activity of functionally substituting for F.VIII than the above-mentioned antibodies were desired.

Prior Art Documents

[Patent Document]

[Patent Document 1] WO 2005/035754

[Patent Document 2] WO 2005/035756

[Patent Document 3] WO 2006/109592

[Non-patent Document]

[Non-patent Document 1] Blood 58, 1-13 (1981)

[Non-patent Document 2] Nature 312, 330-337 (1984)

[Non-patent Document 3] Nature 312, 337-342 (1984)

[Non-patent Document4] Biochim.Biophys.Acta 871, 268-278 (1986)

Summary of the Invention

[Problems to be Solved by the Invention]

An objective of the present invention is to provide multispecific antigen-binding molecules that functionally substitute for F.VIII, a cofactor that enhances enzymatic reactions.

[Means for Solving the Problems]

As a result of dedicated research, the present inventors succeeded in discovering bispecific antibodies having a better F.Xa generation-promoting activity than known antibodies from among various bispecific antibodies that specifically bind to both F.IX/F.IXa and F.X, and substitute for the cofactor function of F.VIII, that is, the function to promote F.X activation by F.IXa (F.Xa generation-promoting function).

Furthermore, the present inventors succeeded in finding the positions in the amino acid sequences of bispecific antibodies having the activity of functionally substituting for F.VIII that are important for improving the F.Xa generation-promoting activity of these antibodies, and thus they successfully obtained bispecific antibodies in which the activity of functionally substituting for F.VIII is further increased by replacing these amino acids. They also succeeded in obtaining bispecific antibodies which not only have a high activity of functionally substituting for F.VIII, but also have a low F.Xase inhibitory action. Satisfying both of these properties is very difficult.

Thus, in a first aspect, the present invention refers to a bispecific antibody comprising a first polypeptide, a second polypeptide, a third polypeptide, and a fourth polypeptide, wherein the bispecific antibody is any one of the following (a) to (u):

(a) a bispecific antibody (Q1-G4k/J268-G4h/L45-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 75, 76, and 77, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 114, 115, and 116, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 141, 142, and 143, respectively;

(b) a bispecific antibody (Q1-G4k/J321-G4h/L45-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 75, 76, and 77, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 120, 121, and 122, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 141, 142, and 143, respectively;

(c) a bispecific antibody (Q31-z7/J326-z107/L2-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 78, 79, and 80, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 123, 124, and 125, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 138, 139, and 140, respectively;

(d) a bispecific antibody (Q64-z55/J344-z107/L45-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 81, 82, and 83, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 132, 133, and 134, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 141, 142, and 143, respectively;

(e) a bispecific antibody (Q64-z7/J326-z107/L334-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 81, 82, and 83, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 123, 124, and 125, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 150, 151, and 152, respectively;

(f) a bispecific antibody (Q64-z7/J344-z107/L406-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 81, 82, and 83, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 132, 133, and 134, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 159, 160, and 161, respectively;

(g) a bispecific antibody (Q85-G4k/J268-G4h/L406-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 84, 85, and 86, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 114, 115, and 116, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 159, 160, and 161, respectively;

(h) a bispecific antibody (Q85-G4k/J321-G4h/L334-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 84, 85, and 86, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 120, 121, and 122, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 150, 151, and 152, respectively;

(i) a bispecific antibody (Q153-G4k/J232-G4h/L406-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 87, 88, and 89, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 108, 109, and 110, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 159, 160, and 161, respectively;

(j) a bispecific antibody (Q354-z106/J259-z107/L324-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 90, 91, and 92, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 111, 112, and 113, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 147, 148, and 149, respectively;

(k) a bispecific antibody (Q360-G4k/J232-G4h/L406-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 93, 94, and 95, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 108, 109, and 110, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 159, 160, and 161, respectively;

(l) a bispecific antibody (Q360-z118/J300-z107/L334-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 93, 94, and 95, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 117, 118, and 119, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 150, 151, and 152, respectively;

(m) a bispecific antibody (Q405-G4k/J232-G4h/L248-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 96, 97, and 98, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 108, 109, and 110, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 144, 145, and 146, respectively;

(n) a bispecific antibody (Q458-z106/J346-z107/L408-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 99, 100, and 101, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise t SEQ ID NOs: 135, 136, and 137, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 162, 163, and 164, respectively;

(o) a bispecific antibody (Q460-z121/J327-z119/L334-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 102, 103, and 104, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 126, 127, and 128, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 150, 151, and 152, respectively;

(p) a bispecific antibody (Q499-z118/J327-z107/L334-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 105, 106, and 107, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 126, 127, and 128, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 150, 151, and 152, respectively;

(q) a bispecific antibody (Q499-z118/J327-z107/L377-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 105, 106, and 107, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 126, 127, and 128, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 153, 154, and 155, respectively;

(r) a bispecific antibody (Q499-z118/J346-z107/L248-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 105, 106, and 107, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 135, 136, and 137, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 144, 145, and 146, respectively;

(s) a bispecific antibody (Q499-z121/J327-z119/L404-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 105, 106, and 107, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 126, 127, and 128, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 156, 157, and 158, respectively;

(t) a bispecific antibody (Q499-z121/J339-z119/L377-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 105, 106, and 107, respectively, the second polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 129, 130, and 131, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 153, 154, and 155, respectively; and

(u) a bispecific antibody (Q153-G4k/J142-G4h/L180-k), wherein the first polypeptide is an H chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 87, 88, and 89, respectively, the second polypeptide is an H chainθ comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs:174, 175, and 176, respectively, and the third polypeptide and the fourth polypeptide are a commonly shared L chain comprising CDRs 1, 2, and 3 that comprise SEQ ID NOs: 177, 178, and 179, respectively.

Thus, in a second aspect, the present invention refers to a nucleic acid encoding the bispecific antibody of the present invention.

Thus, in a third aspect, the present invention refers to a vector inserted with the nucleic acid of the present invention.

Thus, in a fourth aspect, the present invention refers to a cell comprising the nucleic acid of the present invention or the vector of the present invention.

Thus, in a fifth aspect, the present invention refers to a method for producing the bispecific antibody of the present invention by culturing the cell of the present invention.

Thus, in a sixth aspect, the present invention refers to a pharmaceutical composition comprising the bispecific antibody of the present invention, and a pharmaceutically acceptable carrier.

Thus, in a seventh aspect, the present invention refers to the use of the bispecific antibody of the present invention for the manufacture of a pharmaceutical composition for preventing and/or treating bleeding, a disease accompanying bleeding, or a disease caused by bleeding.

Thus, in an eighth aspect, the present invention refers to a kit for use in the prevention and/or treatment of bleeding, a disease accompanying bleeding, or a disease caused by bleeding, which comprises the bispecific antibody of the present invention, or the composition of the present invention.

Specifically, the present ~~invention~~disclosure relates to multispecific antigen-binding molecules that functionally substitute for F.VIII, a cofactor that enhances enzymatic reactions, and pharmaceutical compositions comprising such a molecule as an active ingredient, and specifically relates to the following:

[1] a multispecific antigen-binding molecule that functionally substitutes for blood coagulation

factor VIII;

[24] the composition of [23], wherein the disease that develops and/or progresses due to a decrease or deficiency in the activity of blood coagulation factor VIII and/or activated blood coagulation factor VIII is hemophilia A;

[25] the composition of [23], wherein the disease that develops and/or progresses due to a decrease or deficiency in the activity of blood coagulation factor VIII and/or activated blood coagulation factor VIII is a disease showing emergence of an inhibitor against blood coagulation factor VIII and/or activated blood coagulation factor VIII;

[26] the composition of [23], wherein the disease that develops and/or progresses due to a decrease or deficiency in the activity of blood coagulation factor VIII and/or activated blood coagulation factor VIII is acquired hemophilia;

[27] the composition of [23], wherein the disease that develops and/or progresses due to a decrease in the activity of blood coagulation factor VIII and/or activated blood coagulation factor VIII is von Willebrand disease;

[28] a method for preventing and/or treating bleeding, a disease accompanying bleeding, or a disease caused by bleeding, which comprises the step of administering the multispecific antigen-binding molecule of any one of [1] to [15] or the bispecific antibody of [16], or the composition of any one of [21] to [27]; and

[29] a kit for use in the prevention and/or treatment method of [28], which comprises at least the multispecific antigen-binding molecule of any one of [1] to [15] or the bispecific antibody of [16], or the composition of any one of [21] to [27].

Furthermore, the present ~~invention~~disclosure relates to:

[30] use of the multispecific antigen-binding molecule of any one of [1] to [15], the bispecific antibody of [16], or the composition of any one of [21] to [27] in the manufacture of an agent for preventing and/or treating bleeding, a disease accompanying bleeding, or a disease caused by bleeding; and

[31] the multispecific antigen-binding molecule of any one of [1] to [15], the bispecific antibody of [16], or the composition of any one of [21] to [27] for preventing and/or treating bleeding, a disease accompanying bleeding, or a disease caused by bleeding.

The present ~~invention~~disclosure also relates to bispecific antibodies that functionally substitute for F.VIII, a cofactor that enhances enzymatic reactions, and pharmaceutical compositions comprising the antibody as an active ingredient, and more specifically relates to:

[32] a bispecific antibody that functionally substitutes for blood coagulation factor VIII, which comprises a first antigen-binding site that recognizes blood coagulation factor IX and/or activated blood coagulation factor IX and a second antigen-binding site that recognizes blood coagulation factor X, wherein the bispecific antibody is any of the following (a) to (u):

acid sequences of:

Q1 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 75, 76, and 77, respectively);

Q31 H chain each CDR1, 2, and 3 sequences (SEQ ID NOs: 78, 79, and 80, respectively);

Q64 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 81, 82, and 83, respectively);

Q85 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 84, 85, and 86, respectively);

Q153 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 87, 88, and 89, respectively);

Q354 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 90, 91, and 92, respectively);

Q360 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 93, 94, and 95, respectively);

Q405 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 96, 97, and 98, respectively);

Q458 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 99, 100, and 101, respectively);

Q460 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 102, 103, and 104, respectively); and

Q499 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 105, 106, and 107, respectively)

mentioned in the later-described Examples~~, or antigen-binding sites that are functionally equivalent to them~~.

Preferred embodiments of the antigen-binding site of a second polypeptide specifically include, for example, antigen-binding sites comprising the amino acid sequences of:

J232 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 108, 109, and 110, respectively);

J259 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 111, 112, and 113, respectively);

J268 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 114, 115, and 116, respectively);

J300 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 117, 118, and 119, respectively);

J321 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 120, 121, and 122, respectively);

J326 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 123, 124, and 125, respectively);

J327 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 126, 127, and 128, respectively);

J339 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 129, 130, and 131, respectively);

J344 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 132, 133, and 134, respectively);

J346 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 135, 136, and 137, respectively); and

J142 H chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 174, 175, and 176, respectively)

mentioned in the later-described Examples~~, or antigen-binding sites that are functionally equivalent to them~~.

More specifically, the present invention provides multispecific antigen-binding molecules, wherein the antigen-binding site of the first polypeptide comprises an antigen-binding site which comprises H chain CDRs consisting of any one of the amino acid sequences selected from the following (a1) to (a11)~~, or an antigen-binding site functionally equivalent thereto~~, and the antigen-binding site of the second polypeptide comprises an antigen-binding site which

comprises H chain CDRs consisting of any one of the amino acid sequences selected from the following (b1) to (b11)~~, or an antigen-binding site functionally equivalent thereto~~:

(a1) an antigen-binding site comprising an H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 75, 76, and 77 (H chain CDRs of Q1), respectively;

(a2) an antigen-binding site comprising an H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 78, 79, and 80 (H chain CDRs of Q31), respectively;

(a3) an antigen-binding site comprising an H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 81, 82, and 83 (H chain CDRs of Q64), respectively;

(a4) an antigen-binding site comprising an H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 84, 85, and 86 (H chain CDRs of Q85), respectively;

(a5) an antigen-binding site comprising the H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 87, 88, and 89 (H chain CDRs of Q153), respectively;

(a6) an antigen-binding site comprising an H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 90, 91, and 92 (H chain CDRs of Q354), respectively;

(a7) an antigen-binding site comprising the H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 93, 94, and 95 (H chain CDRs of Q360), respectively;

(a8) an antigen-binding site comprising the of H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 96, 97, and 98 (H chain CDRs of Q405), respectively;

(a9) an antigen-binding site comprising an H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 99, 100, and 101 (H chain CDRs of Q458), respectively;

(a10) an antigen-binding site comprising an H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 102, 103, and 104 (H chain CDRs of Q460), respectively;

(a11) an antigen-binding site comprising an H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 105, 106, and 107 (H chain CDRs of Q499), respectively;

(b1) an antigen-binding site comprising an H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 108, 109, and 110 (H chain CDRs of J232), respectively;

(b2) an antigen-binding site comprising an H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 111, 112, and 113 (H chain CDRs of J259), respectively;

(b3) an antigen-binding site comprising an H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 114, 115, and 116 (H chain CDRs of J268), respectively;

(b4) an antigen-binding site comprising an H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 117, 118, and 119 (H chain CDRs of J300), respectively;

(b5) an antigen-binding site comprising an H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 120, 121, and 122 (H chain CDRs of J321), respectively;

(b6) an antigen-binding site comprising the H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 123, 124, and 125 (H chain CDRs of J326), respectively;

(b7) an antigen-binding site comprising an H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 126, 127, and 128 (H chain CDRs of J327), respectively;

(b8) an antigen-binding site comprising an H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 129, 130, and 131 (H chain CDRs of J339), respectively;

(b9) an antigen-binding site comprising an H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 132, 133, and 134 (H chain CDRs of J344), respectively;

(b10) an antigen-binding site comprising an H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 135, 136, and 137 (H chain CDRs of J346), respectively; and

(b11) an antigen-binding site comprising an H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs: 174, 175, and 176 (H chain CDRs of J142), respectively.

Preferred embodiments of the antigen-binding site of the third and fourth polypeptides specifically include, for example, antigen-binding sites comprising the amino acid sequences of:
L2 L chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 138, 139, and 140, respectively);
L45 L chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 141, 142, and 143, respectively);
L248 L chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 144, 145, and 146, respectively);
L324 L chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 147, 148, and 149, respectively);
L334 L chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 150, 151, and 152, respectively);
L377 L chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 153, 154, and 155, respectively);
L404 L chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 156, 157, and 158, respectively);
L406 L chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 159, 160, and 161, respectively);
L408 L chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 162, 163, and 164, respectively); and
L180 L chain each CDR1, 2, and 3 sequence (SEQ ID NOs: 177, 178, and 179, respectively)
mentioned in the later-described Examples~~, or antigen-binding sites that are functionally equivalent to them~~.

More specifically, the present invention provides multispecific antigen-binding molecules, wherein the antigen-binding sites included in the third polypeptide and the fourth polypeptide comprise an antigen-binding site which comprises L chain CDRs consisting of any one of the amino acid sequences selected from the following (c1) to (c10)~~, or an antigen-binding site functionally equivalent thereto~~:

(c1) an antigen-binding site comprising an L chain CDR1, 2, and 3 amino acid sequences of SEQ ID NOs: 138, 139, and 140 (L chain CDR of L2), respectively;

(c2) an antigen-binding site comprising an L chain CDR1, 2, and 3 amino acid sequences of SEQ ID NOs: 141, 142, and 143 (L chain CDR of L45), respectively;

(c3) an antigen-binding site comprising an L chain CDR1, 2, and 3 amino acid sequences of SEQ ID NOs: 144, 145, and 146 (L chain CDR of L248), respectively;

J326: SEQ ID NO: 51
J327: SEQ ID NO: 52
J339: SEQ ID NO: 53
J344: SEQ ID NO: 54
J346: SEQ ID NO: 55
J142: SEQ ID NO: 172

More specifically, the present invention provides multispecific antigen-binding molecules, wherein the antigen-binding site of the first polypeptide comprises an antigen-binding site which comprises an H chain variable region consisting of any one of the amino acid sequences selected from the following (a1) to (a11)~~, or an antigen-binding site functionally equivalent thereto~~, and the antigen-binding site of the second polypeptide comprises an antigen-binding site which comprises an H chain variable region consisting of any one of the amino acid sequences selected from the following (b1) to (b11)~~, or an antigen-binding site functionally equivalent thereto~~:

(a1) an antigen-binding site comprising an H chain variable region amino acid sequence of SEQ ID NO: 35 (H chain variable region of Q1);
(a2) an antigen-binding site comprising an H chain variable region amino acid sequence of SEQ ID NO: 36 (H chain variable region of Q31);
(a3) an antigen-binding site comprising an H chain variable region amino acid sequence of SEQ ID NO: 37 (H chain variable region of Q64);
(a4) an antigen-binding site comprising an H chain variable region amino acid sequence of SEQ ID NO: 38 (H chain variable region of Q85);
(a5) an antigen-binding site comprising an H chain variable region amino acid sequence of SEQ ID NO: 39 (H chain variable region of Q153);
(a6) an antigen-binding site comprising an H chain variable region amino acid sequence of SEQ ID NO: 40 (H chain variable region of Q354);
(a7) an antigen-binding site comprising an H chain variable region amino acid sequence of SEQ ID NO: 41 (H chain variable region of Q360);
(a8) an antigen-binding site comprising an H chain variable region amino acid sequence of SEQ ID NO: 42 (H chain variable region of Q405);
(a9) an antigen-binding site comprising an H chain variable region amino acid sequence of SEQ ID NO: 43 (H chain variable region of Q458);
(a10) an antigen-binding site comprising an H chain variable region amino acid sequence of SEQ ID NO: 44 (H chain variable region of Q460);
(a11) an antigen-binding site comprising an H chain variable region amino acid sequence of SEQ ID NO: 45 (H chain variable region of Q499);

molecules, wherein the antigen-binding sites included in the third polypeptide and the fourth polypeptide comprise an antigen-binding site which comprises an L chain variable region consisting of any one of the amino acid sequences selected from the following (c1) to (c10)~~, or an antigen-binding site functionally equivalent thereto~~:

(c1) an antigen-binding site comprising an L chain variable region amino acid sequence of SEQ ID NO: 56 (L chain variable region of L2);

(c2) an antigen-binding site comprising an L chain variable region amino acid sequence of SEQ ID NO: 57 (L chain variable region of L45);

(c3) an antigen-binding site comprising an L chain variable region amino acid sequence of SEQ ID NO: 58 (L chain variable region of L248);

(c4) an antigen-binding site comprising an L chain variable region amino acid sequence of SEQ ID NO: 59 (L chain variable region of L324);

(c5) an antigen-binding site comprising an L chain variable region amino acid sequence of SEQ ID NO: 60 (L chain variable region of L334);

(c6) an antigen-binding site comprising an L chain variable region amino acid sequence of SEQ ID NO: 61 (L chain variable region of L377);

(c7) an antigen-binding site comprising an L chain variable region amino acid sequence of SEQ ID NO: 62 (L chain variable region of L404);

(c8) an antigen-binding site comprising an L chain variable region amino acid sequence of SEQ ID NO: 63 (L chain variable region of L406);

(c9) an antigen-binding site comprising an L chain variable region amino acid sequence of SEQ ID NO: 64 (L chain variable region of L408); and

(c10) an antigen-binding site comprising an L chain variable region amino acid sequence of SEQ ID NO: 173 (L chain variable region of L180).

The amino acid sequences of CDR1 to 3 and FR1 to 4 in each of the sequences are as described in Figs. 3A to D

When producing a full-length antibody using the variable regions disclosed in the present invention, without particular limitations, constant regions well known to those skilled in the art may be used. For example, constant regions described in "Sequences of proteins of immunological interest", (1991), U.S. Department of Health and Human Services. Public Health Service National Institutes of Health, or "An efficient route to human bispecific IgG", (1998). Nature Biotechnology vol. 16, 677-681 can be used. Preferred examples of the antibody constant regions of the present invention include the constant regions of IgG antibodies. When using the constant region of an IgG antibody, its type is not limited, and a constant region of IgG subclass such as IgG1, IgG2, IgG3, or IgG4 may be used. Furthermore, amino acid mutations may be introduced into the constant region of these IgG subclasses. Amino acid mutations to be

(d7) an H chain constant region of SEQ ID NO: 71 (G4h);

(d8) an H chain constant region of SEQ ID NO: 72 (z107); and

(d9) an H chain constant region of SEQ ID NO: 73 (z119).

Furthermore, the present invention provides a multispecific antigen-binding molecule, wherein the third and fourth polypeptides comprise an antibody L chain constant region consisting of the following amino acid sequence of:

(e) an L chain constant region of SEQ ID NO: 74 (k).

In the present invention, the phrase "functionally substitute for F.VIII" means that F.IX and/or F.IXa, and F.X is recognized, and activation of F.X is promoted (F.Xa generation is promoted).

In the present invention, "F.Xa generation-promoting activity" can be confirmed by evaluating the multispecific antigen-binding molecules of the present invention using, for example, a measurement system comprising F.XIa (F.IX activating enzyme), F.IX, F.X, F synthetic substrate S-2222 (synthetic substrate of F.Xa), and phospholipids. This measurement system shows the correlation between the severity of the disease and clinical symptoms in hemophilia A cases (Rosen S, Andersson M, Blomba¨ck M *et al.* Clinical applications of a chromogenic substrate method for determination of FVIII activity. Thromb Haemost 1985, 54: 811-23). That is, in the present measurement system, test substances that show higher F.Xa generation-promoting activity are expected to show better hemostatic effects against bleeding episodes in hemophilia A. With these results, if a multispecific antigen-binding molecule having activity of functionally substituting for F.VIII is a molecule having a higher activity than hA69-KQ/hB26-PF/hAL-AQ, it may yield excellent blood coagulation-promoting activity, and excellent effects may be obtained as a pharmaceutical component for preventing and/or treating bleeding, a disease accompanying bleeding, or a disease caused by bleeding. To obtain excellent effects as the above-mentioned pharmaceutical component, for example, F.Xa generation-promoting activity measured under the conditions described in [Example 2] is preferably not less than that of hA69-KQ/hB26-PF/hAL-AQ, and in particular, the activity is more preferably the same as or not less than that of Q153-G4k/J142-G4h/L180-k. Herein, the "F.Xa generation-promoting activity" is the value obtained by subtracting the change in absorbance upon 20 minutes in a solvent from the change in absorbance upon 20 minutes in an antibody solution.

A preferred embodiment of the present invention is a multispecific antibody that functionally substitutes for F.VIII, which recognizes F.IX and/or F.IXa, and F.X.

The above-mentioned multispecific antibodies of the present invention are preferably antibodies which comprise H chain CDRs of anti-F.IX/F.IXa antibodies ~~or CDRs functionally equivalent to them~~, and H chain CDRs of anti-F.X antibodies ~~or CDRs functionally equivalent to them~~.

Furthermore, the antibodies of the present invention are preferably antibodies comprising an antigen-binding site having:

H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 75, 76, and 77 (H chain CDRs of Q1), respectively;

H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 78, 79, and 80 (H chain CDRs of Q31), respectively;

H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 81, 82, and 83 (H chain CDRs of Q64), respectively;

H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 84, 85, and 86 (H chain CDRs of Q85), respectively;

H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 87, 88, and 89 (H chain CDRs of Q153), respectively;

H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 90, 91, and 92 (H chain CDRs of Q354), respectively;

H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 93, 94, and 95 (H chain CDRs of Q360), respectively;

H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 96, 97, and 98 (H chain CDRs of Q405), respectively;

H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 99, 100, and 101 (H chain CDRs of Q458), respectively;

H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 102, 103, and 104 (H chain CDRs of Q460), respectively; or

H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 105, 106, and 107 (H chain CDRs of Q499), respectively,

in an anti-F.IX/IXa antibody~~, or an antigen-binding site functionally equivalent to it~~, and an antigen-binding site comprising:

H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 108, 109, and 110 (H chain CDRs of J232), respectively;

H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 111, 112, and 113 (H chain CDRs of J259), respectively;

H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 114, 115, and 116 (H chain CDRs of J268), respectively;

H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 117, 118, and 119 (H chain CDRs of J300), respectively;

H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 120, 121, and 122 (H chain CDRs of J321), respectively;

H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 123, 124, and 125 (H chain CDRs of J326), respectively;
H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 126, 127, and 128 (H chain CDRs of J327), respectively;
H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 129, 130, and 131 (H chain CDRs of J339), respectively;
H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 132, 133, and 134 (H chain CDRs of J334), respectively;
the amino acid sequences of H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 135, 136, and 137 (H chain CDRs of J346), respectively; or
H chain CDR 1, 2, and 3 amino acid sequences of SEQ ID NOs 174, 175, and 176 (H chain CDRs of J142), respectively,
in an anti-F.X antibody~~, or an antigen-binding site functionally equivalent to it~~.

In the present invention, "antigen-binding sites are functionally equivalent" means that the activities of functionally substituting for F.VIII possessed by the multispecific antigen-binding molecules having the antigen-binding sites are equivalent.

In the present invention, the term "equivalent" does not necessarily have to mean the same degree of activity, and the activity may be enhanced, or the activity may be decreased as long as there is an activity higher than that of hA69-KQ/hB26-PF/hAL-AQ according to the measurement system described above, or preferably F.Xa generation-promoting activity measured under the conditions described in [Example 2] is equivalent to or not less than that of Q153-G4k/J142-G4h/L180-k.

The above-mentioned antibodies may have one or more amino acid substitutions, deletions, additions, and/or insertions in the variable region (CDR sequences and/or FR sequences) of the amino acid sequence as long as they have an activity higher than that of hA69-KQ/hB26-PF/hAL-AQ according to the measurement system described above at page 35, lines 11-30, or preferably F.Xa generation-promoting activity measured under the conditions described in [Example 2] is equivalent to or not less than that of Q153-G4k/J142-G4h/L180-k. A method of introducing mutations into proteins is well known to those skilled in the art as a method for introducing one or more amino acid substitutions, deletions, additions, and/or insertions into an amino acid sequence. For example, those skilled in the art can prepare a desired mutant functionally equivalent to a multispecific polypeptide multimer having the activity of functionally substituting for F.VIII by introducing appropriate mutations into the amino acid sequence using site-directed mutagenesis (Hashimoto-Gotoh, T, Mizuno, T, Ogasahara, Y, and Nakagawa, M. (1995) An oligodeoxyribonucleotide-directed dual amber method for site-directed mutagenesis. Gene 152: 271-275; Zoller, MJ, and Smith, M. (1983) Oligonucleotide-directed

for gene therapy. In addition to direct administration using naked plasmids, methods of administration include administration after packaging into liposomes and such, forming a variety of virus vectors such as retrovirus vectors, adenovirus vectors, vaccinia virus vectors, poxvirus vectors, adeno-associated virus vectors, and HVJ vectors (see Adolph "Viral Genome Methods" CRC Press, Florida (1996)), or coating with carrier beads such as colloidal gold particles (WO 93/17706, and such). However, so long as the antibodies are expressed *in vivo* and their activities are exercised, any method can be used for administration. Preferably, a sufficient dose can be administered by a suitable parenteral route (such as injecting or infusing intravenously, intraperitoneally, subcutaneously, intradermally, intramuscularly, into adipose tissues or mammary glands; inhalation; gas-driven particle bombardment (using electron gun and such); or mucosal route such as nasal drops). Alternatively, the genes encoding the antibodies of the present invention may be administered into blood cells, bone marrow cells, and such *ex vivo* using liposome transfection, particle bombardment (U.S. Patent No. 4,945,050), or viral infection, and the cells may be reintroduced into patients. Any gene encoding an antibody of the present invention may be used in gene therapy, and its examples include genes comprising nucleotide sequences encoding the CDRs of Q1, Q31, Q64, Q85, Q153, Q354, Q360, Q405, Q458, Q460, Q499, J232, J259, J268, J300, J321, J326, J327, J339, J344, J346, J142, L2, L45, L248, L324, L334, L377, L404, L406, L408, and L180 described above.

The present invention also provides methods for preventing and/or treating bleeding, diseases accompanying bleeding, and/or diseases caused by bleeding, such methods comprising the step of administering the antibodies or compositions of the present invention. The antibodies or compositions can be administered, for example, by the above-mentioned methods.

Furthermore, the present invention provides kits to be used for the above-mentioned methods, such kits comprising at least an antibody or composition of the present invention. In addition, the kits may include, packaged therewith, a syringe, injection needle, pharmaceutically acceptable medium, alcohol-soaked cotton, adhesive bandage, instructions describing the method of use, and the like.

The present invention also relates to use of a multispecific antigen-binding molecule, a bispecific antibody, or a composition of the present invention in the manufacture of an agent for preventing and/or treating bleeding, a disease accompanying bleeding, or a disease caused by bleeding.

Furthermore, the present invention relates to a multispecific antigen-binding molecule, a bispecific antibody, or a composition of the present invention for preventing and/or treating bleeding, a disease accompanying bleeding, or a disease caused by bleeding.

~~All prior art references cited herein are incorporated by reference into this description.~~