plasma concentration of antigens bound by the antigen-binding molecules.

[Means for Solving the Problems]

Specifically, the present invention provides antigen-binding molecules comprising (i) an antigen-binding domain whose antigen-binding activity varies depending on ion concentration conditions, (ii) an Fcγ-binding domain having FcγRIIb-selective binding activity, and (iii) an FcRn-binding domain having FcRn-binding activity under an acidic pH range condition, and methods for decreasing plasma concentration of the antigen as compared to before administering the antigen-binding molecule, which comprises the step of administering the molecule. Furthermore, the present invention provides agents for decreasing plasma concentration of the antigen, which comprise an antigen-binding molecule comprising (i) an antigen-binding domain whose antigen-binding activity varies depending on ion concentration conditions, (ii) an Fcγ-binding domain having FcγRIIb-selective binding activity, and (iii) an FcRn-binding domain having FcRn-binding activity under an acidic pH range condition. The present invention also provides pharmaceutical compositions which comprise an antigen-binding molecule comprising (i) an antigen-binding domain whose antigen-binding activity varies depending on ion concentration conditions, (ii) an Fcγ-binding domain having FcγRIIb-selective binding activity, and (iii) an FcRn-binding domain having FcRn-binding activity under an acidic pH range condition. The present invention also provides uses of the antigen-binding molecule for decreasing plasma concentration of the antigen, wherein the molecule comprises (i) an antigen-binding domain whose antigen-binding activity varies depending on ion concentration conditions, (ii) an Fcγ-binding domain having FcγRIIb-selective binding activity, and (iii) an FcRn-binding domain having FcRn-binding activity under an acidic pH range condition. In addition to the above, the present invention provides methods of producing and/or methods of screening for the antigen-binding molecules. Although it is not particularly intended to limit the invention, specifically, the following is provided as a non-limiting embodiment:

[1] use of an antigen-binding molecule for eliminating antigen from plasma, wherein the antigen-binding molecule comprises an antigen-binding domain whose antigen-binding activity varies depending on ion concentration conditions, and an Fc region in which the amino acid at position 238 is Asp and the amino acid at position 271 is Gly as indicated by EU numbering;

[2] the use of [1], wherein the Fc region has an amino acid substitution at at least one or more positions selected from the group consisting of 233, 234, 237, 264, 265, 266, 267, 268, 269, 272, 274, 296, 326, 327, 330, 331, 332, 333, 355, 356, 358, 396, 409, and 419 as indicated by EU numbering;

[3] the use of [2], wherein the amino acids of the Fc region include any one or more of the following amino acids indicated by EU numbering:

those having antibody heavy-chain and light-chain variable regions. Preferred examples of antigen-binding domains include "single chain Fv (scFv)", "single chain antibody", "Fv", "single chain Fv 2 (scFv2)", "Fab", and "F(ab')2".

The antigen-binding domains of antigen-binding molecules of the present invention can bind to an identical epitope. Such epitope can be present, for example, in a protein comprising the amino acid sequence of SEQ ID NO: 1. Alternatively, each of the antigen-binding domains of antigen-binding molecules of the present invention can bind to a different epitope. Herein, the different epitope can be present in, for example, a protein comprising the amino acid sequence of SEQ ID NO: 1.

Specific

With regard to binding of antigen-binding molecules provided by the present invention to an antigen, the term "specific" means that one of the molecules that specifically binds to does not substantially bind to molecules other than its single or plurality of binding partner molecule(s). Herein, "does not substantially bind" refers to showing 80% or less, generally 50% or less, preferably 30% or less and particularly preferably 15% or less binding activity to molecules other than the binding partner molecules compared to the binding activity towards the partner molecule(s), as described in the above-mentioned section on binding activity. Furthermore, "specific" is also used when an antigen-binding domain is specific to a particular epitope among multiple epitopes in an antigen. When an epitope bound by an antigen-binding domain is contained in multiple different antigens, antigen-binding molecules containing the antigen-binding domain can bind to various antigens that have the epitope.

Neutralizing activity

In a non-limiting embodiment of the present invention, a pharmaceutical composition comprising as an active ingredient an antigen-binding molecule having antigen-neutralizing activity is provided, wherein the antigen-binding molecule comprises (i) an antigen-binding domain whose antigen-binding activity varies depending on ion concentration conditions, (ii) an Fcγ-binding domain having FcγRIIb-selective binding activity, and (iii) an FcRn-binding domain having FcRn-binding activity under an acidic pH range condition. Generally, neutralizing activity refers to activity of inhibiting the biological activity of a ligand, such as viruses and toxins, having biological activity on cells. Thus, substances having neutralizing activity refer to substances that bind to the ligand or the receptor to which the ligand binds, and inhibits the binding between the ligand and the receptor. Receptors blocked from binding with the ligand by the neutralizing activity will not be able to exhibit biological activity through this receptor. When the antigen-binding molecule is an antibody, such an antibody having neutralizing activity

structure of the Fc (WT) / FcγRIIb extracellular region complex, pairs of atoms that have a distance of 3.7 Å or less between the extracted FcγRIIb extracellular region and Fc CH2 domain B were compared for comparison of the interatomic interaction between FcγRIIb and Fc (WT) CH2 domain B with the interatomic interaction between FcγRIIb and Fc (P238D) CH2 domain B. As shown in Table 15, the interatomic interactions between Fc CH2 domain B and FcγRIIb in Fc (P238D) and Fc (WT) did not match.

[Table 15]

FcγRIIb ATOM			Fc (P648D) CH2 DOMAIN B INTERACTION PARTNER (DISTANCE BETWEEN ATOMS, Å)				Fc (WT) CH2 DOMAIN B INTERACTION PARTNER (DISTANCE BETWEEN ATOMS, Å)			
Val	116	CG2					Asp	265	OD2	(3.47)
							Gly	237	O	(3.65)
Ser	126	OG	Ser	298	N	(3.31)				
			Ser	298	CB	(3.32)				
			Tyr	296	O	(3.05)				
Lys	128	CA	Ser	298	OG	(3.50)				
Phe	129	CB	Ser	298	O	(3.36)				
Phe	129	CD2					Asn	297	CB	(3.50)
							Asn	297	CG	(3.43)
Lys	128	C	Ser	298	OG	(3.47)				
Phe	129	N	Ser	298	OG	(3.30)				
Phe	129	O	Ser	267	OG	(3.54)				
Arg	131	CB					Val	266	O	(3.02)
Arg	131	CG					Val	266	O	(3.22)
Arg	131	CD					Val	266	CG1	(3.45)
							Val	266	C	(3.55)
							Val	266	O	(3.10)
Arg	131	NE	Ala	327	O	(3.60)	Val	266	C	(3.66)
							Val	266	O	(3.01)
							Val	266	N	(3.49)
Arg	131	CZ	Asp	270	CG	(3.64)	Val	266	N	(3.13)
			Asp	270	OD2	(3.22)				
			Asp	270	OD1	(3.27)				
			Ala	327	CB	(3.63)				
Arg	131	NH1	Asp	270	CG	(3.19)	Val	266	CG1	(3.47)
			Asp	270	OD2	(2.83)	Val	266	N	(3.43)
			Asp	270	OD1	(2.99)	Thr	299	OG1	(3.66)
			Ser	267	CB	(3.56)	Ser	298	O	(3.11)
Arg	131	NH2	Asp	270	CG	(3.20)	Asp	265	CA	(3.16)
			Asp	270	OD2	(2.80)	Val	266	N	(3.37)
			Asp	270	OD1	(2.87)				
			Ala	327	CB	(3.66)				
Tyr	157	CE1					Leu	234	CG	(3.64)
							Leu	234	CD1	(3.61)
Tyr	157	OH					Gly	236	O	(3.62)
							Leu	234	CA	(3.48)
							Leu	234	CG	(3.45)

summarized in Table 13. In the table, alteration refers to the alteration introduced into IL6R-B3 (SEQ ID NO: 63). The template used for producing IL6R-B3, IL6R-G1d/IL6R-L, is indicated with an asterisk (*). Furthermore, KD (IIaR)/KD (IIb) and KD (IIaH)/KD (IIb) in the table respectively represent the value obtained by dividing the KD value of each variant for FcγRIIaR by the KD value of each variant for FcγRIIb, and the value obtained by dividing the KD value of each variant for FcγRIIaH by the KD value of each variant for FcγRIIb. KD (IIb) of the parent polypeptide / KD (IIb) of the altered polypeptide refers to the value obtained by dividing the KD value of the parent polypeptide for FcγRIIb by the KD value of each variant for FcγRIIb. In addition, the KD value for the stronger of the FcγRIIaR- and FcγRIIaH-binding activities of each variant / KD value for the stronger of the FcγRIIaR- and FcγRIIaH-binding activities of the parent polypeptide are shown in Table 17. Here, parent polypeptide refers to the variant which has IL6R-B3 (SEQ ID NO: 63) as the H chain. It was determined that due to weak binding of FcγR to IgG, it was impossible to accurately analyze by kinetic analysis, and thus the gray-filled cells in Table 17 show values calculated by using Equation 2 of Reference Example 2.

[Equation 2]

$$KD = C \bullet R_{max} / (R_{eq} - RI) - C$$

[Table 17]

[X-ray crystal structure analysis of Fc (P208)/FcγRIIaR type extracellular region complex]

The structure of Fc (P208)/FcγRIIaR type extracellular region complex was determined by a molecular replacement method using program Phaser (J. Appl. Cryst. (2007) 40, 658-674). The number of complexes in an asymmetrical unit was estimated to be one from the size of the obtained crystal lattice and the molecular weight of Fc (P208)/FcγRIIaR extracellular region complex. Using, as a search model, the crystallographic structure of Fc (P208)/FcγRIIb extracellular region complex obtained as described in Example (14-1), the orientation and position of Fc (P208)/FcγRIIaR extracellular region complex in the crystal lattices were determined based on the rotation function and translation function. The crystallographic reliability factor R value of the structural model for the data of diffracted intensity at 25 to 3.0 Å was 38.4% and Free R value was 30.0% after rigid body refinement of the obtained initial structural model which moves the two CH2 domains and two CH3 domains of the Fc region, and the extracellular region of FcγRIIaR. Then, structural model refinement was achieved by repeating structural refinement using program REFMAC5 (Acta Cryst. (2011) D67, 355-367) followed by revision of the structural model performed using program Coot (Acta Cryst. (2010) D66, 486-501) with reference to the electron density maps where the coefficients Fo-Fc and 2Fo-Fc were calculated using experimentally determined structural factor Fo, structural factor Fc calculated according to the model, and the phases calculated according to the model. Finally, as a result of incorporation of water molecules into the model based on the electron density maps which use 2Fo-Fc or Fo-Fc as the coefficient, and the following refinement, the crystallographic reliability factor, R values and the Free R value of the model containing 4758 non-hydrogen atoms became 26.3% and 38.0% to 24838 diffraction intensity data from 25 Å to 2.87 Å resolution, respectively.

The three-dimensional structure of the complex of Fc (P208)/FcγRIIaR extracellular region was determined at a resolution of 2.87 Å by structure analysis. A comparison of the crystal structure between the complex of Fc (P208)/FcγRIIaR type extracellular region and the complex of Fc (P208)/FcγRIIb extracellular region described in Example (14-1) detected almost no difference at the level of overall structure (Fig. 36), reflecting the very high amino acid identity between the two Fcγ receptors.

However, a precise observation of the structures at the electron density level detected some differences that can lead to improvement of the selectivity between the FcγRIIb binding and the FcγRIIaR binding of the Fc region. The amino acid residue at position 160 in FcγRIIaR is not Tyr but Phe. As shown in Fig. 37, the hydrogen bond between the main chain of the amino acid residue at position 237 (EU numbering) in the CH2 domain A of the Fc region and Tyr at position 160 in FcγRIIb, though formed upon binding between FcγRIIb and the Fc region with alteration P238D, is expected not to be formed upon binding between FcγRIIb and the Fc region

enhanced mouse FcγRIIb-binding activity and having pH-dependent human IL-6 receptor-binding properties was examined as indicated below.

(26-2) Production of mouse antibodies with selectively enhanced FcγRIIb-binding activity

VH3-mIgG1 (SEQ ID NO: 49) and VL3-mk1 (SEQ ID NO: 50) were produced as the heavy chain and light chain, respectively, of a mouse IgG1 antibody having pH-dependent human IL-6 receptor-binding properties using the method of Reference Example 1. Furthermore, to enhance the mouse FcγRIIb-binding activity of VH3-mIgG1, substitutions of Glu for Thr at position 230, Ala for Val at position 231, Asn for Pro at position 232, Glu for Ser at position 238, and Asp for Ser at position 239 as indicated by EU numbering were carried out to produce VH3-mIgG1-MB367 (SEQ ID NO: 79). Fv4-mIgG1 or Fv4-mIgG1-MB367 comprising VL3-mk1 as the light chain and VH3-mIgG1 or VH3-mIgG1-MB367, respectively, as the heavy chain was expressed and purified using the method of Reference Example 1.

(26-3) Confirmation of mouse FcγR-binding activity

VH3/L(WT)-mIgG1 or VH3/L(WT)-mIgG1-MB367 comprising L(WT)-CK (SEQ ID NO: 42) as the light chain and VH3-mIgG1 or VH3-mIgG1-MB367, respectively, as the heavy chain was expressed and purified using the method of Reference Example 1. Mouse FcγR-binding activities of these antibodies were assessed by the method of Reference Example 2, and the results are shown in Table 31. Furthermore, how much the mouse FcγR-binding activity of each variant is enhanced as compared to mIgG1 before the alteration is shown in Table 32.

[Table 31]

VARIANT NAME	KD (M)	
	mFc γ RIIb	mFc γ RIII
VH3/L (WT)-mIgG1	2.10E-07	2.82E-07
VH3/L (WT)-mIgG1-MB367	1.32E-09	4.54E-08

[Table 32]

VARIANT NAME	BINDING RATIO TO mIgG1	
	mFc γ RIIb	mFc γ RIII
VH3/L (WT)-mIgG1	1.0	1.0
VH3/L (WT)-mIgG1-MB367	158.6	6.2

plasma concentration of antigens bound by the antigen-binding molecules.

[Means for Solving the Problems]

Specifically, the present invention provides antigen-binding molecules comprising (i) an antigen-binding domain whose antigen-binding activity varies depending on ion concentration conditions, (ii) an FcγR-binding domain having FcγRIIb-selective binding activity, and (iii) an FcRn-binding domain having FcRn-binding activity under an acidic pH range condition, and methods for decreasing plasma concentration of the antigen as compared to before administering the antigen-binding molecule, which comprises the step of administering the molecule. Furthermore, the present invention provides agents for decreasing plasma concentration of the antigen, which comprise an antigen-binding molecule comprising (i) an antigen-binding domain whose antigen-binding activity varies depending on ion concentration conditions, (ii) an FcγR-binding domain having FcγRIIb-selective binding activity, and (iii) an FcRn-binding domain having FcRn-binding activity under an acidic pH range condition. The present invention also provides pharmaceutical compositions which comprise an antigen-binding molecule comprising (i) an antigen-binding domain whose antigen-binding activity varies depending on ion concentration conditions, (ii) an FcγR-binding domain having FcγRIIb-selective binding activity, and (iii) an FcRn-binding domain having FcRn-binding activity under an acidic pH range condition. The present invention also provides uses of the antigen-binding molecule for decreasing plasma concentration of the antigen, wherein the molecule comprises (i) an antigen-binding domain whose antigen-binding activity varies depending on ion concentration conditions, (ii) an FcγR-binding domain having FcγRIIb-selective binding activity, and (iii) an FcRn-binding domain having FcRn-binding activity under an acidic pH range condition. In addition to the above, the present invention provides methods of producing and/or methods of screening for the antigen-binding molecules. Although it is not particularly intended to limit the invention, specifically, the following is provided as a non-limiting embodiment:

[1] use of an antigen-binding molecule for eliminating antigen from plasma, wherein the antigen-binding molecule comprises an antigen-binding domain whose antigen-binding activity varies depending on ion concentration conditions, and an Fc region in which the amino acid at position 238 is Asp and the amino acid at position 271 is Gly as indicated by EU numbering;

[2] the use of [1], wherein the Fc region has an amino acid substitution at at least one or more positions selected from the group consisting of 233, 234, 237, 264, 265, 266, 267, 268, 269, 272, 274, 296, 326, 327, 330, 331, 332, 333, 355, 356, 358, 396, 409, and 419 as indicated by EU numbering;

[3] the use of [2], wherein the amino acids of the Fc region include any one or more of the following amino acids indicated by EU numbering:

those having antibody heavy-chain and light-chain variable regions. Preferred examples of antigen-binding domains include "single chain Fv (scFv)", "single chain antibody", "Fv", "single chain Fv 2 (scFv2)", "Fab", and "F(ab')2".

The antigen-binding domains of antigen-binding molecules of the present invention can bind to an identical epitope. Such epitope can be present, for example, in a protein comprising the amino acid sequence of SEQ ID NO: 1. Alternatively, each of the antigen-binding domains of antigen-binding molecules of the present invention can bind to a different epitope. Herein, the different epitope can be present in, for example, a protein comprising the amino acid sequence of SEQ ID NO: 1.

Specific

With regard to binding of antigen-binding molecules provided by the present invention to an antigen, the term "specific" means that one of the molecules that specifically binds to does not substantially bind to molecules other than its single or plurality of binding partner molecule(s). Herein, "does not substantially bind" refers to showing 80% or less, generally 50% or less, preferably 30% or less and particularly preferably 15% or less binding activity to molecules other than the binding partner molecules compared to the binding activity towards the partner molecule(s), as described in the above-mentioned section on binding activity. Furthermore, "specific" is also used when an antigen-binding domain is specific to a particular epitope among multiple epitopes in an antigen. When an epitope bound by an antigen-binding domain is contained in multiple different antigens, antigen-binding molecules containing the antigen-binding domain can bind to various antigens that have the epitope.

Neutralizing activity

In a non-limiting embodiment of the present invention, a pharmaceutical composition comprising as an active ingredient an antigen-binding molecule having antigen-neutralizing activity is provided, wherein the antigen-binding molecule comprises (i) an antigen-binding domain whose antigen-binding activity varies depending on ion concentration conditions, (ii) an FcγR-binding domain having FcγRIIb-selective binding activity, and (iii) an FcRn-binding domain having FcRn-binding activity under an acidic pH range condition. Generally, neutralizing activity refers to activity of inhibiting the biological activity of a ligand, such as viruses and toxins, having biological activity on cells. Thus, substances having neutralizing activity refer to substances that bind to the ligand or the receptor to which the ligand binds, and inhibits the binding between the ligand and the receptor. Receptors blocked from binding with the ligand by the neutralizing activity will not be able to exhibit biological activity through this receptor. When the antigen-binding molecule is an antibody, such an antibody having

structure of the Fc (WT) / FcγRIIb extracellular region complex, pairs of atoms that have a distance of 3.7 Å or less between the extracted FcγRIIb extracellular region and Fc CH2 domain B were compared for comparison of the interatomic interaction between FcγRIIb and Fc (WT) CH2 domain B with the interatomic interaction between FcγRIIb and Fc (P238D) CH2 domain B. As shown in Table 15, the interatomic interactions between Fc CH2 domain B and FcγRIIb in Fc (P238D) and Fc (WT) did not match.

[Table 15]

FcγRIIb ATOM			Fc (P238D) CH2 DOMAIN B INTERACTION PARTNER (DISTANCE BETWEEN ATOMS, Å)				Fc (WT) CH2 DOMAIN B INTERACTION PARTNER (DISTANCE BETWEEN ATOMS, Å)			
Val	116	CG2					Asp	265	OD2	(3.47)
							Gly	237	O	(3.65)
Ser	126	OG	Ser	298	N	(3.31)				
			Ser	298	CB	(3.32)				
			Tyr	296	O	(3.05)				
Lys	128	CA	Ser	298	OG	(3.50)				
Phe	129	CB	Ser	298	O	(3.36)				
Phe	129	CD2					Asn	297	CB	(3.50)
							Asn	297	CG	(3.43)
Lys	128	C	Ser	298	OG	(3.47)				
Phe	129	N	Ser	298	OG	(3.30)				
Phe	129	O	Ser	267	OG	(3.54)				
Arg	131	CB					Val	266	O	(3.02)
Arg	131	CG					Val	266	O	(3.22)
Arg	131	CD					Val	266	CG1	(3.45)
							Val	266	C	(3.55)
							Val	266	O	(3.10)
Arg	131	NE	Ala	327	O	(3.60)	Val	266	C	(3.66)
							Val	266	O	(3.01)
							Val	266	N	(3.49)
Arg	131	CZ	Asp	270	CG	(3.64)	Val	266	N	(3.13)
			Asp	270	OD2	(3.22)				
			Asp	270	OD1	(3.27)				
			Ala	327	CB	(3.63)				
Arg	131	NH1	Asp	270	CG	(3.19)	Val	266	CG1	(3.47)
			Asp	270	OD2	(2.83)	Val	266	N	(3.43)
			Asp	270	OD1	(2.99)	Thr	299	OG1	(3.66)
			Ser	267	CB	(3.56)	Ser	298	O	(3.11)
Arg	131	NH2	Asp	270	CG	(3.20)				
			Asp	270	OD2	(2.80)	Asp	265	CA	(3.16)
			Asp	270	OD1	(2.87)	Val	266	N	(3.37)
			Ala	327	CB	(3.66)				
Tyr	157	CE1					Leu	234	CG	(3.64)
							Leu	234	CD1	(3.61)
Tyr	157	OH					Gly	236	O	(3.62)
							Leu	234	CA	(3.48)
							Leu	234	CG	(3.45)

summarized in Table 17. In the table, alteration refers to the alteration introduced into IL6R-B3 (SEQ ID NO: 63). The template used for producing IL6R-B3, IL6R-G1d/IL6R-L, is indicated with an asterisk (*). Furthermore, KD (IIaR)/KD (IIb) and KD (IIaH)/KD (IIb) in the table respectively represent the value obtained by dividing the KD value of each variant for FcγRIIaR by the KD value of each variant for FcγRIIb, and the value obtained by dividing the KD value of each variant for FcγRIIaH by the KD value of each variant for FcγRIIb. KD (IIb) of the parent polypeptide / KD (IIb) of the altered polypeptide refers to the value obtained by dividing the KD value of the parent polypeptide for FcγRIIb by the KD value of each variant for FcγRIIb. In addition, the KD value for the stronger of the FcγRIIaR- and FcγRIIaH-binding activities of each variant / KD value for the stronger of the FcγRIIaR- and FcγRIIaH-binding activities of the parent polypeptide are shown in Table 17. Here, parent polypeptide refers to the variant which has IL6R-B3 (SEQ ID NO: 63) as the H chain. It was determined that due to weak binding of FcγR to IgG, it was impossible to accurately analyze by kinetic analysis, and thus the gray-filled cells in Table 17 show values calculated by using Equation 2 of Reference Example 2.

[Equation 2]

$$KD = C \bullet R_{max} / (R_{eq} - RI) - C$$

[Table 17]

[X-ray crystal structure analysis of Fc (P208)/FcγRIIaR type extracellular region complex]

The structure of Fc (P208)/FcγRIIaR type extracellular region complex was determined by a molecular replacement method using program Phaser (J. Appl. Cryst. (2007) 40, 658-674). The number of complexes in an asymmetrical unit was estimated to be one from the size of the obtained crystal lattice and the molecular weight of Fc (P208)/FcγRIIaR extracellular region complex. Using, as a search model, the crystallographic structure of Fc (P208)/FcγRIIb extracellular region complex obtained as described in Example (14-1), the orientation and position of Fc (P208)/FcγRIIaR extracellular region complex in the crystal lattices were determined based on the rotation function and translation function. The crystallographic reliability factor R value of the structural model for the data of diffracted intensity at 25 to 3.0 Å was 38.4% and Free R value was 30.0% after rigid body refinement of the obtained initial structural model which moves the two CH2 domains and two CH3 domains of the Fc region, and the extracellular region of FcγRIIaR. Then, structural model refinement was achieved by repeating structural refinement using program REFMAC5 (Acta Cryst. (2011) D67, 355-367) followed by revision of the structural model performed using program Coot (Acta Cryst. (2010) D66, 486-501) with reference to the electron density maps where the coefficients Fo-Fc and 2Fo-Fc were calculated using experimentally determined structural factor Fo, structural factor Fc calculated according to the model, and the phases calculated according to the model. Finally, as a result of incorporation of water molecules into the model based on the electron density maps which use 2Fo-Fc or Fo-Fc as the coefficient, and the following refinement, the crystallographic reliability factor, R values and the Free R value of the model containing 4758 non-hydrogen atoms became 26.3% and 38.0% to 24838 diffraction intensity data from 25 Å to 2.87 Å resolution, respectively.

The three-dimensional structure of the complex of Fc (P208)/FcγRIIaR extracellular region was determined at a resolution of 2.87 Å by structure analysis. A comparison of the crystal structure between the complex of Fc (P208)/FcγRIIaR type extracellular region and the complex of Fc (P208)/FcγRIIb extracellular region described in Example (14-1) detected almost no difference at the level of overall structure (Fig. 36), reflecting the very high amino acid identity between the two Fcγ receptors.

However, a precise observation of the structures at the electron density level detected some differences that can lead to improvement of the selectivity between the FcγRIIb binding and the FcγRIIaR binding of the Fc region. The amino acid residue at position 160 in FcγRIIaR is not Tyr but Phe. As shown in Fig. 37, the hydrogen bond between the main chain of the amino acid residue at position 237 (EU numbering) in the CH2 domain A of the Fc region and Tyr at position 160 in FcγRIIb, though formed upon binding between FcγRIIb and the Fc region with alteration P238D, is expected not to be formed upon binding between FcγRIIaR and the Fc

enhanced mouse FcγRIIb-binding activity and having pH-dependent human IL-6 receptor-binding properties was examined as indicated below.

(26-2) Production of mouse antibodies with selectively enhanced FcγRIIb-binding activity

VH3-mIgG1 (SEQ ID NO: 49) and VL3-mk1 (SEQ ID NO: 50) were produced as the heavy chain and light chain, respectively, of a mouse IgG1 antibody having pH-dependent human IL-6 receptor-binding properties using the method of Reference Example 1. Furthermore, to enhance the mouse FcγRIIb-binding activity of VH3-mIgG1, substitutions of Glu for Thr at position 230, Ala for Val at position 231, Asn for Pro at position 232, Glu for Ser at position 238, and Asp for Ser at position 239 as indicated by EU numbering were carried out to produce VH3-mIgG1-MB367 (SEQ ID NO: 79). Fv4-mIgG1 or Fv4-mIgG1-MB367 comprising VL3-mk1 as the light chain and VH3-mIgG1 or VH3-mIgG1-MB367, respectively, as the heavy chain was expressed and purified using the method of Reference Example 1.

(26-3) Confirmation of mouse FcγR-binding activity

VH3/L(WT)-mIgG1 or VH3/L(WT)-mIgG1-MB367 comprising L(WT)-CK (SEQ ID NO: 42) as the light chain and VH3-mIgG1 or VH3-mIgG1-MB367, respectively, as the heavy chain was expressed and purified using the method of Reference Example 1. Mouse FcγR-binding activities of these antibodies were assessed by the method of Reference Example 2, and the results are shown in Table 31. Furthermore, how much the mouse FcγR-binding activity of each variant is enhanced as compared to mIgG1 before the alteration is shown in Table 32.

[Table 31]

VARIANT NAME	KD (M)	
	mFc γ RIIb	mFc γ RIII
VH3/L (WT)-mIgG1	2.10E-07	2.82E-07
VH3/L (WT)-mIgG1-MB367	1.32E-09	4.54E-08

[Table 32]

VARIANT NAME	BINDING RATIO COMPARED TO mIgG1	
	mFc γ RIIb	mFc γ RIII
VH3/L (WT)-mIgG1	1.0	1.0
VH3/L (WT)-mIgG1-MB367	158.6	6.2