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Summary ~~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 an Fc region variant by introducing an amino acid alteration(s) into an antibody Fc region, which variant has enhanced FcγRIIb-binding activity, and/or enhanced binding selectivity to FcγRIIb compared to FcγRIIa (type R), as compared to when no amino acid alteration has been introduced into the Fc region; a polypeptide comprising the Fc region variant; and a pharmaceutical composition containing the polypeptide.

[Means for Solving the Problems]

The present inventors carried out dedicated research on: an Fc region variant with enhanced FcγRIIb-binding and enhanced binding selectivity to FcγRIIb compared to FcγRIIa (type R), as compared to when the Fc region is unaltered, by introducing amino acid alteration(s) into the Fc region; and a polypeptide comprising the Fc region variant. As a result, the present inventors found that FcγRIIb-binding activity is enhanced and/or binding selectivity to FcγRIIb compared to FcγRIIa (type R) is enhanced by combining an Fc region variant in which the amino acid at position 238 (EU numbering) in the Fc Region has been altered with other amino acid alteration(s). Thus, in one aspect, the present invention refers to a Fc region variant whose amino acid at position 238 according to EU numbering is Asp, and which comprises at least two amino acids selected from the amino acid group consisting of Tyr at amino acid position 234, Phe at amino acid position 235, Ile at amino acid position 264, Glu at amino acid position 265, Phe, Leu, or Met at amino acid position 266, Ala, Glu, Gly, or Gln at amino acid position 267, Gln, or Glu at amino acid position 268, Asp at amino acid position 269, Asp, Phe, Ile, Met, Asn, Pro, or Gln at amino acid position 272, Gln at amino acid position 274, Phe at amino acid position 296, Asp at amino acid position 326, Gly at amino acid position 327, Lys, Arg, or Ser at amino acid position 330, Ser at amino acid position 331, Lys, Arg, Ser, or Thr at amino acid position 332, Lys, Arg, Ser, or Thr at amino acid position 333, Arg, Ser, or Thr at amino acid position 334, Ala or Gln at amino acid position 355, Glu at amino acid position 356, Met at amino acid position 358, Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Gln, Arg, Ser, Thr, Val, Trp, or Tyr at amino acid position 396, Arg at amino acid position 409, and Glu at amino acid position 419, wherein the value for [KD value for FcγRIIa (type R) of a polypeptide comprising an Fc region variant]/[KD value for FcγRIIb of a polypeptide comprising an Fc region variant] is 20.0 or greater. In another aspect, the present invention refers to a Fc region variant consisting of any one amino acid sequence selected from among SEQ ID NOs: 43 to 68, SEQ ID NO: 70, SEQ ID NO: 71, and SEQ ID NOs: 75 to 77. In still another aspect, the present invention refers to a polypeptide comprising at least two Fc region variants of the present invention, wherein the two Fc region variants are associated. The present invention refers also to a pharmaceutical composition comprising the polypeptide of the present invention.

~~More specifically, the present invention relates to~~ Also disclosed herein is the following:

[1] an Fc region variant in which amino acid at position 238 according to EU numbering and at least one amino acid selected from those at positions 233, 234, 235, 237, 264, 265, 266, 267, 268, 269, 271, 272, 274, 296, 326, 327, 330, 331, 332, 333, 334, 355, 356, 358, 396, 409, and 419 according to EU numbering are altered to other amino acids, wherein binding activity of the variant to Fcγ receptors [KD value for FcγRIIb of a polypeptide comprising an Fc region to which an amino acid alteration(s) has not been introduced]/[KD value for FcγRIIb of a

polypeptide comprising the Fc region variant] has a value which is 15.0 or greater;
[2] the Fc region variant of [1], wherein the amino acids at positions 238, 268, and 271 according to EU numbering are altered to other amino acids, and in addition, at least one amino acid selected from amino acids at positions 233, 237, 264, 267, 272, 296, 327, 330, 332, and 396 according to EU numbering are altered to other amino acids;
[3] an Fc region variant whose amino acid at position 238 according to EU numbering is Asp, and which comprises at least one amino acid selected from the amino acid group consisting of Asp at amino acid position 233, Tyr at amino acid position 234, Phe at amino acid position 235, Asp at amino acid position 237, Ile at amino acid position 264, Glu at amino acid position 265, Phe, Leu, or Met at amino acid position 266, Ala, Glu, Gly, or Gln at amino acid position 267, Asp, Gln, or Glu at amino acid position 268, Asp at amino acid position 269, Gly at amino acid position 271, Asp, Phe, Ile, Met, Asn, Pro, or Gln at amino acid position 272, Gln at amino acid position 274, Asp or Phe at amino acid position 296, Ala or Asp at amino acid position 326, Gly at amino acid position 327, Lys, Arg, or Ser at amino acid position 330, Ser at amino acid position 331, Lys, Arg, Ser, or Thr at amino acid position 332, Lys, Arg, Ser, or Thr at amino acid position 333, Arg, Ser, or Thr at amino acid position 334, Ala or Gln at amino acid position 355, Glu at amino acid position 356, Met at amino acid position 358, Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Gln, Arg, Ser, Thr, Val, Trp, or Tyr at amino acid position 396, Arg at amino acid position 409, and Glu at amino acid position 419, wherein binding activity of the variant to Fcγ receptors [KD value for FcγRIIb of a polypeptide comprising an Fc region to

at position 296 is Asp, according to EU numbering, in an Fc region; and

(x) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, and the amino acid at position 296 is Asp, according to EU numbering, in an Fc region;

[11] an Fc region variant consisting of any one amino acid sequence selected from among SEQ ID NOs: 43 to 68, SEQ ID NO: 70, SEQ ID NO: 71, and SEQ ID NOs: 75 to 77;

[12] a polypeptide comprising at least two Fc region variants of any one of [1] to [11], wherein the two Fc region variants are associated;

[13] the polypeptide of [12], wherein the amino acid sequences of the two associated Fc region variants in the polypeptide are the same;

[14] the polypeptide of [12], wherein the amino acid sequences of the two associated Fc region variants in the polypeptide are different;

[15] the polypeptide of [14], wherein the amino acid sequences of the two associated Fc region variants have different amino acid(s) at at least one amino acid position selected from positions 235, 236, 237, 238, and 239 according to EU numbering in the Fc region variant;

[16] the polypeptide of [15], wherein one of the amino acid sequences of the two associated Fc region variants is an amino acid sequence comprising at least one amino acid selected from Asp, Gln, Glu, or Thr at amino acid position 235, Asn at amino acid position 236, Phe or Trp at amino acid position 237, Glu, Gly, or Asn at amino acid position 238, and Asp or Glu at amino acid position 239 according to EU numbering;

[17] the polypeptide of any one of [12] to [16], wherein the polypeptide comprising the Fc region variant is an IgG antibody;

[18] the polypeptide of any one of [12] to [16], wherein the polypeptide comprising the Fc region variant is an Fc fusion protein molecule; and

[19] a pharmaceutical composition comprising the polypeptide of any one of [12] to [18].

Furthermore, the present ~~invention~~ disclosure relates to a method of enhancing FcγRIIb-binding activity of an Fc region and enhancing binding selectivity to FcγRIIb compared to FcγRIIa (type R), by introducing an amino acid alteration(s) into the Fc region ~~of the present invention~~disclosed herein. The present ~~invention~~ disclosure also relates to a method of suppressing production of antibodies against a polypeptide containing an Fc region, by introducing an amino acid alteration(s) ~~of the present invention~~as disclosed herein into the Fc region.

The present disclosure~~invention~~ also relates to a therapeutic or preventive agent for immune inflammatory diseases that comprises a polypeptide ~~of the present invention~~as described herein. Furthermore, the present disclosure~~invention~~ relates to a method for treating or preventing immune inflammatory diseases, which comprises the step of administering a polypeptide ~~of the present invention~~as described herein to a subject. In

addition, the present disclosure~~invention~~ relates to a kit for use in the method ~~of the present invention~~as described herein for treating or preventing immune inflammatory diseases, which comprises a polypeptide of the present invention. The present disclosure~~invention~~ also relates to use of a polypeptide ~~of the present invention~~as described herein in the production of a therapeutic or preventive agent for immune inflammatory diseases. Furthermore, the present disclosure~~invention~~ relates to a polypeptide as described herein~~of the present invention~~ for use in the method for treating or preventing immune inflammatory diseases of the present invention.

The present ~~invention~~ disclosure relates to an activation inhibitor for B cells, mast cells, dendritic cells, and/or basophils, which comprises a polypeptide as described herein~~of the present invention~~. Furthermore, the present disclosure~~invention~~ relates to a method of inhibiting activation of B cells, mast cells, dendritic cells, and/or basophils, which comprises administering a polypeptide as described herein~~of the present invention~~ to a subject. The present disclosure~~invention~~ also relates to a kit for use in the method of inhibiting activation of B cells, mast cells, dendritic cells, and/or basophils, which comprises a polypeptide as described herein~~of the present invention~~. The present ~~invention~~ disclosure relates to a use of a polypeptide as described herein~~of the present invention~~ in producing activation inhibitors for B cells, mast cells, dendritic cells, and/or basophils. The present disclosure~~invention~~ also relates to a polypeptide as described herein~~of the present invention~~ for use in the method as described herein~~of the present invention~~ of inhibiting activation of B cells, mast cells, dendritic cells, and/or basophils.

Furthermore, the present ~~invention~~ disclosure relates to a therapeutic agent for diseases in which a protein necessary for an organism is deficient, wherein the agent comprises a polypeptide as described herein~~of the present invention~~. The present ~~invention~~ disclosure also relates to a method for treating diseases in which a protein necessary for an organism is deficient, which comprises administering a polypeptide as described herein~~of the present invention~~ to a subject. Furthermore, the present ~~invention~~ disclosure relates to a kit for use in the method as described herein~~of the present invention~~ for treating diseases in which a protein necessary for an organism is deficient, wherein the kit comprises a polypeptide as described herein~~of the present invention~~. The present ~~invention~~ disclosure relates to use of a polypeptide as described herein~~of the present invention~~ in producing a therapeutic agent for diseases in which a protein necessary for an organism is deficient. The present ~~invention~~ disclosure also relates to a polypeptide as described herein~~of the present invention~~ for use in the method as described herein~~of the present invention~~ for treating diseases in which a protein necessary for an organism is deficient.

In addition, the present ~~invention~~ disclosure relates to an agent for suppressing virus proliferation, which comprises a polypeptide as described herein~~of the present invention~~. The present ~~invention~~ disclosure also relates to a method of suppressing virus proliferation, which comprises administering a polypeptide as described herein~~of the present invention~~ to a subject. Furthermore, the present ~~invention~~ disclosure relates to a kit as described herein~~of the present invention~~ for use in the method of suppressing virus proliferation, wherein the kit comprises a polypeptide as described herein~~of the present invention~~. The present ~~invention~~ disclosure relates to use of a polypeptide as described herein~~of the~~

~~present invention~~ in producing an agent for suppressing virus proliferation. The present ~~invention~~ disclosure also relates to a polypeptide as described herein~~of the present invention~~ for use in the method as described herein~~of the present invention~~ of suppressing virus proliferation.

[Effects of the Invention]

Fc region variants with enhanced FcγRIIb-binding activity and/or enhanced binding selectivity to FcγRIIb compared to FcγRIIa (type R), as compared to when the Fc region is unaltered, are provided by the present invention. By using the polypeptides containing the Fc region variants, it is possible to enhance inhibitory signals of inflammatory immune responses mediated by phosphorylation of ITIM of FcγRIIb. Also, by conferring an Fc region with the property of selective FcγRIIb binding, it may be possible to suppress anti-drug antibody production. Also, by using an Fc region variant of the present invention as a polypeptide having human FcRn-binding activity under an acidic pH range condition and comprising an antigen-binding domain in which an antigen-binding activity of an antigen-binding molecule changes depending on the ion concentration conditions, it is possible to promote elimination of antigens that bind to the polypeptide, which are present in plasma.

Brief Description of the Drawings

Fig. 1 is a graph showing the results of evaluating platelet aggregation due to the omalizumab-G1d-3/IgE immune complex in a platelet aggregation assay using platelets derived from a donor having FcγRIIa (R/H) polymorphism.

Fig. 2 is a graph showing the results of evaluating platelet aggregation due to the omalizumab-G1d-3/IgE immune complex in a platelet aggregation assay using platelets derived from a donor having FcγRIIa (H/H) polymorphism.

Fig. 3 is a graph showing the results of evaluating CD62p expression on a washed platelet membrane surface. The black-filled curve indicates the result obtained when reaction with PBS was followed by stimulation by ADP addition, and the unfilled curve indicates the result obtained when reaction with an immune complex was followed by stimulation with ADP.

Fig. 4 is a graph showing the results of evaluating active integrin expression on a washed platelet membrane surface. The black-filled curve indicates the result obtained when reaction with PBS was followed by stimulation by ADP addition, and the unfilled curve indicates the result obtained when reaction with an immune complex was followed by stimulation with ADP.

Fig. 5 shows the Fc(P208)/FcγRIIb extracellular region complex as determined by X-ray crystal structure analysis. For each of the Fc-region CH2 domain and CH3 domain, the portion shown on the left was defined as domain A and the portion shown on the right was defined as

CLAIMS

1. A Fc region variant whose amino acid at position 238 according to EU numbering is Asp, and which comprises at least two amino acids selected from the amino acid group consisting of Tyr at amino acid position 234, Phe at amino acid position 235, Ile at amino acid position 264, Glu at amino acid position 265, Phe, Leu, or Met at amino acid position 266, Ala, Glu, Gly, or Gln at amino acid position 267, Gln, or Glu at amino acid position 268, Asp at amino acid position 269, Asp, Phe, Ile, Met, Asn, Pro, or Gln at amino acid position 272, Gln at amino acid position 274, Phe at amino acid position 296, Asp at amino acid position 326, Gly at amino acid position 327, Lys, Arg, or Ser at amino acid position 330, Ser at amino acid position 331, Lys, Arg, Ser, or Thr at amino acid position 332, Lys, Arg, Ser, or Thr at amino acid position 333, Arg, Ser, or Thr at amino acid position 334, Ala or Gln at amino acid position 355, Glu at amino acid position 356, Met at amino acid position 358, Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Gln, Arg, Ser, Thr, Val, Trp, or Tyr at amino acid position 396, Arg at amino acid position 409, and Glu at amino acid position 419, wherein the value for [KD value for FcγRIIa (type R) of a polypeptide comprising an Fc region variant]/[KD value for FcγRIIb of a polypeptide comprising an Fc region variant] is 20.0 or greater.

2. The Fc region variant of claim 1, wherein binding activity of the variant to Fcγ receptors [KD value for FcγRIIb of a polypeptide comprising an Fc region to which an amino acid alteration(s) has not been introduced]/[KD value for FcγRIIb of a polypeptide comprising the Fc region variant] has a value which is 15.0 or greater.

3. The Fc region variant of claim 1 or 2, wherein the value for [KD value for FcγRIIb of a polypeptide comprising an Fc region to which an amino acid alteration(s) has not been introduced]/[KD value for FcγRIIb of a polypeptide comprising an Fc region variant] is 50.0 or more.

4. The Fc region variant of any one of claims 1 to 3, wherein the value for [KD value for FcγRIIb of a polypeptide comprising an Fc region to which an amino acid alteration(s) has not been introduced]/[KD value for FcγRIIb of a polypeptide comprising an Fc region variant] is 100.0 or more.

5. The Fc region variant of any one of claims 1 to 4, wherein the Fc region variant comprises any one of the following set of amino acid alterations of (a) to (x):
(a) amino acid alterations at positions 238, 233, 237, 268, 271, 296, and 330 (EU numbering)

of an Fc region;

(b) amino acid alterations at positions 238, 237, 268, 271, 296, and 330 (EU numbering) of an Fc region;

(c) amino acid alterations at positions 238, 233, 237, 268, 271, 296, 330, and 332 (EU numbering) of an Fc region;

(d) amino acid alterations at positions 238, 233, 237, 264, 267, 268, 271, and 330 (EU numbering) of an Fc region;

(e) amino acid alterations at positions 238, 233, 237, 267, 268, 271, 296, 330, and 332 (EU numbering) of an Fc region;

(f) amino acid alterations at positions 238, 237, 267, 268, 271, 296, 330, and 332 (EU numbering) of an Fc region;

(g) amino acid alterations at positions 238, 233, 237, 268, 271, 296, 327, and 330 (EU numbering) of an Fc region;

(h) amino acid alterations at positions 238, 233, 237, 264, 267, 268, and 271 (EU numbering) of an Fc region;

(i) amino acid alterations at positions 238, 233, 237, 264, 267, 268, 271, 296, and 330 (EU numbering) of an Fc region;

(j) amino acid alterations at positions 238, 233, 237, 264, 267, 268, 271, 296, 330, and 396 (EU numbering) of an Fc region;

(k) amino acid alterations at positions 238, 237, 264, 267, 268, 271, and 330 (EU numbering) of an Fc region;

(l) amino acid alterations at positions 238, 237, 264, 267, 268, 271, 296, and 330 (EU numbering) of an Fc region;

(m) amino acid alterations at positions 238, 264, 267, 268, and 271 (EU numbering) of an Fc region;

(n) amino acid alterations at positions 238, 264, 267, 268, 271, and 296 (EU numbering) of an Fc region;

(o) amino acid alterations at positions 238, 237, 267, 268, 271, 296, and 330 (EU numbering) of an Fc region;

(p) amino acid alterations at positions 238, 233, 237, 264, 267, 268, 271, 330, and 396 (EU numbering) of the Fc region;

(q) amino acid alterations at positions 238, 233, 237, 264, 267, 268, 271, 296, 327, 330, and 396 (EU numbering) of an Fc region;

(r) amino acid alterations at positions 238, 233, 237, 264, 267, 268, 271, 272, and 296 (EU numbering) of an Fc region;

(s) amino acid alterations at positions 238, 237, 264, 267, 268, 271, 272, and 330 (EU numbering) of an Fc region;

(t) amino acid alterations at positions 238, 237, 264, 267, 268, 271, 272, 296, and 330 (EU numbering) of an Fc region;

(u) amino acid alterations at positions 238, 233, 264, 267, 268, and 271 (EU numbering) of an Fc region;

(v) amino acid alterations at positions 238, 237, 267, 268, 271, 296, and 330 (EU numbering) of an Fc region;

(w) amino acid alterations at positions 238, 264, 267, 268, 271, 272, and 296 (EU numbering) of an Fc region; and

(x) amino acid alterations at positions 238, 233, 264, 267, 268, 271, and 296 (EU numbering) of an Fc region.

6. The Fc region variant of any one of claims 1 to 5, wherein the Fc region variant comprises any one of the following amino acid sequences of (b) to (x):

(b) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 237 is Asp, the amino acid at position 268 is Asp or Glu, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(c) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 268 is Asp, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, the amino acid at position 330 is Arg, and the amino acid at position 332 is Thr, according to EU numbering, in an Fc region;

(d) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Gly or Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(e) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, the amino acid at position 330 is Arg, and the amino acid at position 332 is Thr, according to EU numbering, in an Fc region;

(f) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 237 is Asp, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, the amino acid at position 330 is Arg, and the amino acid at position 332 is Thr, according to EU numbering, in an Fc region;

(g) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 268 is Asp, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, the amino acid at position 327 is Gly, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(h) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, and the amino acid at position 271 is Gly, according to EU numbering, in an Fc region;

(i) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(j) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, the amino acid at position 330 is Arg, and the amino acid at position 396 is Met or Leu, according to EU numbering, in an Fc region;

(k) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(l) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(m) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, and the amino acid at position 271 is Gly, according to EU numbering, in an Fc region;

(n) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, and the amino acid at position 296 is Asp, according to EU numbering, in an Fc region;

(o) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at

position 237 is Asp, the amino acid at position 267 is Ala or Gly, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(p) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 330 is Arg, and the amino acid at position 396 is Met or Leu, according to EU numbering, in an Fc region;

(q) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, the amino acid at position 327 is Gly, the amino acid at position 330 is Arg, and the amino acid at position 396 is Met, according to EU numbering, in an Fc region;

(r) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 272 is Asp, and the amino acid at position 296 is Asp, according to EU numbering, in an Fc region;

(s) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 272 is Pro, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(t) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 272 is Pro, the amino acid at position 296 is Asp, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(u) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, and the amino acid at position 271 is Gly, according to EU numbering, in an Fc region;

(v) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 237 is Asp, the amino acid at position 267 is Gly, the amino acid at position 268 is Asp, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(w) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 272 is Asp, and the amino acid at position 296 is Asp, according to EU numbering, in an Fc region; and

(x) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, and the amino acid at position 296 is Asp, according to EU numbering, in an Fc region.

7. An Fc region variant consisting of any one amino acid sequence selected from among SEQ ID NOs: 43 to 68, SEQ ID NO: 70, SEQ ID NO: 71, and SEQ ID NOs: 75 to 77.

8. A polypeptide comprising at least two Fc region variants of any one of claims 1 to 7, wherein the two Fc region variants are associated.

9. The polypeptide of claim 7, wherein the amino acid sequences of the two associated Fc region variants in the polypeptide are the same.

10. The polypeptide of claim 7, wherein the amino acid sequences of the two associated Fc region variants in the polypeptide are different.

11. The polypeptide of claim 10, wherein the amino acid sequences of the two associated Fc region variants have different amino acid(s) at at least one amino acid position selected from positions 235, 236, 237, 238, and 239 according to EU numbering in the Fc region variant.

12. The polypeptide of claim 11, wherein one of the amino acid sequences of the two associated Fc region variants is an amino acid sequence comprising at least one amino acid selected from Asp, Gln, Glu, or Thr at amino acid position 235, Asn at amino acid position 236, Phe or Trp at amino acid position 237, Glu, Gly, or Asn at amino acid position 238, and Asp or Glu at amino acid position 239 according to EU numbering.

13. The polypeptide of any one of claims 8 to 12, wherein the polypeptide comprising the Fc region variant is an IgG antibody.

14. The polypeptide of any one of claims 8 to 12, wherein the polypeptide comprising the Fc region variant is an Fc fusion protein molecule.

15. A pharmaceutical composition comprising the polypeptide of any one of claims 8 to 14.

CLAIMS

1. An Fc region variant in which amino acid at position 238 according to EU numbering and at least one amino acid selected from those at positions 233, 234, 235, 237, 264, 265, 266, 267, 268, 269, 271, 272, 274, 296, 326, 327, 330, 331, 332, 333, 334, 355, 356, 358, 396, 409, and 419 according to EU numbering are altered to other amino acids, wherein binding activity of the variant to Fcγ receptors [KD value for FcγRIIb of a polypeptide comprising an Fc region to which an amino acid alteration(s) has not been introduced]/[KD value for FcγRIIb of a polypeptide comprising the Fc region variant] has a value which is 15.0 or greater.

2. The Fc region variant of claim 1, wherein the amino acids at positions 238, 268, and 271 according to EU numbering are altered to other amino acids, and in addition, at least one amino acid selected from amino acids at positions 233, 237, 264, 267, 272, 296, 327, 330, 332, and 396 according to EU numbering are altered to other amino acids.

3. An Fc region variant whose amino acid at position 238 according to EU numbering is Asp, and which comprises at least one amino acid selected from the amino acid group consisting of Asp at amino acid position 233, Tyr at amino acid position 234, Phe at amino acid position 235, Asp at amino acid position 237, Ile at amino acid position 264, Glu at amino acid position 265, Phe, Leu, or Met at amino acid position 266, Ala, Glu, Gly, or Gln at amino acid position 267, Asp, Gln, or Glu at amino acid position 268, Asp at amino acid position 269, Gly at amino acid position 271, Asp, Phe, Ile, Met, Asn, Pro, or Gln at amino acid position 272, Gln at amino acid position 274, Asp or Phe at amino acid position 296, Ala or Asp at amino acid position 326, Gly at amino acid position 327, Lys, Arg, or Ser at amino acid position 330, Ser at amino acid position 331, Lys, Arg, Ser, or Thr at amino acid position 332, Lys, Arg, Ser, or Thr at amino acid position 333, Arg, Ser, or Thr at amino acid position 334, Ala or Gln at amino acid position 355, Glu at amino acid position 356, Met at amino acid position 358, Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Gln, Arg, Ser, Thr, Val, Trp, or Tyr at amino acid position 396, Arg at amino acid position 409, and Glu at amino acid position 419, wherein binding activity of the variant to Fcγ receptors [KD value for FcγRIIb of a polypeptide comprising an Fc region to which an amino acid alteration(s) has not been introduced]/[KD value for FcγRIIb of a polypeptide comprising the Fc region variant] has a value which is 15.0 or greater.

4. The Fc region variant of claim 3, wherein the amino acid at position 238 is Asp, the amino acid at position 268 is Asp or Glu, and the amino acid at position 271 is Gly, according to EU numbering, and wherein the Fc region variant further comprises at least one amino acid selected

from the amino acid group consisting of Asp at amino acid position 233, Asp at amino acid position 237, Ile at amino acid position 264, Ala or Gly at amino acid position 267, Asp or Pro at amino acid position 272, Asp at amino acid position 296, Gly at amino acid position 327, Arg at amino acid position330, Thr at amino acid position 332, and Leu or Met at amino acid position 396.

5. The Fc region variant of any one of claims 1 to 4, wherein the value for [KD value for FcγRIIb of a polypeptide comprising an Fc region to which an amino acid alteration(s) has not been introduced]/[KD value for FcγRIIb of a polypeptide comprising an Fc region variant] is 50.0 or more.

6. The Fc region variant of any one of claims 1 to 4, wherein the value for [KD value for FcγRIIb of a polypeptide comprising an Fc region to which an amino acid alteration(s) has not been introduced]/[KD value for FcγRIIb of a polypeptide comprising an Fc region variant] is 100.0 or more.

7. The Fc region variant of any one of claims 1 to 6, wherein the value for [KD value for FcγRIIa (type R) of a polypeptide comprising an Fc region variant]/[KD value for FcγRIIb of a polypeptide comprising an Fc region variant] is 10.0 or greater.

8. The Fc region variant of any one of claims 1 to 6, wherein the value for [KD value for FcγRIIa (type R) of a polypeptide comprising an Fc region variant]/[KD value for FcγRIIb of a polypeptide comprising an Fc region variant] is 20.0 or greater.

9. The Fc region variant of any one of claims 1 to 8, wherein the Fc region variant comprises any one of the following set of amino acid alterations of (a) to (x):
(a) amino acid alterations at positions 238, 233, 237, 268, 271, 296, and 330 (EU numbering) of an Fc region;
(b) amino acid alterations at positions 238, 237, 268, 271, 296, and 330 (EU numbering) of an Fc region;
(c) amino acid alterations at positions 238, 233, 237, 268, 271, 296, 330, and 332 (EU numbering) of an Fc region;
(d) amino acid alterations at positions 238, 233, 237, 264, 267, 268, 271, and 330 (EU numbering) of an Fc region;
(e) amino acid alterations at positions 238, 233, 237, 267, 268, 271, 296, 330, and 332 (EU numbering) of an Fc region;

(f) amino acid alterations at positions 238, 237, 267, 268, 271, 296, 330, and 332 (EU numbering) of an Fc region;

(g) amino acid alterations at positions 238, 233, 237, 268, 271, 296, 327, and 330 (EU numbering) of an Fc region;

(h) amino acid alterations at positions 238, 233, 237, 264, 267, 268, and 271 (EU numbering) of an Fc region;

(i) amino acid alterations at positions 238, 233, 237, 264, 267, 268, 271, 296, and 330 (EU numbering) of an Fc region;

(j) amino acid alterations at positions 238, 233, 237, 264, 267, 268, 271, 296, 330, and 396 (EU numbering) of an Fc region;

(k) amino acid alterations at positions 238, 237, 264, 267, 268, 271, and 330 (EU numbering) of an Fc region;

(l) amino acid alterations at positions 238, 237, 264, 267, 268, 271, 296, and 330 (EU numbering) of an Fc region;

(m) amino acid alterations at positions 238, 264, 267, 268, and 271 (EU numbering) of an Fc region;

(n) amino acid alterations at positions 238, 264, 267, 268, 271, and 296 (EU numbering) of an Fc region;

(o) amino acid alterations at positions 238, 237, 267, 268, 271, 296, and 330 (EU numbering) of an Fc region;

(p) amino acid alterations at positions 238, 233, 237, 264, 267, 268, 271, 330, and 396 (EU numbering) of the Fc region;

(q) amino acid alterations at positions 238, 233, 237, 264, 267, 268, 271, 296, 327, 330, and 396 (EU numbering) of an Fc region;

(r) amino acid alterations at positions 238, 233, 237, 264, 267, 268, 271, 272, and 296 (EU numbering) of an Fc region;

(s) amino acid alterations at positions 238, 237, 264, 267, 268, 271, 272, and 330 (EU numbering) of an Fc region;

(t) amino acid alterations at positions 238, 237, 264, 267, 268, 271, 272, 296, and 330 (EU numbering) of an Fc region;

(u) amino acid alterations at positions 238, 233, 264, 267, 268, and 271 (EU numbering) of an Fc region;

(v) amino acid alterations at positions 238, 237, 267, 268, 271, 296, and 330 (EU numbering) of an Fc region;

(w) amino acid alterations at positions 238, 264, 267, 268, 271, 272, and 296 (EU numbering) of an Fc region; and

(x) amino acid alterations at positions 238, 233, 264, 267, 268, 271, and 296 (EU numbering) of an Fc region.

10. The Fc region variant of any one of claims 1 to 8, wherein the Fc region variant comprises any one of the following amino acid sequences of (a) to (x):

(a) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 268 is Asp, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(b) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 237 is Asp, the amino acid at position 268 is Asp or Glu, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(c) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 268 is Asp, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, the amino acid at position 330 is Arg, and the amino acid at position 332 is Thr, according to EU numbering, in an Fc region;

(d) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Gly or Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(e) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, the amino acid at position 330 is Arg, and the amino acid at position 332 is Thr, according to EU numbering, in an Fc region;

(f) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 237 is Asp, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, the amino acid at position 330 is Arg, and the amino acid at position 332 is Thr, according to EU numbering, in an Fc region;

(g) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 268 is Asp, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, the amino acid at

position 327 is Gly, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(h) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, and the amino acid at position 271 is Gly, according to EU numbering, in an Fc region;

(i) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(j) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, the amino acid at position 330 is Arg, and the amino acid at position 396 is Met or Leu, according to EU numbering, in an Fc region;

(k) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(l) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(m) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, and the amino acid at position 271 is Gly, according to EU numbering, in an Fc region;

(n) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, and the amino acid at position 296 is Asp, according to EU numbering, in an Fc region;

(o) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 237 is Asp, the amino acid at position 267 is Ala or Gly, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(p) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 330 is Arg, and the amino acid at position 396 is Met or Leu, according to EU numbering, in an Fc region;

(q) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, the amino acid at position 327 is Gly, the amino acid at position 330 is Arg, and the amino acid at position 396 is Met, according to EU numbering, in an Fc region;

(r) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 272 is Asp, and the amino acid at position 296 is Asp, according to EU numbering, in an Fc region;

(s) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 272 is Pro, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(t) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 237 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, the amino acid at position 272 is Pro, the amino acid at position 296 is Asp, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(u) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, and the amino acid at position 271 is Gly, according to EU numbering, in an Fc region;

(v) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 237 is Asp, the amino acid at position 267 is Gly, the amino acid at position 268 is Asp, the amino acid at position 271 is Gly, the amino acid at position 296 is Asp, and the amino acid at position 330 is Arg, according to EU numbering, in an Fc region;

(w) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu,

the amino acid at position 271 is Gly, the amino acid at position 272 is Asp, and the amino acid at position 296 is Asp, according to EU numbering, in an Fc region; and

(x) an amino acid sequence in which the amino acid at position 238 is Asp, the amino acid at position 233 is Asp, the amino acid at position 264 is Ile, the amino acid at position 267 is Ala, the amino acid at position 268 is Glu, the amino acid at position 271 is Gly, and the amino acid at position 296 is Asp, according to EU numbering, in an Fc region.

11. An Fc region variant consisting of any one amino acid sequence selected from among SEQ ID NOs: 43 to 68, SEQ ID NO: 70, SEQ ID NO: 71, and SEQ ID NOs: 75 to 77.

12. A polypeptide comprising at least two Fc region variants of any one of claims 1 to 11, wherein the two Fc region variants are associated.

13. The polypeptide of claim 12, wherein the amino acid sequences of the two associated Fc region variants in the polypeptide are the same.

14. The polypeptide of claim 12, wherein the amino acid sequences of the two associated Fc region variants in the polypeptide are different.

15. The polypeptide of claim 14, wherein the amino acid sequences of the two associated Fc region variants have different amino acid(s) at at least one amino acid position selected from positions 235, 236, 237, 238, and 239 according to EU numbering in the Fc region variant.

16. The polypeptide of claim 15, wherein one of the amino acid sequences of the two associated Fc region variants is an amino acid sequence comprising at least one amino acid selected from Asp, Gln, Glu, or Thr at amino acid position 235, Asn at amino acid position 236, Phe or Trp at amino acid position 237, Glu, Gly, or Asn at amino acid position 238, and Asp or Glu at amino acid position 239 according to EU numbering.

17. The polypeptide of any one of claims 12 to 16, wherein the polypeptide comprising the Fc region variant is an IgG antibody.

18. The polypeptide of any one of claims 12 to 16, wherein the polypeptide comprising the Fc region variant is an Fc fusion protein molecule.

19. A pharmaceutical composition comprising the polypeptide of any one of claims 12 to 18.