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Summary ~~of the Invention~~

[Problems to be solved by the ~~Invention~~Disclosure]

The above noted circumstances led to the discoveries of the present ~~invention~~disclosure. Accordingly, an objective of the present ~~invention~~disclosure is to provide methods for binding antigen-binding molecules to the antigens multiple times and methods for improving the pharmacokinetics of antigen-binding molecules, as well as antigen-binding molecules that are capable of binding to the antigens multiple times, antigen-binding molecules having improved pharmacokinetics, pharmaceutical compositions containing such antigen-binding molecules, and methods for producing such molecules and compositions.

[Means for Solving the Problems]

Dedicated studies on methods for binding polypeptides having antigen-binding ability, such as antigen-binding molecules, to the antigens multiple times, and methods for improving

the half-lives of such molecules in plasma (blood) (improving their pharmacokinetics) were conducted herein. As a result, it was discovered that if the antigen-binding activity of an antigen-binding molecule at the early endosomal pH is lower than its antigen-binding activiy at the pH of plasma (blood), it would be able to bind to antigens multiple times and have a longer half-life in plasma.

Accordingly, the present ~~invention~~disclosure relates to methods for binding antigen-binding molecules to antigens multiple times, methods for improving the pharmacokinetics of antigen-binding molecules, and methods for producing antigen-binding molecules with improved pharmacokinetics; the present ~~invention~~disclosure also relates to antigen-binding molecules that are capable of binding to antigens multiple times and antigen-binding molecules with improved pharmacokinetics. More specifically, the present ~~invention~~disclosure provides:

[1] an antigen-binding molecule having a KD(pH5.8)/KD(pH7.4) value, defined as the ratio of KD for the antigen at pH 5.8 and KD for the antigen at pH 7.4, of 2 or higher;

[2] the antigen-binding molecule of [1], wherein the KD(pH5.8)/KD(pH7.4) value is 10 or higher;

[3] the antigen-binding molecule of [1], wherein the KD(pH5.8)/KD(pH7.4) value is 40 or higher;

[4] the antigen-binding molecule of any one of [1] to [3], wherein at least one amino acid of the antigen-binding molecule has been substituted with histidine, or at least one histidine has been inserted into the antigen-binding molecule;

[5] the antigen-binding molecule of any one of [1] to [4], wherein the antigen-binding molecule has an antagonistic activity;

[6]. the antigen-binding molecule of any one of [1] to [5], wherein the antigen-binding molecule binds to a membrane antigen or a soluble antigen;

[7] the antigen-binding molecule of any one of [1] to [6], wherein the antigen-binding molecule is an antibody;

[8] a pharmaceutical composition comprising the antigen-binding molecule of any one of [1] to [7];

[9] a method for improving the pharmacokinetics of an antigen-binding molecule by impairing the antigen-binding activity of the antigen-binding molecule at pH 5.8 as compared to that at pH 7.4;

[10] a method for increasing the number of times of antigen-binding for an antigen-binding molecule by impairing the antigen-binding activity of the antigen-binding molecule at pH 5.8 as compared to that at pH 7.4;

[50] a pharmaceutical composition comprising an antigen-binding molecule produced by the production method of any one of [43] to [49].

[Effects of the ~~Invention~~Disclosure]

The present ~~invention~~disclosure provides methods for making single antigen-binding molecules to repeatedly bind to multiple antigen molecules. When an antigen-binding molecule binds to multiple antigen molecules, the pharmacokinetics of the antigen-binding molecule can be improved and such molecule can exert more superior *in vivo* effects than those of ordinary antigen-binding molecules.

In one aspect, there is provided a pharmaceutical composition comprising an antibody having a KD(pH5.8)/KD(pH7.4) value, defined as the ratio of KD for the antigen at pH 5.8 and KD for the antigen at pH 7.4, of 2 or higher and 10000 or less, and having a longer half-life in plasma, wherein at least one amino acid of the variable region has been substituted with histidine, or at least one histidine has been inserted into the variable region.

Brief Description of the Drawings

FIG. 1 is a diagram depicting a degradation pathway of antibodies bound to membrane-bound antigen.

FIG. 2 is a diagram depicting a mechanism by which IgG molecules are salvaged by FcRn.

FIG. 3 is a schematic diagram depicting the re-binding of IgG molecules to new antigen following dissociation from membrane-bound antigen within endosomes.

FIG. 4 is a schematic diagram depicting the re-binding of IgG molecules to new antigen following dissociation from soluble antigen within endosomes.

FIG. 5 is a diagram depicting the process of panning using an antigen-immobilized column.

FIG. 6 presents graphs depicting the results of phage ELISA for clones acquired by column panning. The upper graph depicts WT and the lower graph depicts CL5.

FIG. 7 is a graph depicting the biological neutralization activity of pH-dependently-binding anti-IL-6 receptor antibodies.

FIG. 8 presents graphs depicting results of Biacore sensorgram for binding of pH-dependently-binding anti-IL-6 receptor antibodies to soluble IL-6 receptor at pH 7.4. The top graph depicts WT; the second graph from the top depicts H3pI/L73; the third graph from the top depicts H170/L82; and the bottom depicts CLH5/L73.

FIG. 9 presents graphs depicting results of Biacore sensorgram for binding of pH-dependently-binding anti-IL-6 receptor antibodies to soluble IL-6 receptor at pH 5.8. The top graph depicts WT; the second graph from the top depicts H3pI/L73; the third graph from the top depicts H170/L82; and the bottom graph depicts CLH5/L73.

FIG. 10 presents graphs depicting results of Biacore sensorgram for association (pH 7.4) and dissociation (pH 5.8) of pH-dependently-binding anti-IL-6 receptor antibodies to membrane-type IL-6 receptor. The top graph depicts WT; the second graph from the top

CLAIMS

1. A pharmaceutical composition comprising an antibody having a KD(pH5.8)/KD(pH7.4) value, defined as the ratio of KD for the antigen at pH 5.8 and KD for the antigen at pH 7.4, of 2 or higher and 10000 or less, and having a longer half-life in plasma, wherein at least one amino acid of the variable region has been substituted with histidine, or at least one histidine has been inserted into the variable region.

2. The pharmaceutical composition of claim 1, wherein the antibody has the KD(pH5.8)/KD(pH7.4) value, defined as the ratio of KD for the antigen at pH 5.8 and KD for the antigen at pH 7.4, of 10 or higher.

3. The pharmaceutical composition of claim 1, wherein the antibody has the KD(pH5.8)/KD(pH7.4) value, defined as the ratio of KD for the antigen at pH 5.8 and KD for the antigen at pH 7.4, of 40 or higher.

4. The pharmaceutical composition of any one of claims 1 to 3, wherein the antibody has an antagonistic activity.

5. The pharmaceutical composition of any one of claims 1 to 4, wherein the antibody binds to a membrane antigen or a soluble antigen.

6. The pharmaceutical composition of any one of claims 1 to 5, wherein the antibody binds to an antigen selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-15, IL-31, IL-23, IL-2 receptor, IL-6 receptor, OSM receptor, gp130, IL-5 receptor, CD40, CD4, Fas, osteopontin, CRTH2, CD26, PDGF-D, CD20, monocyte chemotactic factor, CD23, TNF-α, HMGB-1, α4 integrin, ICAM-1, CCR2, CD11a, CD3, IFNγ, BLyS, HLA-DR, TGF-β, CD52, and IL-31 receptor.

CLAIMS

1. An antigen-binding molecule having a KD(pH5.8)/KD(pH7.4) value, defined as the ratio of KD for the antigen at pH 5.8 and KD for the antigen at pH 7.4, of 2 or higher.

2. The antigen-binding molecule of claim 1, wherein the KD(pH5.8)/KD(pH7.4) value is 10 or higher.

3. The antigen-binding molecule of claim 1, wherein the KD(pH5.8)/KD(pH7.4) value is 40 or higher.

4. The antigen-binding molecule of any one of claims 1 to 3, wherein at least one amino acid of the antigen-binding molecule has been substituted with histidine, or at least one histidine has been inserted into the antigen-binding molecule.

5. The antigen-binding molecule of any one of claims 1 to 4, wherein the antigen-binding molecule has an antagonistic activity.

6. The antigen-binding molecule of any one of claims 1 to 5, wherein the antigen-binding molecule binds to a membrane antigen or a soluble antigen.

7. The antigen-binding molecule of any one of claims 1 to 6, wherein the antigen-binding molecule is an antibody.

8. A pharmaceutical composition comprising the antigen-binding molecule of any one of claims 1 to 7.

9. A method for improving the pharmacokinetics of an antigen-binding molecule by impairing the antigen-binding activity of the antigen-binding molecule at pH 5.8 as compared to that at pH 7.4.

10. A method for increasing the number of times of antigen-binding for an antigen-binding molecule by impairing the antigen-binding activity of the antigen-binding molecule at pH 5.8 as compared to that at pH 7.4.

11. A method for increasing the number of antigens that can be bound by an antigen-binding molecule by impairing the antigen-binding activity of the antigen-binding molecule at pH 5.8 as compared to that at pH 7.4.

12. A method for dissociating within a cell an antigen from an extracellularly-bound antigen-binding molecule by impairing the antigen-binding activity of the antigen-binding molecule at pH 5.8 as compared to that at pH 7.4.

13. A method for releasing an antigen-binding molecule, which has been bound to an antigen and internalized into a cell, in an antigen-free form to the outside of the cell by impairing the antigen-binding activity of the antigen-binding molecule at pH 5.8 as compared to that at pH 7.4.

14. A method for increasing the ability of an antigen-binding molecule to eliminate an antigen in plasma by impairing the antigen-binding activity of the antigen-binding molecule at pH 5.8 as compared to that at pH 7.4.

15. The method of any one of claims 9 to 14, wherein the KD(pH5.8)/KD(pH7.4) value, defined as the ratio of KD for the antigen at pH 5.8 and KD for the antigen at pH 7.4, is 2 or higher.

16. The method of any one of claims 9 to 14, wherein the KD(pH5.8)/KD(pH7.4) value is 10 or higher.

17. The method of any one of claims 9 to 14, wherein the KD(pH5.8)/KD(pH7.4) value is 40 or higher.

18. A method for improving the pharmacokinetics of an antigen-binding molecule by substituting at least one amino acid of the antigen-binding molecule with histidine, or inserting at least one histidine into the antigen-binding molecule.

19. A method for increasing the number of times of antigen-binding for an antigen-binding molecule by substituting at least one amino acid of the antigen-binding molecule with histidine, or inserting at least one histidine into the antigen-binding molecule.

20. A method for increasing the number of antigens that can be bound by an antigen-binding molecule by substituting at least one amino acid of the antigen-binding molecule with histidine, or inserting at least one histidine into the antigen-binding molecule.

21. A method for dissociating within a cell an antigen from an extracellularly-bound antigen-binding molecule by substituting at least one amino acid of the antigen-binding molecule with histidine, or inserting at least one histidine into the antigen-binding molecule.

22. A method for releasing an antigen-binding molecule, which has been bound to an antigen and internalized into a cell, in an antigen-free form to the outside of the cell, by substituting at least one amino acid of the antigen-binding molecule with histidine, or inserting at least one histidine into the antigen-binding molecule.

23. A method for increasing the ability of an antigen-binding molecule to eliminate an antigen in plasma by substituting at least one amino acid of the antigen-binding molecule with histidine, or inserting at least one histidine into the antigen-binding molecule.

24. The method of any one of claims 18 to 23, wherein the histidine substitution or insertion increases the KD(pH5.8)/KD(pH7.4) value, defined as the ratio of the antigen-binding activity at pH 5.8 and the antigen-binding activity at pH 7.4, as compared to the KD(pH5.8)/KD(pH7.4) value before the histidine substitution or insertion.

25. The method of any one of claims 9 to 24, wherein the antigen-binding molecule has an antagonistic activity.

26. The method of any one of claims 9 to 25, wherein the antigen-binding molecule binds to a membrane antigen or a soluble antigen.

27. The method of any one of claims 9 to 26, wherein the antigen-binding molecule is an antibody.

28. A pharmaceutical composition comprising an antigen-binding molecule produced by a method comprising the steps of:
(a) determining the antigen-binding activity of an antigen-binding molecule at pH 6.7 to pH 10.0;
(b) determining the antigen-binding activity of the antigen-binding molecule at pH 4.0 to pH 6.5;
(c) selecting the antigen-binding molecule whose antigen-binding activity at pH 6.7 to pH 10.0 is greater than that at pH 4.0 to pH 6.5;
(d) obtaining the gene encoding the antigen-binding molecule selected in (c); and
(e) producing the antigen-binding molecule using the gene obtained in (d).

29. A pharmaceutical composition comprising an antigen-binding molecule produced by a method comprising the steps of:
(a) binding an antigen-binding molecule to an antigen under a condition of pH 6.7 to pH 10.0;
(b) allowing the antigen-binding molecule bound to the antigen of (a) to stand under a condition of pH 4.0 to pH 6.5;
(c) collecting the antigen-binding molecule that dissociated under the condition of pH 4.0 to pH 6.5;
(d) obtaining the gene encoding the antigen-binding molecule obtained in (c); and
(e) producing the antigen-binding molecule using the gene obtained in (d).

30. A pharmaceutical composition comprising an antigen-binding molecule whose binding activity at a first pH is greater than that at a second pH, wherein the antigen-binding molecule produced by a method comprising the steps of:
(a) binding an antigen-binding molecule to an antigen-immobilized column under the first pH condition;
(b) eluting the antigen-binding molecule, which is bound to the column at the first pH, from the column under a second pH condition;
(c) collecting the eluted antigen-binding molecule;
(d) obtaining the gene encoding the antigen-binding molecule obtained in (c); and
(e) producing the antigen-binding molecule using the gene obtained in (d).

31. A pharmaceutical composition comprising an antigen-binding molecule whose binding activity at a first pH is greater than that at a second pH, wherein the antigen-binding molecule is produced by a method comprising the steps of:
(a) binding an antigen-binding molecule library to an antigen-immobilized column under the first pH condition;
(b) eluting the antigen-binding molecule from the column under the second pH condition;
(c) amplifying the gene encoding the eluted antigen-binding molecule;
(d) collecting the eluted antigen-binding molecule;
(e) obtaining the gene encoding the antigen-binding molecule collected in (d); and
(f) producing the antigen-binding molecule using the gene obtained in (e).

32. The pharmaceutical composition of claim 30 or 31, wherein the first pH is 6.7 to 10.0 and the second pH is 4.0 to 6.5.

33. The production method of any one of claims 28 to 32, which further comprises the step of substituting at least one amino acid of the antigen-binding molecule with histidine, or inserting at least one histidine into the antigen-binding molecule.

34. The production method of any one of claims 28 to 33, wherein the antigen-binding molecule is an antibody.