DESCRIPTION

Title of Invention: ANTIBODIES WITH MODIFIED AFFINITY TO FCRN THAT PROMOTE ANTIGEN CLEARANCE

Technical Field

[0001]

The present invention relates to:

methods for facilitating antigen-binding molecule-mediated antigen uptake into cells;

methods for increasing the number of antigens to which a single antigen-binding molecule can bind;

methods for enhancing the reduction of plasma antigen concentration by administering antigen-binding molecules;

methods for improving pharmacokinetics of antigen-binding molecules;

methods for reducing total or free antigen concentration in plasma;

antigen-binding molecules that improve antigen uptake into cells;

antigen-binding molecules that have an increased number of binding antigens;

antigen-binding molecules capable of enhancing the reduction of plasma antigen concentration by administration of the molecules;

antigen-binding molecules with improved pharmacokinetics;

pharmaceutical compositions comprising the antigen-binding molecules;

methods for producing those described above; and the like.

[0002]

Priority

The present invention claims the benefit of Japanese Patent Application No. 2010-079667, filed on March 30, 2010, and Japanese Patent Application No. 2010-250830, filed on November 9, 2010, the entire contents of which are incorporated be reference herein.

[Background Art]

[0003]

Antibodies are drawing attention as pharmaceuticals as they are highly stable in plasma and have few side effects. At present, a number of IgG-type antibody pharmaceuticals are available on the market and many antibody pharmaceuticals are currently under development (NPLs 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 (NPL 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 the 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.

an amino acid substitution of Ala for Val at position 305;
an amino acid substitution of Ala, Asp, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Val, Trp, or Tyr for Thr at position 307;
an amino acid substitution of Ala, Phe, Ile, Leu, Met, Pro, Gln, or Thr for Val at position 308;
an amino acid substitution of Ala, Asp, Glu, Pro, or Arg for Leu or Val at position 309;
an amino acid substitution of Ala, His, or Ile for Gln at position 311;
an amino acid substitution of Ala, or His for Asp at position 312;
an amino acid substitution of Lys, or Arg for Leu at position 314;
an amino acid substitution of Ala, or His for Asn at position 315;
an amino acid substitution of Ala for Lys at position 317;
an amino acid substitution of Gly for Asn at position 325;
an amino acid substitution of Val for Ile at position 332;
an amino acid substitution of Leu for Lys at position 334;
an amino acid substitution of His for Lys at position 360;
an amino acid substitution of Ala for Asp at position 376;
an amino acid substitution of Ala for Glu at position 380;
an amino acid substitution of Ala for Glu at position 382;
an amino acid substitution of Ala for Asn or Ser at position 384;
an amino acid substitution of Asp, or His for Gly at position 385;
an amino acid substitution of Pro for Gln at position 386;
an amino acid substitution of Glu for Pro at position 387;
an amino acid substitution of Ala, or Ser for Asn at position 389;
an amino acid substitution of Ala for Ser at position 424;
an amino acid substitution of Ala, Asp, Phe, Gly, His, Ile, Lys, Leu, Asn, Pro, Gln, Ser, Thr, Val, Trp, or Tyr for Met at position 428;
an amino acid substitution of Lys for His at position 433;
an amino acid substitution of Ala, Phe, His, Ser, Trp, or Tyr for Asn at position 434;
and an amino acid substitution of His or Phe for Tyr at position 436 in EU numbering;
[20] the antigen-binding molecule of any one of [1] to [18], whose human FcRn-binding domain comprises at least one amino acid selected from:
Met at amino acid position 237;
Ala at amino acid position 238;
Lys at amino acid position 239;
Ile at amino acid position 248;
Ala, Phe, Ile, Met, Gln, Ser, Val, Trp, or Tyr at amino acid position 250;
Phe, Trp, or Tyr at amino acid position 252;
Thr at amino acid position 254;

and His or Phe at amino acid position 436 (EU numbering) in the Fc domain of parent IgG;

[21] the antigen-binding molecule of any one of [18] to [20], wherein the parent IgG is selected from an IgG obtained from a non-human animal;

[22] the antigen-binding molecule of any one of [18] to [20], wherein the parent IgG is a human IgG;

[23] the antigen-binding molecule of any one of [1] to [22], which has an antagonistic activity;

[24] the antigen-binding molecule of [1] to [23], which binds to a membrane antigen or soluble antigen;

[25] the antigen-binding molecule of any one of [1] to [24], wherein the antigen-binding domain comprises an artificial ligand which binds to a receptor;

[26] the antigen-binding molecule of any one of [1] to [24], wherein the antigen-binding domain comprises an artificial receptor which binds to a ligand;

[27] the antigen-binding molecule of any one of [1] to [24], which is an antibody;

[28] the antigen-binding molecule of [27], wherein the antibody is selected from a chimeric antibody, a humanized antibody, or human antibody;

[29] a pharmaceutical composition comprising any one of the antigen-binding molecule of [1] to [28];

[30] a method for facilitating antigen-binding molecule-mediated antigen uptake into a cell by increasing its human FcRn-binding activity in the neutral pH range, wherein the antigen-binding molecule comprises an antigen-binding domain and a human FcRn-binding domain, and has a human FcRn-binding activity in the acidic pH range;

[31] a method for facilitating antigen-binding molecule-mediated antigen uptake into a cell by increasing its human FcRn-binding activity in the neutral pH range and reducing its antigen-binding activity in the acidic pH range to less than that in the neutral pH range, wherein the antigen-binding molecule comprises an antigen-binding domain and a human FcRn-binding domain, and has a human FcRn-binding activity in the acidic pH range;

[32] a method for increasing the number of antigens to which a single antigen-binding molecule can bind by increasing its human FcRn-binding activity in the neutral pH range, wherein the antigen-binding molecule comprises an antigen-binding domain and a human FcRn-binding domain, and has a human FcRn-binding activity in the acidic pH range;

[33] a method for increasing the number of antigens to which a single antigen-binding molecule can bind by increasing its human FcRn-binding activity in the neutral pH range and reducing its antigen-binding activity in the acidic pH range to less than that in the neutral pH range, wherein the antigen-binding molecule comprises an antigen-binding domain and a human FcRn-binding domain, and has a human FcRn-binding activity in the acidic pH range;

[34] a method for augmenting the ability of an antigen-binding molecule to eliminate an antigen from plasma by increasing its human FcRn-binding activity in the neutral pH range, wherein the antigen-binding molecule comprises an antigen-binding domain and a human FcRn-binding domain, and has a human FcRn-binding

an amino acid substitution of Lys, or Arg for Leu at position 314;
an amino acid substitution of Ala, or His for Asn at position 315;
an amino acid substitution of Ala for Lys at position 317;
an amino acid substitution of Gly for Asn at position 325;
an amino acid substitution of Val for Ile at position 332;
an amino acid substitution of Leu for Lys at position 334;
an amino acid substitution of His for Lys at position 360;
an amino acid substitution of Ala for Asp at position 376;
an amino acid substitution of Ala for Glu at position 380;
an amino acid substitution of Ala for Glu at position 382;
an amino acid substitution of Ala for Asn or Ser at position 384;
an amino acid substitution of Asp, or His for Gly at position 385;
an amino acid substitution of Pro for Gln at position 386;
an amino acid substitution of Glu for Pro at position 387;
an amino acid substitution of Ala, or Ser for Asn at position 389;
an amino acid substitution of Ala for Ser at position 424;
an amino acid substitution of Ala, Asp, Phe, Gly, His, Ile, Lys, Leu, Asn, Pro, Gln, Ser, Thr, Val, Trp, or Tyr for Met at position 428;
an amino acid substitution of Lys for His at position 433;
an amino acid substitution of Ala, Phe, His, Ser, Trp, or Tyr for Asn at position 434;
and an amino acid substitution of His or Phe for Tyr at position 436 (EU numbering) in the parent IgG Fc domain. Meanwhile, the number of amino acids to be altered is not particularly limited; and it is possible to alter amino acids at only a single site or at two or more sites. Combinations of two or more amino acid alterations include, for example, those shown in Table 3. Meanwhile, combinations of alterations that can potentiate the binding to human FcRn in the acidic pH range as compared to the parent human IgG are shown in Tables 4-1 to 4-5. When appropriate combinations of alterations that can also potentiate the binding to human FcRn in the neutral pH range are selected from the above-described alterations, they are applicable to the present invention. Furthermore, combinations of alterations that can potentiate the binding of Fv4-IgG1 to human FcRn under neutral conditions are shown in Tables 6-1 and 6-2.

[0096]

The symbol "^" in the Tables shows an amino acid insertion after the indicated number in EU numbering. For example, ^281S means that S is inserted between positions 281 and 282 in EU numbering.

[0097]

an amino acid substitution of His for Glu a position 258;
an amino acid substitution of Ala for Asp at position 265;
an amino acid substitution of Phe for Asp at position 270;
an amino acid substitution of Ala, or Glu for Asn at position 286;
an amino acid substitution of His for Thr at position 289;
an amino acid substitution of Ala for Asn at position 297;
an amino acid substitution of Gly for Ser at position 298;
an amino acid substitution of Ala for Val at position 303;
an amino acid substitution of Ala for Val at position 305;
an amino acid substitution of Ala, Asp, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Val, Trp, or Tyr for Thr at position 307;
an amino acid substitution of Ala, Phe, Ile, Leu, Met, Pro, Gln, or Thr for Val at position 308;
an amino acid substitution of Ala, Asp, Glu, Pro, or Arg for Leu or Val at position 309;
an amino acid substitution of Ala, His, or Ile for Gln at position 311;
an amino acid substitution of Ala, or His for Asp at position 312;
an amino acid substitution of Lys, or Arg for Leu at position 314;
an amino acid substitution of Ala, or His for Asn at position 315;
an amino acid substitution of Ala for Lys at position 317;
an amino acid substitution of Gly for Asn at position 325;
an amino acid substitution of Val for Ile at position 332;
an amino acid substitution of Leu for Lys at position 334;
an amino acid substitution of His for Lys at position 360;
an amino acid substitution of Ala for Asp at position 376;
an amino acid substitution of Ala for Glu at position 380;
an amino acid substitution of Ala for Glu t position 382;
an amino acid substitution of Ala for Asn or Ser at position 384;
an amino acid substitution of Asp, or His for Gly at position 385;
an amino acid substitution of Pro for Gln at position 386;
an amino acid substitution of Glu for Pro at position 387;
an amino acid substitution of Ala, or Ser for Asn at position 389;
an amino acid substitution of Ala for Ser at position 424;
an amino acid substitution of Ala, Asp, Phe, Gly, His, Ile, Lys, Leu, Asn, Pro, Gln, Ser, Thr, Val, Trp, or Tyr for Met at position 428;
an amino acid substitution of Lys for His at position 433;
an amino acid substitution of Ala, Phe, His, Ser, Trp, or Tyr for Asn at position 434;
and an amino acid substitution of His or Phe for Tyr at position 436 in EU numbering.

[0147] The number of amino acids to be altered is not particularly limited; it is possible to

Ala at amino acid position 376;
Ala at amino acid position 380;
Ala at amino acid position 382;
Ala at amino acid position 384;
Asp or His at amino acid position 385;
Pro at amino acid position 386;
Glu at amino acid position 387;
Ala or Ser at amino acid position 389;
Ala at amino acid position 424;
Ala, Asp, Phe, Gly, His, Ile, Lys, Leu, Asn, Pro, Gln, Ser, Thr, Val, Trp, or Tyr at amino acid position 428;
Lys at amino acid position 433;
Ala, Phe, His, Ser, Trp, or Tyr at amino acid position 434;
and His or Phe at amino acid position 436 (EU numbering) in the parent IgG Fc domain.

[0149]

An amino acid at a site or amino acids at two or more sites may have these amino acids. Combinations of amino acids at two or more positions include, for example, those shown in Tables 3, 4-1 to 4-5, 6-1, 6-2, and 9.

[0150]

Alternatively, in a preferred embodiment, the antigen-binding molecule whose antigen-binding activity in the acidic pH range is lower than that in the neutral pH range includes antigen-binding molecules in which at least one amino acid in the antigen-binding molecule is replaced with histidine or a non-natural amino acid, or in which at least one histidine or a non-natural amino acid has been inserted. The site into which the histidine or non-natural amino acid mutation is introduced is not particularly limited and may be any site, as long as the antigen-binding activity in the acidic pH range is weaker than that in the neutral pH range (the KD (in the acidic pH range)/KD (in the neutral pH range) value is greater or the k_d (in the acidic pH range)/k_d (in the neutral pH range) value is greater) as compared to before substitution. Examples include variable regions and CDRs of an antibody in the case the antigen-binding molecule is an antibody. The number of amino acids to be replaced with histidine or non-natural amino acid and the number of amino acids to be inserted can be appropriately determined by those skilled in the art. One amino acid may be replaced with histidine or non-natural amino acid, or one amino acid may be inserted, or two or more amino acids may be replaced with histidine or non-natural amino acids, or two or more amino acids may be inserted. Moreover, apart from the substitutions of histidine or non-natural amino acid or insertion of histidine or of non-natural amino acid, deletion, addition, insertion, and/or substitution and such of other amino acids may also be simultaneously carried out. Substitutions of histidine or non-natural amino acid or insertion of histidine or of non-natural amino acid may be carried out at random using

CLAIMS

[Claim 1]
A method for facilitating antigen-binding molecule-mediated antigen uptake into a cell by increasing its human FcRn-binding activity in the neutral pH range, wherein the antigen-binding molecule comprises an antigen-binding domain and a human FcRn-binding domain, and has a human FcRn-binding activity in the acidic pH range.

[Claim 2]
A method for facilitating antigen-binding molecule-mediated antigen uptake into a cell by increasing its human FcRn-binding activity in the neutral pH range and reducing its antigen-binding activity in the acidic pH range to less than that in the neutral pH range, wherein the antigen-binding molecule comprises an antigen-binding domain and a human FcRn-binding domain, and has a human FcRn-binding activity in the acidic pH range.

[Claim 3]
A method for increasing the number of antigens to which a single antigen-binding molecule can bind by increasing its human FcRn-binding activity in the neutral pH range, wherein the antigen-binding molecule comprises an antigen-binding domain and a human FcRn-binding domain, and has a human FcRn-binding activity in the acidic pH range.

[Claim 4]
A method for increasing the number of antigens to which a single antigen-binding molecule can bind by increasing its human FcRn-binding activity in the neutral pH range and reducing its antigen-binding activity in the acidic pH range to less than that in the neutral pH range, wherein the antigen-binding molecule comprises an antigen-binding domain and a human FcRn-binding domain, and has a human FcRn-binding activity in the acidic pH range.

[Claim 5]
A method for improving pharmacokinetics of an antigen-binding molecule by increasing its human FcRn-binding activity in the neutral pH range, wherein the antigen-binding molecule comprises an antigen-binding domain and a human FcRn-binding domain, and has a human FcRn-binding activity in the acidic pH range.

[Claim 6]
A method for improving pharmacokinetics of an antigen-binding molecule by increasing its human FcRn-binding activity in the neutral pH range and reducing its antigen-binding activity in the acidic pH range to less than that in the neutral pH range, wherein the antigen-binding molecule comprises an

antigen-binding domain and a human FcRn-binding domain, and has a human FcRn-binding activity in the acidic pH range.

[Claim 7]
A method for facilitating intracellular dissociation of an antigen bound to an antigen-binding molecule outside the cell from the antigen-binding molecule, by increasing its human FcRn-binding activity in the neutral pH range and reducing its antigen-binding activity in the acidic pH range to less that in the neutral pH range, wherein the antigen-binding molecule comprises an antigen-binding domain and a human FcRn-binding domain, and has a human FcRn-binding activity in the acidic pH range.

[Claim 8]
A method for facilitating extracellular release of the antigen-free form of an antigen-binding molecule taken up into a cell in an antigen-bound form, by increasing its human FcRn-binding activity in the neutral pH range and reducing its antigen-binding activity in the acidic pH range to less than that in the neutral pH range, wherein the antigen-binding molecule comprises an antigen-binding domain and a human FcRn-binding domain, and has a human FcRn-binding activity in the acidic pH range.

[Claim 9]
A method for reducing total or free plasma antigen concentration in plasma, by increasing its human FcRn-binding activity in the neutral pH range, wherein the antigen-binding molecule comprises an antigen-binding domain and a human FcRn-binding domain, and has a human FcRn-binding activity in the acidic pH range.

[Claim 10]
A method for reducing total or free plasma antigen concentration in plasma, by increasing its human FcRn-binding activity in the neutral pH range and reducing its antigen-binding activity in the acidic pH range to less than that in the neutral pH range, wherein the antigen-binding molecule comprises an antigen-binding domain and a human FcRn-binding domain, and has a human FcRn-binding activity in the acidic pH range.

[Claim 11]
The method of any one of claims 1 to 10, wherein the acidic pH range is pH 5.5 to 6.5 and the neutral pH range is pH 7.0 to 8.0.

[Claim 12]
The method of any one of claims 1 to 10, wherein the increase in the human FcRn-binding activity in the neutral pH range is an increase by substituting a different amino acid for at least one amino acid in the parent IgG Fc domain of the human FcRn-binding domain.

[Claim 13]
The method of any one of claims 1 to 10, wherein the increase in the human FcRn-binding activity in the neutral pH range is an increase by substituting a different amino acid for at least one amino acid selected from those at positions 237, 238, 239, 248, 250, 252, 254, 255, 256, 257, 258, 265, 270, 286, 289, 297, 298, 303, 305, 307, 308, 309, 311, 312, 314, 315, 317, 325, 332, 334, 360, 376, 380, 382, 384, 385, 386, 387, 389, 424, 428, 433, 434, and 436 (EU numbering) in the parent IgG Fc domain of the human FcRn-binding domain.

[Claim 14]
The method of any one of claims 2, 4, 6 to 8, and 10, wherein the antigen-binding activity of the antigen-binding molecule in the acidic pH range is reduced to less than that in the neutral pH range by substituting histidine for at least one amino acid of the antigen-binding molecule or inserting at least one histidine.

[Claim 15]
The method of any one of claims 2, 4, 6 to 8, and 10, wherein the antigen-binding domain is obtained from antigen-binding domain library.

[Claim 16]
The method of any one of claims 2, 4, 6 to 8, and 10, wherein the decrease in the antigen-binding activity is represented by an increase in the value of KD (in the acidic pH range) /KD (in the neutral pH range) which is a ratio of antigen-binding activity in the acidic pH range and neutral pH range, relative to before histidine substitution or insertion.