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 residual binding response detected at pH 7.4 is no more than 0.1 nanometer more than that detected using the control Fc-polypeptide.

~~Described here~~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 ~~embodiments~~examples, the insertion does not contain methionine and/or tryptophan residues. The insertion within or adjacent to the variant Fc-

fragment can comprise at least one cysteine among the first four inserted amino acids and at least one cysteine among the last four inserted amino acids. Optionally, the insertion can lack cysteine residues other than any cysteine residues occurring in the first or last four amino acids of the insertion. In some ~~embodiments~~examples, the insertion is within or adjacent to Loop 10 of the variant Fc-fragment, optionally between amino acids 384 and 385 of the variant Fc-fragment using the EU numbering system shown in Table 1. The insertion can be within amino acids 383 to 387 using the EU numbering system. In some ~~embodiments~~examples, the insertion in the variant human Fc-fragment can be between amino acids 382 and 383, 383 and 384, 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 be between amino acids 383 and 387 using the EU numbering system shown in Table 1. The insertion in the variant Fc-fragment can comprise the amino acid sequence of any one of SEQ ID NO:13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 90-356, and 359-379. Further, the insertion in the variant Fc-fragment can comprise the amino acid sequence of any one of the following: SEQ ID NOs:41-67; SEQ ID NOs:90-246; SEQ ID NOs:247-

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. The variant Fc-polypeptide can be a variant Fc fusion protein comprising a non-antibody polypeptide. In ~~particular embodiments~~some examples, a control Fc fusion protein for the variant Fc fusion protein can be alefacept, rilonacept, aflibercept, etanercept, romiplostim, or abatacept. In other ~~embodiments~~examples, the variant Fc-polypeptide can comprise a heavy chain variable region (V_H) and/or a light chain variable region (V_L) of any antibody and may also comprise a first heavy chain constant region (C_H1) and a light chain constant region (C_L). In some ~~embodiments~~examples, the variant Fc-polypeptide can comprise (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). The variant Fc-polypeptide can be monovalent. The variant Fc-polypeptide can be a dimer or can be a tetramer.

In further ~~embodiments~~examples, described herein are nucleic acids encoding the variant Fc-polypeptides or the variant Fc-fragments described herein, as well as the insertions within or adjacent to the loops of the variant Fc-fragments. Also described are host cells containing such nucleic acids.

Thus, in a further aspect, the present invention refers to a nucleic acid encoding the variant Fc-polypeptide of the present invention. In still another aspect, the present invention refers to a host cell containing the nucleic acid of the present invention. Also contemplated are methods of making a variant Fc-polypeptide or variant Fc-fragment 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, wherein the variant Fc-fragment, or the variant Fc-polypeptide that contains a 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, and wherein the variant Fc-polypeptide or Fc-fragment binds to a hFcRn with higher affinity and or higher binding activity at a pH in a range 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 or Fc-fragment and wherein the variant Fc-polypeptide or Fc-fragment 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 or control Fc-fragment, that is, with little or no binding

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 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 ~~embodiment~~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 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 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; and

(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 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

affinity or binding activity compared to the control Fc-fragment, that is, with little or no binding activity, at physiologic pH and/or at a pH of 7.4, 7.5, or 7.6; (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) administering the variant Fc-polypeptide to an animal and administering the control Fc-polypeptide to another animal; and (g)

monitoring the concentrations of the variant and control Fc-polypeptides in peripheral blood over time subsequent to administration, thereby identifying a variant Fc-fragment that confers a longer *in vivo* half life on a variant Fc-polypeptide. The insertion of step (a) can be between positions 384 and 385 using the EU numbering system as illustrated in Table 1. 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 the use of a variant Fc-polypeptide according to the present invention in the manufacture of a medicament for treating a chronic disease.

In another ~~aspect~~example, a method is provided for treating a chronic disease comprising administering to a patient in need thereof a variant Fc-polypeptide as described herein.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Ribbon diagram of the predicted three dimensional structure of portions of human FcRn (hFcRn) and a human IgG1 Fc-fragment that come in closest contact upon binding of FcRn to the Fc-fragment. At top is a ribbon diagram of a portion of the tertiary structure of hFcRn. Below is a ribbon diagram of a portion of a human IgG1 Fc-fragment that comes in closest contact with FcRn when FcRn is bound to it. Loops are shown as strings, whereas alpha helices and beta sheets are shown as ribbons. The six sites at which insertions were made are indicated by the names of the eight libraries that were constructed as described below, *i.e.*, L1, L2A, L2B, L3, L4, L5, L6A, and L6B.

Figure 2: Format of the insertion libraries. Above is shown the sequence of a human IgG1 Fc-fragment (SEQ ID NO:1). Amino acids within loops in which, or adjacent to which, insertions were made are indicated by underlining and boldface type. Library names, *i.e.*, L1, L2A, etc., appear above the region into which insertions were made for that particular library. The format of the insertions is indicated below. At far left are the library names, *i.e.*, L1, L2A, etc. Just to the right of the library names is the original sequence around the insertion site prior to the insertion of any amino acids. Italic letters indicate amino acids that are deleted prior to insertion. A vertical dashed line indicates the insertion site in those libraries in which no amino acids were deleted. At far right is the sequence around the insertion site with the

insertion in place. The designations "$(19R)_5$" or "$(19R)_6$" means five or six, respectively, randomized amino acids, which can be any of the 19 amino acids other than cysteine. The designation "$(20R)_8$" means eight randomized amino acids, which can be any of the twenty amino acids.

Figure 3: Association and dissociation curves at pH 6 and pH 7.4, respectively, of variant Fc-fragments from library L5. The curves at left show the response detected using the ForteBio Octet®

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 within or adjacent to Loop 5, 8, and/or 10 of the variant Fc-fragment is at least six amino acids long.

3. The variant Fc-polypeptide of claim 2, wherein the insertion within or adjacent to Loop 5, 8, and/or 10 of the variant Fc-fragment is from 6 to 16 amino acids long.

4. The variant Fc-polypeptide of any one of claims 1 to 3, wherein the insertion within or adjacent to Loop 5, 8, and/or 10 of the variant Fc-fragment is at least 12 amino acids long.

5. The variant Fc-polypeptide of any one of claims 1 to 4, wherein the insertion within or adjacent to Loop 5, 8, and/or 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 5, wherein the insertion is within or adjacent to Loop 10 of the variant Fc-fragment.

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, 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.

9. The variant Fc-polypeptide of claim 8,

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, 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.

10. The variant Fc-polypeptide of any one of claims 1 to 9, wherein the variant human Fc-fragment is a variant IgG1 Fc-fragment.

11. The variant Fc-polypeptide of any one of claims 1 to 9, wherein the variant human Fc-fragment is a variant IgG2 Fc-fragment.

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

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

15. 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,

wherein the binding activity of the variant Fc-polypeptide to a hFcRn at pH 6.0 is higher than that of a control Fc-polypeptide that is the same 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 hFcRn 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.

16. The variant Fc-polypeptide of claim 15, wherein the insertion is at least six amino acids long.

17. The variant Fc-polypeptide of claim 15 or 16, wherein the insertion contains at least one cysteine among the first four amino acids of the insertion and at least one cysteine among the last four amino acids of the insertion.

18. The variant Fc-polypeptide of any one of claims 15 to 17, wherein the insertion is 6 to 16 amino acids long.

19. The variant Fc-polypeptide of any one of claims 15 to 18, wherein the insertion is at least 12 amino acids long.

20. The variant Fc-polypeptide of any one of claims 15 to 19, wherein the insertion is within amino acids 383 to 387 using the EU numbering system shown in Table 1.

21. The variant Fc-polypeptide of claim 20,

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.

22. The variant Fc-polypeptide of claim 21, wherein the insertion in the variant Fc-fragment is between amino acids 384 and 385 using the EU numbering system shown in Table 1.

23. The variant Fc-polypeptide of any one of claims 15 to 22, wherein the insertion in the variant Fc-fragment has the amino acid sequence of any one of SEQ ID NOs:13-24.

24. The variant Fc-polypeptide of claim 23, 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, and 216.

25. The variant Fc-polypeptide of any one of claims 15-22, wherein the insertion in the variant Fc-fragment comprises the amino acid sequence of SEQ ID NO:54, 55, 56, 57, or 58.

26. The variant Fc-polypeptide of any one of claims 15 to 25, which comprises a human IgG1 variant Fc-fragment.

27. The variant Fc-polypeptide of any one of claims 15 to 25, which comprises a human IgG2 variant Fc-fragment.

28. The variant Fc-polypeptide of any one of claims 15 to 25, which comprises a human IgG4 variant Fc-fragment.

29. The variant Fc-polypeptide of any one of claims 1 to 28, which is a variant Fc fusion protein comprising a non-antibody polypeptide.

30. The variant Fc-polypeptide of claim 29, wherein a control Fc fusion protein for the variant Fc fusion protein is alefacept, rilonacept, aflibercept, etanercept, romiplostim, or abatacept.

31. The variant Fc-polypeptide of any one of claims 1 to 28, comprising a heavy chain variable region (V_H) and/or a light chain variable region (V_L) of an antibody.

32. The variant Fc-polypeptide of claim 31, which further comprises a first heavy chain constant region (C_H1) and a light chain constant region (C_L).

33. The variant Fc-polypeptide of claim 31 or 32, which comprises a V_H region and a V_L region.

34. The variant Fc-polypeptide of any one of claims 39 to 41, comprising

(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).

35. The variant Fc-polypeptide of any one of claims 31 to 34, which is monovalent.

36. The variant Fc-polypeptide of any one of claims 1 to 35, which is a dimer.

37. The variant Fc-polypeptide of any one of claims 31 to 34, which is a tetramer.

38. A nucleic acid encoding the variant Fc-polypeptide of any one of claims 1 to 37.

39. A host cell containing the nucleic acid of claim 38.

40. A 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 5, 8, and/or 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 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.

41. The method of claim 40, wherein the nucleic acid is the nucleic acid of claim 38.

42. 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 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.

43. The method of claim 42, wherein the site is within or adjacent to Loop 10.

44. The method of claim 43, wherein the insertion site is within amino acids 383 to 387 numbered according to the EU numbering system.

45. The method of claim 44,

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.

46. 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 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) administering the variant Fc-polypeptide to an animal and administering the control Fc-polypeptide to another animal; and

(g) monitoring the concentrations of the variant and control Fc-polypeptides in peripheral blood over time subsequent to administration, thereby identifying a variant Fc-fragment that confers a longer *in vivo* half life and greater exposure on a variant Fc-polypeptide.

47. The method of claim 46, wherein the insertion of step (a) is between positions 384 and 385 using the EU numbering system as illustrated in Table 1.

48. A method for treating a chronic disease comprising administering to a patient in need thereof a variant Fc-polypeptide according to any one of claims 1 to 37.

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.