

[00367]FIG. 7. Inhibition of AP pathway-mediated hemolysis by anti-factor Bb mAbs.

[00368]FIG. 8. Inhibition of AP pathway-mediated C3b deposition by anti-Factor Bb mAbs.

[00369]FIG. 9. Comparison of the effects of Factor Bb mAbs on AP activity using three different assays. Non-linear curve fits and IC_{50} s were calculated using Prism software.

Example 6: inhibition of cynomolgus monkey AP by anti-factor Bb mAbs

[00370] Inhibition of complement alternative pathway activity (AP) by anti-factor Bb mAb M10 was measured using the Complement System Alternative Pathway WIESLAB® kit. Normal human serum or cynomolgus (“cyno”) monkey serum (Innovative Research; 5.5%) was incubated with 2-fold serial dilutions of M10 or mouse isotype controls starting at 100 μ g/mL. V_{max} was determined for each concentration and the results are shown in **FIG. 10**. The IC_{50} for M10 in this experiment was 9.79E-8 and 3.67E-7 M for human and cyno serum, respectively.

[00371] Inhibition of AP pathway mediated hemolysis by anti-Factor Bb mAbs was measured using cynomolgus serum and rabbit erythrocytes in buffer containing EGTA to inhibit the classical pathway. Anti-factor Bb mAbs or mouse isotype controls (2-fold dilutions starting at 100 μ g/mL) were incubated with 5% cynomolgus monkey serum and rabbit RBCs at 37°C for one hour. The amount of lysis was determined by measuring the A_{540nm} of the supernatant and subtracting the background absorbance in control wells containing EDTA. Results are shown in **FIG. 11**. M17 does not inhibit cyno serum mediated hemolysis of rabbit red blood cells. IC_{50} values for cyno serum were as follows: 2.497E-8 (M4), 2.202E-7 (M10), 2.824E-8 (M12), 2.319E-7 (M18) and 7.524E-7 M (M20).

[00372]FIG. 10. Inhibition of Wieslab AP Activity in human or monkey serum by anti-Factor Bb mAbs.

[00373]FIG. 11. Inhibition of cyno AP pathway mediated hemolysis of rabbit RBCs by anti-factor Bb mAbs.

Example 7: Sequencing of anti-factor Bb mAbs

[00374] Amino acid sequencing of the VH and VL regions of the factor Bb mAbs was conducted using techniques known to those skilled in the art (LakePharma). Specifically, cell pellets were prepared from the hybridoma cell lines and RNA was extracted. V-regions were amplified by reverse transcription-polymerase chain reaction (RT-PCR) using degenerate primer pools for murine antibody signal sequences together with constant region primers for IgMVH, IgGVH, Ig κ VL and Ig λ VL. The polymerase chain reaction (PCR) products obtained from each of the successful amplifications were purified and cloned into a ‘TA’ cloning vector system from which sequences were obtained. The deduced amino acid sequences of the VH and VL regions, as well as the CDRs, of the factor Bb mAbs are provided in **FIG. 12A-12F**. M10 and M18 VL and VH are identical in sequence, and M4 and M12 VH and VL are identical in sequence.

Example 8: Further characterization of anti-Bb mAbs**Inhibition of AP pathway-mediated hemolysis by Factor Bb antibodies on human RBCs pre-treated with CD55/CD59 antibodies (FIG. 14)**

[00375] Patients with Paroxysmal Nocturnal Hemoglobinuria (PNH) suffer from complement mediated lysis of their red blood cells (RBCs) due to the lack of complement regulators (CD55 and CD59) on the cell surface. Pre-treatment with neutralizing antibodies to CD55 and CD59 renders healthy RBCs susceptible to complement mediated lysis.

[00376] Normal human erythrocytes pre-treated with anti-CD55 and CD59 antibodies were incubated with 20% normal human serum containing varying concentrations of Factor Bb antibodies M4, M10, M17 and M20 as well as mouse IgG2a (isotype control). The reaction buffer contained 10 mM Mg EGTA to block the classical and lectin complement pathways. After 120 minutes at 37°C, the cells were centrifuged. The amount of lysis was determined by measuring the A_{540nm} of the supernatant and subtracting the background absorbance in control wells containing EDTA. The results are shown in FIG. 14. Complete inhibition of hemolysis was only obtained with the M10 antibody.

Production of anti-Factor Bb Chimeric Antibodies

[00377] Utilizing the DNA sequences from example 6, the variable domains for hybridoma clones M4 and M10 were synthesized (DNA 2.0) along with additional flanking sequences needed for cloning. The variable domains were cloned into expression vectors that generated a chimeric antibody composed of mouse variable regions and human IgG4 constant/Fc regions. The constructs were transfected into HEK293 cells and purified from culture supernatants using Protein A Sepharose. The chimeric antibody containing the variable region of M10 is referred to as “chimeric M10.” The chimeric antibody containing the variable region of M4 is referred to as “chimeric M4.”

Inhibition of Complement Alternative Pathway (AP) by Factor Bb Chimeric Antibodies (FIG. 15)

[00378] Inhibition of complement AP activity by Factor Bb chimeric antibodies chimeric M10 and chimeric M4 was measured using the Complement System Alternative Pathway WIESLAB® kit. 11% normal human serum (NHS; Complement Technologies) was incubated with 2-fold serial dilution of the chimeric antibodies or their parental monoclonal mouse antibodies, starting at 300 µg/ml. At the end of the assay, the A405 was measured. The data are shown in FIG. 15. The A405 was proportional to the amount of terminal membrane attack complex (MAC) deposited on the plate. The A405 was plotted versus antibody concentration. The chimeric antibodies were found to have similar potencies in the Wieslab Alternative Pathway assay when compared to their corresponding parental monoclonal mouse antibodies.

CLAIMS

What is claimed is:

1. A humanized antibody specific for complement factor Bb, wherein the antibody comprises (a) light chain complementarity determining regions (CDRs) of an antibody light chain variable (VL) region comprising amino acid sequence SEQ ID NO:7 and (b) heavy chain CDRs of an antibody heavy chain variable (VH) region comprising amino acid sequence SEQ ID NO:8 .
2. A humanized antibody specific for complement factor Bb, wherein the antibody comprises (a) a light chain variable region comprising amino acid sequences SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3 and (b) a heavy chain variable region comprising amino acid sequences SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6.
3. The humanized antibody of claim 2, wherein the antibody comprises a CDR-L1 having amino acid sequence SEQ ID NO:1, a CDR-L2 having amino acid sequence SEQ ID NO:2, a CDR-L3 having amino acid sequence SEQ ID NO:3, a CDR-H1 having amino acid sequence SEQ ID NO:4, a CDR-H2 having amino acid sequence SEQ ID NO:5, and a CDR-H3 having amino acid sequence SEQ ID NO:6 (a).
4. The humanized antibody of any one of claims 1-3, wherein the antibody binds a human complement Bb protein with an affinity of at least 10^{-8} M.
5. The humanized antibody of any one of claims 1-4, wherein the antibody inhibits C3b/Bb-mediated cleavage of C3.
6. The humanized antibody of any one of claims 1-5, wherein the antibody comprises a humanized light chain framework region.
7. The humanized antibody of any one of claims 1-6, wherein the antibody comprises a humanized heavy chain framework region.
8. The humanized antibody of any one of claims 1-7, wherein the antibody comprises a humanized light chain framework region and a humanized heavy chain framework region.

9. The humanized antibody of any one of claims 1-8, wherein the antibody is selected from the group consisting of an Ig monomer, a Fab fragment, a F(ab')₂ fragment, a Fd fragment, a scFv, a scAb, a dAb, a Fv, a single domain heavy chain antibody, and a single domain light chain antibody.
10. The humanized antibody of any one of claims 1-9, wherein the antibody comprises a light chain region and a heavy chain region that are present in separate polypeptides.
11. The humanized antibody of any one of claims 1-9, wherein the antibody comprises a light chain region and a heavy chain region that are present in a single polypeptide.
12. A pharmaceutical composition comprising an antibody of any one of claims 1-11; and a pharmaceutically acceptable excipient.
13. A sterile container comprising the pharmaceutical composition of claim 12.
14. Use of the humanized antibody of any one of claims 1-11 or the pharmaceutical composition of claim 12 in the preparation of a medicament for inhibiting C3b/Bb-mediated cleavage of C3 in an individual in need thereof, wherein an effective amount of the medicament is to be administered to the individual.
15. The use of claim 14, wherein said medicament is to be administered intravenously.
16. The use of claim 14, wherein said medicament is to be administered subcutaneously.
17. The use of claim 14, wherein said medicament is to be administered intramuscularly.
18. Use of the humanized antibody of any one of claims 1-11 or the pharmaceutical composition of claim 12 in the preparation of a medicament for treating an individual having a complement-mediated disease or disorder, wherein an effective amount of the medicament is to be administered to the individual .
19. The use of claim 18, wherein said medicament is to be administered intravenously.

20. The use of claim 18, wherein said medicament is to be administered subcutaneously.
21. The use of claim 18, wherein said medicament is to be administered intramuscularly.
22. The use of any one of claims 14-21, wherein administration of the medicament results in an outcome selected from the group consisting of:
- a) inhibition of complement alternative pathway (AP) activity;
 - b) inhibition of formation of membrane attack complex;
 - c) inhibition of C3b/Bb-mediated cleavage of C3;
 - d) reduction of the level of C3a and/or C3b in a fluid or tissue;
 - e) inhibition of cleavage of factor B;
 - f) inhibition of AP-mediated cell lysis;
 - g) inhibition of AP-mediated hemolysis;
 - h) inhibition of AP-mediated deposition of C3b, C3d, or other C3 split product on a cell or tissue;
 - i) inhibition of AP-mediated deposition of C3b on red blood cells;
 - j) reduction of the amount of factor Bb in circulation in the individual;
 - k) reduction of the amount of factor Bb in plasma in the individual; and
 - l) inhibition of production of an anaphylatoxin.
23. Use of the humanized antibody of any one of claims 1-11 or the pharmaceutical composition of claim 12 in the preparation of a medicament for reducing the amount of complement C3 cleavage product in a body fluid or tissue in an individual in need thereof, wherein an effective amount of the medicament is to be administered to the individual.
24. The use of claim 23, wherein said medicament is to be administered intravenously.
25. The use of claim 23, wherein said medicament is to be administered subcutaneously.
26. The use of claim 23, wherein said medicament is to be administered intramuscularly.