Patent Right	Date	June 26, 2019	Court	Intellectual Property High Court, Third Division
	Case number	2018 (Gyo-Ke) 10043		
- A case in which, with regard to the invention titled "ANTIGEN-BINDING MOLECULE CAPABLE OF BINDING TO TWO OR MORE ANTIGEN MOLECULES REPEATEDLY", the Court has determined that the Detailed Description of the Invention does not conform to the enablement requirement of Article 36, paragraph (4), item (i) of the Patent Act, stating that it cannot be said that the Detailed Description of the Invention provides a description to the extent that allows a person ordinarily skilled in the art to implement the whole range of pharmaceutical composition encompassed by the invention.				

Case type: Rescission of Trial Decision to Maintain

Result: Granted

References: Article 36, paragraph (4), item (i) of the Patent Act

Number of related rights, etc.: Patent No. 4954326

Summary of the Judgment

1 The case is a suit against a trial decision that dismissed a request for an invalidation trial of the patent according to the invention titled "antigen-binding molecule capable of binding to two or more antigen molecules repeatedly".

The issues of the case are: [i] conformity to the clarity requirement; and [ii] conformity to the enablement requirement and the support requirement.

2 The court decision has rescinded the JPO decision, ruling as in the following with regard to [ii] conformity to the enablement requirement.

(1) The scope of the claims of Invention 1 fails to specify an original antibody and the position and number of histidine substitution or insertion. Thus it can also be seen that neither an original antibody nor the position and number of histidine substitution or insertion is limited for an antibody included in a pharmaceutical composition of Invention 1. Therefore, the technical scope of Invention 1 encompasses a pharmaceutical composition comprising every antibody having one or more of histidine substitutions and/or insertions, a certain pH-dependent binding property, and a long half-life in plasma.

Consequently, in order that Invention 1 conforms to the enablement requirement, the Detailed Description of the Invention must provide a description to the extent that allows a person ordinarily skilled in the art to implement the whole range of pharmaceutical compositions encompassed by Invention 1.

(2) The CDR sequence described in [Description of Embodiment] of the description

as a site to be substituted with histidine is only an example, and a desired antibody may likely be obtained by modification of the other site. Thus it does not apply to the whole range of the pharmaceutical composition encompassed by Invention 1.

(3) Example 2 of the description describes a method using homology modeling and conformation model ([0285]).

However, homology modeling is a method that predicts a conformation of a protein with an unknown structure by a computer on the basis of a conformation of a protein with a known structure having amino acid sequence homology. It is a technique that is based on the premise of information of a conformation of a protein homologous in amino acid sequence to a protein to be subjected to structural prediction.

Consequently, the method of Example 2 using homology modeling may not always be utilized when a position of histidine substitution is investigated for general antibodies with unknown structures.

Example 3 of the description describes a method of preliminarily selecting sites where the binding ability was not greatly changed even when a residue of CDR was substituted with histidine by a histidine scanning method to prepare an antibody where any one of the sites is substituted with histidine ([0288] to [0290]). Differing from the method of the above Example 2, this method is applicable to antibodies with unknown structures.

It is indefinite from the description, however, as to whether "sites where the binding ability was not greatly changed even when a residue of CDR was substituted with histidine" of Example 3 ([0289]) always includes a site of an antibody of Invention 1 substituted with histidine. Further, there is no evidence sufficient to find the common technical knowledge that a site of an antibody of Invention 1 substituted with histidine is always encompassed by "sites where the binding ability was not greatly changed even when a residue of CDR was substituted with histidine" as mentioned in the description.

Therefore, the methods of Example 2 and Example 3 are not applicable to the whole range of pharmaceutical composition encompassed by Invention 1.

As described above, it cannot be said that the Detailed Description of the Invention provides a description to the extent that allows a person ordinarily skilled in the art to implement Invention 1 without undue trials and errors on the basis of the description of the Detailed Description of the Invention and the common general knowledge as of the filing.

(4) Paragraph [0029] of the description describes alanine scanning. It is recognized

that alanine scanning was a matter of common technical knowledge as of the filing date as a means for replacing each residue of amino acid sequences one by one with alanine and analyzing the role of each residue (Exhibits Otsu 19 to 23). Therefore, it can be said that a person ordinarily skilled in the art who read the description could have produced an antibody in which each of 220 amino acid residues of variable site of an antibody was exhaustively substituted with histidine one by one on the basis of the common technical knowledge.

Defendant alleges that the identification of a position substituted with histidine after preparing an antibody should be made with the criterion of "a site showing a desired pH dependency (found to be promising or cause pH-dependent binding properties)"; however, the description fails to describe as such, and the criterion for identifying a position substituted with histidine is indefinite from the description or common technical knowledge appeared as evidence to implement the whole range of pharmaceutical composition encompassed into Invention 1.

As seen above, the description has insufficient information on how to identify a position to be substituted with histidine by histidine scanning as Defendant alleges, and thus it cannot be said that the Detailed Description of the Invention provides a description to the extent that allows a person ordinarily skilled in the art to implement Invention 1 without undue trials and errors on the basis of the description of the Detailed Description of the Invention and the common general knowledge as of the filing.

Judgment rendered on June 26, 2019
2018 (Gyo-Ke) 10043 A case of seeking rescission of the JPO decision
Date of conclusion of oral argument: April 15, 2019

Judgment

Plaintiff Alexion pharmaceuticals, incorporated

Defendant CHUGAI PHARMACEUTICAL CO., LTD.

Main text

1 The decision on Invalidation Trial No. 2016-800136 that JPO has made on November 22, 2017 shall be rescinded.

2 The court costs shall be borne by Defendant.

Facts and reasons

No. 1 Claim

The same as the main text

No. 2 Summary of the case (Facts to be found from the evidences listed below and the overall import of oral argument)

1 History of the procedures, etc. in Japan Patent Office

(1) Defendant is a patentee of a patent right according to the invention titled "antigen-binding molecule capable of binding to two or more antigen molecules repeatedly" (Patent No. 4954326, Filing date: April 10, 2009 (claiming priority dates of April 11, 2008, September 26, 2008, March 19, 2009) (hereinafter the filing date is referred to as "the filing date"), Registered Date: March 23, 2012, Number of claims: 6, hereinafter referred to as "the patent right", and the patent according to the patent right is referred to as "the Patent") (Exhibit Ko 16).

(2) Plaintiff requested a trial for patent invalidation with respect to the Patent on December 19, 2016, which was assigned to a collegial body as a case of Invalidation Trial No. 2016-800136 in JPO.

(3) JPO made a trial decision that dismissed the request for a patent invalidation trial on November 22, 2017 (hereinafter referred to as "the trial decision") and its certified copies were served for Plaintiff on November 30. Ninety days are offered as a period for filing a suit.

(4) Plaintiff filed a suit for the case, seeking for the rescission of the trial decision, on March 29, 2018.

2 The recitation of the Claims

The recitation of Claims 1 to 6 of the scope of the claims of the Patent is set forth as below: Hereinafter, the inventions according to the claims are referred to as "Invention 1", "Invention 2", etc. according to the number of the claim, and are collectively referred to as "the Invention". The specification of the Patent (Exhibit Ko 17) is referred to as "the specification", including the drawings.

[Claim 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 to 10000 or less, as well as a longer half-life in plasma, wherein at least one amino acid of a variable region is substituted with histidine, or one histidine is inserted into at least variable region.

[Claim 2] The pharmaceutical composition of Claim 1, wherein 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, is 10 or higher.

[Claim 3] The pharmaceutical composition of Claim 1, wherein 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, is 40 or higher.

[Claim 4] The pharmaceutical composition of any one of Claims 1 to 3, wherein said antibody has an antagonist activity.

[Claim 5] The pharmaceutical composition of any one of Claims 1 to 4, wherein said antibody binds to a membrane antigen or a soluble antigen.

[Claim 6] The pharmaceutical composition of any one of Claims 1 to 5, wherein said antibody is an antibody binding 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.

3 Summary of reasons of trial decision

Plaintiff alleges that [i] nonconformance to the enablement requirement and support requirement with respect to Inventions 1 to 6 (Reason 1 for invalidation), [ii] violation of the provision concerning prior art effect on the grounds of an application corresponding to the following Exhibit Ko 6 (Japanese Patent Application No. 2009-531851, hereinafter referred to as "Prior application 1", which translation relies on

Exhibit Ko 7 document) with respect to the Inventions 1 to 5 (Reason 2 for invalidation), [iii] lack of inventive step on the basis of the following Exhibits Ko 8 to 11 documents and the common general knowledge with respect to Inventions 1, 2, and 4 to 6 (Reason 3 for invalidation), and [iv] nonconformance to the clarity requirement with respect to Inventions 1 to 6 (Reason 4 for invalidation).

The reasons of the trial decision are as per described in the written trial decision (copy) of the attachment. In summary, [i] regarding Reason 1 for invalidation, Inventions 1 to 6 conform to the enablement requirement and support requirement, [ii] regarding Reason 2 for invalidation, it cannot be said that Inventions 1 to 5 are identical to the inventions described in the description of Prior application 1, nor is the prior art effect recognized, [iii] regarding Reason 3 for invalidation, it cannot be said that Inventions 1, 2, and 4 to 6 were easily conceivable on the basis of the following Exhibits Ko 8 to 11 documents and the common general knowledge, nor can it be said that these inventions do not involve inventive step, [iv] regarding Reason 4 for invalidation, Inventions 1 to 6 conform to the clarity requirement.

Exhibit Ko 6: International Publication No. WO2008-043822

Exhibit Ko 7: National Publication of International Patent Application No. 2010-505436

Exhibit Ko 8: Ito et al., FEBS Lett., 1992, 309(1), pp. 85-88

Exhibit Ko 9: U.S. patent publication No. 2006/0141456

Exhibit Ko 10: Junghans et al., Proc. Natl. Acad. Sci. USA (1996) Vol. 93, pp. 5512-5516

Exhibit Ko 11: Sarkar et. al., Nature Biotechnology (2002) Vol 20, pp. 908-913

4 Reasons for rescission

Reason 1 for rescission: Reason 4 for invalidation (Nonconformance to the clarity requirement)

Reason 2 for rescission: Erroneous Determination of Reason 1 for invalidation (Nonconformance to the Enablement requirement and Support requirement)

Reason 3 for rescission: Erroneous Determination of Reason 3 for invalidation (Lack of inventive step)

Reason 4 for rescission: Erroneous Determination of Reason 2 for invalidation (Prior art effect)

(omitted)

No. 5 Judgment of this court

1 The Invention

(1) The recitation of the Claims

The scope description of the claims of the Invention is as per described in the aforesaid No. 2-2.

(2) The description of the specification

The specification has the following descriptions (Exhibit Ko 17):

A. [Technical Field]

[0001] The present invention relates to methods for improving the pharmacokinetics of antigen-binding molecules and methods for increasing the number of times of antigen-binding of antigen-binding molecules, as well as antigen-binding molecules having improved pharmacokinetics, antigen-binding molecules having increased number of times of antigen-binding, and methods for producing such molecules.

B. [Background Art]

[0002] Antibodies are drawing attention as pharmaceuticals as they are highly stable in plasma and have few adverse effects. At present, a number of IgG-type antibody pharmaceuticals are available on the market and many more antibody pharmaceuticals are currently under development In general, the requisite dose of an antibody pharmaceutical is very high. In theory, the dose of an antibody pharmaceutical may be reduced by improving antibody pharmacokinetics or improving the affinity between antibodies and antigens.

[0003] The literature has reported methods for improving antibody pharmacokinetics using artificial substitution of amino acids in constant regions. Similarly, affinity maturation has been reported as a technology for enhancing antigen-binding ability or antigen-neutralizing activity. This technology enables enhancement of antigen-binding activity by introduction of amino acid mutations into the CDR region of a variable region or such. The enhancement of antigen-binding ability enables the reduction of dosage, and further enables improvement of in vivo efficacy.

[0004] Meanwhile, the antigen-neutralizing capacity of a single antibody molecule depends on its affinity. By increasing the affinity, an antigen can be neutralized by smaller amount of an antibody. Various methods can be used to enhance the antibody affinity. Furthermore, if the affinity could be made infinite by covalently binding the antibody to the antigen, a single antibody molecule could neutralize one antigen molecule (a divalent antibody can neutralize two antigen molecules). However, the stoichiometric neutralization

of one antibody against one antigen (one divalent antibody against two antigens) is the limit of pre-existing methods, and thus it is impossible to completely neutralize antigen with an amount of antibody smaller than that of antigen. In other words, the affinity enhancing effect has a limit (Non-Patent Document 9). To prolong the neutralization effect of a neutralizing antibody for a certain period, the antibody must be administered at a dose higher than the amount of antigen produced in the body during the same period. With the improvement of antibody pharmacokinetics or affinity maturation technology alone described above, there is thus a limitation in the reduction of the required antibody dose.

[0005] Accordingly, in order to sustain antibody's antigen-neutralizing effect for a target period with an amount of the antibody smaller than that of antigen, a single antibody must neutralize multiple antigens. Methods for neutralizing multiple antigens with a single antibody include antigen inactivation using catalytic antibodies, which are antibodies conferred with a catalytic function. When the antigen is a protein, it can be inactivated by hydrolyzing its peptide bonds. It is supposed that an antibody can repeatedly neutralize antigens by catalyzing such hydrolysis (Non-Patent Document 8). There are many previous reports published on catalytic antibodies and technologies for producing them. However, there have been no reports of catalytic antibodies having sufficient catalytic activity as a pharmaceutical agent. Specifically, in an antibody in vivo study for a certain antigen, there has been no publication of catalytic antibodies which can produce a comparable or stronger effect even at low doses or produce a more prolonged effect even at a same dose as compared to an ordinary non-catalytic neutralizing antibody.

[0006] As described above, there have been no reports of antibodies that can produce a superior in vivo effect than ordinary neutralizing antibodies through a single antibody neutralizing multiple antigen molecules. Thus, from the viewpoint of dose reduction and sustained effect, there is a need for new technology to produce an antibody having a stronger in vivo effect than ordinary neutralizing antibodies by individually neutralizing multiple antigens with one antibody.

C. [Means for solving problem]

[0010] The present inventors have intensively investigated a method of binding multiple times to an antigen of a polypeptide having an antigen binding property such as an antigen binding molecule, and a method of improving (improving pharmacokinetics) a half-life in plasma (half-life in blood). As a result, the

present inventors have found that an antigen binding molecule with a weak antigen-binding activity at a pH in an earlier stage endosome compared to an antigen-binding activity at a pH in plasma (blood) binds to an antigen multiple times and has a longer half-life in plasma.

D. [Advantageous Effects of Invention]

[0012] The present invention provides a method of repeatedly binding one antigen binding molecule to a plurality of antigens. Binding one antigen binding molecule to a plurality of antigens may improve pharmacokinetics of antigen binding molecule and cause more excellent effects compared to ordinary antigen binding molecule in vivo.

E. [Description of Embodiment]

[0025] In the present invention, the difference in the antigen-binding activity between acidic and neutral pHs is not particularly limited so long as the antigen-binding activity at acidic pH is lower than that at neutral pH. However, the value of KD(pH5.8)/KD(pH7.4), which is a ratio of dissociation constant (KD) against an antigen at pH 5.8 and that at pH 7.4, is preferably 2 or greater, more preferably the value of KD(pH5.8)/KD(pH7.4) is 10 or greater, and still more preferably the value of KD(pH5.8)/KD(pH7.4) is 40 or greater. The upper limit of KD(pH5.8)/KD(pH7.4) value is not particularly limited, and may be any value, for example, 400, 1,000, or 10,000, as long as the molecule can be produced by technologies of a person ordinarily skilled in the art. ...

[0029] The methods for impairing the antigen-binding activity of an antigen-binding molecule at pH 5.8 as compared to that at pH 7.4 (methods for conferring the pH-dependent binding ability) are not particularly limited and may be any methods. Such methods include, for example, methods for impairing the antigen-binding activity at pH 5.8 as compared to that at pH 7.4 by substituting histidine for amino acids in the antigen-binding molecule or inserting histidine into the antigen-binding molecule. It is already known that an antibody can be conferred with a pH-dependent antigen-binding activity by substituting histidine for amino acids in the antibody (FEBS Letter, 309(1), 85-88 (1992)). Such histidine mutation (substitution) or insertion sites are not particularly limited, and any site is acceptable so long as the antigen-binding activity at pH 5.8 is lower than that at pH 7.4 (the value of KD(pH5.8)/KD(pH7.4) gets greater) as compared to before mutation or insertion. When the antigen-binding molecule is an antibody, such sites include, for example, sites within an antibody variable region. The appropriate number of histidine mutation or insertion sites can be

appropriately determined by a person ordinarily skilled in the art. Histidine may be substituted or inserted at a single site, or two or more sites. It is also possible to introduce non-histidine mutation (mutation with amino acids other than histidine) at the same time. Furthermore, histidine mutation may be introduced simultaneously with histidine insertion. It is possible to substitute or insert histidine at random using a method such as histidine scanning, which uses histidine instead of alanine in alanine scanning known to a person ordinarily skilled in the art. Alternatively, antigen-binding molecules whose KD(pH5.8)/KD(pH7.4) is increased as compared to before mutation can be selected from an antigen-binding molecule library with random histidine mutation or insertion.

[0030] When histidine is substituted for amino acids of an antigen-binding molecule or inserted between amino acids of the molecule, it is preferred, but not required, that the antigen-binding activity of the antigen-binding molecule at pH 7.4 after histidine substitution or insertion is comparable to that at pH 7.4 before histidine substitution or insertion. ... When the antigen-binding activity of the antigen-binding molecule has been impaired due to histidine substitution or insertion, the antigen-binding activity may be adjusted by introducing substitution, deletion, addition, and/or insertion of one or more amino acids into the antigen-binding molecule so that the antigen-binding activity becomes comparable to that before histidine substitution or insertion. The present invention also includes such antigen-binding molecules having a comparable binding activity as a result of substitution, deletion, addition, and/or insertion of one or more amino acids after histidine substitution or insertion.

[0070] In the present invention, when the antigen-binding molecule is an antibody, possible sites of histidine or non-natural amino acid substitution include, for example, sites within the CDR sequence or sequence responsible for the CDR structure of an antibody. Such sites include, for example, the sites listed below. The amino acid positions are numbered based on Kabat numbering (Kabat EA et al., (1991) Sequences of Proteins of Immunological Interest, NIH).

[0071] Heavy chain: H27, H31, H32, H33, H35, H50, H58, H59, H61, H62, H63, H64, H65, H99, H100b, and H102 Light chain: L24, L27, L28, L32, L53, L54, L56, L90, L92, and L94

[0072] Among the above sites, H32, H61, L53, L90, and L94 could be universal modification sites.

[0073] When the antigen is the IL-6 receptor (e.g., human IL-6 receptor), preferable modification sites include, but are not particularly limited to, the following.

[0074] Heavy chain: H27, H31, H32, H35, H50, H58, H61, H62, H63, H64, H65, H100b, and H102 Light chain: L24, L27, L28, L32, L53, L56, L90, L92, and L94

[0075] When histidine or non-natural amino acid is substituted at multiple sites, preferred combinations of substitution sites include, for example, the combination of H27, H31, and H35; combination of H27, H31, H32, H35, H58, H62, and H102; combination of L32 and L53; and combination of L28, L32, and L53. In addition, preferred combinations of substitution sites of heavy and light chains include the combination of H27, H31, L32, and L53.

[0076] When the antigen is IL-6 (e.g., human IL-6), preferable modification sites include, but are not particularly limited to, the following.

[0077] Heavy chain: H32, H59, H61, and H99 Light chain: L53, L54, L90, and L94

[0078] When the antigen is the IL-31 receptor (e.g., human IL-31 receptor), preferable modification sites include, but are not particularly limited to, H33.

[0106] In particular, the present inventors noted that the pH in the plasma was different from the pH in the endosomes, and thus discovered that antibodies that strongly bind to antigens under plasma pH condition but that weakly bind to antigens under endosomal pH condition were superior in retention in the plasma, because one antibody molecule could bind to multiple antigens.

[0108] Accordingly, an antigen-binding molecule whose antigen-binding activity at acidic pH is weaker than the antigen-binding activity at neutral pH binds to the antigen in the plasma which have a neutral pH, is taken up into cells, and then dissociates from the antigen in the endosomes which have an acidic pH. The antigen-binding molecule that dissociated from the antigen binds to FcRn, translocates to the cell surface, and returns again in the plasma in a state not bound to antigens. As a result, the antigen-binding molecule can bind to antigens multiple times, thereby improving pharmacokinetics.

[0183] Antigen-binding substances that are screened by the screening methods of the present invention may be prepared by any method. For example, it is possible to use pre-existing antibodies, pre-existing libraries (phage libraries and the like), antibodies and libraries that are prepared from hybridomas obtained by immunizing animals or from B cells of immunized animals, antibodies and

libraries prepared by introducing histidine mutations or non-natural amino acid mutations into the above-described antibodies and libraries (libraries with high content of histidine or non-natural amino acid, libraries introduced with histidine mutations or non-natural amino acid mutations at specific sites, and the like), and so on.

[0191] In addition, the present invention provides libraries in which the histidine content is increased as compared to the original libraries. Libraries containing antigen-binding molecules with increased histidine content can be used in the screening methods described above and the production methods described hereinafter.

[0192] Libraries with increased histidine content can be prepared by methods known to those skilled in the art, which include the following method. 20 types of triplet codons (trinucleotides) encoding 20 types of amino acids can be incorporated at equal frequency when synthesizing nucleic acids to prepare a library by the trinucleotide-method (J Mol Biol. 2008 Feb. 29; 376(4): 1182-200). As a result, the position mutated for the library can be made to contain 20 types of amino acids at equal probability. The frequency of histidine in the position mutated for the library can be increased by increasing the proportion of a histidine-encoding trinucleotide as compared to the remaining amino acids among the 20 types in the synthesis.

[0254]

<Pharmaceutical Compositions>

The present invention also relates to pharmaceutical compositions that include antigen-binding molecules of the present invention, antigen-binding molecules isolated by the screening methods of the present invention, or antigen-binding molecules produced by the production methods of the present invention. The antigen-binding molecules of the present invention and antigen-binding molecules produced by the production methods of the present invention are superior in retention in plasma, and thus, are expected to reduce the administration frequency of the antigen-binding molecules, and are therefore useful as pharmaceutical compositions. The pharmaceutical composition of the present invention may include pharmaceutically acceptable carriers.

F. [Example(s)]

[0264] Herein below, the present invention will be specifically described with reference to Examples, but is not to be construed as being limited thereto.

[0265]

[Example 1] Preparation of modified humanized PM1 antibody ...

[0272]

Preparation of modified humanized IL-6 receptor antibody

In this context, mutations were introduced into the framework sequence and CDR sequence of humanized mouse PM1 antibody (the term "wild type" is abbreviated as WT, the term "wild type H chain" is abbreviated as H(WT) (amino acid sequence of SEQ ID NO: 9), and the term "wild type L chain is abbreviated as L(WT) (amino acid sequence: SEQ ID NO. 10)) described in Cancer Res. 1993, Feb. 15; 53(4): 851-6, to produce modified H chains H53 (amino acid sequence: SEQ ID NO: 1) and PF1H (amino acid sequence: SEQ ID NO: 11), and modified L chains L28 (amino acid sequence: SEQ ID NO: 12) and PF1L (amino acid sequence: SEQ ID NO: 2). ...

[0274]

[Example 2] Production of pH-Dependently-Binding Antibody H3pI/L73

Method for Creating Antibody Capable of Neutralizing Antigen Multiple Times

Since IgG molecules are divalent, a single IgG molecule can neutralize up to two antigen molecules when the two sites bind to the antigens; however, it cannot neutralize three or more antigen molecules. Therefore, to maintain the neutralizing effect of a neutralizing antibody over a certain period, it is necessary to administer an amount of the antibody equal to or greater than the amount of antigen produced during the period. Thus, there is a limitation on the extent to which the required dose of antibody can be reduced by improving the pharmacokinetics or affinity of antibody. Therefore, if it were possible to neutralize two or more antigen molecules with a single IgG molecule, the same dose could improve the duration of neutralizing effect, or alternatively the dose of antibody required to achieve the same duration could be reduced.

[0275] For neutralizing antibodies, there are two types of target antigens: soluble-type antigens, which are present in plasma, and membrane-bound antigens, which are expressed on the surfaces of cells.

[0276] When the antigen is a membrane-bound antigen, an administered antibody binds to the membrane antigen on the cellular surface, and the antibody is subsequently taken up into endosomes within the cell by internalization together with the membrane antigen bound to the antibody. Then, the antibody which is kept bound to the antigen moves to a lysosome where it is degraded by lysosome together with the antigen. The elimination of antibody from the plasma mediated by internalization by membrane antigen is referred to as antigen-

dependent elimination, and this has been reported for numerous antibody molecules (Drug Discov. Today, 2006 January; 11(1-2): 81-8). Since a single IgG antibody molecule binds to two antigen molecules when it divalently binds to antigens, and is then internalized and directly degraded by lysosome, a single ordinary IgG antibody cannot neutralize two or more antigen molecules (FIG. 1).

[0277] The reason for the long retention (slow elimination) of IgG molecules in plasma is that FcRn, known as an IgG molecule salvage receptor, functions (Nat. Rev. Immunol. 2007 September; 7(9): 715-25). IgG molecules that have been taken up into endosomes by pinocytosis bind to FcRn expressed in endosomes under intraendosomal acidic conditions. IgG molecules bound to FcRn move to the cell surface where they dissociate from FcRn under neutral conditions in plasma and return to plasma, while IgG molecules unable to bind to FcRn proceed into lysosomes where they are degraded (FIG. 2).

[0278] IgG molecules bound to a membrane antigen are taken up into intracellular endosomes by internalization, move into lysosomes while bound to the antigen, and undergo degradation. When an IgG antibody divalently binds to antigens, it neutralizes two antigen molecules and undergoes degradation together with the antigens. If the IgG antibody, when taken up into intracellular endosomes by internalization, can dissociate from the antigen under intraendosomal acidic conditions, the dissociated antibody may be able to bind to FcRn expressed in the endosomes. The IgG molecule dissociated from the antigen and bound to FcRn is transferred to the cell surface and then dissociated from FcRn under neutral conditions in the plasma, to thereby return to the plasma again. The IgG molecule that has returned to the plasma is able to bind to a new membrane antigen again. The repetition of this process allows a single IgG molecule to repeatedly bind to membrane antigens, thereby enabling neutralization of multiple antigens with a single IgG molecule (FIG. 3).

[0279] In the case of a soluble antigen, an antibody administered binds to the antigen in the plasma, and remains in the plasma in the form of an antigen-antibody complex. Normally, while the retention of antibody in plasma is very long (elimination rate is very slow) due to the function of FcRn as described above, the retention of antigen in plasma is short (elimination rate is fast). Thus, antibody-bound antigens show retention in plasma comparable to that of antibody (elimination rate is very slow). Antigens are produced in the body at a constant rate and, in the absence of antibody, remain present in plasma at a concentration at which the antigen production rate and the antigen elimination

rate are under equilibrium. In the presence of antibody, most of the antigens are bound to antibodies, resulting in the very slow elimination of antigens. Thus, the antigen concentration in plasma increases as compared with that in the absence of antibody (Kidney Int. 2003, 64, 697-703; J. National Cancer Institute 2002, 94(19), 1484-1493; J. Allergy and Clinical Immunology 1997, 100(1), 110-121; Eur. J. Immunol. 1993, 23; 2026-2029). Even if the affinity of antibody for antigen is infinite, antigen concentration elevates as antibody is slowly eliminated from the plasma, and the neutralizing effect of antibody terminates after the concentrations of antibody and antigen become equal. Although antibodies with a stronger dissociation constant (KD) can neutralize soluble antigens at a lower antibody concentration, antibodies at a concentration half or less than the concentration of antigen present are unable to neutralize antigens regardless of how strong the affinity of antibody is (Biochem. Biophys. Res. Commun. 2005 Sep. 9; 334(4): 1004-13). As is the case with IgG molecules not bound to antigens, IgG molecules bound to antigens in the plasma are also taken up into endosomes by pinocytosis, and bind to FcRn expressed in endosomes under intraendosomal acidic conditions. The IgG molecules bound to FcRn move to the cell surface while the antibody is kept bound to the antigen, and then dissociate from the FcRn under neutral conditions in the plasma. Since the IgG molecules return to the plasma while bound to the antigen, they cannot bind to new antigens in the plasma. In this case, if IgG molecules can dissociate from the antigen under intraendosomal acidic conditions, the dissociated antigen will not be able to bind to FcRn and thereby may be degraded by lysosomes. On the other hand, the IgG molecules can return to the plasma again by binding to FcRn. Since the IgG molecules that have returned to the plasma have already dissociated from the antigen in endosomes, they are able to bind to a new antigen again in the plasma. The repetition of this process allows a single IgG molecule to repeatedly bind to soluble antigens. This enables a single IgG molecule to neutralize multiple antigens (FIG. 4).

[0280] Thus, regardless of whether the antigen is a membrane antigen or a soluble antigen, if the dissociation of IgG antibody from the antigen is possible under intraendosomal acidic conditions, a single IgG molecule would be able to repeatedly neutralize antigens. In order for IgG antibodies to dissociate from antigens under intraendosomal acidic conditions, it is necessary that antigen-antibody binding be considerably weaker under acidic conditions than under neutral conditions. Since membrane antigens on the cell surface need to be

neutralized, antibodies have to strongly bind to antigens at the cell surface pH; namely, pH 7.4. Since the intraendosomal pH has been reported to be typically pH 5.5 to 6.0 (Nat. Rev. Mol. Cell. Biol. 2004 February; 5(2): 121-32), an antibody that weakly binds to an antigen at pH 5.5 to 6.0 is considered to dissociate from the antigen under intraendosomal acidic conditions. More specifically, a single IgG molecule that strongly binds to an antibody at the cell surface pH of 7.4 and weakly binds to the antigen at the intraendosomal pH of 5.5 to 6.0 may be able to neutralize multiple antigens and thereby improve the pharmacokinetics.

[0281] In general, protein-protein interactions consist of hydrophobic interaction, electrostatic interaction, and hydrogen bonding, and the binding strength is typically expressed as a binding constant (affinity) or apparent binding constant (avidity). pH-dependent binding, whose binding strength varies between neutral conditions (pH 7.4) and acidic conditions (pH 5.5 to 6.0), is present in naturally-occurring protein-protein interactions. For example, the above-mentioned binding between IgG molecules and FcRn known as a salvage receptor for IgG molecules is strong under acidic conditions (pH 5.5 to 6.0) but remarkably weak under neutral conditions (pH 7.4). Most of such pH-dependently changing protein-protein interactions are associated with histidine residues. Since the pKa of histidine residue is in the vicinity of pH 6.0 to 6.5, the proton dissociation state of histidine residues varies between neutral conditions (pH 7.4) and acidic conditions (pH 5.5 to 6.0). Specifically, histidine residues are not charged and function as hydrogen atom acceptors under neutral conditions (pH 7.4), while they become positively charged and function as hydrogen atom donors under acidic conditions (pH 5.5 to 6.0). It has been reported that the pH-dependent binding of the above-described IgG-FcRn interaction is also associated with histidine residues present in IgG (Mol. Cell. 2001 April; 7(4): 867-77).

[0282] Therefore, pH-dependence can be imparted to protein-protein interactions by substituting an amino acid residue involved in protein-protein interactions with a histidine residue, or by introducing a histidine into an interaction site. Such attempts have also been made in protein-protein interactions between antibodies and antigens, and a mutant antibody with antigen-binding ability decreased under acidic conditions has been successfully acquired by introducing histidine into the CDR sequence of an anti-egg white lysozyme antibody (FEBS Letter (vol. 309, No. 1, 85-88, 1992)). In addition, an antibody that is prepared by introducing histidine into its CDR sequence and specifically binds to an

antigen at the low pH of cancer tissues but weakly binds under neutral conditions has been reported (WO 2003-105757).

[0283] Although methods for introducing pH dependency into antigen-antibody reactions have been reported as described above, an IgG molecule that neutralizes multiple antigens by strongly binding to antigens at the body fluid pH of 7.4 but weakly binding to antigens at the intraendosomal pH of pH 5.5 to 6.0 has not been reported. In other words, there have been no reports relating to modifications that significantly reduce the binding under acidic conditions while maintaining the binding under neutral conditions such that, as compared to an unmodified antibody, a modified antibody binds to antigens multiple times in vivo and thereby shows improved pharmacokinetics as well as improved duration of the neutralizing effect at the same dose.

[0284] The IL-6 receptor is present in the body in the form of either soluble IL-6 receptor or membrane IL-6 receptor (Nat. Clin. Pract. Rheumatol. 2006 November; 2(11): 619-26). Anti-IL-6 receptor antibodies bind to both the soluble IL-6 receptor and membrane IL-6 receptor, and neutralize their biological action. It is considered that, after binding to the membrane IL-6 receptor, anti-IL-6 receptor antibodies are taken up into intracellular endosomes by internalization while bound to the membrane IL-6 receptor, then move into lysosomes while the antibodies are kept bound to the membrane IL-6 receptor, and undergo degradation by lysosomes together with the membrane IL-6 receptor. In fact, it has been reported that a humanized anti-IL-6 receptor antibody exhibits non-linear clearance, and its antigen-dependent elimination greatly contributes to the elimination of the humanized anti-IL-6 receptor antibody (The Journal of Rheumatology, 2003, 30; 71426-1435). Thus, one humanized anti-IL-6 receptor antibody binds to one or two membrane IL-6 receptors (monovalently or divalently), and is then internalized and degraded in lysosomes. Therefore, if it is possible to produce modified antibodies that exhibit greatly reduced binding ability under acidic conditions but retain the same binding ability as the wild type humanized anti-IL-6 receptor antibody under neutral conditions (pH-dependent binding anti-IL-6 receptor antibody), multiple IL-6 receptors can be neutralized with a single humanized anti-IL-6 receptor antibody. Thus, in comparison with wild type humanized anti-IL-6 receptor antibodies, pH-dependent binding anti-IL-6 receptor antibodies may improve the duration of the neutralizing effect in vivo at the same dosage.

[0285]

Production of pH-Dependently Binding Humanized Anti-IL-6 Receptor Antibody H3pI/L73:

Introduction of histidine into a CDR has been reported as a method for introducing pH-dependent binding to antigen-antibody reaction (FEBS Letter (vol. 309, No. 1, 85-88, 1992)). In order to find amino acid residues exposed on the surface of the variable region of the H53/PF1L produced in Example 1 and possible residues interacting with the antigen, an Fv region model of H53/PF1L was created by homology modeling using MOE software (Chemical Computing Group Inc.). A three-dimensional model constructed on the basis of the sequence information of H53/PF1L was used to select H27, H31, H35, L28, L32, and L53 (Kabat numbering, Kabat, E. A. et al., 1991, Sequences of Proteins of Immunological Interest, NIH) as mutation sites that may introduce pH-dependent antigen-binding by histidine introduction. The product in which the residues at H27, H31, and H35 in H53 produced in Example 1 were substituted with histidines was designated as H3pI (amino acid sequence: SEQ ID NO: 3), and the product in which the residues at L28, L32, and L53 in PF1L produced in Example 1 were substituted with histidines was designated as L73 (amino acid sequence: SEQ ID NO: 6).

[0286] Production, Expression and Purification of H3pI/L73 Expression Vector

Amino acid modification was carried out to produce antibodies modified at the selected sites. Mutations were introduced into H53 (nucleotide sequence: SEQ ID NO: 13) and PF1L (nucleotide sequence: SEQ ID NO: 14) produced in Example 1 to produce H3pI (amino acid sequence: SEQ ID NO: 3) and L73 (amino acid sequence: SEQ ID NO: 6). More specifically, the QuikChange Site-Directed Mutagenesis Kit (Stratagene) was used according to the method described in the instructions provided, and the resulting plasmid fragments were inserted into a mammalian cell expression vector to produce the desired H chain expression vector and L chain expression vector. The nucleotide sequences of the resulting expression vectors were determined using a method known to persons skilled in the art. H3pI/L73 which uses H3pI for the H chain and L73 for the L chain was expressed and purified by the method described in Example 1.

[0287]

[Example 3] Conferring pH-Dependent Antigen Binding Ability by His Modification of CDR Using Phage Display Technology

Production of scFv Molecule of Humanized PM1 Antibody

The humanized PM1 antibody, which is a humanized anti-IL-6R antibody

(Cancer Res. 1993 Feb. 15; 53(4): 851-6), was converted into scFv. The VH and VL regions were amplified by PCR, and humanized PM1 HL scFv having the linker sequence GGGGSGGGGSGGGGS (SEQ ID NO. 15) between VH and VL was produced.

[0288]

Selection of Histidine-Introducible Positions by Histidine Scanning

PCR was performed using the produced humanized PM1 HL scFv DNA as a template to produce a histidine library in which any one of the CDR amino acids is replaced with histidine. The library portions were constructed by PCR using primers in which the codon of an amino acid desired to be mutated for the library was replaced with CAT, a codon corresponding to histidine, and other portions were constructed by normal PCR. These portions were then linked by assemble PCR. The constructed library was digested with SfiI, inserted into a phagemide vector pELBG lacI vector that was also digested with SfiI, and then used to transform XL1-Blue (Stratagene). The resulting colonies were used to evaluate antigen binding by phage ELISA and analyze the sequence of HL scFv. Phage ELISA was carried out using a plate coated with SR344 at 1μg/mL in accordance with J. Mol. Biol. 1992; 227: 381-388. Clones that were found to bind to SR344 were subjected to sequence analysis using specific primers.

[0289] Phage titer was determined by ELISA with an anti-Etag antibody (GE Healthcare) and anti-M13 antibody (GE Healthcare). This value was then used to select sites where the binding ability was not greatly changed even when a residue of CDR was substituted with histidine as compared to humanized PM1 HL scFv, based on the results of phage ELISA for SR344. The selected positions are shown in Table 2. Numbering of each residue was in accordance with Kabat numbering (Kabat, et al., 1991, Sequences of Proteins of Immunological Interest, NIH).

[0290]

[TABLE 2] Positions of Histidine Substitution Not Significantly Affecting Binding Ability

H31, H50, H54, H56, H57, H58, H59, H60, H61, H62, H63, H64, H65, H100a, H100b, H102

L24, L26, L27, L28, L30, L31, L32, L52, L53, L54, L56, L90, L92, L93, L94

[0291]

Construction of Histidine-Modified CDR Library

A library was designed in which the amino acids of CDR residues that did not

significantly alter the binding ability when substituted with histidine as shown in Table 2 (histidine-introducible positions) are their original sequence (wild type sequence) or histidine. The library was constructed based on the sequences of the H chain PF1H and the L chain PF1L produced in Example 1 such that the mutated positions for the library have the original sequences or histidines (either the original sequence or histidines).

[0292] The library portions were constructed by PCR using primers that were designed such that a position desired to be mutated for the library has the original amino acid codon or histidine codon, and other portions were produced by normal PCR, or by PCR using synthetic primers as in the library portions. These portions were then linked by assemble PCR (J. Mol. Biol. 1996; 256: 77-88).

[0293] This library was used to construct a ribosome display library in accordance with J. Immunological Methods 1999; 231: 119-135. In order to carry out Escherichia coli cell-free in vitro translation, an SDA sequence (ribosome binding site) and T7 promoter were added to the 5′ side, and a gene3 partial sequence serving as a linker for ribosome display was ligated to the 3′ side using SfiI.

[0294] Acquisition of pH-Dependent Binding scFv from Library by Bead Panning

In order to concentrate only scFv having the ability to bind to SR344, panning was carried out twice by the ribosome display method in accordance with Nature Biotechnology 2000 December; 18: 1287-1292. The prepared SR344 was biotinylated using NHS-PEO4-Biotin (Pierce) to obtain an antigen. Panning was carried out using 40 nM of the biotinylated antigen.

[0299] However, no clones exhibiting potent pH-dependent binding ability were obtained by this panning using the antigen immobilized on the magnetic beads. Clones that were found to show weak pH-dependent binding ability were subjected to sequence analysis using specific primers. The positions in these clones where histidine was present at a high rate are shown in Table 3.

[0300]

[TABLE 3] Positions of Histidine Substitution Detected Using Phage Library (Magnetic Bead Panning)

H50, H58, H61, H62, H63, H64, H65, H102

L24, L27, L28, L32, L53, L56, L90, L92, L94

[0301]

Acquisition of pH-Dependently Binding scFv from Library by Column Panning

No clones having strong pH-dependent binding ability were obtained by typical panning using the magnetic bead-immobilized antigen. This may be due to the following reasons. In the panning using an antigen immobilized on magnetic beads or a plate, all phages dissociated from the magnetic beads or plate under acidic conditions are collected. Thus, phage clones having weak pH dependency recovered together reduce the likelihood that clones having strong pH dependency are included in the finally concentrated clones.

[0302] Therefore, panning using a column immobilized with an antigen was examined as a more stringent panning method (FIG. 5). ...

[0305]

Evaluation by Phage ELISA

The resulting phages were evaluated by phage ELISA. Clones that were found to have strong pH dependency were subjected to sequence analysis using specific primers. As a result, several clones showing strong pH-dependent binding as compared to WT were obtained. As shown in FIG. 6, clone CL5 (H chain: CLH5, L chain: CLL5) (CLH5: amino acid sequence of SEQ ID NO: 5, CLL5: amino acid sequence of SEQ ID NO: 8) was found to exhibit particularly strong pH-dependent binding as compared to WT. It was thus confirmed that antibodies exhibiting strong pH-dependent binding, while being unable to be obtained by typical panning using the antigen immobilized onto magnetic beads, can be obtained by panning using a column immobilized with the antigen. Therefore, panning using an antigen-immobilized column was found to be a highly effective method for obtaining pH-dependently binding antibodies from a library. The amino acid sequences of the clones showing pH-dependent binding were analyzed, and the positions where histidine was present at a high probability in the concentrated clones are shown in Table 4.

[0306]

[TABLE 4] Positions of Histidine Substitution found by Phage Library (Column Panning)

H31, H50, H58, H62, H63, H65, H100b, H102

L24, L27, L28, L32, L53, L56, L90, L92, L94

[0307]

[Example 4] Expression and Purification of Histidine-Modified Humanized IL-6 Receptor Antibody

Production, Expression, and Purification of Expression Vector of Histidine-

Modified Humanized IL-6 Receptor Antibody

In order to convert clones showing strong pH dependency in phage ELISA to IgG, VH, and VL were respectively amplified by PCR, digested with XhoI/NheI and EcoRI, and inserted to a mammalian cell expression vector. The nucleotide sequence of each DNA fragment was determined by a method known to persons skilled in the art. CLH5/L73, in which CLH5 was used for the H chain and L73 obtained in Example 2 was used for the L chain, was expressed and purified as IgG. Expression and purification were carried out by the method described in Example 1.

[0308] Antibody having even higher pH dependency was produced by combining mutation sites. Based on the locations where His was concentrated in the phage library as well as the structural information and the like, H32, H58, H62, and H102 in H3pI which was obtained as an H chain in Example 2 were substituted with histidine, and H95 and H99 were further substituted with valine and isoleucine, respectively, to produce H170 (SEQ ID NO: 4). The variant production was carried out using the method described in Example 1. In addition, L82 (SEQ ID NO: 7) was produced by substituting the 28th histidine of L73, which was produced as an L chain in Example 2, with aspartic acid. The variant production was carried out using the method described in Example 1. H170/L82, in which H170 was used for the H chain and L82 was used for the L chain, was expressed and purified as IgG using the method described in Example 1.

[0311]

[Example 6] Biacore Analysis of pH-Dependently Binding Antibody

Analysis of Binding of pH-Dependently Binding Clones to Soluble IL-6 Receptor

Kinetic analyses of antigen-antibody reactions at pH 5.8 and pH 7.4 were carried out using Biacore T100 (GE Healthcare) on the four antibodies: humanized PM1 antibody (WT) and H3pI/L73, CLH5/L73, and H170/L82 produced in Examples 2 and 4 (buffer: 10 mM MES (pH 7.4 or pH 5.8), 150 mM NaCl, and 0.05% Tween 20). ...

[0312] As a result of calculating the affinity ratio between pH 5.8 and pH 7.4 for each clone, the pH-dependent binding (affinity) of H3pI/L73, H170/L82, and CLH5/L73 to SR344 was 41-fold, 394-fold, and 66-fold, respectively, each showing the pH-dependent binding more than 15 times higher than that of WT.

[0313] Anti-IL-6 receptor antibodies that strongly bind to the antigen at the

plasma pH of 7.4 but weakly bind to the antigen at the intraendosomal pH of 5.5 to 6.0 have not yet been reported. In this study, antibodies were obtained that retain the biological neutralization activity equivalent to the WT humanized IL-6 receptor antibody and the affinity at pH 7.4, but exhibit the affinity at pH 5.8 that has been specifically lowered by a factor of more than 10 times.

[0314]

[TABLE 5] Comparison of Binding of pH-Dependently Binding Clones Directed Against SR344 to Soluble IL-6 Receptor

	pH7.4			pH5.8			
	ka(1/Ms)	kd(1/s)	KD(M)	ka(1/Ms)	kd(1/s)	KD(M)	KD(pH5.8)/KD(pH7.4)
WT	5.1E+05	1.0E-03	2.1E-09	7.6E+05	3.8E-03	5.0E-09	**2.4**
H3pI/L73	5.4E+05	7.4E-04	1.4E-09	1.7E+05	9.7E-03	5.7E-08	**41.3**
H170/L82	6.8E+05	1.1E-03	1.6E-09	2.6E+04	1.7E-02	6.4E-07	**393.5**
CLH5/L73	7.1E+05	7.9E-04	1.1E-09	3.8E+05	2.8E-02	7.4E-08	**66.1**

[0335]

[Example 10] Improvement of pH-Dependent Binding to Membrane-Type IL-6 Receptor by Optimization of Variable Region

Optimization of Variable Regions H3pI/L73 and CLH5/L82

Antibodies having pH-dependent binding abilities were shown to demonstrate superior effects in Example 9. Therefore, to further improve the pH-dependent binding abilities, mutations were introduced into the CDR sequence of CLH5 obtained in Example 3 to construct VH1-IgG1 (SEQ ID NO: 21) and VH2-IgG1 (SEQ ID NO: 22). In addition, mutations were introduced into the framework sequence and CDR sequence of H3pI to construct the modified H chains VH3-IgG1 (SEQ ID NO: 23) and VH4-IgG1 (SEQ ID NO: 24). Mutations were introduced into the CDR sequences of L73 and L82 to construct the modified L chains VL1-CK (SEQ ID NO: 25), VL2-CK (SEQ ID NO: 26), and VL3-CK (SEQ ID NO: 27). ...

[0336] The antibody having VH2-IgG1 (SEQ ID NO: 22) as H chain and VL2-CK (SEQ ID NO: 26) as L chain was denoted as Fv1-IgG1, the antibody having VH1-IgG1 (SEQ ID NO: 21) as H chain and L82 as L chain was denoted as Fv2-IgG1, the antibody having VH4-IgG1 (SEQ ID NO: 24) as H chain and VL1-CK (SEQ ID NO: 25) as L chain was denoted as Fv3-IgG1, and the antibody having VH3-IgG1 (SEQ ID NO: 23) as H chain and VL3-CK (SEQ ID NO: 27) as L chain was denoted as Fv4-IgG1. Of these, Fv2-IgG1 and Fv4-IgG1 were expressed and purified. The expression and purification were carried out by the method described in Example 1. ...

[0391]

[Example 18] Repetitive Binding to Antigen by pH-Dependently Binding Antibody

Expression and Purification of Antibody Administered to Mice

The four types of humanized IL-6 receptor antibodies described below were prepared. As the antibodies that do not pH-dependently bind to the IL-6 receptor, WT-IgG1 including H (WT) (amino acid sequence: SEQ ID NO: 9) and L (WT) (amino acid sequence: SEQ ID NO: 10), and H54/L28-IgG1 including H54 (amino acid sequence: SEQ ID NO: 70) and L28 (amino acid sequence: SEQ ID NO: 12) were expressed and purified using the method indicated in Example 1. As the antibodies that pH-dependently bind to the IL-6 receptor, H170/L82-IgG1of Example 3 including H170 (amino acid sequence: SEQ ID NO: 4) and L82 (amino acid sequence: SEQ ID NO: 7), and Fv4-IgG1of Example 10 including VH3-IgG1 (SEQ ID NO: 23) and VL3-CK (SEQ ID NO: 27) were expressed and purified using the method indicated in Example 1.

[0392]

Analysis of Binding of Each Type of Antibody to Soluble IL-6 Receptor

Kinetic analysis of antigen-antibody reactions at pH 7.4 and pH 5.8 was carried out using Biacore T100 (GE Healthcare) for the four types of antibodies prepared: WT-IgG1, H54/L28-IgG1, H170/L82-IgG1, and Fv4-IgG1 (buffer: 10 mM MES (pH 7.4 or pH 5.8), 150 mM NaCl, 0.05% Surfactant-P20). The antibodies were bound to a sensor chip on which recomb-protein A/G (Pierce) was immobilized by amine coupling, and SR344 adjusted to an appropriate concentration was injected therein as an analyte. The association with and dissociation from SR344 of each type of antibody were observed on a real-time basis. All the measurements were carried out at 37°C. Association rate constants ka (1/Ms) and dissociation rate constants kd (1/s) were calculated using Biacore T100 Evaluation Software (GE Healthcare), and dissociation constants KD (M) were calculated based on those values (Table 17).

[0393]

[TABLE 17] Comparison of Association Rates (ka), Dissociation Rates (kd), and Dissociation Constants (KD) of Each Type of Antibody Against Soluble IL-6 Receptor (SR344)

Sample	pH7.4			pH5.8			pH dependency	
	ka(1/Ms)	kd(1/s)	KD(M)	ka(1/Ms)	kd(1/s)	KD(M)	kd(pH5.8)/kd(pH7.4)	KD(pH5.8)/KD(pH7.4)
WT-IgG1	4.9E+05	9.4E-04	1.9E-09	8.9E+05	2.7E-03	3.1E-09	2.9	1.6
H54/L28-IgG1	8.3E+05	1.4E-03	1.7E-09	2.4E+06	2.7E-03	1.1E-09	2.0	0.7
H170/L82-IgG1	6.7E+05	1.1E-03	1.6E-09	1.2E+05	1.3E-02	1.0E-07	11.4	61.9
Fv4-IgG1	9.8E+05	9.5E-04	9.7E-10	1.4E+06	3.7E-02	2.6E-08	38.8	27.3

[0394] The ratio of affinity (KD) at pH 5.8 and pH 7.4 for each antibody was calculated. The KD ratio, which indicates the pH-dependent binding to SR344, was 1.6, 0.7, 61.9, and 27.3 for WT-IgG1, H54/L28-IgG1, H170/L82-IgG1, and Fv4-IgG1, respectively. In addition, the ratio of dissociation rate (kd) at pH 5.8 and pH 7.4 for each antibody was calculated. The kd ratio, which indicates the pH-dependent dissociation rate for SR344, was 2.9, 2.0, 11.4, and 38.8 for WT-IgG1, H54/L28-IgG1, H170/L82-IgG1, and Fv4-IgG1, respectively. Thus, it was confirmed that H170/L82-IgG1 and Fv4-IgG1 demonstrate the pH-dependent binding, while the conventional antibodies WT-IgG1 and H54/L28-IgG1 hardly exhibit the ability. In addition, since the affinity (KD) of these antibodies at pH 7.4 was nearly equal, their ability to bind to SR344 in the plasma was thought to be equivalent.

(3) Feature of the Invention

According to the above (1) and (2), the following matters are recognized with regard to the Invention: The present invention relates to methods for improving the pharmacokinetics of antigen-binding molecules and methods for increasing the number of times of antigen-binding of antigen-binding molecules, as well as antigen-binding molecules having improved pharmacokinetics, antigen-binding molecules having increased number of times of antigen-binding, and methods for producing such molecules. ([0001])

In general, the requisite dose of an antibody pharmaceutical is very high. The literature has reported methods for improving antibody pharmacokinetics using artificial substitution of amino acids in constant regions. Similarly, affinity maturation has been reported as a technology for enhancing antigen-binding ability or antigen-neutralizing activity. The enhancement of antigen-binding ability enables the reduction of dosage, and further enables improvement of in vivo efficacy. ([0002], [0003])

The stoichiometric neutralization of one antibody against one antigen (one divalent antibody against two antigens) is the limit of pre-existing methods; in a case of neutralizing antibody, to prolong the neutralization effect of a neutralizing antibody

for a certain period, the antibody must be administered at a dose higher than the amount of antigen produced in the body during the same period. With the improvement of antibody pharmacokinetics or affinity maturation technology alone described above, there is thus a limitation on the reduction of the required antibody dose. ([0004])

From the viewpoint of dose reduction and sustained effect, there is a need for new technology to produce an antibody having a stronger in vivo effect than ordinary neutralizing antibodies by individually neutralizing multiple antigens with one antibody. ([0006])

The present inventors have found that an antigen binding molecule with a weak antigen binding property at a pH in an earlier stage endosome as compared to an antigen binding property at a pH in plasma (blood) binds to an antigen multiple times and has a longer half-life in plasma. ([0010])

Binding one antigen binding molecule to a plurality of antigens may improve pharmacokinetics of antigen binding molecule and cause more excellent effects as compared to ordinary antigen binding molecule in vivo. ([0012])

2 Reasons 2 for rescission (Erroneous Determination of Reason 1 for invalidation (Enablement requirement and Support requirement))

In view of the nature of the case, Reason 2 for rescission is firstly considered in the following.

(1) Enablement requirement

A. Article 36(4)(i) of the Patent Act specifies that the Detailed Description of the Invention is required to disclose matters necessary for those who have an ordinary knowledge in a technical field to which the invention pertains to understand a technical significance of the invention, including a problem to be solved by the invention and a means for solving the problem, definitely and sufficiently to the extent that allowed those who have expert knowledge in the technical field to implement the invention. In order that the description of the Detailed Description of the Invention conforms to the enablement requirement, the Detailed Description of the Invention is required to provide a description to the extent that allows a person ordinarily skilled in the art to implement the invention without undue trials and errors on the basis of the description of the Detailed Description of the Invention and the common general knowledge as of the filing.

B. The scope of the claims of Invention 1 neither specifies an original antibody nor the position and number of histidine substitution or insertion. Thus it can be seen that an antibody included in a pharmaceutical composition of Invention 1

specifies neither an original antibody nor the position and number of histidine substitution or insertion. Therefore, the technical scope of the invention 1 encompasses every pharmaceutical composition comprising every antibody having one or more of histidine substitutions and/or insertions, a certain pH-dependent binding property, and a long half-life in plasma.

Consequently, in order that Invention 1 conforms to the enablement requirement, the Detailed Description of the Invention must provide a description to the extent that allows a person ordinarily skilled in the art to implement the whole range of pharmaceutical compositions encompassed by Invention 1.

(2) The descriptions of the Detailed Description of the Invention

A. Description of [Description of Embodiment]

(A) Regarding a method of weakening an antigen binding activity of an antigen binging molecule at a pH of 7.4 as compared with that at a pH of 5.8 (a method of imparting a pH-dependent binding ability), paragraph [0029] of the description discloses that [i] the position of histidine substitution or insertion is not particularly limited, [ii] the position may include a variable region of an antibody in a case of an antigen binding molecule being an antibody, [iii] the number of histidine substitutions or insertions may be determined as necessary by a person ordinarily skilled in the art, [iv] mutation other than histidine mutation (mutation to amino acid other than histidine) may be introduced simultaneously, [v] histidine substitution and insertion may be done simultaneously. Furthermore, it is possible to substitute or insert histidine at random using a method such as histidine scanning, which uses histidine instead of alanine in alanine scanning known to those skilled in the art. Alternatively, it is described that antigen-binding molecules with increased KD(pH5.8)/KD(pH7.4) compared to before substitution can be selected from an antigen-binding molecule library with random histidine substitution or insertion.

Further, with respect to the site substituted with histidine, paragraphs [0070] to [0078] disclose that CDR sequence of antibody and a sequence for determination of CDR structure are assumed in a case of an antigen binding molecule being an antibody, including, for example, 16 sites for heavy chain and 10 sites for light chain, and furthermore, 4 sites of these are supposed to be universal modifying sites, and some examples of preferable combinations may be given in a case where a plurality of sites are substituted with histidine in combination.

(B) However, the above CDR sequence is only an example, and a desired antibody may be obtained by the modification of another site. Thus it does not

apply to the whole range of the pharmaceutical composition encompassed by Invention 1.

B. Description of [Example]

(A) According to the description of H3pI/L73 in [0285], the description of CLH5/L73 in [0287] to [0291], [0294], [0305], and [0307], the description of HL170/L82 in [0308], the description of H170/L82-IgG1 in [0308] and [0391], and the description of Fv4-IgG1 in [0335], [0336], and [0391], it can be said as the methods of Example 2 and Example 3 for which a method of identifying the position of histidine substitution or insertion with regard to the "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 to 10000 or less, wherein at least one amino acid of a variable region is substituted with histidine, or one histidine is inserted into at least a variable region" of Invention 1; i.e., an antibody with a determined pH-dependent binding property that is substituted with or inserted by histidine.

(B) Example 2

Example 2 describes a method using homology modeling and conformation model ([0285]).

However, homology modeling is a method that predicts a conformation of a protein with unknown structure by a computer on the basis of a conformation of a protein with known structure having amino acid sequence homology. It is a technique that is based on the premise of information of a conformation of a protein homologous in amino acid sequence to a protein to be subjected to structural prediction (no dispute between parties).

Consequently, the method of Example 2 using homology modeling may not always be utilized when a position of histidine substitution is investigated for general antibodies with unknown structures.

Therefore, the method of Example 2 is not applicable to the whole range of pharmaceutical composition encompassed by Invention 1.

(C) Example 3

Example 3 describes a method of preliminarily selecting sites where the binding ability was not greatly changed even when a residue of CDR was substituted with histidine by a histidine scanning method to prepare an antibody where any one of the sites is substituted with histidine ([0288] to [0290]). Differing from the method of Example 2 of the above (B), this method is applicable to antibodies with unknown structures.

It is indefinite from the description, however, as to whether "sites where the binding ability was not greatly changed even when a residue of CDR was substituted with histidine" of Example 3 ([0289]) always includes a site of an antibody of Invention 1 substituted with histidine. Further, there is no evidence sufficient to find the common technical knowledge that a site of an antibody of Invention 1 substituted with histidine is always encompassed by "sites where the binding ability was not greatly changed even when a residue of CDR was substituted with histidine" as mentioned in the description.

Therefore, the method of Example 3 is not applicable to the whole range of pharmaceutical composition encompassed by Invention 1.

C. As described above, it cannot be said that the Detailed Description of the Invention of the description is described to the extent that allows a person ordinarily skilled in the art to implement Invention 1 without undue trials and errors on the basis of the description of the Detailed Description of the Invention and the common general knowledge as of the filing.

(3) Defendant's allegation

A. Defendant alleges that a person ordinarily skilled in the art could implement Invention 1 in view of the description including paragraph [0029] and [0116] and the common general knowledge by [i] a test for preparing an antibody, in which each of 220 amino acid residues of a variable site of an antibody is substituted with histidine, measuring the KD value and identifying the substituted position for identifying a substituted site of histidine (hereinafter referred to as "the first half of test") and [ii] a test of blood kinetics in a case where a desired pH dependency is shown by the above [i] (found to be promising and cause pH-dependent binding properties) (hereinafter referred to as "the second half of test") (histidine scanning as Defendant alleges).

A consideration is given hereinafter. Paragraph [0029] of the description describes alanine scanning. It is recognized that the alanine scanning was a matter of common technical knowledge as of the filing date as a means for replacing each residue of amino acid sequences one by one with alanine and analyzing the role of each residue (Exhibits Otsu 19 to 23). Therefore, it can be said that a person ordinarily skilled in the art who read the description could have produced an antibody in which each of 220 amino acid residues of variable sites of an antibody was exhaustively substituted with histidine one by one on the basis of the common technical knowledge.

Defendant alleges that the identification of a position substituted with histidine

after preparing an antibody is made with the criteria of "a site showing a desired pH dependency (found to be promising or cause pH-dependent binding properties)"; however, the description fails to describe as such, and the criteria for identifying a position substituted with histidine is indefinite from the description or common technical knowledge appearing in evidence to implement the whole range of pharmaceutical composition encompassed by Invention 1.

As seen above, the description has insufficient information on how to identify the position to be substituted with histidine by histidine scanning as alleged by Defendant, and thus it cannot be said that the Detailed Description of the Invention of the description is described to the extent that allows a person ordinarily skilled in the art to implement Invention 1 without undue trials and errors on the basis of the description of the Detailed Description of the Invention and the common general knowledge as of the filing.

B. When it comes to the identification of the position of histidine substitution in a first half test of histidine scanning as Defendant alleges, it can be seen as [i] a site "where KD(pH5.8)/KD(pH7.4) is increased as compared to before mutation" as described in [0029] of the description, or [ii] a site "having certain pH-dependent binding properties" as recited in the scope of claims, it cannot be said that such histidine scanning allows for the implementation of the whole range of pharmaceutical compositions of Invention 1 for the following reasons:

(A) "Antibody" "in which at least one amino acid of a variable region is substituted with histidine, or one histidine is inserted into at least variable region" of Invention 1 includes an antibody substituted with a plurality of histidines. Defendant alleges that it is sufficient to combine the positions of histidine substitution identified by a first half test for identifying the positions of a single histidine substitution of antibodies substituted with a plurality of histidines.

(B) Accordingly, a consideration is given as to whether the position of histidine substitution can be always identified in an antibody of Invention 1 substituted with a plurality of histidines by a method of combining the positions of a single substitution as Defendant alleges.

a. The description fails to show that a site of histidine substitution in an antibody having a certain pH-dependent binding property can always be identified by a first half test of histidine scanning as Defendant alleges, wherein the antibody is substituted with a plurality of histidines. Further, there is no proper evidence showing the common general knowledge as of the

filing for this fact.

Consequently, the Detailed Description of the Invention fails to describe the constitution of the invention to the extent that allows a person ordinarily skilled in the art to implement the case where a plurality of sites are substituted with histidines.

b. In addition, while it is a document after filing date, the description of Exhibit Ko 43 of anti-C5 antibody where a plurality of sites are substituted with histidines (Exhibit Ko 43, [0276], [0281], [0282], Table 3) also supports the above determination.

(a) Specifically, an antibody in which a reference antibody is substituted with histidine at any one site of E62, D66, or S104 for heavy chain and N28, I29, or A55 for light chain has a lower KD(pH5.5)/KD(pH7.4) compared to a value of the reference antibody (2.6). The KD(pH5.5)/KD(pH7.4) is 6.73 to 19.4 for an antibody substituted with histidine at three sites of any one of these sites, heavy chain F100 and light chain S26.

Further, KD(pH5.5)/KD(pH7.4) is 1.83 and 1.8 respectively in a case where a reference antibody is substituted with histidine solely at heavy chain N63 or light chain A51. These values are lower than 2. In contrast, an antibody in which a reference antibody is substituted with histidine at three sites of the above heavy chain N63, heavy chain F100, and light chain S26 has a KD(pH5.5)/KD(pH7.4) of 10.03. An antibody in which a reference antibody is substituted with histidine at three sites of the above light chain A51, heavy chain F100, and light chain S26 has a KD(pH5.5)/KD(pH7.4) of 4.02.

(b) According to this, it is presumed that a site of histidine substitution of an antibody substituted with a plurality of histidines included in Invention 1 may include [i] a site in which a single histidine substitution provides a KD(pH5.8)/KD(pH7.4) value lower than a value of an antibody before substitution, and [ii] a site in which a single histidine substitution may not provide a certain pH-dependent binding property (In addition, the description of the above (a) relates to KD(pH5.5)/KD(pH7.4). This might possibly be a little bit higher than a KD(pH5.8)/KD(pH7.4) value of Invention 1. Thus the above presumption could have been made (Exhibit Otsu 34 and the overall import of oral argument)).

Further, the above sites [i] and [ii] do not apply to the specific criteria of the position of histidine substitution in a first half test ([i] a site

"where KD(pH5.8)/KD(pH7.4) is increased as compared to before mutation", or [ii] a site "having a certain pH-dependent binding properties"). Thus these sites would not be identified as the position of histidine substitution by a first half test.

Therefore, the combination of single substitution positions as Defendant alleges may not implement the pharmaceutical composition according to Invention 1 comprising antibody including these sites of substitution.

(c) Regarding the credibility of Exhibit Ko 43, Defendant alleges that it is only N28H and I29H of light chain that decrease KD(pH5.5)/KD(pH7.4) in a reference antibody and increases KD(pH5.5)/KD(pH7.4) in an antibody where heavy chain F100 and light chain S26 are substituted with histidine; however, a plurality of the other positions can be found as a similar substitution position as in the above (a).

Further, Defendant alleges that a degree of decrease in KD(pH5.5)/KD(pH7.4) by substitution falls within an error range; however, as in the above (a), a reference antibody has a KD(pH5.5)/KD(pH7.4) of 2.6, whereas KD(pH5.5)/KD(pH7.4) is about 1.8 for the substitution of light chain A51H and heavy chain F100H. Thus it is doubtful whether this can be said as falling within an error range.

C. Defendant alleges that single substitution or insertion only has an added effect in most cases for an antibody where a plurality of histidine substitutions or insertions are introduced, and thus is not necessary to reconsider the value of KD(pH5.8)/KD(pH7.4) for a combination of individual substitutions or insertions found to be promising by histidine scanning as Defendant alleges.

However, as explained in the above B, it cannot be said that a plurality of positions of histidine substitution can be always identified by histidine scanning as Defendant alleges even if single substitution or insertion only has an added effect in many cases for an antibody where a plurality of histidine substitutions or insertions are introduced. Thus the Defendant's allegation does not affect the determination of the above (2).

D. Defendant refers to the use of libraries ([0183], [0191], [0192]) and conformation model (Example 2); however, information for identifying histidine substitution position still remains insufficient. Thus such allegation does not affect the determination of the above (2).

(4) As aforementioned, Invention 1 does not conform to the enablement

requirement. Further, Inventions 2 to 6 are inventions depending from Invention 1. Thus the above explanation holds true with respect to the conformity to Inventions 2 to 6 to the enablement requirement. Therefore, the Inventions do not conform to the enablement requirement.

3. As aforementioned, the Invention should be invalidated for the Reason 1 for invalidation. The determination of the trial decision that negates this is erroneous. Thus Reason 2 for rescission has a point, and the trial decision should be rescinded without the determination of the remaining reasons for rescission.

Therefore, the judgment shall be made as described in the main text.

Intellectual Property High Court, Third Division

Presiding Judge TSURUOKA Toshihiko

Judge YAMAKADO Masaru

Judge TAKAHASHI Aya