DESCRIPTION

ANTIGEN-BINDING MOLECULE FOR PROMOTING ELIMINATION OF ANTIGENS

Technical Field

The present invention provides antigen-binding molecules with enhanced intracellular uptake of a bound antigen, antigen-binding molecules in which the number of antigens that can be bound per single molecule is increased, antigen-binding molecules with improved pharmacokinetics, antigen-binding molecules with enchanced intracellular dissociation of extracellularly bound antigen, antigen-binding molecules with enhanced extracellular release in the antigen-unbound state, antigen-binding molecules having the function of decreasing the total antigen concentration or the free antigen concentration in plasma, pharmaceutical compositions comprising such an antigen-binding molecules, and methods for producing them.

Background Art

Antibodies are drawing attention as pharmaceuticals as they are highly stable in plasma and have few side effects. A number of IgG-type antibody pharmaceuticals are now available on the market and many antibody pharmaceuticals are currently under development (Non-patent Documents 1 and 2). Meanwhile, various technologies applicable to second-generation antibody pharmaceuticals have been reported, including those that enhance effector function, antigen-binding ability, pharmacokinetics and stability, and those that reduce the risk of immunogenicity (Non-patent Document 3). In general, the requisite dose of an antibody pharmaceutical is very high. This in turn has led to problems such as high production cost as well as difficulty in producing subcutaneous formulations. In theory, the dose of an antibody pharmaceutical may be reduced by improving antibody pharmacokinetics or improving the affinity between antibodies and antigens.

Literature has reported methods for improving antibody pharmacokinetics using artificial substitution of amino acids in constant regions (Non-patent Documents 4 and 5). Similarly, affinity maturation has been reported as a technology for enhancing antigen-binding ability or antigen-neutralizing activity (Non-patent Document 6). This technology enables enhancement of antigen-binding activity by introduction of amino acid mutations into the CDR of a variable region or such. The enhancement of antigen-binding ability enables improvement of *in vitro* biological activity or reduction of dosage, and further enables improvement of *in vivo* efficacy (Non-patent Document 7).

The antigen-neutralizing capacity of a single antibody molecule depends on its affinity.

[33] The method of [29] or [30], wherein the ion concentration condition is a pH condition.

[34] The method of [33], wherein the antigen-binding domain is an antigen-binding domain in which binding activity to the antigen in a neutral pH range condition is higher than that in an acidic pH range condition.

[35] The method of any one of [29] to [34], wherein the antigen-binding molecule has neutralizing activity against the antigen.

[36] The method of any one of [29] to [35], wherein the Fcγ receptor-binding domain comprises an antibody Fc region.

[37] The method of [36], wherein the Fc region is an Fc region in which at least one or more amino acids selected from the group consisting of amino acids at positions 221, 222, 223, 224, 225, 227, 228, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 243, 244, 245, 246, 247, 249, 250, 251, 254, 255, 256, 258, 260, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 278, 279, 280, 281, 282, 283, 284, 285, 286, 288, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 311, 313, 315, 317, 318, 320, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 339, 376, 377, 378, 379, 380, 382, 385, 392, 396, 421, 427, 428, 429, 434, 436, and 440 in the Fc region site according to EU numbering are different from the amino acids at corresponding sites in the native Fc region.

[38] The method of [33], wherein the Fc region is an Fc region comprising at least one or more amino acids selected from the group consisting of:

either Lys or Tyr at amino acid position 221;

any one of Phe, Trp, Glu, and Tyr at amino acid position 222;

any one of Phe, Trp, Glu, and Lys at amino acid position 223;

any one of Phe, Trp, Glu, and Tyr at amino acid position 224;

any one of Glu, Lys, and Trp at amino acid position 225;

any one of Glu, Gly, Lys, and Tyr at amino acid position 227;

any one of Glu, Gly, Lys, and Tyr at amino acid position 228;

any one of Ala, Glu, Gly, and Tyr at amino acid position 230;

any one of Glu, Gly, Lys, Pro, and Tyr at amino acid position 231;

any one of Glu, Gly, Lys, and Tyr at amino acid position 232;

any one of Ala, Asp, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Gln, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 233;

any one of Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 234;

any one of Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 235;

any one of Ala, Asp, Glu, Phe, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr at

ID NO: 42) as the light chain, were prepared by the method described in Reference Example 2. These antibodies and VH3/L (WT)-IgG1-F1022 were assessed for their mouse FcγR-binding activity by the method described in Reference Example 2. The result is shown in Table 8. In addition, the ratio of the increase in the mouse FcγR-binding activity of each variant relative to the IgG1 before alteration is shown in Table 9.

[Table 8]

VARIANT NAME	KD (M)			
	mFc γ RI	mFc γ RIIb	mFc γ RIII	mFc γ RIV
IgG1	5.3E-08	9.8E-07	2.4E-06	8.6E-08
F1022	7.6E-09	1.0E-08	5.5E-09	1.4E-07
F1087	2.9E-08	5.6E-08	5.2E-08	3.3E-07
F1182	2.4E-09	1.1E-07	4.8E-07	5.3E-10

[Table 9]

VARIANT NAME	RATIO OF BINDING TO mIgG1			
	mFc γ RI	mFc γ RIIb	mFc γ RIII	mFc γ RIV
IgG1	1.0	1.0	1.0	1.0
F1022	7.0	93.6	440.5	0.6
F1087	1.8	17.5	46.2	0.3
F1182	22.1	9.1	5.0	162.3

As shown in Table 9, it was demonstrated that F1087 and F1022 had increased binding activity to mouse FcγRI, mouse FcγRIIb, and mouse FcγRIII as compared to IgG1, whereas their mouse FcγRIV-binding activity was not increased. Regarding the binding activity of F1087 to mouse FcγRI, mouse FcγRIIb, mouse FcγRIII, and mouse FcγRIV, the extent of its increase was revealed to be smaller than that of F1022. Meanwhile, it was shown that the binding activity of F1182 to mouse FcγRI and mouse FcγRIV was considerably increased, whereas the extent of increase in its binding activity to FcγRIIb and FcγRIII was smaller than those of F1022 and F1087. As mentioned above, these three types of variants showed enhanced binding to some mouse FcγRs; however, it was shown that the FcγR to which the binding activity is selectively increased and the extent of the increase vary depending on the variant.

(4-4) The effect of eliminating antigens from the plasma in an individual administered with Fv4-IgG1-F1087 and Fv4-IgG1-F1182

Claims

[Claim 1]

A pharmaceutical composition which comprises an antigen-binding molecule having human-FcRn-binding activity in an acidic pH range condition and comprising an antigen-binding domain whose antigen-binding activity changes depending on an ion-concentration condition and an Fcγ-receptor-binding domain having higher binding activity to the Fcγ receptor in a neutral pH range condition than that of an Fc region of a native human IgG in which the sugar chain bound at position 297 according to EU numbering is a fucose-containing sugar chain, wherein the antigen-binding activity of the antigen-binding domain that changes depending on an ion-concentration condition is defined by either one or both of the following (a) and (b):

(a) the value of KD (Ca 3μM) / KD (Ca 2 mM), which is the ratio of the KD for an antigen at a low-calcium-ion concentration condition to the KD at a high-calcium-ion concentration condition, is 2 or more; and

(b) the value of KD (pH 5.8) / KD (pH 7.4), which is the ratio of the KD for the antigen in an acidic pH range condition to the KD in a neutral pH range condition, is 2 or more.

[Claim 2]

The pharmaceutical composition of claim 1, wherein the antigen is a soluble antigen.

[Claim 3]

The pharmaceutical composition of claim 1 or 2, wherein the antigen-binding molecule has neutralizing activity against the antigen.

[Claim 4]

The pharmaceutical composition of any one of claims 1 to 3, wherein the Fcγ receptor-binding domain comprises an antibody Fc region.

[Claim 5]

The pharmaceutical composition of claim 4, wherein the Fc region is an Fc region in which at least one or more amino acids selected from the group consisting of amino acids at positions 221, 222, 223, 224, 225, 227, 228, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 243, 244, 245, 246, 247, 249, 250, 251, 254, 255, 256, 258, 260, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 278, 279, 280, 281, 282, 283, 284, 285, 286, 288, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 311, 313, 315, 317, 318, 320, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 339, 376, 377, 378, 379, 380, 382, 385, 392, 396, 421, 427, 428, 429, 434, 436, and 440 in the Fc region site according to EU numbering are different from amino acids at corresponding sites in a native Fc region.

[Claim 6]

The pharmaceutical composition of claim 4 or 5, wherein the Fc region is an Fc region which comprises at least one or more amino acids selected from the group consisting of:

either Lys or Tyr at amino acid position 221;

any one of Phe, Trp, Glu, and Tyr at amino acid position 222;

any one of Phe, Trp, Glu, and Lys at amino acid position 223;

any one of Phe, Trp, Glu, and Tyr at amino acid position 224;

any one of Glu, Lys, and Trp at amino acid position 225;

any one of Glu, Gly, Lys, and Tyr at amino acid position 227;

any one of Glu, Gly, Lys, and Tyr at amino acid position 228;

any one of Ala, Glu, Gly, and Tyr at amino acid position 230;

any one of Glu, Gly, Lys, Pro, and Tyr at amino acid position 231;

any one of Glu, Gly, Lys, and Tyr at amino acid position 232;

any one of Ala, Asp, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Gln, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 233;

any one of Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 234;

any one of Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 235;

any one of Ala, Asp, Glu, Phe, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 236;

any one of Asp, Glu, Phe, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 237;

any one of Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Gln, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 238;

any one of Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Thr, Val, Trp, and Tyr at amino acid position 239;

any one of Ala, Ile, Met, and Thr at amino acid position 240;

any one of Asp, Glu, Leu, Arg, Trp, and Tyr at amino acid position 241;

any one of Leu, Glu, Leu, Gln, Arg, Trp, and Tyr at amino acid position 243;

His at amino acid position 244;

Ala at amino acid position 245;

any one of Asp, Glu, His, and Tyr at amino acid position 246;

any one of Ala, Phe, Gly, His, Ile, Leu, Met, Thr, Val, and Tyr at amino acid position 247;

any one of Glu, His, Gln, and Tyr at amino acid position 249;

either Glu or Gln at amino acid position 250;
Phe at amino acid position 251;
any one of Phe, Met, and Tyr at amino acid position 254;
any one of Glu, Leu, and Tyr at amino acid position 255;
any one of Ala, Met, and Pro at amino acid position 256;
any one of Asp, Glu, His, Ser, and Tyr at amino acid position 258;
any one of Asp, Glu, His, and Tyr at amino acid position 260;
any one of Ala, Glu, Phe, Ile, and Thr at amino acid position 262;
any one of Ala, Ile, Met, and Thr at amino acid position 263;
any one of Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Trp, and Tyr at amino acid position 264;
any one of Ala, Leu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 265;
any one of Ala, Ile, Met, and Thr at amino acid position 266;
any one of Asp, Glu, Phe, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Thr, Val, Trp, and Tyr at amino acid position 267;
any one of Asp, Glu, Phe, Gly, Ile, Lys, Leu, Met, Pro, Gln, Arg, Thr, Val, and Trp at amino acid position 268;
any one of Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 269;
any one of Glu, Phe, Gly, His, Ile, Leu, Met, Pro, Gln, Arg, Ser, Thr, Trp, and Tyr at amino acid position 270;
any one of Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Gln, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 271;
any one of Asp, Phe, Gly, His, Ile, Lys, Leu, Met, Pro, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 272;
either Phe or Ile at amino acid position 273;
any one of Asp, Glu, Phe, Gly, His, Ile, Leu, Met, Asn, Pro, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 274;
either Leu or Trp at amino acid position 275;
any one of Asp, Glu, Phe, Gly, His, Ile, Leu, Met, Pro, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 276;
any one of Asp, Glu, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, and Trp at amino acid position 278;
Ala at amino acid position 279;
any one of Ala, Gly, His, Lys, Leu, Pro, Gln, Trp, and Tyr at amino acid position 280;

any one of Asp, Lys, Pro, and Tyr at amino acid position 281;
any one of Glu, Gly, Lys, Pro, and Tyr at amino acid position 282;
any one of Ala, Gly, His, Ile, Lys, Leu, Met, Pro, Arg, and Tyr at amino acid position 283;
any one of Asp, Glu, Leu, Asn, Thr, and Tyr at amino acid position 284;
any one of Asp, Glu, Lys, Gln, Trp, and Tyr at amino acid position 285;
any one of Glu, Gly, Pro, and Tyr at amino acid position 286;
any one of Asn, Asp, Glu, and Tyr at amino acid position 288;
any one of Asp, Gly, His, Leu, Asn, Ser, Thr, Trp, and Tyr at amino acid position 290;
any one of Asp, Glu, Gly, His, Ile, Gln, and Thr at amino acid position 291;
any one of Ala, Asp, Glu, Pro, Thr, and Tyr at amino acid position 292;
any one of Phe, Gly, His, Ile, Leu, Met, Asn, Pro, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 293;
any one of Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 294;
any one of Asp, Glu, Phe, Gly, His, Ile, Lys, Met, Asn, Pro, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 295;
any one of Ala, Asp, Glu, Gly, His, Ile, Lys, Leu, Met, Asn, Gln, Arg, Ser, Thr, and Val at amino acid position 296;
any one of Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 297;
any one of Ala, Asp, Glu, Phe, His, Ile, Lys, Met, Asn, Gln, Arg, Thr, Val, Trp, and Tyr at amino acid position 298;
any one of Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Val, Trp, and Tyr at amino acid position 299;
any one of Ala, Asp, Glu, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, and Trp at amino acid position 300;
any one of Asp, Glu, His, and Tyr at amino acid position 301;
Ile at amino acid position 302;
any one of Asp, Gly, and Tyr at amino acid position 303;
any one of Asp, His, Leu, Asn, and Thr at amino acid position 304;
any one of Glu, Ile, Thr, and Tyr at amino acid position 305;
any one of Ala, Asp, Asn, Thr, Val, and Tyr at amino acid position 311;
Phe at amino acid position 313;
Leu at amino acid position 315;
either Glu or Gln at amino acid position 317;
any one of His, Leu, Asn, Pro, Gln, Arg, Thr, Val, and Tyr at amino acid position 318;

any one of Asp, Phe, Gly, His, Ile, Leu, Asn, Pro, Ser, Thr, Val, Trp, and Tyr at amino acid position 320;
any one of Ala, Asp, Phe, Gly, His, Ile, Pro, Ser, Thr, Val, Trp, and Tyr at amino acid position 322;
Ile at amino acid position 323;
any one of Asp, Phe, Gly, His, Ile, Leu, Met, Pro, Arg, Thr, Val, Trp, and Tyr at amino acid position 324;
any one of Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 325;
any one of Ala, Asp, Glu, Gly, Ile, Leu, Met, Asn, Pro, Gln, Ser, Thr, Val, Trp, and Tyr at amino acid position 326;
any one of Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Arg, Thr, Val, Trp, and Tyr at amino acid position 327;
any one of Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 328;
any one of Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Gln, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 329;
any one of Cys, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 330;
any one of Asp, Phe, His, Ile, Leu, Met, Gln, Arg, Thr, Val, Trp, and Tyr at amino acid position 331;
any one of Ala, Asp, Glu, Phe, Gly, His, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr at amino acid position 332;
any one of Ala, Asp, Glu, Phe, Gly, His, Ile, Leu, Met, Pro, Ser, Thr, Val, and Tyr at amino acid position 333;
any one of Ala, Glu, Phe, Ile, Leu, Pro, and Thr at amino acid position 334;
any one of Asp, Phe, Gly, His, Ile, Leu, Met, Asn, Pro, Arg, Ser, Val, Trp, and Tyr at amino acid position 335;
any one of Glu, Lys, and Tyr at amino acid position 336;
any one of Glu, His, and Asn at amino acid position 337;
any one of Asp, Phe, Gly, Ile, Lys, Met, Asn, Gln, Arg, Ser, and Thr at amino acid position 339;
either Ala or Val at amino acid position 376;
either Gly or Lys at amino acid position 377;
Asp at amino acid position 378;
Asn at amino acid position 379;

any one of Ala, Asn, and Ser at amino acid position 380;
either Ala or Ile at amino acid position 382;
Glu at amino acid position 385;
Thr at amino acid position 392;
Leu at amino acid position 396;
Lys at amino acid position 421;
Asn at amino acid position 427;
either Phe or Leu at amino acid position 428;
Met at amino acid position 429;
Trp at amino acid position 434;
Ile at amino acid position 436; and
any one of Gly, His, Ile, Leu, and Tyr at amino acid position 440,
in the Fc region site according to EU numbering.

[Claim 7]

The pharmaceutical composition of any one of claims 1 to 6, wherein the Fc region of a native human IgG in which the sugar chain bound at position 297 according to EU numbering is a fucose-containing sugar chain, is an Fc region of any one of native human IgG1, native human IgG2, native human IgG3, and native human IgG4 in which the sugar chain bound at position 297 according to EU numbering is a fucose-containing sugar chain.

[Claim 8]

The pharmaceutical composition of any one of claims 1 to 7, wherein the human Fcγ receptor is FcγRIa, FcγRIIa(R), FcγRIIa(H), FcγRIIb, FcγRIIIa(V), or FcγRIIIa(F).

[Claim 9]

The pharmaceutical composition of any one of claims 1 to 8, wherein the human Fcγ receptor is FcγRIIb.

[Claim 10]

A method comprising the step of contacting an antigen-binding molecule with an Fcγ-receptor-expressing cell *ex vivo*, wherein the antigen-binding molecule has human-FcRn-binding activity in an acidic pH range condition and comprises an antigen-binding domain whose antigen-binding activity changes depending on the ion concentration condition and an Fcγ-receptor-binding domain that has higher binding activity to the Fcγ receptor in a neutral pH range condition compared to an Fc region of a native human IgG in which the sugar chain bound at position 297 according to EU numbering is a fucose-containing sugar chain, wherein the antigen-binding activity of the antigen-binding domain that changes depending on an ion-concentration condition is defined by either one or both of the following (a) and (b):

(a) the value of KD (Ca 3μM) / KD (Ca 2 mM), which is the ratio of the KD for an antigen at a low-calcium-ion concentration condition to the KD at a high-calcium-ion concentration condition, is 2 or more; and

(b) the value of KD (pH 5.8) / KD (pH 7.4), which is the ratio of the KD for the antigen in an acidic pH range condition to the KD in a neutral pH range condition, is 2 or more,

wherein the method is a method of any one of the following (i) to (vi):

(i) a method for increasing the number of antigens that can bind to a single antigen-binding molecule;

(ii) a method for eliminating plasma antigens;

(iii) a method for improving antigen-binding molecule pharmacokinetics;

(iv) a method for promoting intracellular dissociation of an antigen from an antigen-binding molecule, wherein the antigen has been extracellularly bound to the antigen-binding molecule;

(v) a method for promoting extracellular release of an antigen-binding molecule not bound to an antigen; and

(vi) a method for decreasing a total antigen concentration or free antigen concentration in plasma.

[Claim 11]

An *ex vivo* method comprising the step of amino acid alteration for enhancing Fcγ-receptor-binding activity in a neutral pH range condition of the Fcγ-receptor-binding domain in an antigen-binding molecule compared to that of a native human IgG Fc region in which the sugar chain bound at position 297 according to EU numbering is a fucose-containing sugar chain, wherein the antigen-binding molecule has human-FcRn-binding activity in an acidic pH range condition and comprises an Fcγ receptor-binding domain and an antigen-binding domain whose antigen-binding activity changes depending on the ion concentration condition, wherein the antigen-binding activity of the antigen-binding domain that changes depending on an ion-concentration condition is defined by either one or both of the following (a) and (b):

(a) the value of KD (Ca 3μM) / KD (Ca 2 mM), which is the ratio of the KD for an antigen at a low-calcium-ion concentration condition to the KD at a high-calcium-ion concentration condition, is 2 or more; and

(b) the value of KD (pH 5.8) / KD (pH 7.4), which is the ratio of the KD for the antigen in an acidic pH range condition to the KD in a neutral pH range condition, is 2 or more,

wherein the method is for the purpose of any one of the following (i) to (vii):

(i) for enhancing the intracellular uptake of the antigen to be bound;

(ii) for increasing the number of antigens that can bind to a single antigen-binding molecule;

(iii) for increasing the ability of an antigen-binding molecule to eliminate plasma antigens;
(iv) for improving antigen-binding molecule pharmacokinetics;
(v) for promoting intracellular dissociation of an antigen from an antigen-binding molecule, wherein the antigen has been extracellularly bound to the antigen-binding molecule;
(vi) for promoting extracellular release of an antigen-binding molecule not bound to an antigen, wherein the antigen-binding molecule has been taken up into a cell in an antigen-bound form; and
(vii) for decreasing a total antigen concentration or free antigen concentration in plasma.
[Claim 12]
An *ex vivo* method for producing an antigen-binding molecule, which comprises the steps of the following (a) to (f):
(a) determining the antigen-binding activity of an antigen-binding domain at a high-calcium-ion concentration condition;
(b) determining the antigen-binding activity of an antigen-binding domain at a low-calcium-ion concentration condition;
(c) selecting the antigen-binding domain for which antigen-binding activity determined in (a) is higher than the antigen-binding activity determined in (b);
(d) linking a polynucleotide encoding the antigen-binding domain selected in (c) to a polynucleotide encoding an Fcγ receptor-binding domain having human-FcRn-binding activity in an acidic pH range condition and having higher binding activity to the Fcγ receptor in a neutral pH range condition than that of an Fc region of a native human IgG in which the sugar chain bound at position 297 according to EU numbering is a fucose-containing sugar chain;
(e) culturing cells introduced with a vector in which the polynucleotide obtained in (d) is operably linked; and
(f) collecting antigen-binding molecules from the cell culture of (e),
wherein the high-calcium-ion concentration condition is between 100 μM and 10 mM and the low-calcium ion-concentration condition is between 0.1 μM and 30 μM.
[Claim 13]
An *ex vivo* method for producing an antigen-binding molecule, which comprises the steps of the following (a) to (f):
(a) determining the antigen-binding activity of an antibody at a high-calcium-ion concentration condition;
(b) determining the antigen-binding activity of an antibody at a low-calcium-ion concentration condition;
(c) selecting the antibody for which antigen-binding activity determined in (a) is higher than the antigen-binding activity determined in (b);

(d) linking a polynucleotide encoding the antigen-binding domain of the antibody selected in (c) to a polynucleotide encoding an Fcγ receptor-binding domain having human-FcRn-binding activity in an acidic pH range and having higher binding activity to the Fcγ receptor in a neutral pH range condition than that of an Fc region of a native human IgG in which the sugar chain bound at position 297 according to EU numbering is a fucose-containing sugar chain;

(e) culturing cells introduced with a vector in which the polynucleotide obtained in (d) is operably linked; and

(f) collecting antigen-binding molecules from the cell culture of (e),

wherein the high-calcium-ion concentration condition is between 100 μM and 10 mM and the low-calcium-ion concentration condition is between 0.1 μM and 30 μM.

[Claim 14]

An *ex vivo* method for producing an antigen-binding molecule, which comprises the steps of the following (a) to (f):

(a) determining the antigen-binding activity of an antigen-binding domain in a neutral pH range condition;

(b) determining the antigen-binding activity of an antigen-binding domain in an acidic pH range condition;

(c) selecting the antigen-binding domain for which antigen-binding activity determined in (a) is higher than the antigen-binding activity determined in (b);

(d) linking a polynucleotide encoding the antigen-binding domain selected in (c) to a polynucleotide encoding an Fcγ receptor-binding domain having human-FcRn-binding activity in an acidic pH range condition and having higher binding activity to the Fcγ receptor in a neutral pH range condition than that of an Fc region of a native human IgG in which the sugar chain bound at position 297 according to EU numbering is a fucose-containing sugar chain;

(e) culturing cells introduced with a vector in which the polynucleotide obtained in (d) is operably linked; and

(f) collecting antigen-binding molecules from the cell culture of (e),

wherein the neutral pH range condition is between pH 6.7 and pH 10.0 and the acidic pH range condition is between pH 4.0 and pH 6.5.

[Claim 15]

An *ex vivo* method for producing an antigen-binding molecule, which comprises the steps of the following (a) to (f):

(a) determining the antigen-binding activity of an antibody in a neutral pH range condition;

(b) determining the antigen-binding activity of an antibody in an acidic pH range condition;

(c) selecting the antibody for which antigen-binding activity determined in (a) is higher than the antigen-binding activity determined in (b);

(d) linking a polynucleotide encoding the antigen-binding domain of the antibody selected in (c) to a polynucleotide encoding an Fcγ receptor-binding domain having human-FcRn-binding activity in an acidic pH range condition and having higher binding activity to the Fcγ receptor in a neutral pH range condition than that of an Fc region of a native human IgG in which the sugar chain bound at position 297 according to EU numbering is a fucose-containing sugar chain;

(e) culturing cells introduced with a vector in which the polynucleotide obtained in (d) is operably linked; and

(f) collecting antigen-binding molecules from the cell culture of (e),

wherein the neutral pH range condition is between pH 6.7 and pH 10.0 and the acidic pH range condition is between pH 4.0 and pH 6.5.