[Non-patent Documents]

[Non-patent Document 1] Monoclonal antibody successes in the clinic, Janice M Reichert, Clark J Rosensweig, Laura B Faden & Matthew C Dewitz, Nature Biotechnology 23, 1073 - 1078 (2005)

[Non-patent Document 2] Pavlou AK, Belsey MJ., The therapeutic antibodies market to 2008., Eur J Pharm Biopharm. 2005 Apr; 59(3): 389-96

[Non-patent Document 3] Kim SJ, Park Y, Hong HJ., Antibody engineering for the development of therapeutic antibodies., Mol Cells. 2005 Aug 31; 20(1): 17-29. Review

[Non-patent Document 4] Hinton PR, Xiong JM, Johlfs MG, Tang MT, Keller S, Tsurushita N., An engineered human IgG1 antibody with longer serum half-life., J Immunol. 2006 Jan 1; 176(1): 346-56

[Non-patent Document 5] Ghetie V, Popov S, Borvak J, Radu C, Matesoi D, Medesan C, Ober RJ, Ward ES., Increasing the serum persistence of an IgG fragment by random mutagenesis., Nat Biotechnol. 1997 Jul; 15(7): 637-40

[Non-patent Document 6] Proc Natl Acad Sci U S A. 2005 Jun 14; 102(24): 8466-71. Epub 2005 Jun 6. A general method for greatly improving the affinity of antibodies by using combinatorial libraries. Rajpal A, Beyaz N, Haber L, Cappuccilli G, Yee H, Bhatt RR, Takeuchi T, Lerner RA, Crea R

[Non-patent Document 7] Wu H, Pfarr DS, Johnson S, Brewah YA, Woods RM, Patel NK, White WI, Young JF, Kiener PA. Development of Motavizumab, an Ultra-potent Antibody for the Prevention of Respiratory Syncytial Virus Infection in the Upper and Lower Respiratory Tract. J Mol Biol. (2007) 368: 652-665

[Non-patent Document 8] Hanson CV, Nishiyama Y, Paul S. Catalytic antibodies and their applications. Curr Opin Biotechnol. 2005 Dec; 16(6): 631-6

[Non-patent Document 9] Rathanaswami P, Roalstad S, Roskos L, Su QJ, Lackie S, Babcook J. Demonstration of an *in vivo* generated sub-picomolar affinity fully human monoclonal antibody to interleukin-8. Biochem Biophys Res Commun. 2005 Sep 9; 334(4): 1004-13

[Summary ~~of the Invention~~]

[Problems to be Solved ~~by the Invention~~]

The present disclosure~~invention~~ was achieved in view of the above circumstances. An objective of the present disclosure~~invention~~ is to provide methods for promoting antigen uptake into cells by using antigen-binding molecules, methods for increasing the number of times of antigen binding by one antigen-binding molecule, methods for promoting the reduction of plasma antigen concentration by administering antigen-binding molecules, methods for improving plasma

retention of antigen-binding molecules, antigen-binding molecules that facilitate antigen uptake into cells, antigen-binding molecules that have an increased number of times of antigen binding, antigen-binding molecules capable of promoting the reduction of plasma antigen concentration by administration, antigen-binding molecules with improved plasma retention, pharmaceutical compositions comprising the antigen-binding molecules, and methods for producing those described above.

[Means for Solving the Problems]

The present inventors conducted dedicated studies on methods for promoting antigen uptake into cells by antigen-binding molecules (molecules such as polypeptides having the antigen-binding activity), methods for increasing the number of times of antigen binding by one antigen-binding molecule, methods for promoting the reduction of plasma antigen concentration by administering antigen-binding molecules, and methods for improving the plasma retention of an antigen-binding molecule. As a result, the present inventors focused on the difference in the calcium concentration between plasma and early endosome, and then discovered that: antigen uptake into cells by antigen-binding molecules could be promoted by using antigen-binding molecules that have antigen-antibody reactivity in a calcium dependent manner; the number of times of antigen binding by one antigen-binding molecule could be increased by repetitive antigen binding of an antigen-binding molecule; the reduction of antigen concentration in plasma could be promoted by administering antigen-binding molecules; and that the plasma retention of antigen-binding molecule could be improved.

In one aspect, there is provided an antigen-binding molecule comprising an antigen-binding domain and a human FcRn-binding domain, whose antigen-binding activity is different under two different calcium concentration conditions and is lower under a low calcium concentration condition than under a high calcium concentration condition, and which has binding activity to human FcRn under a neutral pH condition.

In another aspect, there is provided an antigen-binding molecule comprising an antigen-binding domain and a human FcRn- binding domain, whose antigen-binding activity is different between two different calcium concentration conditions and is lower under a low calcium concentration condition than under a high calcium concentration condition, and wherein a light chain or heavy chain of the antigen-binding domain comprises a calcium-binding motif derived from a human antibody.

In another aspect, there is provided a method of producing an antigen-binding molecule having

at least one function selected from:

(i) function of promoting uptake of an antigen into cells,

(ii) function of binding to an antigen two or more times,

(iii) function of promoting the reduction of plasma antigen concentration, and

(iv) function of excellence in plasma retention,

wherein the method comprises the steps of (a) to (e) below:

(a) determining the antigen-binding activity of an antigen-binding molecule under a low calcium concentration condition;

(b) determining the antigen-binding activity of the antigen-binding molecule under a high calcium concentration condition;

(c) selecting an antigen-binding molecule that has a lower antigen-binding activity under the low calcium concentration condition than under the high calcium concentration condition;

(d) obtaining a gene encoding the antigen-binding molecule selected in step (c); and

(e) producing the antigen-binding molecule using the gene obtained in step (d).

In another aspect, there is provided a method of producing an antigen-binding molecule having at least one function selected from:

(i) function of promoting uptake of an antigen into cells,

(ii) function of binding to an antigen two or more times,

(iii) function of promoting the reduction of plasma antigen concentration, and

(iv) function of excellence in plasma retention,

wherein the method comprises the steps of (a) to (e) below:

(a) contacting an antigen with an antigen-binding molecule or a library of antigen-binding molecules under a high calcium concentration condition;

(b) placing an antigen-binding molecule that binds to the antigen in step (a) under a low calcium concentration condition;

(c) obtaining an antigen-binding molecule that dissociates in step (b);

(d) obtaining a gene encoding the antigen-binding molecule obtained in step (c); and

(e) producing the antigen-binding molecule using the gene obtained in step (d).

In another aspect, there is provided a method of producing an antigen-binding molecule having at least one function selected from:

(i) function of promoting uptake of an antigen into cells,

(ii) function of binding to an antigen two or more times,

(iii) function of promoting the reduction of plasma antigen concentration, and

(iv) function of excellence in plasma retention,

wherein the method comprises the steps of (a) to (f) below:

(a) contacting an antigen with an antigen-binding molecule or a library of antigen-binding molecules under a low calcium concentration condition;

(b) selecting an antigen-binding molecule that does not bind to the antigen in step (a);

(c) allowing the antigen-binding molecule selected in step (b) to bind to the antigen under a high calcium concentration condition;

(d) obtaining an antigen-binding molecule that bound to the antigen in step (c);

(e) obtaining a gene encoding the antigen-binding molecule obtained in step (d); and

(f) producing the antigen-binding molecule using the gene obtained in step (e).

In another aspect, there is provided a pharmaceutical composition comprising:

any one of the antigen-binding molecules described herein or an antigen-binding molecule produced by any one of the the production methods described herein, and a pharmaceutically acceptable carrier.

In another aspect, there is provided a pharmaceutical composition for use in promoting antigen uptake into cells or reduction of plasma antigen concentration, which comprises an antigen-binding molecule comprising an antigen-binding domain and a human FcRn-binding domain, whose antigen-binding activity is different between two different calcium concentrations and is lower under a low calcium concentration condition than under a high calcium concentration condition.

In another aspect, there is provided a method of screening for an antigen-binding molecule that has at least one function selected from:

(i) function of promoting uptake of an antigen into cells,

(ii) function of binding to an antigen two or more times,

(iii) function of promoting the reduction of plasma antigen concentration, and

(iv) function of excellence in plasma retention,

wherein the method comprises the steps of (a) to (c) below:

(a) determining the antigen-binding activity of an antigen-binding molecule under a low calcium concentration condition;

(b) determining the antigen-binding activity of an antigen-binding molecule under a high calcium concentration condition; and

(c) selecting an antigen-binding molecule whose antigen-binding activity is lower under the

low calcium concentration condition than under the high calcium concentration condition.

In another aspect, there is provided a method of screening for an antigen-binding molecule that comprises at least one function selected from:

(i) function of promoting uptake of an antigen into cells,

(ii) function of binding to an antigen two or more times,

(iii) function of promoting the reduction of plasma antigen concentration, and

(iv) function of excellence in plasma retention,

wherein the method comprises the steps of (a) to (c) below:

(a) contacting an antigen with an antigen-binding molecule or a library of antigen-binding molecules under a high calcium concentration condition;

(b) placing an antigen-binding molecule that binds to the antigen in step (a) under a low calcium concentration condition; and

(c) obtaining an antigen-binding molecule that dissociates in step (b).

In another aspect, there is provided a method of screening for an antigen-binding molecule that comprises at least one function selected from:

(i) function of promoting uptake of an antigen into cells,

(ii) function of binding to an antigen two or more times,

(iii) function of promoting the reduction of plasma antigen concentration, and

(iv) function of excellence in plasma retention,

wherein the method comprises the steps of (a) to (d) below:

(a) contacting an antigen with an antigen-binding molecule or a library of antigen-binding molecules under a low calcium concentration condition;

(b) selecting an antigen-binding molecule that does not bind to the antigen in step (a);

(c) allowing the antigen-binding molecule selected in step (b) to bind to the antigen under a high calcium concentration condition; and

(d) obtaining an antigen-binding molecule bound to the antigen in step (c).

In another aspect, there is provided a method for increasing the number of times of antigen binding by one antigen-binding molecule by using any one of the antigen-binding molecules described herein or an antigen-binding molecule produced by any one of the production methods described herein.

In another aspect, there is provided a method for improving plasma retention of an antigen-binding molecule by using any one of the antigen-binding molecules described herein or an

antigen-binding molecule produced by any one of the production methods described herein.

In another aspect, there is provided a method for increasing the number of times of antigen binding by one antigen-binding molecule by using an antigen-binding molecule comprising an antigen-binding domain and a human FcRn-binding domain, whose antigen-binding activity is different between two different calcium concentrations and is lower under a low calcium concentration condition than under a high calcium concentration condition.

In another aspect, there is provided a method for improving plasma retention of an antigen-binding molecule by using an antigen-binding molecule comprising an antigen-binding domain and a human FcRn-binding domain, whose antigen-binding activity is different between two different calcium concentrations and is lower under a low calcium concentration condition than under a high calcium concentration condition.

Specifically, the present disclosure~~invention~~ relates to methods for promoting antigen uptake into cells by using antigen-binding molecules that have antigen-antibody reactivity in a calcium dependent manner, methods for increasing the number of times of antigen binding by one antigen-binding molecule, methods for promoting the reduction of plasma antigen concentration by administering antigen-binding molecules, and methods for improving the plasma retention of antigen-binding molecules, as well as antigen-binding molecules that allow enhanced antigen uptake into cells, antigen-binding molecules with an increased number of times of antigen binding, antigen-binding molecules that can promote the reduction of plasma antigen concentration when administered, antigen-binding molecules with improved plasma retention, pharmaceutical compositions comprising the above antigen-binding molecules, and methods for producing them. More specifically, the present disclosure~~invention~~ relates to the following:
[1] an antigen-binding molecule comprising an antigen-binding domain and a human FcRn-binding domain, whose antigen-binding activity is different under two different calcium concentration conditions and is lower under a low calcium concentration condition than under a high calcium concentration condition, and which has binding activity to human FcRn under a

modification by substituting at least one amino acid with histidine, or inserting at least one histidine into the antigen-binding molecule;

[73] the method of any of [62] to [72], wherein the antigen to which the antigen-binding molecule binds is an antigen selected from the group consisting of IL-6R, IL-6, IgA, human glypican 3, and IgE; and

[74] the method of any of [62] to [73], wherein the antigen-binding molecule is an antibody.

Furthermore, the present disclosure~~invention~~ relates to kits for use in the methods of the present disclosure~~invention~~, which comprise an antigen-binding molecule of the present disclosure~~invention~~ or an antigen-binding molecule produced by production methods of the present disclosure~~invention~~. The present disclosure~~invention~~ also relates to agents for promoting antigen uptake into cells by an antigen-binding molecule, agents for promoting the reduction of plasma antigen concentration, agents for increasing the number of times of antigen binding by one antigen-binding molecule, and agents for improving plasma retention of an antigen-binding molecule, all of which comprise as an active ingredient an antigen-binding molecule of the present disclosure~~invention~~ or an antigen-binding molecule produced by the production method of the present disclosure~~invention~~. Furthermore, the present disclosure~~invention~~ relates to the use of an antigen-binding molecule of the present disclosure~~invention~~ or an antigen-binding molecule produced by the production methods of the present disclosure~~invention~~ in the production of agents for promoting antigen uptake into cells by an antigen-binding molecule, agents for promoting reduction of plasma antigen concentration, agents for increasing the number of times of antigen binding by an antigen-binding molecule, or agents for improving plasma retention of an antigen-binding molecule. The present disclosure~~invention~~ also relates to antigen-binding molecules of the present disclosure~~invention~~ or antigen-binding molecules produced by production methods of the present disclosure~~invention~~ for use in the methods of the present disclosure~~invention~~.

[Effects of the Disclosure~~Invention~~]

The present disclosure~~invention~~ provides methods for promoting antigen uptake into cells by antigen-binding molecules, methods for increasing the number of times of antigen binding by one antigen-binding molecule, methods for promoting the reduction of plasma antigen concentration by administering antigen-binding molecules, and methods for improving the plasma retention of an antigen-binding molecule. Promotion of antigen uptake into cells by antigen-binding molecules enables one to promote reduction of plasma antigen concentration by administering the antigen-binding molecules and also to promote the plasma retention of an antigen-binding molecule. This can increase the number of times of antigen binding by one antigen-binding molecule. Thus, such antigen-binding molecules can produce more superior *in vivo* effects as compared to typical antigen-binding molecules.

is an ionized calcium concentration of 0.1 to 30 μM.

53. The screening method of any one of claims 49 to 51, wherein the high calcium concentration is an ionized calcium concentration of 100 μM to 10 mM.

54. The screening method of any one of claims 49 to 52, wherein the low calcium concentration is an intraendosomal concentration of ionized calcium.

55. The screening method of any one of claims 49 to 51, or 53, wherein the high calcium concentration is a plasma concentration of ionized calcium.

56. The screening method of any one of claims 49 to 55, wherein the antigen to which the antigen-binding molecule binds is an antigen selected from the group consisting of IL-6R, IL-6, IgA, human glypican 3, and IgE.

57. The screening method of any one of claims 49 to 56, wherein the antigen-binding molecule is an antibody.

~~58. A method for promoting antigen uptake into a cell by an antigen-binding molecule by administering the antigen-binding molecule of any one of claims 1 to 24 or an antigen-binding molecule produced by the production method of any one of claims 25 to 36.~~

~~59. A method for promoting the reduction of plasma antigen concentration by administering the antigen-binding molecule of any one of claims 1 to 24 or an antigen-binding molecule produced by the production method of any one of claims 25 to 36.~~

~~60~~58. A method for increasing the number of times of antigen binding by one antigen-binding molecule by using the antigen-binding molecule of any one of claims 1 to 24 or an antigen-binding molecule produced by the production method of any one of claims 25 to 36.

~~61~~59. A method for improving plasma retention of an antigen-binding molecule by using the antigen-binding molecule of any one of claims 1 to 24 or an antigen-binding molecule produced by the production method of any one of claims 25 to 36.

~~62. A method for promoting antigen uptake into a cell by an antigen-binding molecule by administering an antigen-binding molecule comprising an antigen-binding domain and a human~~

~~FcRn-binding domain, whose antigen-binding activity is different between two different calcium concentrations and is lower under a low calcium concentration condition than under a high calcium concentration condition.~~

~~63. A method for promoting the reduction of plasma antigen concentration by administering an antigen-binding molecule comprising an antigen-binding domain and a human FcRn-binding domain, whose antigen-binding activity is different between two different calcium concentrations and is lower under a low calcium concentration condition than under a high calcium concentration condition.~~

~~64~~60. A method for increasing the number of times of antigen binding by one antigen-binding molecule by using an antigen-binding molecule comprising an antigen-binding domain and a human FcRn-binding domain, whose antigen-binding activity is different between two different calcium concentrations and is lower under a low calcium concentration condition than under a high calcium concentration condition.

~~65~~61. A method for improving plasma retention of an antigen-binding molecule by using an antigen-binding molecule comprising an antigen-binding domain and a human FcRn-binding domain, whose antigen-binding activity is different between two different calcium concentrations and is lower under a low calcium concentration condition than under a high calcium concentration condition.

~~66~~62. The method of ~~any one of claims 62 to 65~~claim 60 or 61, wherein the low calcium concentration is an ionized calcium concentration of 0.1 to 30 μM.

~~67~~63. The method of any one of claims ~~62 to 65~~60 to 62, wherein the high calcium concentration is an ionized calcium concentration of 100 μM to 10 mM.

~~68~~64. The method of any one of claims ~~62 to 66~~60 to 63, wherein the low calcium concentration is an intraendosomal concentration of ionized calcium.

~~69~~65. The method of any one of claims ~~62 to 65, or 67~~60 to 64, wherein the high calcium concentration is a plasma concentration of ionized calcium.

~~70~~66. The method of any one of claims ~~62 to 69~~60 to 65, wherein an FcRn-binding domain of the antigen-binding molecule is an Fc region.

~~71~~67. The method of any one of claims ~~62 to 70~~60 to 66, wherein additionally the antigen-binding activity of the antigen-binding molecule is lower under an acidic pH condition than under a neutral pH condition.

~~72~~68. The method of claim ~~71~~67, wherein the amino acid modification in the antigen-binding molecule is modification by substituting at least one amino acid with histidine, or inserting at least one histidine into the antigen-binding molecule.

~~73~~69. The method of any one of claims ~~62 to 72~~60 to 68, wherein the antigen to which the antigen-binding molecule binds is an antigen selected from the group consisting of IL-6R, IL-6, IgA, human glypican 3, and IgE.

~~74~~70. The method of any one of claims ~~62 to 73~~60 to 69, wherein the antigen-binding molecule is an antibody.