two or more of positions L310, P311, P313, or K315 of SEQ ID NO: 1.

[0012] In certain examples, the modified human IgM constant region as provided herein can include amino acid substitutions at positions P311 and P313 of SEQ ID NO: 1. For example, P311 of SEQ ID NO: 1 can be substituted with alanine (P311A), serine (P311S), or glycine (P311G), and P313 of SEQ ID NO: 1 can be substituted with alanine (P313A), serine (P313S), or glycine (P313G). In certain examples, P311 of SEQ ID NO: 1 can be substituted with alanine (P311A) and P313 of SEQ ID NO: 1 can be substituted with serine (P313S), for example the modified IgM constant region can include SEQ ID NO: 4.

[0013] In certain examples, the modified human IgM constant region as provided herein can include amino acid substitutions at positions L310 and K315 of SEQ ID NO: 1.

For example, L310 can be substituted with alanine (L310A) or serine (L310S) and K315 can be substituted with alanine (K315A) or serine (K315S), for example the modified IgM constant region can include SEQ ID NO: 17 or SEQ ID NO: 18.

[0014] In certain examples, the modified human IgM constant region as provided herein can include amino acid substitutions at positions L310 and P311 of SEQ ID NO: 1. For example, L310 can be substituted with alanine (L310A) and P311 can be substituted with alanine (P311A), for example the modified IgM constant region can include SEQ ID NO: 19.

[0015] In certain examples, the modified human IgM constant region as provided herein can include amino acid substitutions at positions L310 and P313 of SEQ ID NO: 1. For example, L310 can be substituted with alanine (L310A) and P313 can be substituted with serine (P313S), for example the modified IgM constant region can include SEQ ID NO: 20.

[0016] In certain examples, the modified human IgM constant region as provided herein can include amino acid substitutions at positions P311 and K315 of SEQ ID NO: 1. For example, P311 can be substituted with alanine (P311A) and K315 can be substituted with alanine (K315A), for example the modified IgM constant region can include SEQ ID NO: 21.

[0017] In certain examples, the modified human IgM constant region as provided herein can include amino acid substitutions at positions P313 and K315 of SEQ ID NO: 1. For example, P313 can be substituted with serine (P313S) and K315 can be substituted with alanine (K315A), for example the modified IgM constant region can include SEQ ID NO: 22.

[0018] In certain examples, the maximum CDC activity achieved in a dose response assay by a target-specific IgM antibody that includes the modified human IgM constant region as provided herein is reduced by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% relative to a corresponding wild-type IgM antibody identical except for the modified human IgM constant region.

[0019] In certain examples, the antibody concentration effecting 50% CDC activity (EC_{50}) of a target-specific IgM antibody that includes the modified human IgM constant region as provided herein is increased by at least 2-fold, at least 5-fold, at least 10-fold, at least 20-fold, and least 30-fold, at least 40-fold, at least 50-fold, at least 60-fold, at least

70-fold, at least 80-fold, at least 90-fold, or at least 100-fold relative to a corresponding wild-type IgM antibody identical except for the modified human IgM constant region.

[0020] The disclosure further provides a modified human IgM antibody that includes the modified human IgM constant region as provided herein and further includes a heavy chain variable region (VH) situated amino terminal to the modified human IgM constant region, where the modified human IgM antibody specifically binds to a target antigen and exhibits reduced complement-dependent cytotoxicity (CDC) of cells expressing the target antigen relative to a corresponding wild-type human IgM antibody. In certain examples, the modified human IgM antibody can be a pentameric or a hexameric antibody that includes five or six bivalent IgM binding units, respectively, where each binding unit includes two IgM heavy chains each including a VH situated amino terminal to the modified human IgM constant region, and two immunoglobulin light chains each including a light chain variable domain (VL) situated amino terminal to a human immunoglobulin light chain constant region. In certain examples, the modified human IgM antibody as provided herein can be pentameric, further including a J-chain, or functional fragment thereof, or a functional variant thereof. In certain examples the J-chain can include amino acids 23 to 158 of SEQ ID NO: 7 or functional fragment thereof, or a functional variant thereof. In certain examples the J-chain or fragment or variant thereof can be a modified J-chain that further includes a heterologous polypeptide, where the heterologous polypeptide is directly or indirectly fused to the J-chain or fragment or variant thereof. In certain examples the heterologous polypeptide can be fused to the J-chain or fragment thereof via a peptide linker, e.g., a peptide linker includes at least 5 amino acids, but no more than 25 amino acids. In certain examples the peptide linker can comprise, consist essentially of, or consist of GGGGSGGGGSGGGGS (SEQ ID NO: 12). In certain examples, the heterologous polypeptide can be fused to the N-terminus of the J-chain or fragment or variant thereof, the C-terminus of the J-chain or fragment or variant thereof, or to both the N-terminus and C-terminus of the J-chain or fragment or variant thereof. In certain examples, the heterologous polypeptide can include a binding domain, e.g., an antibody or antigen-binding fragment thereof, e.g., an Fab fragment, an Fab' fragment, an F(ab')₂ fragment, an Fd fragment, an Fv fragment, a single-chain Fv (scFv) fragment, a disulfide-linked Fv (sdFv) fragment, or any combination thereof. In certain examples the modified J-chain can include a scFv fragment. In certain examples, the heterologous polypeptide can specifically bind to CD3ε. In certain examples, the modified

J-chain can include the amino acid sequence SEQ ID NO: 9 (V15J) or SEQ ID NO: 11 (J15V). In certain examples the modified J-chain can further include a signal peptide, e.g., the modified J-chain can include the amino acid sequence SEQ ID NO: 8 (V15J) or SEQ ID NO: 10 (J15V). In certain examples the modified human IgM antibody as provided herein can direct T-cell-mediated killing of a cell expressing the target antigen at an activity level equivalent to that of a corresponding IgM antibody that is identical to the modified IgM antibody except for the modified IgM constant region. In certain examples, the cell expressing the target antigen is a eukaryotic cell.

[0021] In another aspect, this disclosure further provides a polynucleotide that includes a nucleic acid sequence that encodes the modified human IgM constant region as provided herein, or a heavy chain polypeptide subunit of the modified human IgM antibody as provided herein. In yet another aspect, also provided is a composition that includes the provided polynucleotide. In certain examples the composition further includes a nucleic acid sequence that encodes a light chain polypeptide subunit. In certain examples, the light chain polypeptide subunit includes a human antibody light chain constant region or fragment thereof fused to the C-terminal end of a VL. In certain examples the nucleic acid sequence encoding the heavy chain polypeptide subunit and the nucleic acid sequence encoding the light chain polypeptide subunit can be on separate vectors. In certain examples, the nucleic acid sequence encoding the heavy chain polypeptide subunit and the nucleic acid sequence encoding the light chain polypeptide subunit can be on a single vector. In certain examples, the composition further includes a nucleic acid sequence that encodes a J-chain, or functional fragment thereof, or a functional variant thereof. In certain examples, the J-chain or fragment or variant thereof is a modified J-chain that further includes a heterologous polypeptide, where the heterologous polypeptide is directly or indirectly fused to the J-chain or fragment thereof. In certain examples, the nucleic acid sequence encoding the heavy chain polypeptide subunit, the nucleic acid sequence encoding the light chain polypeptide subunit, and the nucleic acid sequence encoding the J-chain can be on a single vector. In certain examples, the nucleic acid sequence encoding the heavy chain polypeptide subunit, the nucleic acid sequence encoding the light chain polypeptide subunit, and the nucleic acid sequence encoding the J-chain can be each on separate vectors. In a further aspect, all such vector or vectors are further provided by this disclosure. In yet another aspect, the disclosure further provides a host cell that includes

the provided polynucleotides, compositions, vector, or vectors wherein the host cell can express the modified human IgM constant region as provided herein, or the modified human IgM antibody as provided herein, or any functional fragment thereof. In yet another aspect, the disclosure further provides a method of producing the modified human IgM constant region as provided herein or the modified human IgM antibody as provided herein, where the method includes culturing the provided host cell, and recovering the constant region or antibody.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0022] **FIGURE 1** shows an SDS PAGE gel run under non-reducing conditions to show assembly of pentamers and hexamers. Gel patterns are depicted for the controls, IgM (pure), IgM + J (pure), and supernatant preparations (all without J chain) of IgM, and the IgM mutants, S283N, P313S, P311A, and D294G.

WHAT IS CLAIMED IS:

1. A modified human IgM constant region comprising one or more amino acid substitutions relative to a wild-type human IgM constant region, wherein at least one amino acid substitution is at a position in the C μ 3 domain ranging from T302 of SEQ ID NO: 1 to K322 of SEQ ID NO: 1, and wherein a modified IgM antibody comprising the modified IgM constant region and a heavy chain variable region specific for a target antigen exhibits reduced complement-dependent cytotoxicity (CDC) of cells expressing the target antigen relative to a corresponding wild-type human IgM antibody.

2. The modified human IgM constant region of claim 1, wherein the at least one amino acid substitution is at amino acid T302, C303, T304, V305, T306, H307, T308, D309, L310, P311, S312, P313, L314, K315, Q316, T317, I318, S319, R320, P321, and/or K322 of SEQ ID NO: 1.

3. The modified human IgM constant region of claim 2, wherein:

(a) the amino acid substitution is at position L310 of SEQ ID NO: 1, and wherein L310 of SEQ ID NO: 1 is substituted with alanine (L310A), serine (L310S), aspartic acid (L310D) or glycine (L310G);

(b) the amino acid substitution is at position P311 of SEQ ID NO: 1, and wherein P311 of SEQ ID NO: 1 is substituted with alanine (P311A), serine (P311S), or glycine (P311G);

(c) the amino acid substitution is at position P313 of SEQ ID NO: 1, and wherein P313 of SEQ ID NO: 1 is substituted with alanine (P313A), serine (P313S), or glycine (P313G); and/or

(d) the amino acid substitution is at position K315 of SEQ ID NO: 1, and wherein K315 of SEQ ID NO: 1 is substituted with alanine (K315A), serine (K315S), aspartic acid (K315D), glutamine (K315Q), or glycine (P313G).

4. The modified human IgM constant region of claim 2 or claim 3, comprising SEQ ID NO: 15, SEQ ID NO: 23, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 16, SEQ ID NO: 24, SEQ ID NO: 25.

5. The modified human IgM constant region of claim 2 or claim 3, comprising:

(a) amino acid substitutions at positions P311 and P313 of SEQ ID NO: 1, wherein P311 of SEQ ID NO: 1 is substituted with alanine (P311A), serine (P311S), or glycine (P311G), and wherein P313 of SEQ ID NO: 1 is substituted with alanine (P313A), serine (P313S), or glycine (P313G);

(b) amino acid substitutions at positions P311 and P313 of SEQ ID NO: 1, wherein P311 of SEQ ID NO: 1 is substituted with alanine (P311A) and P313 of SEQ ID NO: 1 is substituted with serine (P313S);

(c) amino acid substitutions at positions L310 and K315 of SEQ ID NO: 1, wherein L310 is substituted with alanine (L310A) or serine (L310S) and K315 is substituted with alanine (K315A) or serine (K315S);

(d) amino acid substitutions at positions L310 and P311 of SEQ ID NO: 1, wherein L310 is substituted with alanine (L310A) and P311 is substituted with alanine (P311A);

(e) amino acid substitutions at positions L310 and P313 of SEQ ID NO: 1, wherein L310 is substituted with alanine (L310A) and P313 is substituted with serine (P313S);

(f) amino acid substitutions at positions P311 and K315 of SEQ ID NO: 1, wherein P311 is substituted with alanine (P311A) and K315 is substituted with alanine (K315A); or

(g) comprising amino acid substitutions at positions P313 and K315 of SEQ ID NO: 1, wherein P313 is substituted with serine (P313S) and K315 is substituted with alanine (K315A).

6. The modified human IgM constant region of claim 5, comprising SEQ ID NO: 4, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, or SEQ ID NO: 22.

7. A modified human IgM antibody comprising the modified human IgM constant region of any one of claims 1 to 6, and a heavy chain variable region (VH) situated amino terminal to the modified human IgM constant region, wherein the modified human IgM antibody specifically binds to a target antigen and exhibits reduced complement-dependent cytotoxicity (CDC) of cells expressing the target antigen relative to a corresponding wild-type human IgM antibody.

8. The modified human IgM antibody of claim 7, which is a pentameric or a hexameric antibody comprising five or six bivalent IgM binding units, respectively, wherein each binding unit comprises two IgM heavy chains each comprising a VH situated amino

terminal to the modified human IgM constant region, and two immunoglobulin light chains each comprising a light chain variable domain (VL) situated amino terminal to a human immunoglobulin light chain constant region.

9. The modified human IgM antibody of claim 8, which is a pentameric antibody, and further comprises a J-chain, or functional fragment thereof, or a functional variant thereof.

10. The modified human IgM antibody of claim 9, wherein J-chain or fragment thereof comprises amino acids 23 to 158 of SEQ ID NO: 7 or a functional fragment thereof, or a functional variant thereof.

11. The modified human IgM antibody of claim 9 or 10, wherein the J-chain or fragment or variant thereof is a modified J-chain further comprising a heterologous polypeptide, wherein the heterologous polypeptide is directly or indirectly fused to the J-chain or fragment or variant thereof.

12. The modified human IgM antibody of claim 11, wherein the heterologous polypeptide is fused to the J-chain or fragment thereof via a peptide linker.

13. The modified human IgM antibody of claim 12, wherein the peptide linker comprises at least 5 amino acids, but no more than 25 amino acids or wherein the peptide linker consists of GGGGSGGGGSGGGGS (SEQ ID NO: 12).

14. The modified human IgM antibody of any one of claims 11 to 13, wherein the heterologous polypeptide is fused to the N-terminus of the J-chain or fragment or variant thereof, the C-terminus of the J-chain or fragment or variant thereof, or to both the N-terminus and C-terminus of the J-chain or fragment or variant thereof.

15. The modified human IgM antibody of any one of claims 11 to 14, wherein the heterologous polypeptide comprises a binding domain.

16. The modified human IgM antibody of claim 15, wherein the binding domain of the heterologous polypeptide is an antibody or antigen-binding fragment thereof.

17. The modified human IgM antibody of claim 16, wherein the antigen-binding fragment comprises an Fab fragment, an Fab' fragment, an F(ab')₂ fragment, an Fd fragment, an Fv fragment, a single-chain Fv (scFv) fragment, a disulfide-linked Fv (sdFv) fragment, or any combination thereof.

18. The modified human IgM antibody of any one of claims 15 to 17, wherein the heterologous polypeptide can specifically bind to CD3ε.

19. The modified human IgM antibody of any one of claims 7 to 18, wherein the maximum CDC activity achieved by a target-specific IgM antibody comprising the modified human IgM constant region in a dose-response assay is reduced by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% relative to a corresponding wild-type IgM antibody identical except for the modified human IgM constant region.

20. The modified human IgM antibody of any one of claims 7 to 18, wherein the antibody concentration of a target-specific IgM antibody comprising the modified human IgM constant region effecting 50% CDC activity (EC₅₀) is increased by at least 2-fold, at least 5-fold, at least 10-fold, at least 20-fold, and least 30-fold, at least 40-fold, at least 50-fold, at least 60-fold, at least 70-fold, at least 80-fold, at least 90-fold, or at least 100-fold relative to a corresponding wild-type IgM antibody identical except for the modified human IgM constant region.

21. A polynucleotide comprising a nucleic acid sequence that encodes the modified human IgM constant region of any one of claims 1 to 6, or a heavy chain polypeptide subunit of the modified human IgM antibody of any one of claims 7 to 20.

22. A composition comprising the polynucleotide of claim 21.

23. The composition of claim 22, further comprising a nucleic acid sequence that encodes a light chain polypeptide subunit comprising a human antibody light chain constant region or fragment thereof fused to the C-terminal end of a VL.

24. The composition of claim 23, wherein the nucleic acid sequence encoding the heavy chain polypeptide subunit and the nucleic acid sequence encoding the light chain polypeptide subunit are on separate vectors or are on a single vector.

25. The composition of any one of claims 22 to 24, further comprising a nucleic acid sequence that encodes a J-chain, or functional fragment thereof, or a functional variant thereof.

26. The composition of claim 25, wherein the nucleic acid sequence encoding the heavy chain polypeptide subunit, the nucleic acid sequence encoding the light chain polypeptide subunit, and the nucleic acid sequence encoding the J-chain are on a single vector or are each on separate vectors.

27. The vector or vectors of claim 24 to 26.

28. A host cell comprising the polynucleotide of claim 21, the composition of any one of claims 22 to 26, or the vector or vectors of claim 27, wherein the host cell can express the modified human IgM constant region of any one of claims 1 to 6, or the modified human IgM antibody of any one of claims 7 to 20 or a functional fragment thereof.

29. A method of producing the modified human IgM constant region of any one of claims 1 to 6, or the modified human IgM antibody of any one of claims 7 to 20, comprising culturing the host cell of claim 28, and recovering the constant region or antibody.