DESCRIPTION

ANTIGEN-BINDING MOLECULE FOR PROMOTING ~~LOSS~~ 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 ~~any~~ at least one or more amino acids ~~of~~ 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

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 ~~IgG1~~ 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

in which binding activity to the antigen under high calcium ion concentration conditions is higher than that under low calcium ion concentration conditions.

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

34. The method of claim 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 claims 29 to 34, wherein the antigen-binding molecule has neutralizing activity against the antigen.

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

37. The method of claim 36, wherein the Fc region is an Fc region in which ~~any~~ at least one or more amino acids ~~of~~ 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 claim 37, 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;