[0035] Several anti-TFPI antibodies were identified in a previous study and described in PCT Application No. PCT/US2009/052702 filed 4 August 2009, hereby incorporated by reference for all purposes. These anti-TFPI antibodies may be further optimized, for example by improving their affinity and blocking activity to TFPI. Such optimization can be performed for example by utilizing site saturation mutagenesis of the complementarity determining regions (CDRs) or residues in close proximity to the CDRs, i.e. about 3 or 4 residues adjacent to the CDRs, of the antibodies.

[0036] Also provided are monoclonal antibodies having increased or high affinity to TFPI. In some embodiments, the anti-TFPI antibodies have a binding affinity of at least about 10^7M^{-1}, in some embodiments at least about 10^8M^{-1}, in some embodiments at least about 10^9M^{-1}, $10^{10}M^{-1}$, $10^{11}M^{-1}$, or greater, e.g. up to $10^{13}M^{-1}$ or greater.

[0037] Site saturation mutagenesis in and adjacent to the CDRs of two anti-TFPI parental antibodies, designated herein as 2A8 and 4B7, was utilized to optimize the antibodies for affinity and functional activity. It is also contemplated that the same optimization may be performed on any of the antibodies described in the previous PCT/US2009/052702.

[0038] In some embodiments, site saturation mutagenesis of the CDRs may be done on the anti-TFPI antibodies. For 2A8, the CDRs in the heavy chain shown in SEQ ID NO:1 correspond to residues FTFRSYGMS (residues 27 to 35) (SEQ ID NO: 35), SIRGSSSSTYYADSVKG (residues 50 to 66) (SEQ ID NO: 36), and KYRYWFDY (residues 99 to 106) (SEQ ID NO: 37). For the 2A8 light chain shown in SEQ ID N0:2, the CDRs correspond to residues SGDNLRNYYAH (residues 23 to 33) (SEQ ID NO: 38), YYDNNRPS (residues 48 to 55) (SEQ ID NO: 39), and QSWDDGVPV (residues 88 to 96) (SEQ ID NO: 40). For 4B7, the CDRs in the heavy chain shown in SEQ ID N0:3 correspond to residues DSVSSNSAAWS (residues 27 to 37) (SEQ ID NO: 41), IIYKRSKWYNDYAVSVKS (residues 52 to 70) (SEQ ID NO: 42), and WHSDKHWGFDY (residues 102 to 112) (SEQ ID NO: 43). For the 4B7 light chain shown in SEQ ID N0:4, the CDRs correspond to residues RSSQSLVFSDGNTYLN (residues 24 to 39) (SEQ ID NO: 44), KGSNRAS (residues 55 to 61) (SEQ ID NO: 45), and QQYDSYPLT (residues 94 to 102) (SEQ ID NO: 35) of SEQ ID N0:4. A modification may be made in any of the six CDRs individually or combinations of modifications may be made. Further, two or more modifications may be made in a single CDR. In other embodiments, modifications may also be introduced in close proximity to the CDRs, for example about 3 or 4 residues on either side of each CDR.

[0039] Briefly, single and/or multiple amino acid modifications were introduced and analyzed to optimize, e.g. improve the affinity, of parental antibodies 2A8 and 4B7. First, single amino acid modifications were introduced into the six CDRs or adjacent to the CDRs of each antibody followed by analysis of TFPI-binding properties. Modifications that

CLAIMS

1. An isolated human monoclonal IgG antibody that binds specifically to human tissue factor pathway inhibitor, wherein the antibody comprises:

(a) a human heavy chain comprising:

a CDR 1 region with the amino acid sequence shown in SEQ ID NO: 35, optionally comprising a substitution relative to SEQ ID NO: 1 of S35D;

a CDR2 region with the amino acid sequence of shown in SEQ ID NO: 36, optionally comprising a substitution relative to SEQ ID NO: 1 of S55R;

a CDR3 region with the amino acid sequence shown in SEQ ID NO: 37, optionally comprising a substitution relative to SEQ ID NO: 1 of K99L,and

(b) a human light chain comprising:

a CDR1 region with the amino acid sequence shown in SEQ ID NO: 38;

a CDR2 region with the amino acid sequence shown in SEQ ID NO: 39, comprising at least one substitution relative to SEQ ID NO: 2 selected from the group consisting of Y48F and N51V and combinations thereof;

a CDR3 region with the amino acid sequence shown in SEQ ID NO: 40, comprising a substitution relative to SEQ ID NO: 2 of D91W.

2. The isolated human monoclonal IgG antibody of claim 1, wherein

the human heavy chain comprises SEQ ID NO: 7 and

the human light chain comprises SEQ ID NO: 14.

3. An isolated human monoclonal IgG antibody that binds specifically to human tissue factor pathway inhibitor, wherein the antibody comprises:

(a) a human heavy chain comprising:

a CDR 1 region with the amino acid sequence shown in SEQ ID NO: 35, comprising at least one substitution relative to SEQ ID NO: 1 selected from the group consisting of R30S and S35D and combinations thereof;

a CDR2 region with the amino acid sequence shown in SEQ ID NO: 36, comprising at least one substitution relative to SEQ ID NO: 1 selected from the group consisting of S55R and S56G and combinations thereof;

a CDR3 region with an amino acid sequence shown in SEQ ID NO: 37, optionally comprising a substitution relative to SEQ ID NO: 1 of K99L, and

(b) a human light chain comprising:

a CDR1 region with the amino acid sequence shown in SEQ ID NO: 38, comprising substitution relative to SEQ ID NO: 2 of N29K;

a CDR2 region with the amino acid sequence shown in SEQ ID NO: 39, comprising at least one substitution relative to SEQ ID NO: 2 selected from the group consisting Y48F and N51V and combinations thereof;

a CDR3 region with the amino acid sequence shown in SEQ ID NO: 40, comprising at least one substitution relative to SEQ ID NO: 2 selected from the group consisting of S89A, D91W, D92S, G93S, and V94T and combinations thereof.

4. The isolated human monoclonal IgG antibody of claim 3, wherein

the substitutions relative to SEQ ID NO: 1 in the heavy chain are comprised of R30S, S35D, S55R, S56G, and K99L.

5. The isolated human monoclonal IgG antibody of claim 4, wherein

the substitutions relative to SEQ ID NO: 2 in the light chain are comprised of N29K, Y48F, N51V, S89A, D91W, D92S, G93S, and V94T.

6. The isolated human monoclonal IgG antibody of claim 3, wherein

(a) the human heavy chain comprises the amino acid sequence as shown in SEQ ID NO: 10; and

(b) the human light chain comprises the amino acid sequence as shown in SEQ ID NO: 17.

7. An isolated human monoclonal antibody that binds specifically to human tissue factor pathway (TFPI) inhibitor, wherein the antibody comprises;

(a) a human heavy chain comprising:

a CDR 1 region with the amino acid sequence shown in SEQ ID NO: 35, comprising at least one substitution relative to SEQ ID NO: 1 selected from the group consisting of R30S and S35D and combinations thereof;

a CDR2 region with the amino acid sequence shown in SEQ ID NO: 36, comprising at least one substitution relative to SEQ ID NO: 1 selected from the group consisting of S55R and S56G and combinations thereof;

a CDR3 region with an amino acid sequence shown in SEQ ID NO: 37, comprising a substitution relative to SEQ ID NO: 1 of K99L, and

(b) a human light chain comprising:

a CDR1 region with the amino acid sequence shown in SEQ ID NO: 38, comprising at least one substitution relative to SEQ ID NO: 2 selected from the group consisting of

R28P and N29K;

a CDR2 region with the amino acid sequence shown in SEQ ID NO: 39, comprising at least one substitution relative to SEQ ID NO: 2 selected from the group consisting Y48F and N51V and combinations thereof;

a CDR3 region with the amino acid sequence shown in SEQ ID NO: 40, comprising at least one substitution relative to SEQ ID NO: 2 selected from the group consisting of S89A, D91W, D92S, G93S, and V94T and combinations thereof.

8. An isolated human monoclonal antibody that binds specifically to human tissue factor pathway inhibitor, wherein the antibody comprises:

(a) a human heavy chain comprising:

a CDR1 region with the amino acid sequence shown in SEQ ID NO: 35, wherein the arginine at the amino acid position corresponding to position 30 of SEQ

ID NO: 1 is replaced with a serine, and wherein the serine at the amino acid position corresponding to position 35 of SEQ ID NO: 1 is replaced with an aspartic acid;

a CDR2 region with the amino acid sequence shown in SEQ ID NO: 36, wherein the serine at the amino acid position corresponding to position 55 of SEQ ID NO: 1 is replaced with an arginine, and wherein the serine at the amino acid position corresponding to position
56 of SEQ ID NO: 1 is replaced with a glycine; and

a CDR3 region with an amino acid sequence shown in SEQ ID NO: 37, wherein the lysine at the amino acid position corresponding to position 99 of SEQ ID NO: 1 is replaced with a leucine; and

(b) a human light chain comprising:

a CDRI region with the amino acid sequence shown in SEQ ID NO: 38, wherein the arginine at the amino acid position corresponding to position 28 of SEQ ID NO: 2 is
replaced with a proline, and wherein the asparagine at the amino acid position corresponding to position 29 of SEQ ID NO: 2 is replaced with a lysine;

a CDR2 region with the amino acid sequence shown in SEQ ID NO: 39, wherein the tyrosine at the amino acid position corresponding to position 48 of SEQ ID NO: 2 is
replaced with a phenylalanine, and wherein the asparagine at the amino acid position corresponding to position 51 of SEQ ID NO: 2 is replaced with a valine;

a CDR3 region with the amino acid sequence shown in SEQ ID NO: 40, wherein the serine at the amino acid position corresponding to position 89 of SEQ ID NO: 2 is replaced with an alanine, wherein the aspartic acid at the amino acid position corresponding to position 91 of SEQ ID NO: 2 is replaced with a tryptophan, wherein the aspartic acid at the amino acid position corresponding to position 92 of SEQ ID NO: 2 is replaced with a serine, wherein the glycine at the amino acid position corresponding to position 93 of SEQ ID NO:

2 is replaced with a serine, and wherein the valine at the amino acid position corresponding to position 94 of SEQ ID NO: 2 is replaced with a threonine.

9. An isolated human monoclonal antibody that binds specifically to human tissue factor pathway inhibitor, wherein the antibody comprises: (a) a human heavy chain comprising the amino acid sequence as shown in SEQ ID NO: 10; and (b) a human light chain comprising the amino acid sequence as shown in SEQ ID NO: 17.

10. The isolated antibody of claim 7 or claim 8, wherein the antibody binds to human tissue factor pathway inhibitor with an affinity of at least 10^8 M^{-1}.

11. The isolated antibody of claim 7 or claim 8, wherein the antibody binds to human tissue factor pathway inhibitor with an affinity of at least 10^9 M^{-1}.

12. The isolated antibody of claim 7 or claim 8, wherein the antibody binds to human tissue factor pathway inhibitor with an affinity of at least 10^{10} M^{-1}.

13. The isolated antibody of claim 7 or claim 8, wherein the antibody binds to human tissue factor pathway inhibitor with an affinity of at least 10^{11} M^{-1}.

14. The isolated antibody of any one of claims 7 to 9, wherein the antibody is an IgGantibody.

15. The isolated antibody of claim 14, wherein the antibody is an IgG_2 antibody.