CLAIMS

1. A method for prevention of recognition of an antibody Fab fragment by preexisting anti-Fab antibodies consisting in masking the C-terminal extremity of the Fab fragment by addition of a molecule.

2. The method of claim 1, wherein the molecule is selected from the group consisting of His-His-His-His-His (SEQ ID NO:35), Gly-Gly-Gly-Gly-Ser (SEQ ID NO:36) and $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_2$ (SEQ ID NO:37).

3. The method of claim 1 or 2, wherein the molecule is added to the C-terminus of the heavy chain of the antibody Fab fragment.

4. The method of any one of claims 1 to 3, wherein the antibody Fab fragment is an anti-GPVI antibody Fab fragment.

5. The method of claim 4, wherein the antibody Fab fragment specifically binds to human GPVI and induces GPVI depletion phenotype.

6. The method of claim 5, wherein the antibody Fab fragment binds a conformational epitope of human GPVI and contacts human GPVI residues comprising Ser 43, Arg 67 and Asp 81.

7. The method of claim 5 or 6, wherein the antibody Fab fragment comprises
(a) complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having amino acid sequences defined by SEQ ID NO:18, SEQ ID NO:19, and SEQ ID NO:20; and
(b) complementarity determining regions (CDRs) of a light chain variable region (LCVR) having amino acid sequences defined by SEQ ID NO:21, SEQ ID NO:22, and SEQ ID NO:23; and
wherein at least 2 amino acid residues of each CDR can be changed to another amino acid residue without directly disrupting a contact with a GPVI epitope residue.

8. The method of any one of claims 5 to 7, wherein the antibody Fab fragment comprises a heavy chain of SEQ ID NO:6 and a light chain of SEQ ID NO:8 or sequences having at least 80% identity with these sequences, as far as the antibody Fab fragment binding specificity is maintained.

9. The method of any one of claims 5 to 8, wherein the antibody Fab fragment is humanized.

10. The method of claim 9, wherein the humanized antibody Fab fragment comprises a combination of a heavy chain variable region (HCVR) amino acid sequence and a light chain variable region (LCVR) amino acid sequence, selected from the group consisting of

(a) LCVR (SEQ ID NO:15) and HCVR (SEQ ID NO:10);
(b) LCVR (SEQ ID NO:15) and HCVR (SEQ ID NO:11);
(c) LCVR (SEQ ID NO:15) and HCVR (SEQ ID NO:12);
(d) LCVR (SEQ ID NO:15) and HCVR (SEQ ID NO:13);
(e) LCVR (SEQ ID NO:15) and HCVR (SEQ ID NO:14);
(f) LCVR (SEQ ID NO:16) and HCVR (SEQ ID NO:11);
(g) LCVR (SEQ ID NO:17) and HCVR (SEQ ID NO:11); and
(h) LCVR (SEQ ID NO:17) and HCVR (SEQ ID NO:13).

11. An engineered antibody Fab fragment comprising a combination of a humanized heavy chain (HC) amino acid sequence and a humanized light chain (LC) amino acid sequence, wherein the humanized heavy chain further comprises a C-terminal extension comprising additional amino acid residues and wherein the C-terminal extension prevents recognition by anti-Fab antibodies.

12. The engineered antibody Fab fragment of claim 11, wherein the C-terminal extension is selected from the group consisting of His-His-His-His-His (SEQ ID NO:35), Gly-Gly-Gly-Gly-Ser (SEQ ID NO:36) and $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_2$ (SEQ ID NO:37).

13. The engineered antibody Fab fragment of claim 11 or 12, wherein the antibody Fab fragment is as defined in any one of claims 4 to 10.

14. A pharmaceutical composition comprising an engineered antibody Fab fragment as defined in any one of claims 11 to 13 and a pharmaceutically acceptable carrier or excipients.

15. A method for the preparation of an engineered antibody Fab fragment as defined any one of claims 11 to 13 comprising the steps of:
(a) Culture of a cell line expressing an engineered antibody Fab fragment as defined in any one of claims 11 to 13;
(b) Purification of the engineered antibody Fab fragment expressed in the culture medium; and
(c) Formulation of the engineered antibody Fab fragment in a convenient form.

16. An engineered antibody Fab fragment as defined in claim 13 for use in preventing or treating thrombotic and vascular diseases.

17. Use of an engineered antibody Fab fragment as defined in claim 13 in the manufacture of a medicament for the prevention or treatment of thrombotic and vascular diseases.

CLAIMS

1. An antibody Fab fragment that specifically binds to human GPVI and induces GPVI depletion phenotype.

2. The antibody Fab fragment of claim 1, wherein the antibody Fab fragment binds a conformational epitope of human GPVI and contacts human GPVI residues comprising Ser 43, Arg 67 and Asp 81.

3. The antibody Fab fragment of claim 1 or 2, wherein the Fab fragment comprises

(a) complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having amino acid sequences defined by SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20; and

(b) complementarity determining regions (CDRs) of a light chain variable region (LCVR) having amino acid sequences defined by SEQ ID NO: 21, SEQ ID NO: 22, and SEQ ID NO: 23; and

wherein at least 2 amino acid residues of each CDR can be changed to another amino acid residue without directly disrupting a contact with a GPVI epitope residue.

4. The antibody Fab fragment of any one of claims 1 to 3, wherein the antibody Fab fragment comprises a heavy chain of SEQ ID NO.6 and a light chain of SEQ ID NO.8 or sequences having at least 80% identity with these sequences, as far as the antibody Fab fragment binding specificity is maintained.

5. The antibody Fab fragment of any preceding claim which is a humanized.

6. The antibody Fab fragment of claim 5, wherein the humanized Fab fragment comprises a combination of a heavy chain variable region (HCVR) amino acid sequence and a light chain variable region (LCVR) amino acid sequence, selected from the group consisting of

(a) LCVR (SEQ ID NO.15) and HCVR (SEQ ID NO.10);
(b) LCVR (SEQ ID NO.15) and HCVR (SEQ ID NO.11);
(c) LCVR (SEQ ID NO.15) and HCVR (SEQ ID NO.12);
(d) LCVR (SEQ ID NO.15) and HCVR (SEQ ID NO.13);

(e) LCVR (SEQ ID NO.15) and HCVR (SEQ ID NO.14);
(f) LCVR (SEQ ID NO.16) and HCVR (SEQ ID NO.11);
(g) LCVR (SEQ ID NO.17) and HCVR (SEQ ID NO.11); and
(h) LCVR (SEQ ID NO.17) and HCVR (SEQ ID NO.13).

7. An engineered Fab fragment comprising a combination of a humanized heavy chain (HC) amino acid sequence and a humanized light chain (LC) amino acid sequence, wherein the humanized heavy chain further comprises a c-terminal extension comprising additional amino acid residues and wherein the c-terminal extension prevents recognition by anti-Fab antibodies.

8. The engineered Fab fragment of claim 7, wherein the c-terminal extension is selected from the group consisting of SEQ ID NO:35, SEQ ID NO:36 and SEQ ID NO:37.

9. The engineered Fab fragment of claim 7 or 8, wherein the Fab fragment is as defined in any one of claims 1 to 6.

10. A pharmaceutical composition comprising an antibody Fab fragment as defined in any one of claims 1 to 9 and a pharmaceutically acceptable carrier or excipients.

11. A polynucleotide encoding a polypeptide selected from the group consisting in SEQ ID NO.6, SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.12, SEQ ID NO.13, SEQ ID NO.14, SEQ ID NO.15, SEQ ID NO.16, and SEQ ID NO.17 or a sequence having at least 80% identity with these sequences, as far as the polypeptide retains its binding specificity.

12. A method for the preparation of an antibody Fab fragment as defined any one of claims 1 to 9 comprising the steps of:

a. Culture of a cell line expression an antibody Fab fragment as defined in claims 1 to 9
b. Purification of Fab fragment expressed in the culture medium
c. Formulation of the Fab fragment in a convenient form

13. An antibody Fab fragment as defined in claims 1 to 9 for use to prevent or treat thrombotic and vascular diseases.

14. A method for prevention of recognition of an antibody Fab fragments by preexisting antibodies consisting in masking the c-terminal extremity of the antibody Fab fragment by addition of a molecule.

15. A method for prevention of platelet activation when an anti-GPVI Fab is used consisting in masking the c-terminal extremity of the antibody Fab fragment by addition of a molecule.