

[Means for Solving the Problems]

To solve the above-mentioned problems, the present inventors produced substances that neutralize the activity of the bispecific antibody and by targeting the test items that measure the reactivity of FVIII, searched for measurement conditions that ensure accuracy even in the presence of the bispecific antibody. As a result, the present inventors found out that by using neutralizing antibodies against the bispecific antibody at appropriate concentrations (for example, concentrations at which the bispecific antibody can be sufficiently neutralized), the FVIII activity in the plasma of hemophilia A patients can be evaluated accurately by APTT-based one-stage clotting assay, and also found out that the FVIII inhibitor titer in the plasma of a hemophilia A patient carrying the FVIII inhibitor can be evaluated accurately by APTT-based Bethesda assay. Furthermore, the present inventors successfully discovered kits containing neutralizing antibodies against the bispecific antibody having an FVIII-substituting activity for use in the measurement. The present invention is based on these findings and provides the following:

[1] an antibody that neutralizes a substance having an activity of functionally substituting for coagulation factor VIII;

[2] the antibody of [1], wherein the substance having an activity of functionally substituting for coagulation factor VIII is a bispecific antibody that binds to coagulation factor IX and/or activated coagulation factor IX and coagulation factor X and/or activated blood coagulation factor X;

[3] the antibody of [2], wherein the bispecific antibody is any one of the antibodies recited below, in which a first polypeptide is associated with a third polypeptide and a second polypeptide is associated with a fourth polypeptide:

a bispecific antibody in which the first polypeptide is an H chain consisting of the amino acid sequence of SEQ ID NO: 9, the second polypeptide is an H chain consisting of the amino acid sequence of SEQ ID NO: 11, and the third polypeptide and the fourth polypeptide are common L chains of SEQ ID NO: 10 (Q499-z121/J327-z119/L404-k);

a bispecific antibody in which the first polypeptide is an H chain consisting of the amino acid sequence of SEQ ID NO: 36, the second polypeptide is an H chain consisting of the amino acid sequence of SEQ ID NO: 37, and the third polypeptide and the fourth polypeptide are common L chains of SEQ ID NO: 38 (Q153-G4k/J142-G4h/L180-k);

[4] the antibody of any one of [1] to [3], wherein the neutralizing antibody is an antibody that binds to Fab comprising an antigen-binding site that binds to coagulation factor IX and/or activated coagulation factor IX, and which comprises

a) a heavy chain variable region comprising a CDR1 that consists of the amino acid sequence of SEQ ID NO: 12, a CDR2 that consists of the amino acid sequence of SEQ ID NO:

13, and a CDR3 that consists of the amino acid sequence of SEQ ID NO: 14; and

b) a light chain variable region comprising a CDR1 that consists of the amino acid sequence of SEQ ID NO: 18, a CDR2 that consists of the amino acid sequence of SEQ ID NO: 19, and a CDR3 that consists of the amino acid sequence of SEQ ID NO: 20;

5 [5] the antibody of any one of [1] to [4], wherein the neutralizing antibody is an antibody comprising

a) a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 1; and

b) a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 2;

[6] the antibody of any one of [1] to [3], wherein the neutralizing antibody is an antibody that binds to Fab comprising an antigen-binding site that binds to coagulation factor X and/or activated coagulation

10 factor X, and which comprises

a) a heavy chain variable region comprising a CDR1 that consists of the amino acid sequence of SEQ ID NO: 24, a CDR2 that consists of the amino acid sequence of SEQ ID NO: 25, and a CDR3 that consists of the amino acid sequence of SEQ ID NO: 26; and

b) a light chain variable region comprising a CDR1 that consists of the amino acid sequence of SEQ ID NO: 30, a CDR2 that consists of the amino acid sequence of SEQ ID NO: 31, and a CDR3 that consists of the amino acid sequence of SEQ ID NO: 32;

[7] the antibody of any one of [1] to [3] and [6], wherein the neutralizing antibody is an antibody comprising

a) the heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 3 and

20 b) the light chain variable region consisting of the amino acid sequence of SEQ ID NO: 4;

[8] a nucleic acid encoding the antibody of any one of [1] to [7];

[9] a vector inserted with the nucleic acid of [8];

[10] a cell comprising the nucleic acid of [8] or the vector of [9];

[11] a method for producing an antibody by culturing the cell of [10];

25 [12] an antibody that binds to Fab comprising an antigen-binding site that binds to coagulation factor IX and/or activated coagulation factor IX, and which comprises

a) a heavy chain variable region comprising a CDR1 that consists of the amino acid sequence of SEQ ID NO: 12, a CDR2 that consists of the amino acid sequence of SEQ ID NO: 13, and a CDR3 that consists of the amino acid sequence of SEQ ID NO: 14; and

30 b) a light chain variable region comprising a CDR1 that consists of the amino acid sequence of SEQ ID NO: 18, a CDR2 that consists of the amino acid sequence of SEQ ID NO: 19, and a CDR3 that consists of the amino acid sequence of SEQ ID NO: 20;

[13] the antibody of [12] comprising

a) a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 1; and

35 b) a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 2;

[14] an antibody that binds to Fab comprising an antigen-binding site that binds to coagulation factor X and/or activated coagulation factor X, and which comprises

a) a heavy chain variable region comprising a CDR1 that consists of the amino acid sequence of SEQ ID NO: 24, a CDR2 that consists of the amino acid sequence of SEQ ID NO: 25, and a CDR3 that consists of the amino acid sequence of SEQ ID NO: 26;

b) a light chain variable region comprising a CDR1 that consists of the amino acid sequence of SEQ ID NO: 30, a CDR2 that consists of the amino acid sequence of SEQ ID NO: 31, and a CDR3 that consists of the amino acid sequence of SEQ ID NO: 32;

[15] the antibody of [14], which comprises

a) a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 3; and

b) a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 4;

[16] a nucleic acid encoding the antibody of any one of [12] to [15];

[17] a vector inserted with the nucleic acid of [16];

[18] a cell comprising the nucleic acid of [16] or the vector of [17]; and

[19] a method for producing an antibody by culturing the cell of [18].

[Effects of the Invention]

The present invention provides antibodies used for measuring FVIII activity and FVIII inhibitor titer without being influenced by the activity of a substance having an FVIII-substituting activity. A substance having an FVIII-substituting activity includes a bispecific antibody that binds to FIX and/or FIXa and FX and/or FXa.

Brief Description of the Drawings

Fig. 1 shows the results of one-stage clotting assay performed under neutralization of the anti-FIXa/FX bispecific antibody using rAQ8-mIgG2b and rAJ540-rbIgG. When FVIII-deficient plasma containing 10 U/dL or 100 U/dL recombinant FVIII supplemented with an anti-FIXa/FX bispecific antibody, ACE910, was diluted with a buffer (#3 and #7), the FVIII activities were shown to be above the range of the calibration curve. On the other hand, when FVIII-deficient plasma containing 10 U/dL or 100 U/dL recombinant FVIII supplemented with the anti-FIXa/FX bispecific antibody, ACE910, was diluted with a buffer containing the two types of antibodies against the anti-FIXa/FX bispecific antibody (#4 and #8), the FVIII activities were shown to be similar to those of the groups without addition of the anti-FIXa/FX bispecific antibody (#1 and #5). When FVIII-deficient plasma containing 10 U/dL or 100 U/dL recombinant FVIII was diluted with a buffer containing only the two types of antibodies against the anti-FIXa/FX bispecific antibody (#2 and #6), the FVIII activities were shown to be similar to those of the groups without addition of the anti-FIXa/FX bispecific antibody (#1 and #5).

CLAIMS

1. An antibody that neutralizes a substance having an activity of functionally substituting for coagulation factor VIII.

2. The antibody of claim 1, wherein the substance having an activity of functionally substituting for coagulation factor VIII is a bispecific antibody that binds to coagulation factor IX and/or activated coagulation factor IX and coagulation factor X and/or activated blood coagulation factor X.

3. The antibody of claim 2, wherein the bispecific antibody is any one of the antibodies recited below, in which a first polypeptide is associated with a third polypeptide and a second polypeptide is associated with a fourth polypeptide:

a bispecific antibody in which the first polypeptide is an H chain consisting of the amino acid sequence of SEQ ID NO: 9, the second polypeptide is an H chain consisting of the amino acid sequence of SEQ ID NO: 11, and the third polypeptide and the fourth polypeptide are common L chains of SEQ ID NO: 10 (Q499-z121/J327-z119/L404-k);

a bispecific antibody in which the first polypeptide is an H chain consisting of the amino acid sequence of SEQ ID NO: 36, the second polypeptide is an H chain consisting of the amino acid sequence of SEQ ID NO: 37, and the third polypeptide and the fourth polypeptide are common L chains of SEQ ID NO: 38 (Q153-G4k/J142-G4h/L180-k).

4. The antibody of any one of claims 1 to 3, wherein the neutralizing antibody is an antibody that binds to Fab comprising an antigen-binding site that binds to coagulation factor IX and/or activated coagulation factor IX, and which comprises

a) a heavy chain variable region comprising a CDR1 that consists of the amino acid sequence of SEQ ID NO: 12, a CDR2 that consists of the amino acid sequence of SEQ ID NO: 13, and a CDR3 that consists of the amino acid sequence of SEQ ID NO: 14; and

b) a light chain variable region comprising a CDR1 that consists of the amino acid sequence of SEQ ID NO: 18, a CDR2 that consists of the amino acid sequence of SEQ ID NO: 19, and a CDR3 that consists of the amino acid sequence of SEQ ID NO: 20.

5. The antibody of any one of claims 1 to 4, wherein the neutralizing antibody is an antibody comprising

a) a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 1; and

b) a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 2.

6. The antibody of any one of claims 1 to 3, wherein the neutralizing antibody is an antibody that binds to Fab comprising an antigen-binding site that binds to coagulation factor X and/or activated coagulation factor X, and which comprises

a) a heavy chain variable region comprising a CDR1 that consists of the amino acid sequence of SEQ ID NO: 24, a CDR2 that consists of the amino acid sequence of SEQ ID NO: 25, and a CDR3 that consists of the amino acid sequence of SEQ ID NO: 26; and

b) a light chain variable region comprising a CDR1 that consists of the amino acid sequence of SEQ ID NO: 30, a CDR2 that consists of the amino acid sequence of SEQ ID NO: 31, and a CDR3 that consists of the amino acid sequence of SEQ ID NO: 32.

7. The antibody of any one of claims 1 to 3 and 6, wherein the neutralizing antibody is an antibody comprising

a) a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 3; and

b) a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 4.

8. A nucleic acid encoding the antibody of any one of claims 1 to 7.

9. A vector inserted with the nucleic acid of claim 8.

10. A cell comprising the nucleic acid of claim 8 or the vector of claim 9.

11. A method for producing an antibody by culturing the cell of claim 10.