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Summary

In one aspect, there is provided an IgG2 constant region that comprises substitutions of the amino acids at positions 14 (position 131 in the EU numbering system) and 102 (position 219 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 with other amino acids. In another aspect, there is provided an antibody comprising the constant region of the present invention. In a further aspect, there is provided a pharmaceutical composition comprising the

constant region of the present invention.

Disclosure of the Invention

[Problems to be Solved by the Invention]

The present invention was achieved in view of the above circumstances. An objective of the present invention is to provide antibody constant regions that have improved physicochemical properties (stability and homogeneity), immunogenicity, safety, and pharmacokinetics (retention in plasma (blood)) by amino acid alteration.

Table 3

	KD (μM)		
	WT	H0L0	DNS
IgG1	1.42	1.07	1.36
M58	1.03	0.91	1.03

As shown in Table 3, it was demonstrated that as a result of conversion of the constant region from the IgG1 type to M58, as with anti-IL-6 receptor antibody WT, the bindings of both the anti-IL-31 receptor antibody H0L0 and anti-RANKL antibody DNS to human FcRn were improved. This suggests the possibility that regardless of the type of antigen or sequence of antibody variable region, the pharmacokinetics in human is improved by converting the constant region from the IgG1 type to M58.

[Example 10] Effect of cysteine in the CH1 domain on heterogeneity and stability

As described in Example 2, cysteines in the hinge region and CH1 domain of IgG2 were substituted to decrease the heterogeneity of natural IgG2. Assessment of various altered antibodies revealed that heterogeneity could be reduced without decreasing stability by using SKSC (SEQ ID NO: ~~15~~438). SKSC (SEQ ID NO: 38) is an altered constant region obtained by substituting serine for cysteine at position 131 and lysine for arginine at position 133, EU numbering, in the H-chain CH1 domain, and serine for cysteine at position 219, EU numbering, in the H-chain upper hinge of the wild type IgG2 constant region sequence.

Meanwhile, another possible method for decreasing heterogeneity is a single substitution of serine for cysteine at position 219, or serine for cysteine at position 220, EU numbering, in the H-chain upper hinge. The altered IgG2 constant region SC (SEQ ID NO: 39) was prepared by substituting serine for cysteine at position 219 and CS (SEQ ID NO: 40) was prepared by substituting serine for cysteine at position 220, EU numbering, in IgG2. WT-SC (SEQ ID NO: 41) and WT-CS (SEQ ID NO: 42) were prepared to have SC and CS, respectively, and compared with WT-IgG1, WT-IgG2, WT-SKSC, and WT-M58 in terms of heterogeneity and thermal stability. Furthermore, F2H/L39-IgG1, F2H/L39-IgG2, F2H/L39-SC, F2H/L39-CS, F2H/L39-SKSC, and F2H/L39-M14, which have the constant region of IgG1 (SEQ ID NO: 1), IgG2 (SEQ ID NO: 2), SC (SEQ ID NO: 39), CS (SEQ ID NO: 40), SKSC (SEQ ID NO: 38), or M14 (SEQ ID NO: 5), respectively, were prepared from F2H/L39 (the amino acid sequences of F2H/L39_VH and F2H/L39_VL as set forth in SEQ ID NOs: 29 and 30, respectively), which is an anti IL-6 receptor antibody different from WT. The antibodies were compared with regard to heterogeneity.

WT-IgG1, WT-IgG2, WT-SC, WT-CS, WT-SKSC, WT-M58, F2H/L39-IgG1,

CLAIMS

1. An IgG2 constant region that comprises substitutions of the amino acids at positions 14 (position 131 in the EU numbering system) and 102 (position 219 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 with other amino acids.

2. The IgG2 constant region according to claim 1, wherein the heterogeneity originated from the hinge region of IgG2 is reduced.

3. The IgG2 constant region according to claim 1, wherein the heterogeneity originated from the hinge region of IgG2 is reduced, compared to that of the wild type IgG2 constant region.

4. The IgG2 constant region according to any one of claims 1 to 3 that comprises a substitution of the amino acid at position 16 (position 133 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 with another amino acid.

5. The IgG2 constant region according to any one of claims 1 to 4 that comprises substitutions of the amino acids at positions 20 (position 137 in the EU numbering system) and 21 (position 138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 with other amino acids.

6. The IgG2 constant region according to any one of claims 1 to 4 that comprises substitutions of the amino acids at positions 209 (position 330 in the EU numbering system), 210 (position 331 in the EU numbering system), 218 (position 339 in the EU numbering system), 276 (position 397 in the EU numbering system), 20 (position 137 in the EU numbering system), and 21 (position 138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 with other amino acids.

7. The IgG2 constant region according to claim 6 that comprises deletions of both Gly at position 325 (position 446 in the EU numbering system) and Lys at position 326 (position 447 in the EU numbering system).

8. The IgG2 constant region according to any one of claims 1 to 4 that comprises substitutions of the amino acids at positions 276 (position 397 in the EU numbering

system), 20 (position 137 in the EU numbering system), and 21 (position 138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 with other amino acids.

9. The IgG2 constant region according to claim 8 that comprises deletions of both Gly at position 325 (position 446 in the EU numbering system) and Lys at position 326 (position 447 in the EU numbering system).

10. The IgG2 constant region according to any one of claims 1 to 4 that comprises substitutions of Glu at position 20 (position 137 in the EU numbering system), Ser at position 21 (position 138 in the EU numbering system), His at position 147 (position 268 in the EU numbering system), Arg at position 234 (position 355 in the EU numbering system), and Gln at position 298 (position 419 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 with other amino acids.

11. The IgG2 constant region according to claim 10 that comprises deletions of both Gly at position 325 (position 446 in the EU numbering system) and Lys at position 326 (position 447 in the EU numbering system).

12. The IgG2 constant region according to any one of claims 1 to 4 that comprises substitutions of Glu at position 20 (position 137 in the EU numbering system), Ser at position 21 (position 138 in the EU numbering system), His at position 147 (position 268 in the EU numbering system), Arg at position 234 (position 355 in the EU numbering system) and Gln at position 298 (position 419 in the EU numbering system) and Asn at position 313 (position 434 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 with other amino acids.

13. The IgG2 constant region according to claim 12 that comprises deletions of both Gly at position 325 (position 446 in the EU numbering system) and Lys at position 326 (position 447 in the EU numbering system).

14. The IgG2 constant region according to any one of claims 1 to 4 that comprises substitutions of Ala at position 209 (position 330 in the EU numbering system), Pro at position 210 (position 331 in the EU numbering system), Thr at position 218 (position 339 in the EU numbering system), Glu at position 20 (position 137 in the EU numbering system), and Ser at position 21 (position 138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 with other amino acids.

15. The IgG2 constant region according to claim 14 that comprises deletions of both Gly at position 325 (position 446 in the EU numbering system) and Lys at position 326 (position 447 in the EU numbering system).

16. An antibody comprising the constant region of any one of claims 1 to 15.

17. The antibody according to claim 16, wherein the antibody is an anti-IL-6 receptor antibody, anti-IL-31 receptor antibody, or anti-RANKL antibody.

18. A pharmaceutical composition comprising the constant region of any one of claims 1 to 15.

CLAIMS

1. A human antibody constant region of any one of:
(a) a human antibody constant region that comprises deletions of both Gly at position 329 (position 446 in the EU numbering system) and Lys at position 330 (position 447 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 1;
(b) a human antibody constant region that comprises deletions of both Gly at position 325 (position 446 in the EU numbering system) and Lys at position 326 (position 447 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2; and
(c) a human antibody constant region that comprises deletions of both Gly at position 326 (position 446 in the EU numbering system) and Lys at position 327 (position 447 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 3.

2. An IgG2 constant region in which the amino acids at positions 209 (position 330 in the EU numbering system), 210 (position 331 in the EU numbering system), and 218 (position 339 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 have been substituted with other amino acids.

3. An IgG2 constant region in which the amino acid at position 276 (position 397 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 has been substituted with another amino acid.

4. An IgG2 constant region in which the amino acids at positions 14 (position 131 in the EU numbering system), 102 (position 219 in the EU numbering system), and/or 16 (position 133 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 have been substituted with another amino acid.

5. The IgG2 constant region of claim 4, in which the amino acids at positions 20 (position 137 in the EU numbering system) and 21 (position 138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 have been substituted with other amino acids.

6. An IgG2 constant region in which His at position 147 (position 268 in the EU numbering system), Arg at position 234 (position 355 in the EU numbering system), and/or Gln at position 298 (position 419 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 have been substituted with other amino acids.

7. An IgG2 constant region in which the amino acids at positions 209 (position 330 in the EU numbering system), 210 (position 331 in the EU numbering system), 218 (position 339 in the EU numbering system), 276 (position 397 in the EU numbering system), 14 (position 131 in the EU numbering system), 16 (position 133 in the EU numbering system), 102 (position 219 in the EU numbering system), 20 (position 137 in the EU numbering system), and 21 (position 138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 have been substituted with other amino acids.

8. The IgG2 constant region of claim 7, which further comprises deletions of both Gly at position 325 (position 446 in the EU numbering system) and Lys at position 326 (position 447 in the EU numbering system).

9. An IgG2 constant region in which the amino acids at positions 276 (position 397 in the EU numbering system), 14 (position 131 in the EU numbering system), 16 (position 133 in the EU numbering system), 102 (position 219 in the EU numbering system), 20 (position 137 in the EU numbering system), and 21 (position 138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 have been substituted with other amino acids.

10. The IgG2 constant region of claim 9, which further comprises deletions of both Gly at position 325 (position 446 in the EU numbering system) and Lys at position 326 (position 447 in the EU numbering system).

11. An IgG2 constant region in which Cys at position 14 (position 131 in the EU numbering system), Arg at position 16 (position 133 in the EU numbering system), Cys at position 102 (position 219 in the EU numbering system), Glu at position 20 (position 137 in the EU numbering system), Ser at position 21 (position 138 in the EU numbering system), His at position 147 (position 268 in the EU numbering system), Arg at position 234 (position 355 in the EU numbering system), and Gln at position 298 (position 419 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 have been substituted with other amino acids.

12. The IgG2 constant region of claim 11, which further comprises deletions of both Gly at position 325 (position 446 in the EU numbering system) and Lys at position 326 (position 447 in the EU numbering system).

13. An IgG2 constant region in which Cys at position 14 (position 131 in the EU numbering

system), Arg at position 16 (position 133 in the EU numbering system), Cys at position 102 (position 219 in the EU numbering system), Glu at position 20 (position 137 in the EU numbering system), Ser at position 21 (position 138 in the EU numbering system), His at position 147 (position 268 in the EU numbering system), Arg at position 234 (position 355 in the EU numbering system), Gln at position 298 (position 419 in the EU numbering system), and Asn at position 313 (position 434 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 have been substituted with other amino acids.

14. The IgG2 constant region of claim 13, which further comprises deletions of both Gly at position 325 (position 446 in the EU numbering system) and Lys at position 326 (position 447 in the EU numbering system).

15. An IgG4 constant region in which the amino acid at position 289 (position 409 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 3 has been substituted with another amino acid.

16. An IgG4 constant region in which the amino acids at position 289 (position 409 in the EU numbering system), positions 14, 16, 20, 21, 97, 100, 102, 103, 104, and 105 (positions 131, 133, 137, 138, 214, 217, 219, 220, 221, and 222 in the EU numbering system, respectively), and positions 113, 114, and 115 (positions 233, 234, and 235 in the EU numbering system, respectively), have been substituted with other amino acids, and the amino acid at position 116 (position 236 in the EU numbering system) has been deleted from the amino acid sequence of SEQ ID NO: 3.

17. The IgG4 constant region of claim 16, which further comprises deletions of both Gly at position 326 (position 446 in the EU numbering system) and Lys at position 327 (position 447 in the EU numbering system).

18. An IgG2 constant region in which Ala at position 209 (position 330 in the EU numbering system), Pro at position 210 (position 331 in the EU numbering system), Thr at position 218 (position 339 in the EU numbering system), Cys at position 14 (position 131 in the EU numbering system), Arg at position 16 (position 133 in the EU numbering system), Cys at position 102 (position 219 in the EU numbering system), Glu at position 20 (position 137 in the EU numbering system), and Ser at position 21 (position 138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 have been substituted with other amino acids.

19. The IgG2 constant region of claim 18, which further comprises deletions of both Gly at position 325 (position 446 in the EU numbering system) and Lys at position 326 (position 447 in the EU numbering system).

20. An IgG2 constant region in which Cys at position 14 (position 131 in the EU numbering system), Arg at position 16 (position 133 in the EU numbering system), Cys at position 102 (position 219 in the EU numbering system), Glu at position 20 (position 137 in the EU numbering system), and Ser at position 21 (position 138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 2 have been substituted with other amino acids.

21. The IgG2 constant region of claim 20, which further comprises deletions of both Gly at position 325 (position 446 in the EU numbering system) and Lys at position 326 (position 447 in the EU numbering system).

22. A human antibody constant region comprising the amino acid sequence of SEQ ID NO: 5.

23. A human antibody constant region comprising the amino acid sequence of SEQ ID NO: 7.

24. A human antibody constant region comprising the amino acid sequence of SEQ ID NO: 9.

25. A human antibody constant region comprising the amino acid sequence of SEQ ID NO: 35.

26. A human antibody constant region comprising the amino acid sequence of SEQ ID NO: 36.

27. A human antibody constant region comprising the amino acid sequence of SEQ ID NO: 37.

28. A human antibody constant region comprising the amino acid sequence of SEQ ID NO: 43.

29. A human antibody constant region comprising the amino acid sequence of SEQ ID NO: 57 (M40ΔGK).

30. A human antibody constant region comprising the amino acid sequence of SEQ ID NO: 55 (M86).

31. An antibody comprising the constant region of any one of claims 1 to 30.

32. An anti-IL-6 receptor antibody comprising the constant region of any one of claims 1 to 30.

33. A pharmaceutical composition comprising the constant region of any one of claims 1 to 30.