

DESCRIPTION

Title of Invention: NOVEL ANTI-HUMAN Ig β ANTIBODY

5 Technical Field

[0001]

The present invention relates to a novel anti-human Ig β antibody which is useful as an active ingredient of a pharmaceutical composition.

10 Background Art

[0002]

A B cell receptor (BCR) is composed of membrane immunoglobulin (mIg) molecules assembled with heterodimers of Ig α (CD79A) and Ig β (CD79B). An antigen is bound to the mIg and allow the receptors to aggregate, and an Ig α /Ig β subunit transmits
15 a signal to the inside of a B cell (Mol. Immunol., Vol. 41, p. 599-613, 2004).

[0003]

As for a protein family of an Fc γ receptor (Fc γ R) which is an Fc receptor against an IgG antibody, Fc γ RIa (CD64A), Fc γ RIIa (CD32A), and Fc γ RIIIa (CD16A) which have immunoactive functions, and Fc γ RIIb (CD32B) which has immunosuppressive functions
20 have been reported. It has been reported that when BCR and Fc γ RIIb on B cells are crosslinked through an IgG immune complex, an activity of the B cells is suppressed and thus a proliferation of the B cells and antibody production are suppressed (Nat. Rev. Immunol., Vol. 10, p. 328-343, 2010; Nat. Rev. Immunol., Vol. 8, p. 34-47, 2008; Nat. Rev. Immunol., Vol. 2, p. 580-592, 2002).

25 [0004]

It has been reported that control of the activity of B cells through such Fc γ RIIb is deeply involved in the pathology of autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus.

[0005]

30 As for the relation to rheumatoid arthritis, it has been reported that in an Fc γ RIIb knockout mouse, humoral immunity is not appropriately controlled (Nature, Vol. 379, p. 346- 349, 1996; J. Immunol., Vol. 163, p. 618- 622, 1999) and susceptibility to collagen-induced arthritis is increased (J. Exp. Med., Vol. 189, p. 187-194, 1999). Further, it has been confirmed that expression of Fc γ RIIb in memory B cells of rheumatoid arthritis
35 patients is decreased (J. Immunol., Vol. 190, p. 6015-6022, 2013).

[0006]

As for the relation to systemic lupus erythematosus, it has been reported that onset of a systemic lupus erythematosus disease is significantly suppressed in a transgenic mouse

in which expression of FcγRIIb is enhanced specifically in B cells (J. Exp. Med., Vol. 205, p. 883-895, 2008). It has been confirmed that in regard to a knockout mouse of FcγRIIb, self-reactive B cells or plasma cells appear and the disease condition of systemic lupus erythematosus develops spontaneously (Immunity, Vol. 13, p. 277-285, 2000; J. Exp. Med., Vol. 207, p. 2767-2778, 2010). Further, a decrease in expression of FcγRIIb in memory B cells of systemic lupus erythematosus patients (J. Exp. Med., Vol. 203, p. 2157-2164, 2006; J. Immunol., Vol. 178, p. 3272-3280, 2007) and relevance between genetic polymorphism in a cell transmembrane region of FcγRIIb and frequency of onsets of systemic lupus erythematosus (Arthritis Rheum., Vol. 46, p. 1242-1254, 2002) have been reported.

[0007]

Further, suppression of antibody production by controlling an activity of B cells through FcγRIIb is effective for treating an autoimmune disease in which an autoantibody is related to the pathological condition.

[0008]

Idiopathic thrombocytopenic purpura is an autoimmune disease in which an autoantibody against platelets of a patient causes platelet destruction (Autoimmun. Rev., Vol. 13, p. 577-583, 2014). It has been reported that in an animal to which an antiplatelet antibody is administered, thrombopenia is induced (Br. J. Haematol., Vol. 167, p. 110-120, 2014) and a decrease in an autoantibody are effective for the treatment of idiopathic thrombocytopenic purpura (Ther. Apher. Dial. Vol. 16, p. 311-320, 2012; Lupus, Vol. 22, p. 664-674, 2013).

[0009]

Therefore, if a monoclonal antibody that crosslinks BCR and FcγRIIb and increases an immunosuppressive function of FcγRIIb can be developed, it is expected that such monoclonal antibody is useful for prevention or treatment of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and idiopathic thrombocytopenic purpura.

[0010]

As an antibody that crosslinks BCR and FcγRIIb, DART which is a bispecific antibody against Igβ and FcγRIIb (Patent Document 1 and Non-Patent Document 1), and anti-CD19 S267E/L328F which has a variable region binding to CD19 which is a part of a BCR complex and an Fc region whose affinity for FcγRIIb is increased (Patent Document 2 and Non-Patent Documents 2 and 3) are reported. Among these, anti-CD19 S267E/L328F is specifically examined, and its inhibitory action with respect to the activity of B cells in which BCR is stimulated and its lowering action of human blood antibody titer concentration in a mouse to which human peripheral blood mononuclear cells (PBMC) are transplanted are confirmed (Patent Document 2 and Non-Patent Documents 2 and 3).

Related Art

Patent Document

[0011]

5 [Patent Document 1] WO 2012/018687

[Patent Document 2] WO 2008/150494

Non-Patent Document

[0012]

[Non-Patent Document 1] Arthritis & Rheumatism (US) 2010; 62(7): 1933-1943

10 [Non-Patent Document 2] Molecular Immunology (US) 2008; 45(15): 3926-3933

[Non-Patent Document 3] The Journal of Immunology (US) 2011; 186(7): 4223-4233

Disclosure of Invention

15 Problems to Be Solved by the Invention

[0013]

An object of the present invention is to provide an anti-human Ig β antibody which crosslinks BCR and Fc γ RIIb and has an immunosuppressive function more enhanced than that of an antibody in the prior art.

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Means for Solving the Problems

[0014]

As a result of intensive research on preparation of an anti-human Ig β antibody by the present inventors, a plurality of anti-human Ig β antibodies comprising a heavy chain variable region comprising CDR1 consisting of the amino acid sequence of amino acid numbers 31 to 35 of SEQ ID NO: 2, CDR2 consisting of the amino acid sequence of amino acid numbers 50 to 65 of SEQ ID NO: 2, and CDR3 consisting of the amino acid sequence of amino acid numbers 98 to 108 of SEQ ID NO: 2, and a light chain variable region comprising CDR1 consisting of the amino acid sequence of amino acid numbers 24 to 38 of SEQ ID NO: 4, CDR2 consisting of the amino acid sequence of amino acid numbers 54 to 60 of SEQ ID NO: 4, and CDR3 consisting of the amino acid sequence of amino acid numbers 93 to 101 of SEQ ID NO: 4, in which a heavy chain constant region of the antibody is a human Ig γ 1 constant region having amino acid mutations of S239D, H268D, and L328W were prepared (Examples 1 to 3), and it was found that these antibodies bind to human Ig β on human B cells (Examples 4 and 5) and inhibit activation of the human B cells induced by an anti-IgM antibody (Example 6). As a result, the above-described anti-human Ig β antibody is provided, thereby completing the present invention. Further, it was found that the antibody suppresses the plasma human antibody titer in a human PBMC

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transfer NOG mouse model (Example 7) and suppresses an antigen-specific antibody without being affected by the total antibody titers in plasma in a monkey TTx antigen sensitization model (Example 8).

[0015]

5 That is, the present invention includes the following invention as a material or a method which is medically or industrially applicable.

(1) An anti-human Ig β antibody comprising a heavy chain variable region comprising CDR1 consisting of the amino acid sequence of amino acid numbers 31 to 35 of SEQ ID NO: 2, CDR2 consisting of the amino acid sequence of amino acid numbers 50 to 65 of
10 SEQ ID NO: 2, and CDR3 consisting of the amino acid sequence of amino acid numbers 98 to 108 of SEQ ID NO: 2, a light chain variable region comprising CDR1 consisting of the amino acid sequence of amino acid numbers 24 to 38 of SEQ ID NO: 4, CDR2 consisting of the amino acid sequence of amino acid numbers 54 to 60 of SEQ ID NO: 4, and CDR3 consisting of the amino acid sequence of amino acid numbers 93 to 101 of SEQ
15 ID NO: 4, and a heavy chain constant region which is a human Ig γ 1 constant region having amino acid mutations of S239D, H268D, and L328W.

(2) The anti-human Ig β antibody of (1) above which is a humanized antibody.

(3) The anti-human Ig β antibody of (1) above, selected from the group consisting of the following 1) to 4):

20 1) an anti-human Ig β antibody comprising a heavy chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 6, a light chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 8, and a heavy chain constant region which is a human Ig γ 1 constant region having amino acid mutations of S239D, H268D, and L328W;

25 2) an anti-human Ig β antibody comprising a heavy chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 2, a light chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 4, and a heavy chain constant region which is a human Ig γ 1 constant region having amino acid mutations of S239D, H268D, and L328W;

30 3) an anti-human Ig β antibody comprising a heavy chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 10, a light chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 12, and a heavy chain constant region which is a human Ig γ 1 constant region having amino acid mutations of S239D, H268D, and L328W; and

35 4) an anti-human Ig β antibody which is derived from posttranslational modification of the anti-human Ig β antibody of any one of (1) to (3) above.

(4) The anti-human Ig β antibody of (3) above, comprising a heavy chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 6, a

light chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 8, and a heavy chain constant region which is a human Igγ1 constant region having amino acid mutations of S239D, H268D, and L328W.

5 (5) The anti-human Igβ antibody of (3) above, comprising a heavy chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 2, a light chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 4, and a heavy chain constant region which is a human Igγ1 constant region having amino acid mutations of S239D, H268D, and L328W.

10 (6) The anti-human Igβ antibody of (3) above, comprising a heavy chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 10, a light chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 12, and a heavy chain constant region which is a human Igγ1 constant region having amino acid mutations of S239D, H268D, and L328W.

15 (7) An anti-human Igβ antibody which is derived from posttranslational modification of the anti-human Igβ antibody of any one of (4) to (6) above.

(8) The anti-human Igβ antibody of (3) or (7) above, wherein the posttranslational modification is pyroglutamylation at the N terminal of the heavy chain variable region and/or deletion of lysine at the C terminal of the heavy chain.

20 (9) The anti-human Igβ antibody of any one of (1) to (8) above, comprising a light chain constant region which is a human Igκ constant region.

(10) The anti-human Igβ antibody of (1) above, comprising a heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 6 and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 8.

25 (11) The anti-human Igβ antibody of (1), comprising a heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 2 and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 4.

(12) The anti-human Igβ antibody of (1) above, comprising a heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 10 and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 12.

30 (13) An anti-human Igβ antibody which is derived from posttranslational modification of the anti-human Igβ antibody of any one of (10) to (12) above.

(14) The anti-human Igβ antibody of (13) above, wherein the posttranslational modification is pyroglutamylation at the N terminal of the heavy chain variable region and/or deletion of lysine at the C terminal of the heavy chain.

35 (15) The anti-human Igβ antibody of (13) above, comprising a heavy chain consisting of the amino acid sequence of amino acid numbers of 1 to 448 of SEQ ID NO: 6 in which glutamine of amino acid number 1 is modified to pyroglutamic acid and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 8.

(16) The anti-human Ig β antibody of (13) above, comprising a heavy chain consisting of the amino acid sequence of amino acid numbers 1 to 448 of SEQ ID NO:2 and a light chain consisting of the amino acid sequence shown by SEQ ID NO:4.

5 (17) The anti-human Ig β antibody of (13) above, comprising a heavy chain consisting of the amino acid sequence of amino acid numbers 1 to 448 of SEQ ID NO:10 and a light chain consisting of the amino acid sequence shown by SEQ ID NO:12.

(18) A polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of any one of (1) to (6) above.

10 (19) A polynucleotide comprising a base sequence encoding the light chain of the anti-human Ig β antibody of any one of (1) to (6) above.

(20) An expression vector comprising the polynucleotide of (18) and/or (19) above.

(21) A host cell transformed with the expression vector of (20) above, selected from the group consisting of the following (a) to (d):

15 (a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of any one of (1) to (6) above and a polynucleotide comprising a base sequence encoding the light chain of the antibody;

20 (b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of any one of (1) to (6) above and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody;

(c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of any one of (1) to (6) above; and

25 (d) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the anti-human Ig β antibody of any one of (1) to (6) above.

(22) A host cell transformed with the expression vector of (20) above, selected from the group consisting of the following (a) to (d):

30 (a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of any one of (10) to (13) above and a polynucleotide comprising a base sequence encoding the light chain of the antibody;

35 (b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of any one of (10) to (13) above and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody;

(c) a host cell transformed with an expression vector comprising a polynucleotide

comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of any one of (10) to (13) above; and

(d) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the anti-human Ig β antibody of any one of (10) to (13) above.

(23) A method for producing an anti-human Ig β antibody comprising culturing host cell(s) selected from the group consisting of the following (a) to (c) to express the anti-human Ig β antibody:

(a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of any one of (1) to (6) above and a polynucleotide comprising a base sequence encoding the light chain of the antibody;

(b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of any one of (1) to (6) above and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody; and

(c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of any one of (1) to (6) above and a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody.

(24) A method for producing an anti-human Ig β antibody comprising culturing host cell(s) selected from the group consisting of the following (a) to (c) to express the anti-human Ig β antibody:

(a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of any one of (10) to (13) above and a polynucleotide comprising a base sequence encoding the light chain of the antibody;

(b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of any one of (10) to (13) above and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody; and

(c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of any one of (10) to (13) above and a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody.

(25) An anti-human Ig β antibody which is produced by the method of (23) above.

- (26) An anti-human Ig β antibody which is produced by the method of (24) above.
- (27) A pharmaceutical composition comprising the anti-human Ig β antibody of any one of (1) to (17), (25), and (26) above and a pharmaceutically acceptable excipient.
- (28) A pharmaceutical composition comprising the anti-human Ig β antibody of (10) above,
 5 the anti-human Ig β antibody of (15) above, and a pharmaceutically acceptable excipient.
- (29) The pharmaceutical composition of (27) or (28) above, which is a pharmaceutical composition for preventing or treating an autoimmune disease.
- (30) The pharmaceutical composition of (29) above, wherein the autoimmune disease is systemic lupus erythematosus, rheumatoid arthritis, or idiopathic thrombocytopenic
 10 purpura.
- (31) A method for preventing or treating an autoimmune disease, comprising administering a therapeutically effective amount of the anti-human Ig β antibody of any one of (1) to (17), (25), and (26) above.
- (32) The method of (1) to (17), (25), and (26) above, wherein the autoimmune disease is
 15 systemic lupus erythematosus, rheumatoid arthritis, or idiopathic thrombocytopenic purpura.
- (33) The anti-human Ig β antibody of any one of (1) to (17), (25), and (26) above for use in preventing or treating an autoimmune disease.
- (34) The anti-human Ig β antibody of (33) above, wherein the autoimmune disease is
 20 systemic lupus erythematosus, rheumatoid arthritis, or idiopathic thrombocytopenic purpura.
- (35) Use of the anti-human Ig β antibody of any one of (1) to (17), (25), and (26) above for manufacture of a pharmaceutical composition for preventing or treating an autoimmune disease.
- 25 (36) The use of (35) above, wherein the autoimmune disease is systemic lupus erythematosus, rheumatoid arthritis, or idiopathic thrombocytopenic purpura.

Effects of the Invention

[0016]

- 30 An anti-human Ig β of the present invention has an excellent immunosuppressive action by means of inhibiting activation of B cells and can be used as an agent for preventing or treating of autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and idiopathic thrombocytopenic purpura.

35 Brief Description of the Drawings

[0017]

Fig. 1 shows an inhibitory effect of a humanized anti-Ig β antibody against anti-IgM antibody-induced cell proliferation in human B cells. The vertical axis indicates a

rate of proliferation of B cells and the horizontal axis indicates added antibody concentration ($\mu\text{g/mL}$).

[0018]

Fig. 2 shows an inhibitory action of a humanized anti-Ig β antibody against an increase in human IgM antibody titers in plasma induced by transfer of human PBMC into an NOG mouse. The vertical axis indicates the human IgM antibody titer in plasma ($\mu\text{g/mL}$) and the horizontal axis indicates the time (day) from transferring the human PBMC into the NOG mouse.

[0019]

Fig. 3 shows an inhibitory action of a humanized anti-Ig β antibody against an increase in human IgE antibody titers in plasma induced by transfer of human PBMC into an NOG mouse. The vertical axis indicates the human IgE antibody titer in plasma (ng/mL) and the horizontal axis indicates the time (day) from transferring the human PBMC into the NOG mouse.

[0020]

Fig. 4 shows an inhibitory action of a humanized anti-Ig β antibody against an increase in anti-adsorbed tetanus toxoid in plasma caused by immunizing adsorbed tetanus toxoid to a monkey. The vertical axis indicates the anti-adsorbed tetanus toxoid antibody titer in plasma (U/mL) and the horizontal axis indicates the time (day) from immunizing adsorbed tetanus toxoid to a monkey.

[0021]

Fig. 5 shows an action of the humanized anti-Ig β antibody against total IgM antibody titer in plasma of the monkey immunized by adsorbed tetanus toxoid. The vertical axis indicates the total IgM antibody titer in plasma (U/mL) and the horizontal axis indicates the time (day) from immunizing adsorbed tetanus toxoid to a monkey.

[0022]

Fig. 6 shows an action of the humanized anti-Ig β antibody against total IgA antibody titer in plasma of the monkey immunized by adsorbed tetanus toxoid. The vertical axis indicates the total IgA antibody titer in plasma (U/mL) and the horizontal axis indicates the time (day) from immunizing adsorbed tetanus toxoid to a monkey.

[0023]

Fig. 7 shows an action of the humanized anti-Ig β antibody against total IgG antibody titer in plasma of the monkey immunized by adsorbed tetanus toxoid. The vertical axis indicates the total IgG antibody titer in plasma (U/mL) and the horizontal axis indicates the time (day) from immunizing adsorbed tetanus toxoid to a monkey.

Embodiments for Carrying Out the Invention

[0024]

Hereinafter, the present invention will be described in detail.

[0025]

There are five classes of IgG, IgM, IgA, IgD, and IgE in an antibody. The basic structure of an antibody molecule is configured of heavy chains having a molecular weight of 50000 to 70000 and light chains having a molecular weight of 20000 to 30000 in each of the classes in common. Heavy chain usually consists of a polypeptide chain comprising approximately 440 amino acids, has a distinctive structure for each of the classes, and is referred to as Ig γ , Ig μ , Ig α , Ig δ , and Ig ϵ corresponding to IgG, IgM, IgA, IgD, and IgE, respectively. Further, four subclasses of IgG1, IgG2, IgG3, and IgG4 are present in IgG, and the heavy chains respectively corresponding thereto are referred to as Ig γ 1, Ig γ 2, Ig γ 3, and Ig γ 4. Light chain usually consists of a polypeptide chain comprising 220 amino acids, two types of which, type L and type K are known, and are referred to as Ig λ and Ig κ . In a peptide configuration of the basic structure of antibody molecules, two homologous heavy chains and two homologous light chains are bound by disulfide bonds (S-S bond) and non-covalent bonds, and the molecular weight thereof is 150000 to 190000. Two kinds of light chains can be paired with any heavy chain. The respective antibody molecules typically consist of two identical light chains and two identical heavy chains.

[0026]

With regard to intrachain S-S bonds, four of the S-S bonds are present in the heavy chain (five in μ and ϵ chains) and two of them are present in the light chain; one loop is formed per 100 to 110 amino acid residues, and this steric structure is similar among the loops and are referred to as a structural unit or a domain. The domain located at the amino terminal side (N terminal side) in both of the heavy chain and the light chain, whose amino acid sequence is not constant even in a case of a sample from the same class (sub class) of the same kind of animal is referred to as a variable region, and respective domains are referred to as a heavy chain variable region and a light chain variable region. The amino acid sequence of the carboxy terminal side (C terminal side) from the variable region is nearly constant in each class or subclass and is referred to as a constant region.

[0027]

An antigenic binding site of an antibody is configured of the heavy chain variable region and the light chain variable region, and the binding specificity depends on the amino acid sequence of this site. On the other hand, biological activities such as binding to complements and various cells reflect differences in the constant region structures among each class Ig. It is understood that the variability of variable regions of the light chains and the heavy chains is mostly limited to three small hypervariable regions present in both chains and these regions are referred to as complementarity determining regions (CDR: CDR1, CDR2, and CDR3 from the N terminal side). The remaining portion of the variable region is referred to as a framework region (FR) and is relatively constant.

[0028]

<Anti-Human Ig β Antibody of the Present Invention>

The anti-human Ig β antibody of the present invention includes an anti-human Ig β antibody having the following characteristics.

5 An anti-human Ig β antibody comprising a heavy chain variable region comprising CDR1 consisting of the amino acid sequence of amino acid numbers 31 to 35 of SEQ ID NO: 2, CDR2 consisting of the amino acid sequence of amino acid numbers 50 to 65 of SEQ ID NO: 2, and CDR3 consisting of the amino acid sequence of amino acid numbers 98 to 108 of SEQ ID NO: 2, a light chain variable region comprising CDR1 consisting of
10 the amino acid sequence of amino acid numbers 24 to 38 of SEQ ID NO: 4, CDR2 consisting of the amino acid sequence of amino acid numbers 54 to 60 of SEQ ID NO: 4, and CDR3 consisting of the amino acid sequence of amino acid numbers 93 to 101 of SEQ ID NO: 4, and a heavy chain constant region which is a human Ig γ 1 constant region having amino acid mutations of S239D, H268D, and L328W.

15 [0029]

In one embodiment, the anti-human Ig β antibody of the present invention is a humanized antibody. The "humanized antibody" in the present specification means an antibody in a form comprising CDRs derived from a mouse antibody and other antibody portions derived from a human antibody. A method for preparing a humanized antibody
20 is a known in the art and can be prepared with reference to USP Nos. 5225539, 6180370, and the like.

[0030]

In one embodiment, the anti-human Ig β antibody of the present invention is an anti-human Ig β antibody described in any one of the following 1) to 3):

25 1) an anti-human Ig β antibody comprising a heavy chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 2, a light chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 4, and a heavy chain constant region which is a human Ig γ 1 constant region having amino acid mutations of S239D, H268D, and L328W;

30 2) an anti-human Ig β antibody comprising a heavy chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 6, a light chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 8, and a heavy chain constant region which is a human Ig γ 1 constant region having amino acid mutations of S239D, H268D, and L328W; and

35 3) an anti-human Ig β antibody comprising a heavy chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 10, a light chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 12, and a heavy chain constant region which is a human Ig γ 1

constant region having amino acid mutations of S239D, H268D, and L328W.

[0031]

The number of residue regarding introduction of amino acid mutations in an antibody constant region used in the present specification follows the EU index (Kabat et al. 1991, Sequences of Proteins of Immunological Interest, 5th Ed., United States Public Health Service, National Institute of Health, Bethesda). S239D is replacement of serine at 239th position of the amino acid according to the EU index of Kabat et al. in the human Igγ1 constant region with aspartic acid. H268D is replacement of histidine at 268th position of the amino acid according to the EU index of Kabat et al. in the human Igγ1 constant region with aspartic acid. L328W is replacement of leucine at 328th position of the amino acid according to the EU index of Kabat et al. in the human Igγ1 constant region with triptophan. Examples of the human Igγ1 constant region having amino acid mutations of S239D, H268D, and L328W include a human Igγ1 constant region consisting of the amino acid sequence of amino acid numbers 120 to 449 of SEQ ID NO: 2.

[0032]

As the light chain constant region of the anti-human Igβ antibody of the present invention, any one of constant region of Igλ and Igκ can be selected, but a human Igκ constant region is preferable. Examples of the human Igκ constant region include a human Igκ constant region consisting of amino acid sequence of amino acid numbers 113 to 218 of SEQ ID NO: 4.

[0033]

In one embodiment, the anti-human Igβ antibody of the present invention is an anti-human Igβ antibody selected from any one of the following i) to iii):

i) an anti-human Igβ antibody comprising a heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 2 and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 4;

ii) an anti-human Igβ antibody comprising a heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 6 and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 8; and

iii) an anti-human Igβ antibody comprising a heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 10 and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 12.

[0034]

It is known that when an antibody is expressed in cells, the antibody is modified after translation. Examples of the posttranslational modification include cleavage of lysine at the C terminal of the heavy chain by a carboxypeptidase; modification of glutamine or glutamic acid at the N terminal of the heavy chain and the light chain to pyroglutamic acid by pyroglutamylation; glycosylation; oxidation; deamidation; and

glycation, and it is known that such posttranslational modifications occur in various antibodies (Journal of Pharmaceutical Sciences, Vol. 97, p. 2426-2447, 2008).

[0035]

The anti-human Ig β antibody of the present invention includes an anti-human Ig β antibody which has undergone posttranslational modification. Examples of the anti-human Ig β antibody of the present invention which undergoes posttranslational modification include anti-human Ig β antibodies which have undergone pyroglutamylation at the N terminal of the heavy chain variable region and/or deletion of lysine at the C terminal of the heavy chain. It is known in the field that such posttranslational modification due to pyroglutamylation at the N terminal and deletion of lysine at the C terminal does not have any influence on the activity of the antibody (Analytical Biochemistry, Vol. 348, p. 24-39, 2006).

[0036]

For example, the anti-human Ig β antibodies of the present invention include an anti-human Ig β antibody described in any one of the following 1) to 3):

- 1) an anti-human Ig β antibody comprising a heavy chain consisting of the amino acid sequence of SEQ ID NO: 2 in which glutamic acid of amino acid number 1 is modified to pyroglutamic acid and/or lysine of amino acid number 449 is deleted and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 4;
- 2) an anti-human Ig β antibody comprising a heavy chain consisting of the amino acid sequence of SEQ ID NO: 6 in which glutamic acid of amino acid number 1 is modified to pyroglutamic acid and/or lysine of amino acid number 449 is deleted and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 8; and
- 3) an anti-human Ig β antibody comprising a heavy chain consisting of the amino acid sequence of SEQ ID NO: 10 in which glutamic acid of amino acid number 1 is modified to pyroglutamic acid and/or lysine of amino acid number 449 is deleted and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 12.

[0037]

In one embodiment, the anti-human Ig β antibody of the present invention is an anti-human Ig β antibody selected from any one of the following i) to iii):

- i) an anti-human Ig β antibody comprising a heavy chain consisting of the amino acid sequence of amino acid numbers of 1 to 448 of SEQ ID NO: 2 and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 4;
- ii) an anti-human Ig β antibody comprising a heavy chain consisting of the amino acid sequence of amino acid numbers 1 to 448 of SEQ ID NO: 6 in which glutamine of amino acid number 1 is modified to pyroglutamic acid and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 8; and
- iii) an anti-human Ig β antibody comprising a heavy chain consisting of the amino

acid sequence of amino acid numbers 1 to 448 of SEQ ID NO: 10 and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 12.

[0038]

Any person skilled in the art can prepare a fused form of an antibody and another peptide or protein and can also prepare a modified form to which a modifying agent binds on the basis of the present invention, and the antibody of the present invention includes the antibody in these forms. Other peptides or proteins used for the fusion is not particularly limited as long as the binding activity of the antibody is not decreased, and examples thereof include human serum albumin, various tag peptides, artificial helix motif peptide, maltose-binding proteins, glutathione S transferase, various toxins, other peptides or proteins capable of promoting multimerization, and the like. The modifying agent used for the modification is not particularly limited as long as the binding activity of the antibody is not decreased, and examples thereof include polyethylene glycol, sugar chains, phospholipids, liposomes, low-molecular compounds, and the like.

[0039]

The "anti-human Ig β antibody" in the present specification means an antibody binding to a human Ig β . Whether the "anti-human Ig β antibody" binds to a human Ig β is confirmed by using a known binding activity measurement method. Examples of the binding activity measurement method include a method of Enzyme-Linked ImmunoSorbent Assay (ELISA) and the like. In a case of using the ELISA, for example, human Ig β -Flag protein (for example, encoded by the base sequence of SEQ ID NO: 13) is solidified on the ELISA Plate and a test antibody is added thereto to be reacted. After the reaction, a secondary antibody such as an anti-IgG antibody, labeled with an enzyme such as horseradish peroxidase (HRP) or the like, is reacted, and washed off, and then it is possible to confirm whether the test antibody binds to the human Ig β by identifying binding of the secondary antibody through activity measurement using a reagent detecting the activity (for example, in a case of HRP labeling, BM-Chemiluminescence ELISA Substrate (POD) (Roche Diagnostics Inc.)). As a specific measurement method, the method described in Example 4 below can be used.

[0040]

The anti-human Ig β antibody of the present invention includes, in addition to binding to human Ig β , an antibody binding to Ig β derived from other animals (for example, monkey Ig β), as long as the antibody binds to human Ig β .

[0041]

As a method for evaluating the activity of the anti-human Ig β antibody of the present invention, the binding activity on human B cells or the activity of inhibiting activation of the human B cells induced by BCR stimulation may be evaluated. As the methods of evaluating such activity, the methods described in Examples 5 and 6 below can

be used. Preferably, the anti-human Ig β antibody of the present invention has an activity of binding to human Ig β and inhibiting activation of human B cells induced by BCR stimulation.

[0042]

5 The anti-human Ig β antibody of the present invention can be easily prepared by a person skilled in the art using a known method in the field, based on sequence information on the heavy chain and the light chain of the antibody of the present invention, which is disclosed in the present specification. The anti-human Ig β antibody of the present invention is not particularly limited, but can be produced according to the method
10 described in the section of <Method of producing anti-human Ig β antibody of the present invention, and anti-human Ig β antibody produced by the method> described below.

[0043]

The anti-human Ig β antibody of the present invention is further purified as needed, formulated according to a conventional method, and may be used for the prevention or the
15 treatment of autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, idiopathic thrombocytopenic purpura, myasthenia gravis, Grave's disease, optic neuromyelitis, autoimmune hemolytic anemia, pemphigus, antiphospholipid antibody syndrome, ANCA associated vasculitis, Sjogren's syndrome, Hashimoto's disease, chronic inflammatory demyelinating polyneuropathy, or chronic fatigue syndrome.

20 [0044]

<Polynucleotide of the Present Invention>

The polynucleotide of the present invention includes a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of the present invention and a polynucleotide comprising a base sequence encoding the light chain of the
25 anti-human Ig β antibody of the present invention.

[0045]

In one embodiment, the polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of the present invention is a polynucleotide comprising a base sequence encoding the heavy chain consisting of the amino acid
30 sequence shown by SEQ ID NO: 2, a polynucleotide comprising a base sequence encoding the heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 6, or a polynucleotide comprising a base sequence encoding the heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 10.

[0046]

35 Examples of the polynucleotide comprising a base sequence encoding the heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 2 include a polynucleotide comprising the base sequence shown by SEQ ID NO: 1 or 15. Examples of the polynucleotide comprising a base sequence encoding the heavy chain consisting of

the amino acid sequence shown by SEQ ID NO: 6 include a polynucleotide comprising the base sequence shown by SEQ ID NO: 5. Examples of the polynucleotide comprising a base sequence encoding the heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 10 include a polynucleotide comprising the base sequence shown by SEQ ID NO: 9.

[0047]

In one embodiment, the polynucleotide comprising a base sequence encoding the light chain of the anti-human Ig β antibody of the present invention is a polynucleotide comprising a base sequence encoding the light chain consisting of the amino acid sequence shown by SEQ ID NO: 4, a polynucleotide comprising a base sequence encoding the light chain consisting of the amino acid sequence shown by SEQ ID NO: 8, or a polynucleotide comprising a base sequence encoding the light chain consisting of the amino acid sequence shown by SEQ ID NO: 12.

[0048]

Examples of the polynucleotide comprising a base sequence encoding the light chain consisting of the amino acid sequence shown by SEQ ID NO: 4 include a polynucleotide comprising the base sequence shown by SEQ ID NO: 3. Examples of the polynucleotide comprising a base sequence encoding the light chain consisting of the amino acid sequence shown by SEQ ID NO: 8 include a polynucleotide comprising the base sequence shown by SEQ ID NO: 7. Examples of the polynucleotide comprising a base sequence encoding the light chain consisting of the amino acid sequence shown by SEQ ID NO: 12 include a polynucleotide comprising the base sequence shown by SEQ ID NO: 11.

[0049]

The polynucleotide of the present invention can be easily prepared by a person skilled in the art using a known method in the field based on the base sequence. For example, the polynucleotide of the present invention can be synthesized using a known gene synthesis method in the field. As the gene synthesis method, various methods such as a synthesis method of antibody genes described in WO90/07861 known by a person skilled in the art can be used.

[0050]

<Expression vector of the present invention>

An expression vector of the present invention includes an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of the present invention, an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the anti-human Ig β antibody of the present invention, and an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of

the present invention and a polynucleotide comprising a base sequence encoding the light chain of the antibody.

[0051]

5 The expression vector used to express the polynucleotide of the present invention are not particularly limited as long as a polynucleotide comprising the base sequence encoding the heavy chain of the anti-human Ig β antibody of the present invention and/or a polynucleotide comprising the base sequence encoding the light chain of the anti-human Ig β antibody of the present invention can be expressed in various host cells of eukaryotic cells (for example, animal cells, insect cells, plant cells, and yeast) and/or prokaryotic cells (for example, *Escherichia coli*), and the polypeptides encoded by these can be produced. 10 Examples of the expression vector include plasmid vectors, viral vectors (for example, adenovirus or retrovirus), and the like. Preferably pEE6.4 or pEE12.4 (Lonza Biologics, Inc.) can be used.

[0052]

15 The expression vector of the present invention may include a promoter that is operably linked to the polynucleotide of the present invention. Examples of the promoter for expressing the polynucleotide of the invention with animal cells include a virus-derived promoter such as CMV, RSV, or SV40, an actin promoter, an EF (elongation factor) 1 α promoter, and a heat shock promoter. Examples of promoters for expressing the 20 polynucleotide of the invention by bacteria (for example, *Escherichia*) include a trp promoter, a lac promoter, λ PL promoter, and tac promoter. Further, examples of promoters for expressing the polynucleotide of the invention by yeast include a GAL1 promoter, a GAL10 promoter, a PH05 promoter, a PGK promoter, a GAP promoter, and an ADH promoter.

25 [0053]

In the case of using an animal cell, an insect cell, or yeast as the host cell, the expression vector of the present invention may comprise initiation codon and termination codon. In this case, the expression vector of the present invention may comprise an enhancer sequence, an untranslated region on the 5' side and the 3' side of genes encoding 30 the antibody of the present invention or the heavy chain or the light chain, a secretory signal sequence, a splicing junction, a polyadenylation site, or a replicable unit. When *Escherichia coli* is used as the host cell, the expression vector of the present invention may comprise an initiation codon, a termination codon, a terminator region, and a replicable unit. In this case, the expression vector of the present invention may comprise a selection 35 marker (for example, tetracycline resistant genes, ampicillin resistant genes, kanamycin resistant genes, neomycin resistant genes, or dihydrofolate reductase genes) which is generally used according to the necessity.

[0054]

<Transformed host cell of the present invention>

The transformed host cell of the present invention includes a host cell transformed with the expression vector of the present invention, which is selected from the group consisting of the following (a) to (d):

5 (a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of the present invention and a polynucleotide comprising a base sequence encoding the light chain of the antibody;

10 (b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of the present invention and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody;

15 (c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of the present invention; and

(d) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the anti-human Ig β antibody of the present invention.

[0055]

20 Examples of the preferred transformed host cell of the present invention include a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of the present invention and a polynucleotide comprising a base sequence encoding the light chain of the antibody, and a host cell transformed with an expression vector comprising a
25 polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of the present invention and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody.

[0056]

30 The transformed host cell is not particularly limited as long as the host cell is appropriate for the expression vector being used, transformed with the expression vector, and can express the antibody. Examples of the transformed host cell include various cells such as natural cells or artificially established cells which are generally used in the field of the present invention (for example, animal cells (for example, CHO-K1SV cells), insect cells (for example, Sf9), bacteria (for example, Escherichia), yeast (for example, Saccharomyces or Pichia) or the like). Preferably cultured cells such as CHO-K1SV
35 cells, CHO-DG 44 cells, 293 cells, or NS0 cells can be used.

[0057]

A method of transforming the host cell is not particularly limited, but, for example,

a calcium phosphate method or an electroporation method can be used.

[0058]

< Method of producing anti-human Ig β antibody of the present invention, and anti-human Ig β antibody produced by the method>

5 The method for producing the anti-human Ig β antibody of the present invention include a method for producing an anti-human Ig β antibody by culturing host cell(s) selected from the group consisting of the following (a) to (c) to express the anti-human Ig β antibody:

10 (a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of the present invention and a polynucleotide comprising a base sequence encoding the light chain of the antibody;

15 (b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of the present invention and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody; and

20 (c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody of the present invention and a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody.

[0059]

25 The method for producing the anti-human Ig β antibody of the present invention is not particularly limited as long as it includes a step of culturing the transformed host cells of the present invention to express the anti-human Ig β antibody. Examples of the preferred host cells used in the method include the preferred transformed host cells of the present invention as described above.

[0060]

30 The transformed host cell can be cultured by known methods. Culture conditions, for example, the temperature, pH of culture medium, and the culture time are appropriately selected. In a case where the host cell is an animal cell, examples of the culture medium include MEM culture medium supplemented with approximately 5% to 20% of fetal bovine serum (Science, Vol. 130, p. 432-437, 1959), DMEM culture medium (Virology, Vol. 8, p. 396, 1959), RPMI1640 culture medium (J. Am. Med. Assoc., Vol. 199, p. 519, 1967), and a 199 culture medium (Exp. Biol. Med., Vol. 73, p. 1-8, 1950). The pH
35 of the culture medium is preferably approximately 6 to 8, and the culture is generally carried out at approximately 30°C to 40°C for approximately 15 hours to 72 hours while air ventilating and stirring if necessary. In a case where the host cell is an insect cell, as the culture medium, for example, Grace's culture medium (Proc. Natl. Acad. Sci. USA,

Vol. 82, p. 8404, 1985) supplemented with fetal bovine serum can be used. The pH of the culture medium is preferably approximately 5 to 8, and the culture is generally carried out at approximately 20°C to 40°C for approximately 15 hours to 100 hours while air ventilating and stirring if necessary. In a case where the host cell is *Escherichia coli* or yeast, as the culture medium, for example, liquid culture medium supplemented with a source of nutrients is appropriate. It is preferable that the nutrient culture medium include a carbon source, an inorganic nitrogen source, or an organic nitrogen source necessary for the growth of the transformed host cell. Examples of the carbon source include glucose, dextran, soluble starch, and sucrose and examples of the inorganic nitrogen source or the organic nitrogen source include ammonium salts, nitrate salts, amino acids, corn steep liquor, peptone, casein, meat extract, soybean meal, and potato extract. Other nutrients (for example, inorganic salts (for example, calcium chloride, sodium dihydrogen phosphate, and magnesium chloride), vitamins), and antibiotics (for example, tetracycline, neomycin, ampicillin, and kanamycin) may be included as desired. The pH of the culture medium is preferably approximately 5 to 8. In a case where the host cell is *Escherichia coli*, preferred examples of the culture medium include LB culture medium and M9 culture medium (Mol. Clo., Cold Spring Harbor Laboratory, Vol. 3, A2.2). The culture is generally carried out at approximately 14°C to 43°C for approximately 3 hours to 24 hours while air ventilating and stirring if necessary. In a case where the host cell is yeast, as the culture medium, for example, Burkholder minimal medium (Proc. Natl. Acad. Sci. USA, Vol. 77, p. 4505, 1980) can be used. The culture is generally carried out at approximately 20°C to 35°C for approximately 14 hours to 144 hours while air ventilating and stirring if necessary. By carrying out the culture in the above-described manner, it is possible to express the anti-human Ig β antibody of the present invention.

[0061]

The method of producing the anti-human Ig β antibody of the present invention may include recovering, preferably isolating or purifying the anti-human Ig β antibody from the transformed host cell in addition to culturing the transformed host cell of the present invention to express the anti-human Ig β antibody. Examples of the isolation or purification method include methods using solubility such as salting-out and the solvent precipitation method, methods using the difference in molecular weight such as dialysis, ultrafiltration, and gel filtration, methods using an electric charge such as ion exchange chromatography and hydroxylapatite chromatography, methods using specific affinity such as affinity chromatography, methods using the difference in hydrophobicity such as reverse phase high performance liquid chromatography, and methods using the difference in the isoelectric point such as isoelectric focusing phoresis. Preferably, the antibody accumulated in a culture supernatant can be purified by various chromatographies, for example, column chromatography using Protein A column or Protein G column.

[0062]

The anti-human Ig β antibody of the present invention also includes an anti-human Ig β antibody produced by the method for producing the anti-human Ig β antibody of the present invention.

5

[0063]

<Pharmaceutical composition of the present invention>

The pharmaceutical compositions of the present invention include a pharmaceutical composition comprising the anti-human Ig β antibody of the present invention and pharmaceutically acceptable excipients. The pharmaceutical composition of the present invention can be prepared by a method being generally used with excipients, that is, excipients for medicine or carriers for medicine being generally used in the field. Examples of dosage forms of the pharmaceutical compositions include parenteral drug such as an injection drug and a drip infusion drug, and these can be administered by intravenous administration, subcutaneous administration, or the like. In drug preparation, excipients, carriers, and additives in accordance with the dosage forms can be used within the pharmaceutically acceptable range.

10

15

[0064]

The pharmaceutical compositions of the present invention may include plural kinds of anti-human Ig β antibody of the present invention. For example, the present invention includes a pharmaceutical composition comprising an antibody which does not undergo posttranslational modification and an antibody derived from posttranslational modification of the antibody.

20

[0065]

In one embodiment, the pharmaceutical composition of the present invention comprises an anti-human Ig β antibody selected from the group consisting of the following (1) to (3) and an anti-human Ig β antibody derived from posttranslational modification of the anti-human Ig β antibody:

25

30

(1) an anti-human Ig β antibody comprising a heavy chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 6, a light chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 8, and a heavy chain constant region which is a human Ig γ 1 constant region having amino acid mutations of S239D, H268D, and L328W;

35

(2) an anti-human Ig β antibody comprising a heavy chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 2, a light chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 4, and a heavy chain constant region which is a human Ig γ 1 constant region having amino acid mutations of S239D, H268D, and L328W; and

(3) an anti-human Ig β antibody comprising a heavy chain variable region

consisting of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 10, a light chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 12, and a heavy chain constant region which is a human Ig γ 1 constant region having amino acid mutations of S239D, H268D, and L328W.

5 [0066]

The pharmaceutical compositions of the present invention include a pharmaceutical composition comprising an antibody in which lysine at the C terminal of the heavy chain is deleted, an antibody which has undergone post-translational modification to the N terminal, an antibody in which lysine at the C terminal of the heavy chain is deleted and which has undergone post-translation modification to N terminal, and/or an antibody which has lysine at the C terminal of the heavy chain and does not undergo post-translational modification to the N terminal.

[0067]

In one embodiment, the pharmaceutical composition of the present invention comprising an anti-human Ig β antibody includes a pharmaceutical composition comprising two or more anti-human Ig β antibodies selected from the following (1) to (4):

(1) an anti-human Ig β antibody comprising a heavy chain consisting of the amino acid sequence of amino acid numbers 1 to 448 of SEQ ID NO: 2 and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 4;

20 (2) an anti-human Ig β antibody comprising a heavy chain consisting of the amino acid sequence of SEQ ID NO: 2 in which glutamic acid of amino acid number 1 is modified to pyroglutamic acid and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 4;

(3) an anti-human Ig β antibody comprising a heavy chain consisting of the amino acid sequence of amino acid numbers 1 to 448 of SEQ ID NO: 2 in which glutamic acid of amino acid number 1 is modified to pyroglutamic acid and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 4; and

(4) an anti-human Ig β antibody comprising a heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 2 and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 4.

30 [0068]

In one embodiment, the pharmaceutical composition of the present invention comprising an anti-human Ig β antibody includes a pharmaceutical composition comprising two or more anti-human Ig β antibodies selected from the following (1) to (4):

35 (1) an anti-human Ig β antibody comprising a heavy chain consisting of the amino acid sequence of amino acid numbers 1 to 448 of SEQ ID NO: 6 and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 8;

(2) an anti-human Ig β antibody comprising a heavy chain consisting of the amino

acid sequence of SEQ ID NO: 6 in which glutamine of amino acid number 1 is modified to pyroglutamic acid and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 8;

(3) an anti-human Ig β antibody comprising a heavy chain consisting of the amino acid sequence of amino acid numbers 1 to 448 of SEQ ID NO: 6 in which glutamine of amino acid number 1 is modified to pyroglutamic acid and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 8; and

(4) an anti-human Ig β antibody comprising a heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 6 and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 8.

[0069]

In one embodiment, the pharmaceutical composition of the present invention comprising an anti-human Ig β antibody includes a pharmaceutical composition comprising two or more anti-human Ig β antibodies selected from the following (1) to (4):

(1) an anti-human Ig β antibody comprising a heavy chain consisting of the amino acid sequence of amino acid numbers 1 to 448 of SEQ ID NO: 10 and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 12;

(2) an anti-human Ig β antibody comprising a heavy chain consisting of the amino acid sequence of SEQ ID NO: 10 in which glutamic acid of amino acid number 1 is modified to pyroglutamic acid and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 12;

(3) an anti-human Ig β antibody comprising a heavy chain consisting of the amino acid sequence of amino acid numbers 1 to 448 of SEQ ID NO: 10 in which glutamic acid of amino acid number 1 is modified to pyroglutamic acid and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 12; and

(4) an anti-human Ig β antibody comprising a heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 10 and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 12.

[0070]

Further, in one embodiment, the pharmaceutical composition of the present invention is a pharmaceutical composition described below:

a pharmaceutical composition comprising an anti-human Ig β antibody comprising a heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 6 and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 8, an anti-human Ig β antibody comprising a heavy chain consisting of the amino acid sequence of amino acid numbers 1 to 448 of SEQ ID NO: 6 in which glutamine of amino acid number 1 is modified to pyroglutamic acid and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 8, and a pharmaceutically acceptable excipient.

[0071]

The addition amount of the anti-human Ig β antibody of the present invention in formulation varies depending on the degree of a patient's symptoms, the age of a patient, a dosage form of the drug to be used, the binding titer of the antibody, or the like, and for
5 example, an addition amount of approximately 0.001 mg/kg to 100 mg/kg can be used.

[0072]

The pharmaceutical composition of the present invention can be used as an agent for treating autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, idiopathic thrombocytopenic purpura, myasthenia gravis, Grave's disease, optic
10 neuromyelitis, autoimmune hemolytic anemia, pemphigus, antiphospholipid antibody syndrome, ANCA associated vasculitis, Sjogren's syndrome, Hashimoto's disease, chronic inflammatory demyelinating polyneuropathy, chronic fatigue syndrome, or the like.

[0073]

The present invention includes a pharmaceutical composition for preventing or
15 treating systemic lupus erythematosus, rheumatoid arthritis, or idiopathic thrombocytopenic purpura comprising the anti-human Ig β antibody of the present invention. Further, the present invention includes a method for preventing or treating systemic lupus erythematosus, rheumatoid arthritis, or idiopathic thrombocytopenic purpura comprising administering a therapeutically effective amount of the anti-human Ig β
20 antibody of the present invention. Further, the present invention includes the anti-human Ig β antibody of the present invention for use in preventing or treating systemic lupus erythematosus, rheumatoid arthritis, or idiopathic thrombocytopenic purpura. In addition, the present invention includes use of the anti-human Ig β antibody of the present invention for manufacture of a pharmaceutical composition for preventing or treating systemic lupus
25 erythematosus, rheumatoid arthritis, or idiopathic thrombocytopenic purpura.

[0074]

The present invention has been described and specific examples referred to for better understanding will be provided, but these are merely examples and the present invention is not limited thereto.

30 [Examples]

[0075]

With regard to parts using commercially available kits or reagents, the tests are performed according to the attached protocol unless otherwise noted.

[0076]

35 (Example 1: acquisition of human and monkey Ig β -Flag proteins)

A protein in which a Flag tag binds to human Ig β (human Ig β -Flag protein) and a protein in a Flag tag binds to which monkey Ig β (monkey Ig β -Flag protein) were acquired. A human Ig β -Flag gene (SEQ ID NO: 13) was introduced into a GS vector pEE6.4 (Lonza

Biologics, Inc.). A monkey Ig β -Flag gene (SEQ ID NO: 14) was introduced into a GS vector pEE6.4 (Lonza Biologics, Inc.). The respective prepared vectors were gene-transferred to FreeStyle 293 cells (Life Technologies, Inc.) using a FreeStyle MAX Reagent (Life Technologies, Inc.). Respective cells were cultured in a serum-free culture system using a FreeStyle 293 Expression medium (Life Technologies, Inc.) for 1 week and culture supernatants respectively containing human Ig β -Flag protein and monkey Ig β -Flag protein were acquired. The proteins were purified using an anti-Flag M2 antibody affinity gel (SIGMA-ALDRICH Corporation) from the acquired culture supernatants and then used for the following test.

10 [0077]

(Example 2: acquisition of anti-human Ig β antibody)

In order to acquire an anti-human Ig β antibody, the human Ig β -Flag protein and the monkey Ig β -Flag protein acquired in Example 1 were injected to a C3H/HeJmsSlc-lpr/lpr mouse (Japan SLC, Inc.) together with an adjuvant for causing an immune reaction to perform immunization. The mouse was immunized several times and final immunization was performed. According to the conventional method, a spleen and a lymph node of the immunized mouse was extracted, and lymphocytes were collected and cell-fused with mouse myeloma cells SP2/0 (ATCC CRL-1581), thereby preparing a hybridoma. A limiting dilution sample of the hybridoma was prepared and the hybridoma was monocloned. Respective clones were expanded and cultured, the culture medium was changed to Hybridoma SFM (Life Technologies, Inc.), which is a serum-free culture medium, and then the clones were cultured for 3 to 5 days. An antibody was purified using an antibody purifying kit (Protein G Purification kit; Proteus, Inc.) from the obtained culture supernatant.

25 [0078]

In regard to the antibodies obtained from respective clones, the binding activity on human and monkey Ig β -Flag proteins and the binding activity on human and monkey B cells were evaluated. As a result, it was found that an antibody referred to as CL6_40 was bound to both of the human and monkey Ig β -Flag proteins and had a high binding activity with respect to both of the human and monkey B cells. In regard to CL6_40, genes encoding a heavy chain and a light chain from Hybridoma were cloned and sequence determination was performed.

[0079]

(Example 3: preparation of humanized antibody)

35 CDRs of the heavy chain and the light chain of CL6_40 were transplanted to other human antibodies, and a plurality of genes of heavy chains and light chains of humanized antibodies were prepared. An expression vector comprising both genes of a heavy chain and a light chain of respective humanized antibodies was constructed using a GS vector

(Lonza Biologics, Inc.). Specifically, genes encoding signal sequences (N. Whittle et al., Protein Eng., Vol. 1, p. 499-505, 1987) and the constant region gene of human Igγ1

(consisting of the base sequence of base numbers 358 to 1350 of SEQ ID NO: 1) having amino acid mutations of S239D, H268D, and L328W were respectively ligated to the 5'

5 side and the 3' side of the heavy chain variable region genes of respective humanized antibodies, and then the heavy chain genes were inserted into a GS vector pEE6.4.

Further, genes encoding signal sequences (N. Whittle et al., mentioned above) and the constant region genes of a human κ chain (consisting of the base sequence of base numbers

337 to 657 of SEQ ID NO: 3) were respectively ligated to the 5' side and the 3' side of the
10 light chain variable region genes of the respective humanized antibodies, and then the light chain genes were inserted into a GS vector pEE12.4.

[0080]

The base sequence of the heavy chain of the prepared humanized antibody CL6_40m12_DDW is shown by SEQ ID NOS: 1 and 15, the amino acid sequence encoded

15 by the base sequence is shown by SEQ ID NO: 2, the base sequence of the light chain of the antibody is shown by SEQ ID NO: 3, and the amino acid sequence encoded by the base sequence is shown by SEQ ID NO: 4. The heavy chain variable region shown by SEQ ID

NO: 2 consists of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 2, and the CDR1, CDR2, and CDR3 of the heavy chain each consist of the amino acid
20 sequence of amino acid numbers 31 to 35, 50 to 65, and 98 to 108 of SEQ ID NO: 2. The light chain variable region shown by SEQ ID NO: 4 consists of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 4, and the CDR1, CDR2, and CDR3 of the light chain each consist of the amino acid sequence of amino acid numbers 24 to 38, 54 to 60, and 93 to 101 of SEQ ID NO: 4.

25 [0081]

The base sequence of the heavy chain of the prepared humanized antibody CL6_40m14_DDW is shown by SEQ ID NO: 5, the amino acid sequence encoded by the

base sequence is shown by SEQ ID NO: 6, the base sequence of the light chain of the antibody is shown by SEQ ID NO: 7, and the amino acid sequence encoded by the base
30 sequence is shown by SEQ ID NO: 8. The variable region of the heavy chain shown by SEQ ID NO: 6 consists of the amino acid sequence of amino acid numbers 1 to 119 of

SEQ ID NO: 6, and the CDR1, CDR2, and CDR3 of the heavy chain respectively consist of the amino acid sequence of amino acid numbers 31 to 35, 50 to 65, and 98 to 108 of
35 SEQ ID NO: 6. The variable region of the light chain shown by SEQ ID NO: 8 consists of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 8, and the CDR1, CDR2, and CDR3 of the light chain respectively consist of the amino acid sequence of amino acid numbers 24 to 38, 54 to 60, and 93 to 101 of SEQ ID NO: 8.

[0082]

The base sequence of the heavy chain of the prepared humanized antibody CL6_40m16_DDW is shown by SEQ ID NO: 9, the amino acid sequence encoded by the base sequence is shown by SEQ ID NO: 10, the base sequence of the light chain of the antibody is shown by SEQ ID NO: 11, and the amino acid sequence encoded by the base sequence is shown by SEQ ID NO: 12. The variable region of the heavy chain shown by SEQ ID NO: 10 consists of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 10, and the CDR1, CDR2, and CDR3 of the heavy chain respectively consist of the amino acid sequence of amino acid numbers 31 to 35, 50 to 65, and 98 to 108 of SEQ ID NO: 10. The variable region of the light chain shown by SEQ ID NO: 12 consists of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 12, and the CDR1, CDR2, and CDR3 of the light chain respectively consist of the amino acid sequence of amino acid numbers 24 to 38, 54 to 60, and 93 to 101 of SEQ ID NO: 12.

[0083]

CDR1, CDR2, and CDR3 of each of heavy chains shown by SEQ ID NOS: 6 and 10 are the same as CDR1, CDR2, and CDR3 of the heavy chain shown by SEQ ID NO: 2, and CDR1, CDR2, and CDR3 of each of light chains shown by SEQ ID NOS: 8 and 12 are the same as CDR1, CDR2, and CDR3 of the light chain shown by SEQ ID NO: 4.

[0084]

In order to prepare each humanized antibody, the above-described GS vector into which the genes of the heavy chain and the light chain of each antibody were respectively inserted was cleaved with a restriction enzyme by NotI and PvuI, and ligation was performed using a Ligation-Convenience Kit (NIPPONGENE Co., Ltd.), thereby constructing a Double-Gene vector into which both genes of the heavy chain and the light chain were inserted. Next, the Double-Gene vector was transfected using an ExpiFectamine 293 (Life Technologies, Inc.), and cultured for 5 days with respect to Expi 293 cells (Life Technologies, Inc.) cultured in an Expi 293 Expression medium (Life Technologies, Inc.) at approximately 3000000 cells/mL. Next, purified antibodies of respective humanized antibodies were obtained using Protein G (GE Healthcare Japan Corporation) from the obtained culture supernatants. In regard to constitutive expression, antibodies were expressed by transfecting the above-described Double-Gene vector to CHO-K1SV cells (Lonza Biologics, Inc.). Then, purified antibodies of respective humanized antibodies were obtained using MabSelect SuRe (GE Healthcare Japan Corporation) from the culture supernatants. As a result of analyzing amino acid modification of the respective purified humanized antibodies, in most of the purified antibodies, deletion of lysine at the C terminal of the heavy chain occurred in CL6_40m12_DDW, pyroglutamylation at the N terminal of the heavy chain and deletion of lysine at the C terminal of the heavy chain occurred in CL6_40m14_DDW, and deletion of lysine at the C terminal of the heavy chain occurred in CL6_40m16_DDW.

[0085]

(Example 4: ELISA assay with respect to antigen)

In order to measure the antigen binding activity of the humanized antibody, antigen ELISA was used. The human Ig β -Flag protein acquired in Example 1 was prepared with a tris-buffered saline (TBS; Wako Pure Chemical Industries, Ltd.) so as to have a concentration of 5000 ng/mL, added to a NUNC MaxiSorp white 384 plate (Maxisorp 384 plate: Nunc Corporation) by an amount of 15 μ L per well, and then solidified at room temperature for 1 hour. The resultant was washed with TBS-T (0.05% Tween-20 containing TBS: Wako Pure Chemical Industries, Ltd.) twice, 120 μ L of a blocking agent (Blocking One: Nacalai tesque, Inc.) was added thereto, the resultant was left at room temperature for 1 hour, and the solution was removed. A dilution series (8 steps with a final concentration of 0.46 ng/mL to 1 μ g/mL) of respective humanized antibodies obtained in Example 3 was prepared using a dilute solution obtained by adding the same amount of the blocking agent and TBS and then added thereto by an amount of 15 μ L. The resultant was left at room temperature for 1 hour, washed with a TBS-T washing liquid three times, and 20 μ L of an horseradish peroxidase (HRP)-labeled rabbit anti-human Ig antibody (Dako Ltd.) which had been diluted 3000-fold with a diluted solution was added thereto. Thereafter, the resultant was left at room temperature for 1 hour and then washed with a TBS-T washing liquid three times. Next, 30 μ L of BM-Chemiluminescence ELISA Substrate (POD) (Roche Diagnostics Inc.) which is a chemiluminescence detection reagent was added thereto, and the amount of chemiluminescence thereof was measured by an EnVision counter (PerkinElmer, Co., Ltd.). Using the same method, antigen ELISA assay was performed using the monkey Ig β -Flag protein acquired in Example 1. When the binding activities in respective concentrations of the test antibodies were calculated, the measuring amount of a well to which a test antibody was not added was set to 0% and the convergence value of the maximum activity of the test antibody was set to 100%. The calculated binding activities were analyzed and the EC50 values of the test antibodies were calculated by fitting a curve.

30 [0086]

As a result, the EC50 values with respect to human and monkey Ig β -Flag proteins of CL6_40m12_DDW were respectively 128 ng/mL and 183 ng/mL. The EC50 values with respect to human and monkey Ig β -Flag proteins of CL6_40m14_DDW were respectively 100 ng/mL and 106 ng/mL. The EC50 values with respect to human and monkey Ig β -Flag proteins of CL6_40m16_DDW were respectively 132 ng/mL and 118 ng/mL. It was confirmed that all of the respective humanized antibodies had high binding activities with respect to both of the human and monkey Ig β -Flag proteins.

[0087]

(Example 5: FACS analysis with respect to human and monkey PBMC)

In order to evaluate the binding activities of humanized antibodies with respect to human and monkey cells, Fluorescence Activated Cell Sorting (FACS) analysis was performed on human and monkey PBMC with an index of CD20 which is a B cell marker using B cells contained in the PBMC as a target. The monkey PBMC was prepared by diluting the blood of a monkey in the same amount of PBS (Life Technologies, Inc.), laminating the diluted blood on the same amount of Ficoll (GE Healthcare Japan Corporation), and performing a centrifugal treatment at room temperature and at 1500 rpm for 30 minutes. Next, human PBMC (AllCells, Inc.) or monkey PBMC was seeded by an amount of 200000 per well in a 96-well plate (Greiner Bio-One) in a state of being suspended in 30 μ L of Stain Buffer (Becton, Dickinson Company). A dilution series (4 steps with a final concentration of 0.03 ng/mL to 30 μ g/mL) of each of the humanized antibodies acquired in Example 3 was prepared using Stain Buffer and 30 μ L of the dilution series was added thereto. The resultant was left on ice for 30 minutes, washed with Stain Buffer three times, and 40 μ L of a solution having a phycoerythrin-labeled goat anti-human IgG Fc γ fragment (JACKSON, Inc.) which was diluted 200-fold with Stain Buffer and an allophycocyanin-labeled mouse anti-CD20 antibody (Becton, Dickinson Company) diluted 8-fold with Stain Buffer was added thereto. The resultant was left on ice for 30 minutes and washed with Stain Buffer twice, the fluorescence intensity was measured using FACSArray (Becton, Dickinson Company), and then the mean fluorescence intensity: MFI) was calculated. FlowJo (TOMY DIGITAL BIOLOGY Co., Ltd.) was used for analysis.

[0088]

As a result, it was confirmed that all of the respective humanized antibodies had high binding activities with respect to both of the human and monkey B cells.

[0089]

(Example 6: evaluation of anti-IgM antibody-induced cell proliferation activity)

In order to evaluate the inhibitory effect of a humanized antibodies with respect to activation of human B cells due to BCR stimulation, anti-IgM antibody-induced cell proliferation activity in human B cells was evaluated. The anti-IgM antibody activates B cells by allowing BCR to aggregate. An antibody binding to both of BCR and Fc γ RIIb mobilizes Fc γ RIIb to BCR and thus proliferation of B cells can be inhibited. In this Example, anti-CD19 S267E/L328F (Patent Document 2) was used as a comparative antibody. As a control antibody, a human IgG1 antibody (anti-KLH Ab) against KLH (keyhole limpet hemocyanin) which is an antigen not existing in a living body was used (WO 2013/094723). Next, human B cells (AllCells, Inc.) were seeded by an amount of 30000 per well in a 96-well plate (Iwaki, Co., Ltd.) using a 60 μ L of RPMI culture medium (SIGMA-ALDRICH Corporation). Subsequently, dilution series (3 steps with a final

concentration of 0.3 ng/mL to 30 µg/mL) of the respective full human antibodies acquired in Example 3, anti-CD19 S267E/L328F, or anti-KLH Ab were prepared and added thereto by an amount of 20 µL using the RPMI culture medium. 20 µL of the anti-IgM antibody (JACKSON, Inc.) prepared such that the final concentration thereof in the RPMI culture medium was adjusted to 5 µg/mL was added and incubated in a CO₂ incubator for 4 days. Next, cell proliferation analysis was performed using CellTiter-Glo (Promega K.K.). In addition, in Example here, a test antibody non-added/anti-IgM antibody non-added group and a test antibody non-added/anti-IgM antibody added group were respectively prepared as a negative control and a positive control and then a test was performed. Respective test antibodies were tested in duplicate.

[0090]

Fig. 1 shows the results of the proliferation rate of human B cells. The proliferation rates of a test antibody administration group was calculated by setting a test antibody non-added/anti-IgM antibody non-added group as a negative control (proliferation rate: 0%) and a test antibody non-added/anti-IgM antibody added group as a positive control (proliferation rate: 100%). This means that the inhibitory activity with respect to BCR of a test antibody is stronger when the value of the proliferation rate thereof is smaller.

[0091]

As shown in Fig. 1, while the proliferation rate in 30 µg/mL of anti-CD19 S267E/L328F was 52.3%, the proliferation rates in 30 µg/mL of CL6_40m12_DDW, CL6_40m14_DDW, and CL6_40m16_DDW were respectively 2.8%, 20.2%, and 23.2%. Therefore, it is evident that all of the above-described full human antibodies have strong inhibitory activities with respect to anti-IgM antibody-induced cell proliferation in human B cells compared to anti-CD19 S267E/L328F.

[0092]

(Example 7: evaluation of drug efficacy in human PBMC transfer NOG mouse model)

For the purpose of verifying the effectiveness of a humanized antibody with respect to in vivo antibody production, an action of various antibodies in administration for treatment with respect to an increase in human antibody titers induced by transferring human PBMC into an NOG mouse was evaluated. In the present model, it is considered that the human B cells violently activated by foreign object (mouse) recognition differentiate into plasma (blast) cells in the body of the mouse, and the present model is appropriate for evaluating a pharmacological action of a test drug with respect to the activity of human B series cells.

[0093]

Human PBMC (AllCells, Inc.) was suspended at 10000000 cells/mL in PBS

(Wako Pure Chemical Industries, Ltd.) and administered to the tail vein of a 11-week-old male NOG mouse (In-vivo Science, Inc.) by an amount of 0.25 mL (2500000 cells). On the 34th day (34th day after PBMC transfer), the weight was measured and blood was sampled. The plasma human IgM and IgE antibody titer was measured using ELISA (Bethyl Laboratories, Inc.). Grouping was performed based on the plasma human IgM, the IgE antibody titer, and the weight data.

[0094]

In this Example, as a comparative antibody, anti-CD19 S267E/L328F was used. As a control antibody, anti-KLH Ab was used. 10 mg/10 mL/kg of a test antibody was administered to a mouse by subcutaneous administration on the 35th and the 42nd days. Blood sampling was performed on the 42nd and the 49th days and the plasma human IgM and IgE antibody titer was measured using ELISA (Bethyl Laboratories, Inc.). The test was performed in a unit of a group of 4 or 5 animals. The test results are shown by "average value $\pm$ standard error." A significant difference test of an anti-KLH Ab group and various test antibody groups was performed using a Student's t-test, and a case where the p value was less than 0.05 was regarded as statistically significant. The above-described test was performed using a GraphPad Prism (version 5.04).

[0095]

Fig. 2 shows an action of a test antibody with respect to the plasma human IgM antibody titer. The plasma human IgM antibody titer was significantly decreased by CL6_40m12_DDW and CL6_40m14_DDW compared to anti-KLH Ab. An action of decreasing CL6_40m12_DDW and CL6_40m14_DDW with respect to the plasma human IgM antibody titer was expressed exceedingly early and recognized from the first week after the administration was started (42nd day). Meanwhile, in anti-CD19 S267E/L328F, an action of a decrease with respect to the plasma human IgM antibody titer was significant only after 2 weeks after administration was started (49th day).

[0096]

Next, Fig. 3 shows an action of a test antibody with respect to the plasma human IgE antibody titer. In CL6_40m12_DDW and CL6_40m14_DDW, the plasma human IgE antibody titer was rapidly and significantly decreased compared to anti-KLH Ab. Meanwhile, in anti-CD19 S267E/L328F, the plasma human IgE antibody titer was not decreased.

[0097]

As shown in Figs. 2 and 3, it is evident that both of the above-described CL6_40m12_DDW and CL6_40m14_DDW have strong inhibitory activities with respect to an increase in the human antibody titer compared to anti-CD19 S267E/L328F.

[0098]

(Example 8: evaluation of drug efficacy in monkey TTx antigen sensitization)

model)

TTx antigen-specific IgG was produced by sensitizing an adsorbed tetanus toxoid (TTx) antigen to a monkey once. In the present model, the total antibody titers in plasma can be evaluated in addition to TTx antigen-specific IgG in plasma. Accordingly, in the present model, safety can be evaluated in addition to effectiveness thereof when autoimmune diseases are treated.

[0099]

Using a male cynomolgus monkey (producing area: China, 3 years old or older), 2 mg/kg to 5 mg/kg (0.05 mL/kg: USP Corporation) of zolazepam hydrochloride and 2.5 mg/kg to 5 mg/kg of tiletamine hydrochloride were mixed under anesthesia and TTx was sensitized (the sensitization day was set to Day 0). The sensitization of TTx was performed by injecting 0.6 mL/monkey of tetanus toxoid (TTx, 10 Lf/mL, Denka Seika Co., Ltd.) to thigh muscle and 0.6 mL/monkey (respectively 50 μ L to 12 places) to the intradermal back portion. As treated groups, a Vehicle group (solvent (20mM of sodium citrate buffer/120 mM, NaCl (pH: 6.0); KOHJIN BIO Co., Ltd.) 1 mL/kg, n = 3) and an antibody administration group (10 mg/1 mL/kg, humanized antibody CL6_40m14_DDW (diluted with solvent), n = 3) were used. The timing of administration was set to the 14th day after TTx sensitization and the administration to the vein was performed with a dosage of 1 mL/kg when awakening.

[0100]

After CL6_40m14_DDW was administered to the above-described cynomolgus monkey, blood was sampled with time, for example, after 4 hours, 72 hours, 168 hours, and 336 hours, and was subjected to a centrifugal treatment, and then plasma was recovered. The concentration of drugs in the plasma was measured using GyrolabTM xP workstation (Gyros AB). As the method and the disc, 200-3W-001-A and Bioaffy 200 compact discs (Gyros AB) were used. In addition, as a solidified antigen and a detection antibody, biotin-labeled Recombinant Human CD79B (Novoprotein, Inc.) and alexa-labeled Goat Anti-Human IgG (Southern Biotechnology Associates, Inc.) were used. As listed in Table 1, the concentration of drugs in plasma of CL6_40m14_DDW was maintained during the evaluation period of the model.

Table 1: Transition of concentration of drugs in plasma with respect to humanized anti-Ig β antibody in monkey

[Table 1]

	Concentration of drugs in plasma of individual 1 ($\mu\text{g/mL}$)	Concentration of drugs in plasma of individual 2 ($\mu\text{g/mL}$)	Concentration of drugs in plasma of individual 3 ($\mu\text{g/mL}$)
After 4 hours	252.111	263.242	258.271
After 72 hours	143.586	144.095	114.605
After 168 hours	120.070	112.865	95.848
After 336 hours	76.854	62.281	53.305

[0101]

Blood was sampled from the above-described cynomolgus monkey with time on the 13th day (13 days after the cynomolgus was immunized by adsorbed tetanus toxoid), the 14th day, the 17th day, the 21st day, and the 28th day, a centrifugal treatment was carried out, and plasma was recovered. In order to measure anti-adsorbed tetanus toxoid (anti-TTx IgG) in the recovered plasma, the antigen ELISA was used. The adsorbed tetanus toxoid (Denka Seika Co., Ltd.) was diluted 20-fold with a phosphate-buffered saline (PBS; Wako Pure Chemical Industries, Ltd.), was added to a NUNC MaxiSorp 96 plate (Maxisorp 96 plate: Nunc Corporation) by an amount of 100 μL per well, and then solidified at 4°C for one night. The resultant was washed with PBS-T (0.05% Tween-20 containing PBS: Thermo Scientific, Inc.) four times, 200 μL of a blocking agent (Blocker Casein In PBS; Life Technologies, Inc.) was added thereto, the resultant was left at room temperature for 2 hour, and the solution was removed. Next, 100 μL of the recovered plasma and 100 μL of a sample for a calibration curve were respectively added thereto. As the sample for a calibration curve, a sample mixed with plasma collected 21 days and 23 days later from immunization of the cynomolgus monkey by adsorbed tetanus toxoid was used, the amount thereof was adjusted to 100 U/mL, and a dilution series (0.488 mU/mL to 500 mU/mL) prepared using a blocking agent as a diluted solution was used. The resultant was left at room temperature for 2 hour, washed with a PBS-T washing liquid four times, and 100 μL of a horseradish peroxidase (HRP)-labeled goat anti-monkey IgG antibody (Nordic, Inc.) which had been diluted 3000-fold with a blocking agent was added thereto. Thereafter, the resultant was left at room temperature for 1 hour and then washed with the PBS-T washing liquid four times. Next, measurement was performed using TMB Microwell Peroxidase Substrate System (KPL, Inc.). The absorbance thereof was measured by SpectraMax (Molecular Devices, Inc.).

[0102]

Fig. 4 shows an action of the test antibody with respect to the adsorbed tetanus toxoid antibody titer in plasma. The adsorbed tetanus toxoid antibody titer in plasma (anti-TTx IgG) was decreased in CL6-40m14_DDW compared to the Vehicle group.

[0103]

The total antibody titers (IgM, IgA, and IgG) in plasma recovered from the cynomolgus monkey on the 14th day, the 17th day, the 21st day, and the 28th day were measured using the following method. A rabbit anti-monkey IgM polyclonal antibody (COVANCE, Inc.) and a rabbit anti-human IgA polyclonal antibody (Bethyl Laboratories, Inc.) were diluted 100-fold, 500-fold, and 1000-fold with a phosphate-buffered saline (PBS: Wako Pure Chemical Industries, Ltd.), added to a NUNC MaxiSorp 96 plate (Maxisorp 96 plate: Nunc Corporation) by an amount of 100 μ L, and then solidified at 4°C for one night. The resultant was washed with PBS-T (0.05% Tween-20 containing PBS: Thermo Scientific, Inc.) four times, 200 μ L of a blocking agent (Blocker Casein In PBS; Life Technologies, Inc.) was added thereto, and the resultant was left at room temperature for 1 hour and washed with the PBS-T washing liquid for times. A dilution series of a sample for a calibration curve and collected plasma of a monkey was prepared using a blocking agent as a diluted solution and 100 μ L of the dilution series was added thereto. As the sample for a calibration curve, plasma prepared from a normal cynomolgus monkey was diluted and then used. The resultant was left at room temperature for 2 hour, washed with a PBS-T washing liquid four times, and an horseradish peroxidase (HRP)-labeled anti-monkey IgM antibody (KPL, Inc.), an horseradish peroxidase (HRP)-labeled anti-human IgA antibody (Bethyl Laboratories, Inc.), and a horseradish peroxidase (HRP)-labeled anti-monkey IgG antibody (KPL, Inc.) were respectively diluted 1000-fold, 5000-fold, and 3000-fold with a blocking agent and added thereto by an amount of 100 μ L respectively. Thereafter, the resultant was left at room temperature for 2 hours and then washed with a PBS-T washing liquid four times. Next, measurement was performed using TMB Microwell Peroxidase Substrate System (KPL, Inc.). The absorbance thereof was measured by SpectraMax (Molecular Devices, Inc.).

[0104]

Figs. 5, 6, and 7 show actions of the test antibodies with respect to the total antibody titers (IgM, IgA, and IgG) in plasma. CL6_40m14_DDW did not affect the total antibody titers (IgM, IgA, and IgG) in plasma compared to the Vehicle group.

[0105]

From the results described above, it is evident that CL6_40m14_DDW suppresses an antigen-specific antibody without affecting the total antibody titers in plasma. Further, it is also evident that CL6_40m14_DDW has an excellent profile in terms of safety in addition to effectiveness at the time of treatment of autoimmune diseases.

Industrial Applicability

[0106]

The anti-human Ig β antibody of the present invention is useful for preventing and

treating autoimmune diseases. Further, the polynucleotide, the expression vectors, the transformed host cell, and the methods for producing the antibody of the present invention are useful for producing the anti-human Ig β antibody.

5 Sequence List Free Text

[0107]

In the number heading <223> of the sequence list, description of "Artificial Sequence" is made. Specifically, the base sequences shown by SEQ ID NOS: 1 and 3 of the sequence list are the base sequences of the heavy chain and the light chain of the CL6_40m12_DDW, respectively, and the amino acid sequences shown by SEQ ID NOS: 2 and 4 are the amino acid sequences of the heavy chain and the light chain encoded by the SEQ ID NOS: 1 and 3, respectively. The base sequences shown by SEQ ID NOS: 5 and 7 of the sequence list are the base sequences of the heavy chain and the light chain of the CL6_40m14_DDW, respectively, and the amino acid sequences shown by SEQ ID NOS: 6 and 8 are the amino acid sequences of the heavy chain and the light chain encoded by the SEQ ID NOS: 5 and 7, respectively. The base sequences shown by SEQ ID NOS: 9 and 11 of the sequence list are the base sequences of the heavy chain and the light chain of the CL6_40m16_DDW, respectively, and the amino acid sequences shown by SEQ ID NOS: 10 and 12 of the sequence list are the amino acid sequences of the heavy chain and the light chain encoded by the SEQ ID NOS: 9 and 11, respectively. The base sequence shown by SEQ ID NO: 13 of the sequence list is the base sequence of the human Ig β -Flag gene and the base sequence shown by SEQ ID NO: 14 of the sequence list is the base sequence of the monkey Ig β -Flag gene. The base sequence shown by SEQ ID NO: 15 of the sequence list is the base sequence of the heavy chain of the CL6_40m12_DDW.

25

Claims

[Claim 1] An anti-human Ig β antibody comprising a heavy chain variable region comprising CDR1 consisting of the amino acid sequence of amino acid numbers 31 to 35 of SEQ ID NO: 2, CDR2 consisting of the amino acid sequence of amino acid numbers 50 to 65 of SEQ ID NO: 2, and CDR3 consisting of the amino acid sequence of amino acid numbers 98 to 108 of SEQ ID NO: 2, a light chain variable region comprising CDR1 consisting of the amino acid sequence of amino acid numbers 24 to 38 of SEQ ID NO: 4, CDR2 consisting of the amino acid sequence of amino acid numbers 54 to 60 of SEQ ID NO: 4, and CDR3 consisting of the amino acid sequence of amino acid numbers 93 to 101 of SEQ ID NO: 4, and a heavy chain constant region which is a human Ig γ 1 constant region having amino acid mutations of S239D, H268D, and L328W.

[Claim 2] The anti-human Ig β antibody according to claim 1 which is a humanized antibody.

[Claim 3] The anti-human Ig β antibody according to claim 1, selected from the group consisting of the following (1) to (4):

(1) an anti-human Ig β antibody comprising a heavy chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 6, a light chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 8, and a heavy chain constant region which is a human Ig γ 1 constant region having amino acid mutations of S239D, H268D, and L328W;

(2) an anti-human Ig β antibody comprising a heavy chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 2, a light chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 4, and a heavy chain constant region which is a human Ig γ 1 constant region having amino acid mutations of S239D, H268D, and L328W;

(3) an anti-human Ig β antibody comprising a heavy chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 10, a light chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 12, and a heavy chain constant region which is a human Ig γ 1 constant region having amino acid mutations of S239D, H268D, and L328W; and

(4) an anti-human Ig β antibody which is derived from posttranslational modification of the anti-human Ig β antibody of any one of (1) to (3) above.

[Claim 4] The anti-human Ig β antibody according to claim 3, comprising a heavy chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 6, a light chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 8, and a heavy chain constant region which is a human Ig γ 1 constant region having amino acid mutations of S239D, H268D,

and L328W.

[Claim 5] The anti-human Ig β antibody according to claim 3, comprising a heavy chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 2, a light chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 4, and a heavy chain constant region which is a human Ig γ 1 constant region having amino acid mutations of S239D, H268D, and L328W.

[Claim 6] The anti-human Ig β antibody according to claim 3, comprising a heavy chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 119 of SEQ ID NO: 10, a light chain variable region consisting of the amino acid sequence of amino acid numbers 1 to 112 of SEQ ID NO: 12, and a heavy chain constant region which is a human Ig γ 1 constant region having amino acid mutations of S239D, H268D, and L328W.

[Claim 7] An anti-human Ig β antibody which is derived from posttranslational modification of the anti-human Ig β antibody according to any one of claims 4 to 6.

[Claim 8] The anti-human Ig β antibody according to claim 3 or 7, wherein the posttranslational modification is pyroglutamylation at the N terminal of the heavy chain variable region and/or deletion of lysine at the C terminal of the heavy chain.

[Claim 9] The anti-human Ig β antibody according to any one of claims 1 to 8, comprising a light chain constant region which is a human Ig κ constant region.

[Claim 10] The anti-human Ig β antibody according to claim 1, comprising a heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 6 and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 8.

[Claim 11] The anti-human Ig β antibody according to claim 1, comprising a heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 2 and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 4.

[Claim 12] The anti-human Ig β antibody according to claim 1, comprising a heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 10 and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 12.

[Claim 13] An anti-human Ig β antibody which is derived from posttranslational modification of the anti-human Ig β antibody according to any one of claims 10 to 12.

[Claim 14] The anti-human Ig β antibody according to claim 13, wherein the posttranslational modification is pyroglutamylation at the N terminal of the heavy chain variable region and/or deletion of lysine at the C terminal of the heavy chain.

[Claim 15] The anti-human Ig β antibody according to claim 13, comprising a heavy chain consisting of the amino acid sequence of amino acid numbers of 1 to 448 of SEQ ID NO: 6 in which glutamine of amino acid number 1 is modified to pyroglutamic acid and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 8.

[Claim 16] The anti-human Ig β antibody according to claim 13, comprising a heavy chain consisting of the amino acid sequence of amino acid numbers 1 to 448 of SEQ ID NO:2 and a light chain consisting of the amino acid sequence shown by SEQ ID NO:4.

5 [Claim 17] The anti-human Ig β antibody according to claim 13, comprising a heavy chain consisting of the amino acid sequence of amino acid numbers 1 to 448 of SEQ ID NO:10 and a light chain consisting of the amino acid sequence shown by SEQ ID NO:12.

[Claim 18] A polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody according to any one of claims 1 to 6.

10 [Claim 19] A polynucleotide comprising a base sequence encoding the light chain of the anti-human Ig β antibody according to any one of claims 1 to 6.

[Claim 20] An expression vector comprising the polynucleotide according to claim 18 and/or 19.

[Claim 21] A host cell transformed with the expression vector according to claim 20, selected from the group consisting of the following (a) to (d):

15 (a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody according to any one of claims 1 to 6 and a polynucleotide comprising a base sequence encoding the light chain of the antibody;

20 (b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody according to any one of claims 1 to 6 and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody;

25 (c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody according to any one of claims 1 to 6; and

(d) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the anti-human Ig β antibody according to any one of claims 1 to 6.

30 [Claim 22] A host cell transformed with the expression vector according to claim 20, selected from the group consisting of the following (a) to (d):

(a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody according to any one of claims 10 to 13 and a polynucleotide comprising a base sequence encoding the light chain of the antibody;

35 (b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody according to any one of claims 10 to 13 and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody;

(c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody according to any one of claims 10 to 13; and

5 (d) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the anti-human Ig β antibody according to any one of claims 10 to 13.

[Claim 23] A method for producing an anti-human Ig β antibody comprising culturing host cell(s) selected from the group consisting of the following (a) to (c) to express the anti-human Ig β antibody:

10 (a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody according to any one of claims 1 to 6 and a polynucleotide comprising a base sequence encoding the light chain of the antibody;

15 (b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody according to any one of claims 1 to 6 and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody; and

20 (c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody according to any one of claims 1 to 6 and a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody.

[Claim 24] A method for producing an anti-human Ig β antibody comprising culturing host cell(s) selected from the group consisting of the following (a) to (c) to express the anti-human Ig β antibody:

25 (a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody according to any one of claims 10 to 13 and a polynucleotide comprising a base sequence encoding the light chain of the antibody;

30 (b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody according to any one of claims 10 to 13 and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody; and

35 (c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Ig β antibody according to any one of claims 10 to 13 and a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody.

- [Claim 25] An anti-human Ig β antibody which is produced by the method according to claim 23.
- [Claim 26] An anti-human Ig β antibody which is produced by the method according to claim 24.
- 5 [Claim 27] A pharmaceutical composition comprising the anti-human Ig β antibody according to any one of claims 1 to 17, 25, and 26 and a pharmaceutically acceptable excipient.
- [Claim 28] A pharmaceutical composition comprising the anti-human Ig β antibody according to claim 10, the anti-human Ig β antibody according to claim 15, and a
10 pharmaceutically acceptable excipient.
- [Claim 29] The pharmaceutical composition according to claim 27 or 28, which is a pharmaceutical composition for preventing or treating an autoimmune disease.
- [Claim 30] The pharmaceutical composition according to claim 29, wherein the autoimmune disease is systemic lupus erythematosus, rheumatoid arthritis, or idiopathic
15 thrombocytopenic purpura.
- [Claim 31] A method for preventing or treating an autoimmune disease, comprising administering a therapeutically effective amount of the anti-human Ig β antibody according to any one of claims 1 to 17, 25, and 26.
- [Claim 32] The method according to claim 31, wherein the autoimmune disease is
20 systemic lupus erythematosus, rheumatoid arthritis, or idiopathic thrombocytopenic purpura.
- [Claim 33] The anti-human Ig β antibody according to any one of claims 1 to 17, 25, and 26 for use in preventing or treating an autoimmune disease.
- [Claim 34] The anti-human Ig β antibody according to claim 33, wherein the
25 autoimmune disease is systemic lupus erythematosus, rheumatoid arthritis, or idiopathic thrombocytopenic purpura.
- [Claim 35] Use of the anti-human Ig β antibody according to any one of claims 1 to 17, 25, and 26 for manufacture of a pharmaceutical composition for preventing or treating an autoimmune disease.
- 30 [Claim 36] The use according to claim 35, wherein the autoimmune disease is systemic lupus erythematosus, rheumatoid arthritis, or idiopathic thrombocytopenic purpura.

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Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Asp Glu Asp
260 265 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
11

305		310		315		320									
Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Trp	Pro	Ala	Pro	Ile	Glu	Lys
				325					330					335	
Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr
			340					345					350		
Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr
		355					360					365			
Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu
	370					375					380				
Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu
385					390					395					400
Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys
				405					410					415	
Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu
			420					425					430		
Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly
		435					440					445			

Lys

<210> 7
 <211> 657
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> L-chain of anti-human Ig-beta antibody

<220>
 <221> CDS
 <222> (1).. (657)

<400>	7														
gac	atc	cag	ctg	acc	cag	tcc	ccc	tcc	agc	ctg	tcc	gcc	tct	gtg	ggc
Asp	Ile	Gln	Leu	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly
1				5					10					15	

48

gac aga gtg acc atc aca tgc aag gcc tcc cag tcc gtg gac tac gac Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp	96
20 25 30	
ggc gac tcc tac atg aac tgg tat cag cag aag ccc ggc aag gcc ccc Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro	144
35 40 45	
aag ctg ctg atc tac gcc gcc tcc aac ctg gaa tcc ggc gtg ccc tcc Lys Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser Gly Val Pro Ser	192
50 55 60	
aga ttc tcc ggc tcc ggc tct ggc acc gac ttc acc ttc acc atc tcc Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser	240
65 70 75 80	
agc ctg cag ccc gag gat atc gcc acc tac tac tgc cag cag tcc aac Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Ser Asn	288
85 90 95	
gag gac ccc ctg acc ttc ggc cag ggc acc aag gtg gaa atc aag cgt Glu Asp Pro Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg	336
100 105 110	
acg gtg gct gca cca tct gtc ttc atc ttc ccg cca tct gat gag cag Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln	384
115 120 125	
ttg aaa tct gga act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr	432
130 135 140	
ccc aga gag gcc aaa gta cag tgg aag gtg gat aac gcc ctc caa tcg Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser	480
145 150 155 160	
ggt aac tcc cag gag agt gtc aca gag cag gac agc aag gac agc acc Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr	528
165 170 175	
tac agc ctc agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys	576
180 185 190	
cac aaa gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro	624
195 200 205	
gtc aca aag agc ttc aac agg gga gag tgt tag Val Thr Lys Ser Phe Asn Arg Gly Glu Cys	657
210 215	

<210> 8
 <211> 218
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 8

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30

Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
35 40 45

Lys Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser
65 70 75 80

Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Ser Asn
85 90 95

Glu Asp Pro Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215

<210> 9
 <211> 1350
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> H-chain of anti-human Ig-beta antibody

<220>
 <221> CDS
 <222> (1).. (1350)

<400> 9
 gaa gtg cag ctg gtg gag tcc ggg gga ggt ctg gtg cag ccc ggg ggt 48
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 tct ctg cgt ctg tct tgt gcc gtg tct ggg ttt agt ctg tcc agc tat 96
 Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Phe Ser Leu Ser Ser Tyr
 20 25 30
 agt gtg cac tgg ttc cga aag gct ccc ggc aaa gga ctg gag tgg ctg 144
 Ser Val His Trp Phe Arg Lys Ala Pro Gly Lys Gly Leu Glu Trp Leu
 35 40 45
 ggc gga atc tgg tca ggg ggt tcc att cat tat acc cct gca ctg tct 192
 Gly Gly Ile Trp Ser Gly Gly Ser Ile His Tyr Thr Pro Ala Leu Ser
 50 55 60
 agt aga ctg aca gtg agc cgc gac atc tct aag aac aca gtc tac ctg 240
 Ser Arg Leu Thr Val Ser Arg Asp Ile Ser Lys Asn Thr Val Tyr Leu
 65 70 75 80
 cag atg aat agc ctg agg gcc gag gat acc gct gtc tac tat tgc gca 288
 Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95
 aga tac gac cgg tat gaa act tac gcc atg gat tac tgg ggc cag ggc 336
 Arg Tyr Asp Arg Tyr Glu Thr Tyr Ala Met Asp Tyr Trp Gly Gln Gly
 100 105 110
 acc ctg gtc acc gtc tcc tca gcc tcc acc aag ggc cca tcg gtc ttc 384
 Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
 115 120 125
 ccc ctg gca ccc tcc tcc aag agc acc tct ggg ggc aca gcg gcc ctg 432
 Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
 130 135 140
 ggc tgc ctg gtc aag gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg 480
 Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp

145	150	155	160	
aac tca ggc gcc ctg acc agc ggc gtg cac acc ttc ccg gct gtc cta Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu	165	170	175	528
cag tcc tca gga ctc tac tcc ctt agt agc gtg gtg acc gtg ccc tcc Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser	180	185	190	576
agc agc ttg ggc acc cag acc tac atc tgc aac gtg aat cac aag ccc Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro	195	200	205	624
agc aac acc aag gtg gac aag aaa gtt gag ccc aaa tct tgt gac aaa Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys	210	215	220	672
act cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro	225	230	235	720
gac gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc Asp Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser	245	250	255	768
cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc gac gaa gac Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Asp Glu Asp	260	265	270	816
cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn	275	280	285	864
gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgt gtg Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val	290	295	300	912
gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu	305	310	315	960
tac aag tgc aag gtc tcc aac aaa gcc tgg cca gcc ccc atc gag aaa Tyr Lys Cys Lys Val Ser Asn Lys Ala Trp Pro Ala Pro Ile Glu Lys	325	330	335	1008
acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr	340	345	350	1056
ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr	355	360	365	1104
tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu	370	375	380	1152

agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg	1200
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu	
385 390 395 400	
gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag	1248
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys	
405 410 415	
agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag	1296
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu	
420 425 430	
gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt	1344
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly	
435 440 445	
aaa tga	1350
Lys	

<210> 10
 <211> 449
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 10

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Phe Ser Leu Ser Ser Tyr
20 25 30

Ser Val His Trp Phe Arg Lys Ala Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Gly Ile Trp Ser Gly Gly Ser Ile His Tyr Thr Pro Ala Leu Ser
50 55 60

Ser Arg Leu Thr Val Ser Arg Asp Ile Ser Lys Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Tyr Asp Arg Tyr Glu Thr Tyr Ala Met Asp Tyr Trp Gly Gln Gly
17

100					105					110					
Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe
	115						120					125			
Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu
	130					135					140				
Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp
145					150					155					160
Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu
				165					170					175	
Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser
			180					185					190		
Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro
		195					200					205			
Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys
	210					215					220				
Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro
225					230					235					240
Asp	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser
				245					250					255	
Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	Asp	Glu	Asp
			260					265					270		
Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn
	275					280						285			
Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val
	290					295					300				
Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu
305					310					315					320
Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Trp	Pro	Ala	Pro	Ile	Glu	Lys
				325					330					335	

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

Lys

<210> 11
<211> 657
<212> DNA
<213> Artificial Sequence

<220>
<223> L-chain of anti-human Ig-beta antibody

<220>
<221> CDS
<222> (1).. (657)

<400> 11
gac atc gtg ctg acc cag tcc ccc tcc agc ctg tcc gcc tct gtg ggc 48
Asp Ile Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

gac aga gtg acc atc aca tgc aag gcc tcc cag tcc gtg gac tac gac 96
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30

ggc gac tcc tac atg aac tgg tat cag cag aag ccc ggc aag gcc ccc 144
19

Gly	Asp	Ser	Tyr	Met	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro		
		35					40					45					
aag	ctg	ctg	atc	tac	gcc	gcc	tcc	aac	ctg	gaa	tcc	ggc	gtg	ccc	tcc		192
Lys	Leu	Leu	Ile	Tyr	Ala	Ala	Ser	Asn	Leu	Glu	Ser	Gly	Val	Pro	Ser		
	50					55					60						
aga	ttc	tcc	ggc	tcc	ggc	tct	ggc	acc	gac	ttc	acc	ctg	acc	atc	tcc		240
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser		
65					70					75					80		
agc	ctg	cag	ccc	gag	gac	ttc	gcc	acc	tac	tac	tgc	cag	cag	tcc	aac		288
Ser	Leu	Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Ser	Asn		
				85					90					95			
gag	gac	ccc	ctg	acc	ttc	ggc	cag	ggc	acc	aag	gtg	gaa	atc	aag	cgt		336
Glu	Asp	Pro	Leu	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	Arg		
			100					105					110				
acg	gtg	gct	gca	cca	tct	gtc	ttc	atc	ttc	cgc	cca	tct	gat	gag	cag		384
Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln		
		115					120					125					
ttg	aaa	tct	gga	act	gcc	tct	gtt	gtg	tgc	ctg	ctg	aat	aac	ttc	tat		432
Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr		
	130					135					140						
ccc	aga	gag	gcc	aaa	gta	cag	tgg	aag	gtg	gat	aac	gcc	ctc	caa	tcg		480
Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser		
145					150					155					160		
ggt	aac	tcc	cag	gag	agt	gtc	aca	gag	cag	gac	agc	aag	gac	agc	acc		528
Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr		
				165					170					175			
tac	agc	ctc	agc	agc	acc	ctg	acg	ctg	agc	aaa	gca	gac	tac	gag	aaa		576
Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys		
			180					185					190				
cac	aaa	gtc	tac	gcc	tgc	gaa	gtc	acc	cat	cag	ggc	ctg	agc	tcg	ccc		624
His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro		
		195				200						205					
gtc	aca	aag	agc	ttc	aac	agg	gga	gag	tgt	tag							657
Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys								
	210					215											

<210> 12
 <211> 218
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 12

Asp Ile Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15
 Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
 20 25 30
 Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
 35 40 45
 Lys Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser Gly Val Pro Ser
 50 55 60
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
 65 70 75 80
 Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Asn
 85 90 95
 Glu Asp Pro Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
 100 105 110
 Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 115 120 125
 Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
 130 135 140
 Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
 145 150 155 160
 Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
 165 170 175
 Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
 180 185 190
 His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
 195 200 205
 Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215

<210> 13
 <211> 450
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Human Ig-beta-Flag fusion gene

<220>
 <221> CDS
 <222> (1).. (447)

<400> 13
 gcc aga tcg gag gac cgg tac cgg aat ccc aaa ggt agt gct tgt tog 48
 Ala Arg Ser Glu Asp Arg Tyr Arg Asn Pro Lys Gly Ser Ala Cys Ser
 1 5 10 15
 cgg atc tgg cag agc cca cgt ttc ata gcc agg aaa cgg ggc ttc acg 96
 Arg Ile Trp Gln Ser Pro Arg Phe Ile Ala Arg Lys Arg Gly Phe Thr
 20 25 30
 gtg aaa atg cac tgc tac atg aac agc gcc tcc ggc aat gtg agc tgg 144
 Val Lys Met His Cys Tyr Met Asn Ser Ala Ser Gly Asn Val Ser Trp
 35 40 45
 ctc tgg aag cag gag atg gac gag aat ccc cag cag ctg aag ctg gaa 192
 Leu Trp Lys Gln Glu Met Asp Glu Asn Pro Gln Gln Leu Lys Leu Glu
 50 55 60
 aag ggc cgc atg gaa gag tcc cag aac gaa tct ctc gcc acc ctc acc 240
 Lys Gly Arg Met Glu Glu Ser Gln Asn Glu Ser Leu Ala Thr Leu Thr
 65 70 75 80
 atc caa ggc atc cgg ttt gag gac aat ggc atc tac ttc tgt cag cag 288
 Ile Gln Gly Ile Arg Phe Glu Asp Asn Gly Ile Tyr Phe Cys Gln Gln
 85 90 95
 aag tgc aac aac acc tcg gag gtc tac cag ggc tgc ggc aca gag ctg 336
 Lys Cys Asn Asn Thr Ser Glu Val Tyr Gln Gly Cys Gly Thr Glu Leu
 100 105 110
 cga gtc atg gga ttc agc acc ttg gca cag ctg aag cag agg aac acg 384
 Arg Val Met Gly Phe Ser Thr Leu Ala Gln Leu Lys Gln Arg Asn Thr
 115 120 125
 ctg aag gat tcg tca gca gac ctg gtt ccg cgc gga tcc gac tac aag 432
 Leu Lys Asp Ser Ser Ala Asp Leu Val Pro Arg Gly Ser Asp Tyr Lys
 130 135 140
 gac gat gac gat aaa tga 450
 Asp Asp Asp Asp Lys
 145

<210> 14
 <211> 453

<212> DNA
 <213> Artificial Sequence

<220>
 <223> Monkey Ig-beta-Flag fusion gene

<220>
 <221> CDS
 <222> (1).. (450)

<400> 14
 gcc aaa tca gag gac ctg tac ccg aat ccc aaa ggt agt gct tgt tct 48
 Ala Lys Ser Glu Asp Leu Tyr Pro Asn Pro Lys Gly Ser Ala Cys Ser
 1 5 10 15
 cgg atc tgg cag agc cca cgt ttc ata gcc agg aaa cgg ggc ttc acg 96
 Arg Ile Trp Gln Ser Pro Arg Phe Ile Ala Arg Lys Arg Gly Phe Thr
 20 25 30
 gtg aaa atg cac tgc tac gtg acc aac agc acc ttc agc atc gtg agc 144
 Val Lys Met His Cys Tyr Val Thr Asn Ser Thr Phe Ser Ile Val Ser
 35 40 45
 tgg ctc cgg aag cgg gag acg gac aag gag ccc caa cag gtg aac ctg 192
 Trp Leu Arg Lys Arg Glu Thr Asp Lys Glu Pro Gln Gln Val Asn Leu
 50 55 60
 gag cag ggc cac atg cat cag acc caa aac agc tct gtc acc acc ctc 240
 Glu Gln Gly His Met His Gln Thr Gln Asn Ser Ser Val Thr Thr Leu
 65 70 75 80
 atc atc caa gac atc cgg ttt gag gac aac ggc atc tac ttc tgt cag 288
 Ile Ile Gln Asp Ile Arg Phe Glu Asp Asn Gly Ile Tyr Phe Cys Gln
 85 90 95
 cag gag tgc agc aag acc tcg gag gtc tac cgg ggc tgc ggc acg gag 336
 Gln Glu Cys Ser Lys Thr Ser Glu Val Tyr Arg Gly Cys Gly Thr Glu
 100 105 110
 ctg cga gtc atg ggg ttc agc acc ttg gca cag ctg aag cag agg aac 384
 Leu Arg Val Met Gly Phe Ser Thr Leu Ala Gln Leu Lys Gln Arg Asn
 115 120 125
 acg ctg aag gat tcg tca gca gac ctg gtt ccg cgc gga tcc gac tac 432
 Thr Leu Lys Asp Ser Ser Ala Asp Leu Val Pro Arg Gly Ser Asp Tyr
 130 135 140
 aag gac gat gac gat aaa tga 453
 Lys Asp Asp Asp Asp Lys
 145 150

<210> 15
 <211> 1350
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> H-chain of anti-human Ig-beta antibody

<220>
 <221> CDS
 <222> (1)..(1350)

<400> 15		
gag gtg cag atg gtg gaa tcc ggc gga ggc ctg gtg cag cct ggc ggc		48
Glu Val Gln Met Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly		
1 5 10 15		
tct ctg aga ctg tcc tgc gcc gtg tcc ggc ttc agc ctg tcc tcc tac		96
Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Phe Ser Leu Ser Ser Tyr		
20 25 30		
tcc gtg cac tgg gtc cga cag gcc cct ggc aag gga ctg gaa tgg gtg		144
Ser Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val		
35 40 45		
gcc ggc att tgg agc ggc ggc tcc atc cac tac acc cct gcc ctg tcc		192
Ala Gly Ile Trp Ser Gly Gly Ser Ile His Tyr Thr Pro Ala Leu Ser		
50 55 60		
tcc cgg ttc acc gtg tcc cgg gac gac tcc aag aac acc gtg tac ctg		240
Ser Arg Phe Thr Val Ser Arg Asp Asp Ser Lys Asn Thr Val Tyr Leu		
65 70 75 80		
cag atg aac tcc ctg cgg gcc gag gac acc gcc gtg tac tac tgc gcc		288
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala		
85 90 95		
cgc tac gac aga tac gag aca tac gcc atg gac tac tgg ggc cag ggc		336
Arg Tyr Asp Arg Tyr Glu Thr Tyr Ala Met Asp Tyr Trp Gly Gln Gly		
100 105 110		
acc ctg gtg aca gtg tcc tcc gcc tcc acc aag ggc ccc tcc gtg ttc		384
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe		
115 120 125		
cct ctg gcc ccc tcc agc aag tcc acc tct ggc ggc acc gct gcc ctg		432
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu		
130 135 140		
ggc tgc ctg gtg aaa gac tac ttc ccc gag ccc gtg acc gtg tcc tgg		480
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp		
145 150 155 160		
aac tct ggc gcc ctg acc tcc ggc gtg cac acc ttc cct gcc gtg ctg		528
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu		
165 170 175		
cag tcc tcc ggc ctg tac tcc ctg tcc agc gtg gtg acc gtg ccc tcc		576
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser		
180 185 190		

agc tct ctg ggc acc cag acc tac atc tgc aac gtg aac cac aag ccc Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro 195 200 205	624
tcc aac acc aag gtg gac aag aag gtg gaa ccc aag tcc tgc gac aag Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys 210 215 220	672
acc cac acc tgt ccc cct tgc cct gcc cct gag ctg ctg ggc gga ccc Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro 225 230 235 240	720
gat gtg ttt ctg ttc ccc cca aag ccc aag gac acc ctg atg atc tcc Asp Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser 245 250 255	768
cgg acc ccc gaa gtg acc tgc gtg gtg gtg gac gtg tcc gac gag gac Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Asp Glu Asp 260 265 270	816
cct gaa gtg aag ttc aat tgg tac gtg gac ggc gtg gaa gtg cac aac Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn 275 280 285	864
gcc aag acc aag ccc aga gag gaa cag tac aac tcc acc tac cgg gtg Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val 290 295 300	912
gtg tcc gtg ctg acc gtg ctg cac cag gac tgg ctg aac ggc aaa gaa Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu 305 310 315 320	960
tac aag tgc aag gtt tcc aac aag gcc tgg cct gcc ccc atc gaa aag Tyr Lys Cys Lys Val Ser Asn Lys Ala Trp Pro Ala Pro Ile Glu Lys 325 330 335	1008
acc atc tcc aag gcc aag ggc cag ccc cgc gag ccc cag gtg tac acc Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr 340 345 350	1056
ctg ccc cct agc cgg gac gag ctg acc aag aac cag gtg tcc ctg acc Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr 355 360 365	1104
tgt ctg gtg aaa ggc ttc tac ccc tcc gat atc gcc gtg gaa tgg gag Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu 370 375 380	1152
tcc aac ggc cag ccc gag aac aac tac aag acc acc ccc cct gtg ctg Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu 385 390 395 400	1200
gac tcc gac ggc tca ttc ttc ctg tac tcc aag ctg acc gtg gac aag Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys 405 410 415	1248

tcc	cgg	tgg	cag	cag	ggc	aac	gtg	ttc	tcc	tgc	tcc	gtg	atg	cac	gag	1296
Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	
			420					425					430			
gcc	ctg	cac	aat	cac	tac	acc	cag	aag	tcc	ctg	tcc	ctg	agc	ccc	ggc	1344
Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	
			435				440					445				
aag	tga															1350
Lys																