containing variant Fc-fragments with the binding properties mentioned above also have longer *in vivo* half lives than control Fc-polypeptides. Further provided are nucleic acids that encode these Fc-fragments and Fc-polypeptides and methods of making these proteins using these nucleic acids. Also included are methods for extending the *in vivo* half life of an Fc-polypeptide and methods for identifying variant Fc-fragments that bind to FcRn with higher affinity at pH 5-6 and bind FcRn with comparable or lower affinity at physiologic pH as compared to a control Fc-fragment.

In one aspect, the present invention refers to a variant Fc-polypeptide comprising a human IgG1, IgG2, IgG3, or IgG4 variant Fc-fragment,

wherein the variant Fc-fragment comprises an insertion of 3 to 20 amino acids within or adjacent to Loop ~~5, 8, and/or~~ 10 of the variant Fc-fragment,

wherein the variant Fc-polypeptide binds to a human neonatal Fc receptor (hFcRn) with higher binding activity at pH 6.0 than a control Fc-polypeptide that has the same amino acid sequence as the variant Fc-polypeptide except that it does not contain the insertion within or adjacent to Loop ~~5, 8, and/or~~ 10 and

wherein the variant Fc-polypeptide has little or no binding activity for binding to nFcRn at pH 7.4 and ~~the~~ any residual binding response detected at pH 7.4 is no more than 0.1 nanometer more than that detected using the control Fc-polypeptide.

Also disclosed herein is a variant Fc-polypeptide comprising a human IgG1, IgG2, IgG3, or IgG4 variant Fc-fragment, wherein the variant Fc-fragment comprises an insertion of 3 to 20, 10 to 20, 20 to 40, 40 to 60, or 60 to 80 amino acids within or adjacent to Loop 5, 8, and/or 10 of the variant Fc-fragment, wherein the variant Fc-polypeptide binds to a human neonatal Fc receptor (hFcRn) with higher affinity and/or higher binding activity at a pH from about 5.0 to about 6.0 and/or at a pH of about 5.0, 5.2, 5.5, 5.7, or 6.0 than a control Fc-polypeptide that has the same amino acid sequence as the variant Fc-polypeptide except that it does not contain the insertion within or adjacent to Loop 5, 8, and/or 10 and wherein the variant Fc-polypeptide binds to the human neonatal Fc receptor (hFcRn) with approximately the same or lower affinity or binding activity as compared to the control Fc-polypeptide, that is, little or no binding activity, at a physiologic pH and/or at a pH of about 7.4, 7.5, or 7.6. The insertion within or adjacent to the variant Fc-fragment can be at least six amino acids long, not more than 25 amino acids long, from 6 to 16 amino acids long, and/or at least 12 amino acids long. In some examples, the insertion does not contain methionine and/or tryptophan residues. The insertion within or adjacent to the variant Fc-

activity, at a physiologic pH and/or at a pH of about 7.4, 7.5, or 7.6. Therefore, in one aspect, the present invention refers to an *in vitro* method for making a variant Fc-polypeptide comprising

introducing a nucleic acid encoding the variant Fc-polypeptide into a host cell,

culturing the host cell comprising the nucleic acid under conditions such that the nucleic acid is expressed, and

recovering the expressed variant Fc-polypeptide from the culture medium or the cell mass,

wherein the variant Fc-polypeptide comprises a variant Fc-fragment comprising an insertion of 3 to 20 amino acids within or adjacent to Loop 10 of the variant Fc-fragment, and

wherein the variant Fc-polypeptide has higher binding activity for binding to an hFcRn at pH 6.0 than a control Fc-polypeptide, and

wherein the variant Fc-polypeptide has little or no binding activity at pH 7.4 and any residual binding response detected at pH 7.4 is no more than 0.1 nanometer more than that detected using the control Fc-polypeptide.~~culturing the host cell comprising the nucleic acid under conditions such that the nucleic acid is expressed, and recovering the expressed variant Fc-polypeptide from the culture medium or the cell mass.~~

Also described are methods for making any of the variant Fc-fragments or Fc-polypeptides described herein comprising (a) introducing a nucleic acid encoding the variant Fc-polypeptide or Fc-fragment into a host cell, (b) culturing the host cell comprising the nucleic acid under conditions such that the nucleic acid is expressed, and (c) recovering the expressed variant Fc-polypeptide or Fc-fragment from the culture medium or the cell mass.

In a further example, described herein is a method for extending the half life of an Fc-polypeptide comprising a human IgG Fc-fragment comprising the following steps: selecting a site within or adjacent to Loop 5, 8, and/or 10 for insertion; and inserting a peptide into the selected site, wherein the peptide comprises an amino acid sequence selected from the group consisting of: SEQ ID NOs:41-67; SEQ ID NOs:90-246; SEQ ID NOs:247-356; SEQ ID NOs:367, 369, 372, 373, and 375-379; amino acids 4-9 of any one of SEQ ID NOs:41-53, 67, 90-162, 90-163, 165-215, 164-214, 217-247, 216-246, 359-379 and 392-399, 401-409, 411-426, 428-439, 441-447, 449-453, 456, 459, 461, 464, and 470, 475, 477-489, 491-496; amino acids 4-10 of SEQ ID NO:164, 252, and 490, and amino acids 4-8 of SEQ ID NO:215 or 216; amino acids 4-11 of any one of SEQ ID NOs: 248-288, 290-306, 342, 400, 410, 427, 440, 448, 454, 455, 457, 458, 460, 465-469, 471-474, and 476; amino acids 4-12 of SEQ ID NO:289 or 307; and amino acids 4-13 of any one of SEQ ID NOs:308-341 and 343-356. In one aspect, the present invention refers to a method for extending the half life of an Fc-polypeptide comprising a human IgG Fc-fragment, the method comprising ~~the following steps~~:

selecting a site within or adjacent to Loop ~~5, 8, and/or~~ 10 for insertion; and

inserting a peptide into the selected site, wherein the peptide comprises an amino acid sequence selected from the group consisting of:

(a) SEQ ID NOs:41-67, 90-356, 359-367, 369, 372, 373, and 375-379;

(b) amino acids 4-8 of SEQ ID NO:216;

(c) amino acids 4-9 of and one of SEQ ID NOs: 41-53, 67, 90-163, 165-215, and 217-247, and 359-378;

(d) amino acids 4-10 of SEQ ID NO:164;

(e) amino acids 4-11 of any one of SEQ ID NOs:248-288, 290-306, and 342;

(f) amino acids 4-12 of SEQ ID NO:289 or 307; and

(g) amino acids 4-13 of any one of SEQ ID NOs:308-341 and 343-356.

The selected site can be within or adjacent to Loop 10, and the insertion site can be between amino acids 384 and 385, numbered according to the EU numbering system. The insertion site can be within amino acids 383 to 387 using the EU numbering system. The insertion in the variant Fc-fragment can be between amino acids 382 and 383, 383 and 384, 384 and 385, 385 and 386, 386 and 387, 387 and 388, 388 and 389, 389 and 390, or 390 and 391 using the EU numbering system shown in Table 1.

Alternatively amino acids 384-386 can be deleted, and the insertion can occur between amino acids 383 and 387, using the EU numbering system.

In another aspect, the present invention refers to an *in vitro* method for identifying a human IgG variant Fc-fragment that confers a longer *in vivo* half life on an ~~variant~~ Fc-polypeptide that comprises the human IgG variant Fc-fragment, as compared to a control Fc-polypeptide, the method comprising ~~the following steps~~:

~~(a)~~ creating a library of nucleic acids encoding Fc-fragments containing an insertion comprising 4-20 randomized amino acids within or adjacent to Loop 10;

~~(b)~~ screening Fc-fragments encoded by the library to identify the variant Fc-fragments that (i) bind to hFcRn with higher binding activity at pH 6 than a control Fc-fragment and (ii) have little or no binding activity for binding to hFcRn at pH 7.4;

~~(c)~~ constructing a nucleic acid encoding a variant Fc-polypeptide comprising an identified variant Fc-fragment ~~identified in (b)~~, wherein the concentration of a control Fc-polypeptide, which comprises a control Fc-fragment rather than the variant Fc-fragment, is known to decrease linearly over time when administered to an animal *in vivo*;

~~(d)~~ introducing the constructed nucleic acid ~~of (c)~~ into a host cell and culturing the host cell under conditions such that the variant Fc-polypeptide encoded by the nucleic acid can be expressed;

~~(e)~~ recovering the variant Fc-polypeptide from the cell mass or cell culture medium; and

~~(f)~~ monitoring over time the concentrations of the variant and control Fc-polypeptides in peripheral blood sample obtained from an animal after which it had been administered the variant Fc-polypeptide and another animal after which it had been administered the control Fc-polypeptide, ~~over time in samples obtained from an animal after which it had been administered the variant Fc polypeotide and another animal after which it had been administered the control Fc polypeptide,~~ thereby identifying a variant Fc-fragment that confers a longer *in vivo* half life and greater exposure on a variant Fc-polypeptide. Further provided herein is a method for identifying a human IgG variant Fc-fragment that confers a longer *in vivo* half life on a variant Fc-polypeptide that comprises the variant Fc-fragment, as compared to a control Fc-polypeptide, comprising the following steps: (a) creating a library of nucleic acids encoding Fc-fragments containing an insertion comprising 4-20, 10 to 20, 20-40, 40-60, or 60-80 randomized amino acids within or adjacent to Loop 10; (b) screening Fc-fragments encoded by the library to identify the variant Fc-fragments that (i) bind to human FcRn with higher affinity and/or higher binding activity at pH 5.5, and/or at a pH from about 5-6, than a control Fc-fragment and (ii) bind to human FcRn at the same or lower

What is claimed is:

1. A variant Fc-polypeptide comprising a human IgG1, IgG2, IgG3, or IgG4 variant Fc-fragment,

wherein the variant Fc-fragment comprises an insertion of 3 to 20 amino acids within or adjacent to Loop 10 of the variant Fc-fragment,

wherein the variant Fc-polypeptide binds to a human neonatal Fc receptor (hFcRn) with higher binding activity at pH 6.0 than a control Fc-polypeptide that has the same amino acid sequence as the variant Fc-polypeptide except that it does not contain the insertion within or adjacent to Loop 10, and

wherein the variant Fc-polypeptide has little or no binding activity for binding to nFcRn at pH 7.4 and any residual binding response detected at pH 7.4 is no more than 0.1 nanometer more than that detected using the control Fc-polypeptide.

2. The variant Fc-polypeptide of claim 1, wherein the insertion within or adjacent to Loop 10 of the variant Fc-fragment is 10 to 20 amino acids long.

3. The variant Fc-polypeptide of claim 1, wherein the insertion within or adjacent to Loop 10 of the variant Fc-fragment is at least 6 amino acids long.

4. The variant Fc-polypeptide of claim 3, wherein the insertion within or adjacent to Loop 10 of the variant Fc-fragment is 6 to 16 amino acids long.

5. The variant Fc-polypeptide of any one of claims 1 to 4, wherein the insertion within or adjacent to Loop 10 of the variant Fc-fragment comprises at least one cysteine among the first four inserted amino acids and at least one cysteine among the last four inserted amino acids.

6. The variant Fc-polypeptide of any one of claims 1 to 4, wherein the insertion within or adjacent to Loop 10 of the variant Fc-fragment is at least 12 amino acids long.

7. The variant Fc-polypeptide of claim 6, wherein the insertion is within amino acids 383 to 387 using the EU numbering system shown in Table 1.

8. The variant Fc-polypeptide of claim 7,

wherein the insertion in the variant human Fc-fragment is between amino acids 382 and 383, amino acids 383 and 384, amino acids 384 and 385, amino acids 385 and 386, amino acids 386 and

387, amino acids 387 and 388, amino acids 388 and 389, amino acids 389 and 390, or amino acids 390 and 391 of the variant Fc-fragment, using the EU numbering system shown in Table 1, or

wherein amino acids 384-386 are deleted and the insertion is between amino acids 383 and 387, using the EU numbering system.

9. The variant Fc-polypeptide of claim 7,

wherein the insertion in the variant human Fc-fragment is between amino acids 382 and 383, amino acids 383 and 384, amino acids 384 and 385, amino acids 385 and 386, amino acids 386 and 387, or amino acids 387 and 388 of the variant Fc-fragment using the EU numbering system shown in Table 1, or

wherein amino acids 384-386 are deleted and the insertion is between amino acids 383 and 387 using the EU numbering system.

10. The variant Fc-polypeptide of claim 7,

wherein the insertion in the variant human Fc-fragment is between amino acids 382 and 383, amino acids 384 and 385, or amino acids 385 and 386 of the variant Fc-fragment using the EU numbering system shown in Table 1, or

wherein amino acids 384-386 are deleted and the insertion is between amino acids 383 and 387 using the EU numbering system.

11. The variant Fc-polypeptide of any one of claims 1-10,

wherein the insertion in the variant human Fc-fragment is between amino acids 384 and 385, using the EU numbering system shown in Table 1.

12. The variant Fc-polypeptide of any one of claims 1 to 4 and 11, wherein the insertion in the variant Fc-fragment comprises the amino acid sequence of any one of SEQ ID NO:13-24.

13. The variant Fc-polypeptide of any one of claims 1 to 4 and 11, wherein the insertion in the variant Fc-fragment comprises an amino acid sequence of any one of SEQ ID NOs:41-67, 90-356, 359-367, 369, 372, 373, and 375-379.

14. The variant Fc-polypeptide of claim 13, wherein the insertion in the variant Fc-fragment comprises the amino acid sequence of any one of SEQ ID NOs:41, 42, 43, 44, 45, 50, 97, 180, 200, and 216.

15. The variant Fc-polypeptide of claim 13, wherein the insertion in the variant Fc-fragment comprises the amino acid sequence of any one of SEQ ID NO:54, 55, 56, 57, and 58.

16. The variant Fc-polypeptide of any one of claims 1-4 and 11, wherein the insertion in the variant Fc-fragment comprises an amino acid sequence selected from the group consisting of:

(a) amino acids 4-8 of SEQ ID NO:216;

(b) amino acids 4-9 of SEQ ID NOs: 41-53, 67, 90-163, 165-215, and 217-247, and 359-378;

(c) amino acids 4-10 of SEQ ID NO:164;

(d) amino acids 4-11 of any one of SEQ ID NOs:248-288, 290-306, and 342;

(e) amino acids 4-12 of SEQ ID NO:289 or 307; and

(f) amino acids 4-13 of any one of SEQ ID NOs:308-341 and 343-356.

17. The variant Fc-polypeptide of any one of claims 1 to 4 and 11, wherein the variant human Fc-fragment is a variant IgG1 Fc-fragment.

18. The variant Fc-polypeptide of any one of claims 1 to 4 and 11, wherein the variant human Fc-fragment is a variant IgG2 Fc-fragment.

19. The variant Fc-polypeptide of any one of claims 1 to 4 and 11, wherein the variant human Fc-fragment is a variant IgG4 Fc-fragment.

20. The variant Fc-polypeptide of any one of claims 1 to 4 and 11, wherein the variant Fc-polypeptide comprises a variant Fc fusion protein comprising a non-antibody polypeptide.

21. The variant Fc-polypeptide of claim 20, wherein a control Fc fusion protein for the variant Fc fusion protein is alefacept, rilonacept, aflibercept, etanercept, romiplostim, or abatacept.

22. The variant Fc-polypeptide of any one of claims 1 to 4 and 11, wherein the variant Fc-polypeptide comprises a heavy chain variable region (V_H) and/or a light chain variable region (V_L) of an antibody.

23. The variant Fc-polypeptide of claim 22, wherein the variant Fc-polypeptide further comprises a first heavy chain constant region (C_H1) and a light chain constant region (C_L).

24. The variant Fc-polypeptide of claim 22, wherein the variant Fc-polypeptide comprises a V_H region and a V_L region.

25. The variant Fc-polypeptide of claim 23, wherein the variant Fc-polypeptide comprises:

(a) a heavy chain comprising a V_H region, a first heavy chain constant region (C_H1), a hinge region, a C_H2 region, and a C_H3 region, and

(b) a light chain comprising a V_L region and a light chain constant region (C_L).

26. The variant Fc-polypeptide of claim 22, wherein the variant Fc-polypeptide is monovalent.

27. The variant Fc-polypeptide of claim 22, wherein the variant Fc-polypeptide is a dimer.

28. The variant Fc-polypeptide of claim 22, wherein the variant Fc-polypeptide is a tetramer.

29. A nucleic acid encoding the variant Fc-polypeptide of any one of claims 1 to 28.

30. A host cell containing the nucleic acid of claim 29.

31. An *in vitro* method for making a variant Fc-polypeptide comprising:

introducing a nucleic acid encoding the variant Fc-polypeptide into a host cell,

culturing the host cell comprising the nucleic acid under conditions such that the nucleic acid is expressed, and

recovering the expressed variant Fc-polypeptide from the culture medium or the cell mass,

wherein the variant Fc-polypeptide comprises a variant Fc-fragment comprising an insertion of 3 to 20 amino acids within or adjacent to Loop 10 of the variant Fc-fragment, and

wherein the variant Fc-polypeptide has higher binding activity for binding to an hFcRn at pH 6.0 than a control Fc-polypeptide, and

wherein the variant Fc-polypeptide has little or no binding activity at pH 7.4 and any residual binding response detected at pH 7.4 is no more than 0.1 nanometer more than that detected using the control Fc-polypeptide.

32. The method of claim 31, wherein the nucleic acid encoding the variant Fc-polypeptide comprises the nucleic acid of claim 29.

33. A method for extending the half life of an Fc-polypeptide comprising a human IgG Fc-fragment, the method comprising:

selecting a site within or adjacent to Loop 10 for insertion; and

inserting a peptide into the selected site, wherein the peptide comprises an amino acid sequence selected from the group consisting of:

(a) SEQ ID NOs:41-67, 90-356, 359-367, 369, 372, 373, and 375-379;

(b) amino acids 4-8 of SEQ ID NO:216;

(c) amino acids 4-9 of any one of SEQ ID NOs: 41-53, 67, 90-163, 165-215, and 217-247, and 359-378;

(d) amino acids 4-10 of SEQ ID NO:164;

(e) amino acids 4-11 of any one of SEQ ID NOs:248-288, 290-306, and 342;

(f) amino acids 4-12 of SEQ ID NO:289 or 307; and

(g) amino acids 4-13 of any one of SEQ ID NOs:308-341 and 343-356.

34. The method of claim 33, wherein the insertion site is within amino acids 383 to 387 numbered according to the EU numbering system.

35. The method of claim 33,

wherein the insertion is between amino acids 382 and 383, amino acids 383 and 384, amino acids 384 and 385, or amino acids 385 and 386 of the variant Fc-fragment using the EU numbering system, or

wherein amino acids 384-386 are deleted and the insertion is between amino acids 383 and 387 using the EU numbering system.

36. An *in vitro* method for identifying a human IgG variant Fc-fragment that confers a longer *in vivo* half-life on an Fc-polypeptide that comprises the human IgG variant Fc-fragment, as compared to a control Fc-polypeptide, the method comprising:

creating a library of nucleic acids encoding Fc-fragments containing an insertion comprising 4-20 randomized amino acids within or adjacent to Loop 10;

screening Fc-fragments encoded by the library to identify the variant Fc-fragments that (i) bind to hFcRn with higher binding activity at pH 6 than a control Fc-fragment and (ii) have little or no binding activity for binding to hFcRn at pH 7.4;

constructing a nucleic acid encoding a variant Fc-polypeptide comprising an identified variant Fc-fragment, wherein the concentration of a control Fc-polypeptide, which comprises a control Fc-fragment rather than the variant Fc-fragment, is known to decrease linearly over time when administered to an animal *in vivo*;

introducing the constructed nucleic acid into a host cell and culturing the host cell under conditions such that the variant Fc-polypeptide encoded by the nucleic acid can be expressed;

recovering the variant Fc-polypeptide from the cell mass or cell culture medium; and

monitoring over time the concentrations of the variant and control Fc-polypeptides in peripheral blood sample obtained from an animal after which it had been administered the variant Fc-polypeptide and another animal after which it had been administered the control Fc-polypeptide, thereby identifying a variant Fc-fragment that confers a longer *in vivo* half-life and greater exposure on a variant Fc-polypeptide.

37. The method of claim 36, wherein the insertion comprising 4-20 randomized amino acids within or adjacent to Loop 10 is between positions 384 and 385 using the EU numbering system as illustrated in Table 1.

38. Use of a variant Fc-polypeptide according to any one of claims 1 to 28 for the manufacture of a medicament for treating a chronic disease.

ABSTRACT

Described herein are variant Fc-fragments that contain an insertion within or adjacent to a loop that bind to the neonatal Fc receptor (FcRn) with higher affinity and/or higher binding activity at pH 5-6 and approximately the same or lower affinity at a physiologic pH as compared to a control Fc-fragment, that is, little or no binding activity at a physiologic pH. Also described are variant Fc-polypeptides that comprise these variant Fc-fragments. Further described are methods of making and identifying such Fc-fragments and methods for making and using such Fc-polypeptides.

What is claimed is:

1. A variant Fc-polypeptide comprising a human IgG1, IgG2, IgG3, or IgG4 variant Fc-fragment,

wherein the variant Fc-fragment comprises an insertion of 3 to 20 amino acids within or adjacent to Loop 5, 8, and/or 10 of the variant Fc-fragment,

wherein the variant Fc-polypeptide binds to a human neonatal Fc receptor (hFcRn) with higher binding activity at pH 6.0 than a control Fc-polypeptide that has the same amino acid sequence as the variant Fc-polypeptide except that it does not contain the insertion within or adjacent to Loop 5, 8, and/or 10 and

wherein the variant Fc-polypeptide has little or no binding activity for binding to nFcRn at pH 7.4 and the residual binding response detected at pH 7.4 is no more than 0.1 nanometer more than that detected using the control Fc-polypeptide.

2. The variant Fc-polypeptide of claim 1, wherein the insertion is

(a) at least six amino acids long,

(b) from 6 to 16 amino acids long, and/or

(c) at least 12 amino acids long.

3. The variant Fc-polypeptide of claim 1 or 2, wherein the insertion is within or adjacent to Loop 10 of the variant Fc-fragment.

4. The variant Fc-polypeptide of claim 3,

wherein the insertion in the variant human Fc-fragment is between amino acids 382 and 383, amino acids 383 and 384, amino acids 384 and 385, amino acids 385 and 386, amino acids 386 and 387, amino acids 387 and 388, amino acids 388 and 389, amino acids 389 and 390, or amino acids 390 and 391 of the variant Fc-fragment, using the EU numbering system shown in Table 1, or

wherein amino acids 384-386 are deleted and the insertion is between amino acids 383 and 387, using the EU numbering system.

5. The variant Fc-polypeptide of claim 4,

wherein the insertion in the variant human Fc-fragment is between amino acids 384 and 385, using the EU numbering system.

6. The variant Fc-polypeptide of any one of claims 1 to 5, wherein the insertion comprises at least one cysteine among the first four inserted amino acids and at least one cysteine among the last four inserted amino acids.

7. The variant Fc-polypeptide of claim 6, wherein the first three amino acids of the insertion are Gly-Gly-Cys and the last three amino acids of the insertion are Cys-Gly-Gly.

8. The variant Fc-polypeptide of any one of claims 1 to 6, wherein the insertion in the variant Fc-fragment comprises the amino acid sequence of any one of SEQ ID NOs:13-24, 41-67, 90-356, 359-367, 369, 372, 373, 375-379, amino acids 4-8 of SEQ ID NO:216, amino acids 4-9 of any one of SEQ ID NOs: 41-53, 67, 90-163, 165-215, 217-247, and 359-378, amino acids 4-10 of SEQ ID NO:164, amino acids 4-11 of any one of SEQ ID NOs:248-288, 290-306, and 342, amino acids 4-12 of SEQ ID NO:289 or 307, and amino acids 4-13 of any one of SEQ ID NOs:308-341 and 343-356.

9. The variant Fc-polypeptide of claims 8, wherein the insertion in the variant Fc-fragment has the amino acid sequence of any one of SEQ ID NOs: 41- 45, 54- 58, 97, 127, 180, 208, 216, and 221.

10. The variant Fc-polypeptide of any one of claims 1-6, wherein the insertion is between amino acids 384 and 385, using the EU numbering system, and wherein the insertion comprises the amino acid sequence of any one of SEQ ID NOs:13-24, 41-67, 90-356, 359-367, 369, 372, 373, 375-379, amino acids 4-8 of SEQ ID NO:216, amino acids 4-9 of any one of SEQ ID NOs: 41-53, 67, 90-163, 165-215, 217-247, and 359-378, amino acids 4-10 of SEQ ID NO:164, amino acids 4-11 of any one of SEQ ID NOs:248-288, 290-306, and 342, amino acids 4-12 of SEQ ID NO:289 or 307, and amino acids 4-13 of any one of SEQ ID NOs:308-341 and 343-356.

11. The variant Fc-polypeptide of any one of claims 1 to 10,

which is a variant Fc fusion protein comprising a non-antibody polypeptide,

which is an antibody comprising a V_H region, a V_L region, a C_H2 region, and a C_H3 region,

which is monovalent or divalent, and/or

which is a dimer or a tetramer.

12. A nucleic acid encoding the variant Fc-polypeptide of any one of claims 1 to 11.

13. A host cell containing the nucleic acid of claim 12.

14. An *in vitro* method for making a variant Fc-polypeptide comprising

culturing the host cell of claim 13 under conditions such that the nucleic acid is expressed, and

recovering the expressed variant Fc-polypeptide from the culture medium or the cell mass.

15. A method for extending the half life of an Fc-polypeptide comprising a human IgG Fc-fragment comprising the following steps:

selecting a site within or adjacent to Loop 5, 8, and/or 10 for insertion; and

inserting a peptide into the selected site, wherein the peptide comprises an amino acid sequence selected from the group consisting of:

(a) SEQ ID NOs:41-67, 90-356, 359-367, 369, 372, 373, and 375-379;

(b) amino acids 4-8 of SEQ ID NO:216;

(c) amino acids 4-9 of any one of SEQ ID NOs: 41-53, 67, 90-163, 165-215, and 217-247, and 359-378;

(d) amino acids 4-10 of SEQ ID NO:164;

(e) amino acids 4-11 of any one of SEQ ID NOs:248-288, 290-306, and 342;

(f) amino acids 4-12 of SEQ ID NO:289 or 307; and

(g) amino acids 4-13 of any one of SEQ ID NOs:308-341 and 343-356.

16. The method of claim 15, wherein the site is within or adjacent to Loop 10.

17. The method of claim 16,

wherein the insertion is between amino acids 382 and 383, amino acids 383 and 384, amino acids 384 and 385, or amino acids 385 and 386 of the variant Fc-fragment using the EU numbering system or

wherein amino acids 384-386 are deleted and the insertion is between amino acids 383 and 387 using the EU numbering system.

18. An *in vitro* method for identifying a human IgG variant Fc-fragment that confers a longer *in vivo* half life on a variant Fc-polypeptide that comprises the variant Fc-fragment, as compared to a control Fc-polypeptide, comprising the following steps:

(a) creating a library of nucleic acids encoding Fc-fragments containing an insertion comprising 4-20 randomized amino acids within or adjacent to Loop 10;

(b) screening Fc-fragments encoded by the library to identify the variant Fc-fragments that (i) bind to hFcRn with higher binding activity at pH 6 than a control Fc-fragment and (ii) have little or no binding activity for binding to hFcRn at pH 7.4;

(c) constructing a nucleic acid encoding a variant Fc-polypeptide comprising a variant Fc-fragment identified in (b), wherein the concentration of a control Fc-polypeptide, which comprises a control Fc-fragment rather than the variant Fc-fragment, is known to decrease linearly over time when administered to an animal *in vivo*;

(d) introducing the nucleic acid of (c) into a host cell and culturing the host cell under conditions such that the variant Fc-polypeptide encoded by the nucleic acid can be expressed;

(e) recovering the variant Fc-polypeptide from the cell mass or cell culture medium;

(f) monitoring the concentrations of the variant and control Fc-polypeptides in peripheral blood over time in samples obtained from an animal after which it had been administered the variant Fc-polypeptide and another animal after which it had been administered the control Fc-polypeptide, thereby identifying a variant Fc-fragment that confers a longer *in vivo* half life and greater exposure on a variant Fc-polypeptide.

19. The method of claim 18, wherein the insertion of step (a) is between positions 384 and 385 using the EU numbering system as illustrated in Table 1.

20. Use of a variant Fc-polypeptide according to any one of claims 1 to 11 for the manufacture of a medicament for treating a chronic disease.