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(54) Titre : ANTICORPS ANTI-FACTEUR B HUMANISE
(54) Title: HUMANEERED ANTI-FACTOR B ANTIBODY

(57) Abrégé/Abstract:
This invention relates to humaneered anti-factor B antibodies and antigen-binding fragments thereof with reduced immunogenicity. The humaneered anti-factor B antibodies and antigen- binding fragments thereof are derived from murine monoclonal antibody 1379, which binds factor B in the third short consensus repeat ("SCR") domain and selectively inhibits activation of the alternative complement pathway by preventing formation of the C3bBb complex. The invention also relates to methods of treating diseases or disorders in which activation of the alternative complement pathway plays a role, and methods of selectively inhibiting activation of the alternative complement pathway in an individual in need thereof.

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(54) Title: HUMANEERED ANTI-FACTOR B ANTIBODY

(57) Abstract: This invention relates to humaneered anti-factor B antibodies and antigen-binding fragments thereof with reduced immunogenicity. The humaneered anti-factor B antibodies and antigen- binding fragments thereof are derived from murine monoclonal antibody 1379, which binds factor B in the third short consensus repeat ("SCR") domain and selectively inhibits activation of the alternative complement pathway by preventing formation of the C3bBb complex. The invention also relates to methods of treating diseases or disorders in which activation of the alternative complement pathway plays a role, and methods of selectively inhibiting activation of the alternative complement pathway in an individual in need thereof.

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HUMANEERED ANTI-FACTOR B ANTIBODY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 60/906,816, filed on March 14, 2007, the disclosure of which is incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was supported in part by Grant Nos. AI47469, HL-36577, HL-61005, and AI-31105, each awarded by the National Institutes of Health; and by Grant No. R825702 awarded by the Environmental Protection Agency. Thus, the government has certain rights to this invention.

REFERENCE TO A COMPACT DISC APPENDIX

[0003] Not applicable.

FIELD OF THE INVENTION

[0004] The present invention relates to novel engineered forms of a monoclonal antibody and antigen-binding fragments thereof that bind complement protein factor B and selectively inhibit the alternative complement pathway. The invention also generally relates to the use of such antibodies and antigen-binding fragments thereof to treat diseases in which the alternative complement pathway plays a role. In particular, the invention relates to the use of such antibodies and antigen-binding fragments thereof to inhibit activation of the alternative complement pathway, and to treat diseases in which activation of the alternative complement pathway is implicated. Such disorders include, but are not limited to, airway hyperresponsiveness and airway inflammation, ischemia-reperfusion injury, and related disorders in animals, including humans.

BACKGROUND OF THE INVENTION

[0005] Certain cells of the immune system produce proteins called antibodies or immunoglobulins ("Ig") in response to the presence of foreign proteins in the body, such as bacterial or viral proteins. Antibodies bind and neutralize foreign proteins in the body.

[0006] Antibodies generally bind their target protein antigens tightly and specifically, making them potentially useful therapeutics for treating a wide range of diseases characterized by altered protein expression. Many protein targets suitable for antibody-mediated disease therapy have been identified using non-human antibody molecules. For many therapeutic applications, however, the efficacy and safety of non-human antibodies is compromised because non-human Ig molecules are themselves immunogenic (*i.e.*, capable of inducing an immune response). Thus, before antibodies can be approved for therapeutic use, they normally must be modified to reduce or eliminate their immunogenicity. Antibody Humaneering™ produces antibodies modified to reduce immunogenicity while retaining the ability to specifically bind their target antigen.

[0007] The present application describes the "humaneering" of a murine monoclonal antibody that binds factor B and selectively blocks the alternative complement pathway. The alternative complement pathway is usually activated by bacteria, parasites, viruses or fungi, although IgA antibodies and certain Ig light chains have also been reported to activate the pathway. Alternative pathway activation is initiated when circulating factor B binds to activated C3 (either C3b or $C3H_2O$). This complex is then cleaved by circulating factor D to yield an enzymatically active fragment, either C3bBb or C3(H_2O)Bb. These two enzymes can cleave circulating C3 generating C3b, which drives inflammation and also further amplifies the activation process, generating a positive feedback loop. Factor B is required to enable activation of the alternative pathway.

[0008] Recent studies have shown that the alternative pathway of complement plays an important role in the pathogenesis of several animal models of disease. Complement activation within the kidney after ischemia/reperfusion injury is mediated almost exclusively by the alternative pathway and the alternative pathway plays a critical role in the development of arthritis. Perhaps most surprisingly, mice deficient in the alternative pathway have been demonstrated to be protected from nephritis in the MRL/lpr model of lupus nephritis and from anti-phospholipid mediated fetal loss, disease models that would traditionally have been assumed to be mediated by the classical complement pathway.

[0009] The murine anti-factor B antibody from which the humaneered variants described herein were derived was produced by injecting factor B deficient mice ("$fB^{-/-}$") with a fusion protein comprising the second and third short consensus repeat ("SCR") domains of factor B fused to an immunoglobulin. The mice were then screened for antibodies to factor B. Spleen cells from an injected mouse producing anti-factor B antibodies were fused to myeloma cells according to standard procedures known in the art. One of the resulting hybridoma cells, number 1379, produced an IgG_1 antibody ("mAb 1379") that completely inhibits activation of the alternative complement pathway *in vitro* and *in vivo*. Antigen-binding Fab' fragments of mAb 1379 also completely inhibit activation of the alternative complement pathway. The hybridoma cell line that produces mAb 1379 has been deposited with the American Type Culture Collection ("ATCC") under Deposit No. PTA-6230.

[0010] Epitope mapping showed that mAb 1379 binds to factor B within the third SCR domain. Further experiments demonstrated that mAb 1379 inhibits alternative complement activation by preventing formation of the C3bBb complex. Finally, mAB 1379 binds an epitope conserved across multiple mammalian species, as shown by its ability to inhibit alternative complement activation in serum from a number of different species, including mice, rats, humans, baboons, rhesus monkeys, cynomolgous monkeys, pigs, rabbits, and horses. The production and characterization of anti-factor B antibody mAb 1379 is described in greater detail in U.S. Patent Publication No. US 2005/0260198 A1, which is incorporated herein by reference.

[0011] All references cited herein, including patent applications and publications, are hereby incorporated by reference in their entirety.

BRIEF SUMMARY OF THE INVENTION

[0012] In one aspect, the present invention provides a humaneered anti-factor B antibody or antigen-binding fragment thereof derived from murine monoclonal antibody 1379 ("mAb 1379") that selectively binds to factor B within the third short consensus repeat ("SCR") domain and prevents formation of the C3bBb complex, wherein the humaneered antibody or antigen-binding fragment thereof has an equilibrium dissociation constant ("K_D") between about 1.0×10^{-8} M and about 1.0×10^{-10} M. In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof has a K_D between about 1.0×10^{-9} M and 9.0×10^{-9} M, or between about 3.0×10^{-9} M and 7.0×10^{-9} M. In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof has a K_D of about 3.7×10^{-9} M or less, about 4.5×10^{-9} M or less, about 5.4×10^{-9} M or less, or about 6.5×10^{-9} M or less.

[0013] In a related aspect, the present invention provides a humaneered anti-factor B antibody or antigen-binding fragment thereof derived from murine monoclonal antibody 1379 ("mAb 1379") that selectively binds to factor B within the third short consensus repeat ("SCR") domain and prevents formation of the C3bBb complex, wherein the humaneered antibody or antigen-binding fragment thereof has a K_D between about 1.0×10^{-8} M and about 1.0×10^{-10} M. In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragments thereof comprises a V_κ-region polypeptide selected from the group consisting of SEQ ID NO: 14 (TA10 reference antibody), SEQ ID NO: 16 (TA101-1 Fab'), SEQ ID NO: 18 (TA102-4 Fab'), and SEQ ID NO: 20 (TA103-2 Fab'), and a V_H-region polypeptide selected from the group consisting of SEQ ID NO: 15 (TA10 reference antibody), SEQ ID NO: 17 (TA101-1 Fab'), SEQ ID NO: 19 (TA102-4 Fab'), and SEQ ID NO: 21 (TA103-2 Fab'). In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region polypeptide comprising SEQ ID NO: 14 (TA10 reference antibody) and a V_H-region polypeptide comprising SEQ ID NO: 15 (TA10 reference antibody), and has a K_D of 6.55×10^{-9} M or less. In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region polypeptide comprising SEQ ID NO: 16 (TA101-1 Fab') and a V_H-region polypeptide comprising SEQ ID NO: 17 (TA101-1 Fab'), and has a K_D of 4.53×10^{-9} M or less. In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region polypeptide comprising SEQ ID NO: 18 (TA102-4 Fab') and a V_H-region polypeptide comprising SEQ ID NO: 19 (TA102-4 Fab'), and has a K_D of

5.40×10^{-9} M or less. In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region polypeptide comprising SEQ ID NO: 20 (TA103-2 Fab') and a V_H-region polypeptide comprising SEQ ID NO: 21 (TA103-2 Fab'), and has a K_D of 3.73×10^{-9} M or less. In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises an antigen-binding fragment selected from the group consisting of Fab', $(Fab')_2$, Fv, scFv, and diabodies. In certain embodiments, the antigen-binding fragment of a humaneered anti-factor B antibody is a Fab'. In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof has a K_D of about 3.7×10^{-9} M or less, about 4.5×10^{-9} M or less, about 5.4×10^{-9} M or less, or about 6.5×10^{-9} M or less.

[0014] In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragments thereof comprises a V_κ-region polypeptide selected from the group consisting of SEQ ID NO: 16 (TA101-1 Fab'), SEQ ID NO: 18 (TA102-4 Fab'), and SEQ ID NO: 20 (TA103-2 Fab'), and a V_H-region polypeptide selected from the group consisting of SEQ ID NO: 35 (TA101-1 Fab'), SEQ ID NO: 36 (TA102-4 Fab'), and SEQ ID NO: 37 (TA103-2 Fab'). In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region polypeptide comprising SEQ ID NO: 16 (TA101-1 Fab') and a V_H-region polypeptide comprising SEQ ID NO: 35 (TA101-1 Fab'). In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region polypeptide comprising SEQ ID NO: 18 (TA102-4 Fab') and a V_H-region polypeptide comprising SEQ ID NO: 36 (TA102-4 Fab'). In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region polypeptide comprising SEQ ID NO: 20 (TA103-2 Fab') and a V_H-region polypeptide comprising SEQ ID NO: 37 (TA103-2 Fab').

[0015] In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region polypeptide selected from the group consisting of SEQ ID NO: 14 (TA10 reference antibody), SEQ ID NO: 16 (TA101-1 Fab'), SEQ ID NO: 18 (TA102-4 Fab'), and SEQ ID NO: 20 (TA103-2 Fab'), wherein the amino acid sequence of the V_κ-region polypeptide is about 80% identical to the closest human germline V_κ-region polypeptide, about 85% identical to the closest human germline V_κ-region polypeptide, about 90% identical to the closest human germline V_κ-region polypeptide, or about 95% identical to the closest human germline V_κ-region polypeptide. In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_H-region polypeptide selected from the group consisting of SEQ ID NO: 15 (TA10 reference antibody), SEQ ID NO: 17 (TA101-1 Fab'), SEQ ID NO: 19 (TA102-4 Fab'), and SEQ ID NO: 21 (TA103-2 Fab'), wherein the amino acid sequence of the V_H-region polypeptide is about 80% identical to the closest human germline V_H-region polypeptide, about 85% identical to the closest human germline V_H-region polypeptide, about 90% identical to the closest human germline V_H-region polypeptide, or about 95% identical to the closest human germline V_H-region polypeptide. In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragments thereof comprises a V_κ-region polypeptide selected from the group consisting of SEQ ID NO: 14 (TA10 reference antibody), SEQ ID NO: 16 (TA101-1 Fab'), SEQ ID NO: 18 (TA102-4 Fab'), and SEQ ID NO: 20 (TA103-2 Fab'), and a V_H-region polypeptide selected from the group consisting of SEQ ID NO: 15 (TA10 reference antibody), SEQ ID NO: 17 (TA101-1 Fab'), SEQ

ID NO: 19 (TA102-4 Fab'), and SEQ ID NO: 21 (TA103-2 Fab'), wherein the amino acid sequence of the V_κ-region polypeptide and the amino acid sequence of the V_H-region polypeptide are about 80% identical to the closest human germline V_κ-region polypeptide and the closest human germline V_H-region polypeptide, about 85% identical to the closest human germline V_κ-region polypeptide and the closest human germline V_H-region polypeptide, about 90% identical to the closest human germline V_κ-region polypeptide and the closest human germline V_H-region polypeptide, or about 95% identical to the closest human germline V_κ-region polypeptide and the closest human germline V_H-region polypeptide.

[0016] In a related aspect, the present invention provides a humaneered anti-factor B antibody or antigen-binding fragment thereof derived from murine monoclonal antibody 1379 ("mAb 1379") that selectively binds to factor B within the third short consensus repeat ("SCR") domain and prevents formation of the C3bBb complex, wherein the humaneered antibody or antigen-binding fragment thereof has a K_D between about 1.0×10^{-8} M and about 1.0×10^{-10} M. In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragments thereof comprises a Vκ-region comprising a binding specificity determinant ("BSD") derived from the third complementarity determining region ("CDR3") and the fourth framework region ("FR4") selected from the group consisting of SEQ ID NO: 22 (TA10 reference antibody), SEQ ID NO: 24 (TA101-1 Fab'), SEQ ID NO: 26 (TA102-4 Fab'), and SEQ ID NO: 28 (TA103-2 Fab'), and the V_H-region of the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a BSD derived from the CDR3-FR4 region selected from the group consisting of SEQ ID NO: 23 (TA10 reference antibody), SEQ ID NO: 25 (TA101-1 Fab'), SEQ ID NO: 27 (TA102-4 Fab'), and SEQ ID NO: 29 (TA103-2 Fab'). In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region BSD polypeptide comprising SEQ ID NO: 22 (TA10 reference antibody) and a V_H-region BSD polypeptide comprising SEQ ID NO: 23 (TA10 reference antibody), and has a K_D of 6.55×10^{-9} M. In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region BSD polypeptide comprising SEQ ID NO: 24 (TA101-1 Fab') and a V_H-region BSD polypeptide comprising SEQ ID NO: 25 (TA101-1 Fab'), and has a K_D of 4.53×10^{-9} M. In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region BSD polypeptide comprising SEQ ID NO: 26 (TA102-4 Fab') and a V_H-region BSD polypeptide comprising SEQ ID NO: 27 (TA102-4 Fab'), and has a K_D of 5.40×10^{-9} M. In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region BSD polypeptide comprising SEQ ID NO: 28 (TA103-2 Fab') and a V_H-region BSD polypeptide comprising SEQ ID NO: 29 (TA103-2 Fab'), and has a K_D of 3.73×10^{-9} M. In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises an antigen-binding fragment selected from the group consisting of Fab', $(Fab')_2$, Fv, scFv, and diabodies. In certain embodiments, the antigen-binding fragment of a humaneered anti-factor B antibody is a Fab'.

[0017] In another aspect, the present invention provides a humaneered anti-factor B antibody or antigen-binding fragment thereof derived from murine monoclonal antibody 1379 ("mAb 1379") that selectively binds to factor B within the third short consensus repeat ("SCR") domain and prevents formation of the C3bBb complex comprising a V_κ-region polypeptide selected from the group consisting of SEQ ID NO: 14 (TA10 reference antibody), SEQ ID NO: 16 (TA101-1 Fab'), SEQ ID NO: 18 (TA102-4 Fab'), and SEQ ID NO: 20 (TA103-2 Fab'), and a V_H-region polypeptide selected from the group consisting of SEQ ID NO: 15 (TA10 reference antibody), SEQ ID NO: 17 (TA101-1 Fab'), SEQ ID NO: 19 (TA102-4 Fab'), and SEQ ID NO: 21 (TA103-2 Fab'). In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region polypeptide comprising SEQ ID NO: 14 (TA10 reference antibody) and a V_H-region polypeptide comprising SEQ ID NO: 15 (TA10 reference antibody). In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region polypeptide comprising SEQ ID NO: 16 (TA101-1 Fab') and a V_H-region polypeptide comprising SEQ ID NO: 17 (TA101-1 Fab'). In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region polypeptide comprising SEQ ID NO: 18 (TA102-4 Fab') and a V_H-region polypeptide comprising SEQ ID NO: 19 (TA102-4 Fab'). In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region polypeptide comprising SEQ ID NO: 20 (TA103-2 Fab') and a V_H-region polypeptide comprising SEQ ID NO: 21 (TA103-2 Fab'). In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region polypeptide selected from the group consisting of SEQ ID NO: 14 (TA10 reference antibody), SEQ ID NO: 16 (TA101-1 Fab'), SEQ ID NO: 18 (TA102-4 Fab'), and SEQ ID NO: 20 (TA103-2 Fab'). In certain embodiments, the humaneered anti-factor B antibody or antigen binding fragment thereof comprises a V_H-region polypeptide selected from the group consisting of SEQ ID NO: 15 (TA10 reference antibody), SEQ ID NO: 17 (TA101-1 Fab'), SEQ ID NO: 19 (TA102-4 Fab'), and SEQ ID NO: 21 (TA103-2 Fab'). In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises an antigen-binding fragment selected from the group consisting of Fab', $(Fab')_2$, Fv, scFv, and diabodies. In certain embodiments, the antigen-binding fragment of a humaneered anti-factor B antibody is a Fab'.

[0018] In another aspect, the present invention provides a humaneered anti-factor B antibody or antigen-binding fragment thereof derived from murine monoclonal antibody 1379 ("mAb 1379") that selectively binds to factor B within the third short consensus repeat ("SCR") domain and prevents formation of the C3bBb complex, wherein the V_κ-region of the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a binding specificity determinant ("BSD") derived from the third complementarity determining region ("CDR3") and the fourth framework region ("FR4") selected from the group consisting of SEQ ID NO: 22 (TA10 reference antibody), SEQ ID NO: 24 (TA101-1 Fab'), SEQ ID NO: 26 (TA102-4 Fab'), and SEQ ID NO: 28 (TA103-2 Fab'), and the V_H-region of the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a BSD derived from the CDR3-FR4 region selected from the group consisting of SEQ ID NO: 23 (TA10 reference antibody), SEQ ID NO: 25 (TA101-1 Fab'), SEQ ID NO: 27 (TA102-4 Fab'), and SEQ ID NO: 29 (TA103-2 Fab'). In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region BSD polypeptide comprising SEQ ID NO: 22 (TA10 reference antibody) and a V_H-region BSD polypeptide comprising SEQ ID NO: 23 (TA10 reference antibody). In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region BSD polypeptide comprising SEQ ID NO: 24 (TA101-1 Fab') and a V_H-region BSD polypeptide comprising SEQ ID NO: 25 (TA101-1 Fab'). In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_κ-region BSD polypeptide comprising SEQ ID NO: 26 (TA102-4 Fab') and a V_H-

region BSD polypeptide comprising SEQ ID NO: 27 (TA102-4 Fab'). In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a V_{κ}-region BSD polypeptide comprising SEQ ID NO: 28 (TA103-2 Fab') and a V_H-region BSD polypeptide comprising SEQ ID NO: 29 (TA103-2 Fab'). In certain embodiments, the V_{κ}-region of the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a binding specificity determinant ("BSD") derived from the third complementarity determining region ("CDR3") and the fourth framework region ("FR4") selected from the group consisting of SEQ ID NO: 22 (TA10 reference antibody), SEQ ID NO: 24 (TA101-1 Fab'), SEQ ID NO: 26 (TA102-4 Fab'), and SEQ ID NO: 28 (TA103-2 Fab'). In certain embodiments, the V_H-region of the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a BSD derived from the CDR3-FR4 region selected from the group consisting of SEQ ID NO: 23 (TA10 reference antibody), SEQ ID NO: 25 (TA101-1 Fab'), SEQ ID NO: 27 (TA102-4 Fab'), and SEQ ID NO: 29 (TA103-2 Fab'). In certain embodiments, the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises an antigen-binding fragment selected from the group consisting of Fab', $(Fab')_2$, Fv, scFv, and diabodies. In certain embodiments, the antigen-binding fragment of a humaneered anti-factor B antibody is a Fab'.

[0019] In another aspect, the present invention provides methods of treating a disease or disorder in which activation of the alternative complement pathway plays a role, comprising administering a humaneered anti-factor B antibody or antigen-binding fragment thereof derived from murine monoclonal antibody 1379 ("mAb 1379") that selectively binds to factor B within the third short consensus repeat ("SCR") domain and prevents formation of the C3bBb complex, wherein the humaneered antibody or antigen-binding fragment thereof has an equilibrium dissociation constant ("K_D") between about 1.0×10^{-8} M and about 1.0×10^{-10} M, to an individual that has, or is at risk of developing such a disease or disorder. In certain embodiments, the disease or disorder is airway hyperresponsiveness ("AHR") or airway inflammation. In certain embodiments, any of the humaneered anti-factor B antibodies or antigen-binding fragments thereof are administered to the individual in an amount effective to measurably reduce AHR in the animal as compared to before administration of the antibody or antigen-binding fragment thereof. In certain embodiments, AHR or airway inflammation is associated with a disease selected from the group consisting of asthma, chronic obstructive pulmonary disease (COPD), allergic bronchopulmonary aspergillosis, hypersensitivity pneumonia, eosinophilic pneumonia, emphysema, bronchitis, allergic bronchitis bronchiecstasis, cyctic fibrosis, tuberculosis, hypersensitivity pneumonitis, occupational asthma, sarcoid, reactive airway disease syndrome, interstitial lung disease, hyper-eosinophilic syndrome, rhinitis, sinusitis, exercise-induced asthma, pollution-induced asthma, cough variant asthma, parasitic lung disease, respiratory syncytial virus ("RSV") infection, parainfluenza virus ("PIV") infection, rhinovirus ("RV") infection, and adenovirus infection. In certain embodiments, the AHR or airway inflammation is associated with allergic inflammation, asthma, or COPD.

[0020] In another aspect, the present invention provides methods of inhibiting activation of the alternative complement pathway in an individual that has, or is at risk of developing a condition or disease in which activation of the alternative complement pathway contributes to the condition or disease, exacerbates at least one symptom of the condition or disease, or causes the condition or disease, comprising administering any of the humaneered anti-factor B antibodies or antigen-binding fragments thereof disclosed herein to an individual in need thereof.

[0021] In another aspect, the present invention provides a composition comprising an effective amount of the humaneered anti-factor B antibody or antigen-binding fragments thereof disclosed herein and a pharmaceutically acceptable carrier. In certain embodiments, the pharmaceutically acceptable carrier is selected from the group consisting of: a dry, dispersible powder; anhydrous ethanol; small capsules; liposomes; a nebulized spray; and an injectable excipient.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] FIG. 1 is an agarose gel showing double-stranded cDNA products generated with degenerate V-region-specific primer sets using a template of first strand cDNA prepared from mRNA isolated from the hybridoma producing mAb 1379.

[0023] FIG. 2 is a comparison of amino acid sequences derived from V_H and V_κ cDNA sequences cloned from the hybridoma cell line producing mAb 1379.

[0024] FIG. 3 is a comparison of amino-terminal amino acid sequences derived from the cloned V_H and V_κ cDNA sequences to amino-terminal amino acid sequences determined from mAb 1379.

[0025] FIG. 4 is a comparison of factor B binding between the cloned Fab' TA003 and a Fab' derived from mAb 1379 by papain digestion.

[0026] FIG. 5 shows the kinetics of Fab' fragment binding to recombinant human factor B analyzed with the FortéBio Octet system by bio-layer interferometry.

[0027] FIG. 6 is a comparison of amino acid sequences derived from the sequence of humaneered antibody isolates TA101-1, TA102-4, and TA103-2 to the corresponding sequences from the reference antibody TA10 and from the closest human germline light and heavy chain variable domain genes ("V_L-" and "V_H-gene") and joining segments ("J-segment") (human V_H1-02/J_H4 and V_κIV-B3/J_κ2).

DETAILED DESCRIPTION OF THE INVENTION

[0028] Humaneered anti-factor B antibodies or antigen-binding fragments thereof that selectively bind to complement factor B and selectively inhibit activation of the alternative complement pathway may be used to treat any disease or disorder involving the alternative complement pathway in animals, including humans. In particular, such antibodies or antigen-binding fragments thereof may be used to treat any disease or disorder in animals, including humans, in which activation of the alternative complement pathway plays a role. Such diseases or disorders include, for example, allergic asthma and the accompanying airway inflammation and airway hyperresponsiveness ("AHR"), chronic obstructive pulmonary disease ("COPD"), allergic bronchopulmonary aspergillosis, hypersensitivity pneumonia, eosinophilic pneumonia, emphysema, bronchitis, allergic bronchitis, bronchiectasis, cystic fibrosis, tuberculosis, hypersensitivity pneumonitis, occupational asthma, sarcoid, reactive airway disease syndrome, interstitial lung disease, hyper-eosinophilic syndrome, rhinitis, sinusitis, exercise-induced asthma, pollution-induced asthma, cough variant asthma, parasitic lung disease, respiratory syncytial virus ("RSV") infection, parainfluenza virus ("PIV") infection, rhinovirus ("RV") infection and adenovirus infection, and ischemia-reperfusion injury. *See, e.g.*, U.S. Patent Publication No. US 2005/0260198 A1, which is incorporated herein by reference.

[0029] Allergic asthma is a common syndrome associated with airway inflammation and AHR. In patients with allergic asthma, exposure to inhaled allergen leads to an increase in AHR and airway inflammation. Studies have shown increased levels of biologically active fragments derived from the complement C3, C4 and C5 family of proteins, especially C3a and C5a in bronchoalveolar lavage ("BAL") fluid. This suggests that in these patients, activation of the complement pathway through an allergen-induced mechanism occurs in the lung after allergen exposure. Animal models have provided further insight in the role of complement for the development of allergic airway disease. Animals deficient in C3 or C3a receptor appear protected from the development of allergen induced airway disease. *See, e.g.*, U.S. Patent Publication No. US 2005/0260198 A1, which is incorporated herein by reference.

Definitions

[0030] As used herein, the term "antibody" or "immunoglobulin" refers to glycoproteins of the immunoglobulin ("Ig") superfamily of proteins. An antibody or immunoglobulin ("Ig") molecule is tetrameric, comprising two identical light chain polypeptides and two identical heavy chain polypeptides (the terms "light chain polypeptide" and "light chain" or "heavy chain polypeptide" and "heavy chain" are used interchangeably herein to describe the polypeptides of an Ig molecule). The two heavy chains are linked together by disulfide bonds, and each heavy chain is linked to a light chain by a disulfide bond. Each full-length Ig molecule contains at least two binding sites for a specific target or antigen.

[0031] The immune system produces several different classes of Ig molecules ("isotypes"), including IgA, IgD, IgE, IgG, and IgM, each distinguished by the particular class of heavy chain polypeptide present: alpha ("α") found in IgA, delta ("δ") found in IgD, epsilon ("ε") found in IgE, gamma ("γ") found in IgG, and mu ("μ") found in IgM. There are at least five different γ heavy chain polypeptides ("isotypes") found in IgG. In contrast, there are only light chain polypeptide isotypes, referred to as kappa ("κ") and lambda ("λ") chains. The distinctive characteristics of antibody isotypes are defined by sequences of the constant domains of the heavy chain.

[0032] An IgG molecule comprises two light chains (either κ or λ form) and two heavy chains (γ form) bound together by disulfide bonds. The κ and λ forms of IgG light chain both contain a domain of relatively variable amino acid sequences, called the variable region (variously referred to as a "V_L-," "V_κ-," or "V_λ-region") and a domain of relatively conserved amino acid sequences, called the constant region ("C_L-region"). Similarly, each IgG heavy chain contains a variable region ("V_H-region") and one or more conserved regions: a complete IgG heavy chain contains three constant domains ("C_H1-," "C_H2-," and "C_H3-regions") and a hinge region. Within each V_L- or V_H-region, hypervariable regions, also known as complementarity-determining regions ("CDR"), are interspersed between relatively conserved framework regions ("FR"). Generally, the variable region of a light or heavy chain polypeptide contains four FR and three CDR arranged in the following order along the polypeptide: NH_2-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-COOH. Together the CDR and FR determine the three-dimensional structure of the IgG binding site and thus, the specific target protein or antigen to which that IgG molecule binds. Each IgG molecule is dimeric, able to bind two antigen molecules. Cleavage of a dimeric IgG with the protease papain produces two identical antigen-binding fragments ("Fab'") and an "Fc" fragment, so named because is readily crystallized.

[0033] As used herein, the term "antigen-binding fragment" refers to a fragment of an antibody or immunoglobulin molecule that retains the ability to specifically bind its cognate antigen. Antigen-binding fragments generally lack part or all of one or more functional domains present in full-length antibody or Ig molecules, such as those that confer the ability to fix complement and stimulate antibody-dependent cell-mediated cytoxicity ("ADCC"). Antigen-binding fragments can be prepared from full-length antibody isolates, for example, by digestion with proteases such as papain (which produces two identical monovalent antigen-binding fragments ("Fab'") comprising the variable and constant regions of an antibody light chain and the variable and first constant region of an antibody heavy chain) or pepsin (which produces a single bivalent antigen-binding fragment ("Fab')$_2$" comprising a pair of Fab' fragments covalently linked near their carboxyl termini).

[0034] Other antigen-binding fragments may be produced using standard recombinant DNA methodology, such as "Fv"fragments, single chain Fv antibodies ("scFv"), bi-specific antibodies, diabodies, humanized or humaneered antibodies, and the like. An "Fv" fragment is an antibody fragment that contains a complete antigen recognition and binding site, comprising a dimer of one V_H-region and one V_L-region. An "scFv" antibody fragment comprises the V_H-region and one V_L-region of an antibody in a single polypeptide chain. A "diabody" is a small antibody fragment with two antigen-binding sites, comprising a heavy chain variable domain connected to a light chain variable domain in the same polypeptide. By using a linker too short to allow the V_H- and V_L-regions of the same polypeptide to pair, the domains are forced to pair with complementary domains of a second polypeptide, creating two antigen-binding sites.

[0035] As used herein, the term "binding specificity determinant" or "BSD" refers to all or a portion of the amino acid sequence of the third complementarity determining region ("CDR3") and the fourth framework region ("FR4") of an IgG V_L or V_H polypeptide that mediates antigen-binding specificity of a particular Ig molecule. BSDs function in heavy chain and light chain pairs, such that a particular BSD comprises the amino acid sequence of CDR3-

FR4 from a V_L-region paired with the amino acid sequence of CDR3-FR4 from a cognate V_H-region.

[0036] As used herein, the term "epitope" refers to a site on a larger molecule, such as a given protein, polypeptide, or antigen (*i.e.*, factor B), to which an antibody, immunoglobulin, or antigen-binding fragment thereof will bind, and against which an antibody will be produced. The term "epitope" can be used interchangeably with the terms "antigenic determininant," "antibody binding site," or "conserved binding surface" of a given protein, polypeptide, or antigen. More specifically, an epitope can be defined by both the amino acid residues involved in antibody binding and also by their conformation in three dimensional space (*e.g.*, a conformational epitope or the conserved binding surface). An epitope can be included in peptides as small as about 4-6 amino acid residues, or can be included in larger segments of a protein, and need not be comprised of contiguous amino acid residues when referring to a three dimensional structure of an epitope, particularly with regard to an antibody-binding epitope. Antibody-binding epitopes are frequently conformational epitopes rather than a sequential or linear epitope, or, in other words, an epitope defined by amino acid residues arrayed in three dimensions on the surface of a protein or polypeptide to which an antibody binds. As mentioned above, the conformational epitope is not comprised of a contiguous sequence of amino acid residues, but instead, the residues are perhaps widely separated in the primary protein sequence, and are brought together to form a binding surface by the way the protein folds in its native conformation in three dimensions.

[0037] The epitope recognized by the mAb 1379, and shared by the humaneered variants described herein, is a conformational epitope that is not a linear epitope located within the three-dimensional structure of a portion of the third SCR domain of factor B. *See, e.g.*, US

2005/0260198 A1, which is incorporated herein by reference in its entirety. Human factor B is expressed as a 764 amino acid preproprotein containing a twenty-five (25) amino acid signal peptide spanning amino acids 1-25 of its amino terminus. The amino acid sequence for human factor B preproprotein is found in NCBI Database Accession No. P00751. Mature human factor B comprises the amino acid sequence of Accession No. P00751 lacking the twenty-five (25) amino acid signal peptide (*i.e.*, SEQ ID NO: 30). The third SCR domain of mature human factor B extends from about position 137 to about position 195 of SEQ ID NO: 30. The portion that contains the epitope is the three-dimensional structure of factor B that is defined by substantially all of (*e.g.*, at least about 90% of) amino acid positions Ala137-Ser192 of SEQ ID NO: 30, or equivalent positions in a non-human factor B sequence, when such sequence is conformationally arranged as it occurs in the natural full-length factor B sequence.

[0038] The murine mAb 1379 and the humaneered variants described herein bind to an epitope or conserved binding surface within or containing a part of the third SCR domain comprising an epitope of human factor B that includes at least a portion of the sequence comprising from about position Tyr139 to about position Ser185 of the mature human factor B protein (SEQ ID NO: 30), to an epitope of human factor B that includes at least a portion of the sequence comprising from about position Tyr139 to about position Ser141 of the mature human factor B protein (SEQ ID NO: 30), to an epitope of human factor B that includes at least a portion of the sequence comprising from about position Glu182 to about position Ser185 with respect to the mature human factor B protein (SEQ ID NO: 30), to an epitope of factor B that includes at least a portion of human factor B (SEQ ID NO: 30) comprising any one or more of the following positions or their equivalent positions in a non-human factor B sequence: Ala137, Tyr139, Cys 140, Ser141, Glu182, Gly184, or Ser185, or to an epitope of factor B that includes at least a portion of the equivalent positions with respect to non-human animal species. In another aspect, the epitope is within or containing a part of portion of the third SCR domain of factor B that includes all or substantially all of (at least five, six, or seven of) the following amino acid positions of SEQ ID NO: 30, or their equivalent positions in a non-human factor B sequence: Ala137, Tyr139, Ser141, Glu182, Ser185, Thr189, Glu190, and Ser192.

[0039] One of skill in the art can readily align the sequence of human factor B with the sequence of factor B from another animal species and determine the positions of the SCR regions and the specific portions of the third SCR regions corresponding to the amino acid positions above. For example, two specific sequences can be aligned to one another using BLAST 2 sequence as described in Tatusova and Madden, (1999), "Blast 2 sequences--a new tool for comparing protein and nucleotide sequences", *FEMS Microbiol. Lett.* 174:247-250, which is incorporated herein by reference in its entirety.

[0040] As used herein, the term "selectively binds to" refers to the specific binding of one protein to another (*e.g.*, an antibody, antigen-binding fragment thereof, or binding partner to an antigen), wherein the level of binding, as measured by any standard assay (*e.g.*, an immunoassay), is statistically significantly higher than the background control for the assay. For example, when performing an immunoassay, controls typically include a reaction well or tube that contains antibody or antigen binding fragment alone (*i.e.*, in the absence of antigen), wherein an amount of reactivity (*e.g.*, non-specific binding to the well or tube) by the antibody or antigen-binding fragment thereof in the absence of the antigen is considered to be background signal. Binding can be measured using a variety of methods standard in the art, including, but not limited to, Western blot, immunoblot, enzyme-linked immunosorbent assay ("ELISA"), radioimmunoassay ("RIA"), immunoprecipitation, surface plasmon resonance, chemiluminescence, fluorescent polarization, phosphorescence, immunohistochemical analysis, matrix-assisted laser desorption/ionization time-of-flight ("MALDI-TOF") mass spectrometry, microcytometry, microarray, microscopy, fluorescence activated cell sorting ("FACS"), and flow cytometry.

[0041] As used herein, "treating" or "to treat" a disease is defined as administering a humaneered variant of mAb 1379 as described above, such as TA101-1, TA102-4, and TA103-2, or antigen-binding fragments thereof, with or without other therapeutic agents, in order to palliate, ameliorate, stabilize, reverse, slow, delay, prevent, reduce, or eliminate either the disease or a symptom of a disease, or to retard or stop the progression of a disease or a symptom of a disease. An "effective amount" of a composition is an amount sufficient to treat a disease.

[0042] As used herein, "to inhibit" the alternative complement pathway in an individual refers to inhibiting the expression and/or the biological activity of at least one protein that is part of the alternative complement pathway. Such proteins include, but are not limited to, factor B, factor D or properdin. To "selectively" inhibit the alternative complement pathway means that the method of the present invention preferentially or exclusively inhibits the alternative complement pathway, but does not inhibit or at least does not substantially inhibit other pathways for complement activation, including the classical complement pathway or the lectin pathway. For example, the humaneered factor B antibodies and antigen-binding fragments thereof of the present invention are one example of a reagent that selectively inhibits the alternative complement pathway. This definition applies to other methods described herein wherein the alternative complement pathway is selectively inhibited.

[0043] An "individual" is a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, farm animals, sport animals, pets, primates, mice and rats. In some embodiments, the individual is human. In some embodiments, the individual is an individual other than a human. In some embodiments, the individual is an animal model for the study of a disease in which the alternative complement pathway is implicated. Individuals amenable to treatment include those who are presently asymptomatic but who are at risk of developing a symptomatic disorder in which the alternative complement pathway plays a role, or in which activation of the alternative complement pathway plays a role.

[0044] General reference to "the composition" or "compositions" includes and is applicable to compositions of the invention.

[0045] As used herein, the singular forms "a," "an," and "the" include the plural references unless clearly indicated otherwise. For example, the term "a V_H-region" includes one or more V_H-regions.

[0046] Reference to "about" a value or parameter herein includes and describes embodiments that are directed to that value or parameter *per se*. For example, description referring to "about X" includes description of "X."

[0047] It is understood that aspects and embodiments of the invention described herein include "consisting" and/or "consisting essentially of" aspects and embodiments.

1. Introduction

[0048] Antibody Humaneering™ generates engineered human antibodies with variable region ("V-region") sequences close to the human germ-line sequences while retaining the binding specificity and affinity of a reference antibody. *See, e.g.*, U.S. Patent Publication No. US 2005/0255552 A1; and U.S. Patent Publication No. US 2006/0134098 A1. The process identifies the minimal sequence information required to determine antigen-binding specificity from the V-region of a reference antibody and transfers that information to a library of partial human V-region gene sequences to generate an epitope-focused library of human antibody V-regions. Members of the library are expressed as antibody Fab' fragments using a microbial-based secretion system. The library is then screened for antigen-binding Fab' fragments using a colony lift binding assay. Positive clones are further characterized to identify those with the highest binding affinity for the target antigen. The resulting engineered human Fab' fragments retain the binding specificity of the parent murine antibody, and preferably have equivalent or higher binding affinity for antigen than the parent antibody. Preferably, the engineered Fab' fragments also have heavy and light chain V-regions with a high degree of amino acid sequence identity compared to the closest human germline antibody genes.

[0049] The minimum binding specificity determinant ("BSD") required to generate the epitope-focused library is typically represented by a sequence within CDR3 of the antibody heavy chain ("CDR_H3") and a sequence within CDR3 of the antibody light chain ("CDR_L3"). In some cases, the epitope-focused library is constructed from human V-segment sequences (the "V-segment" contains FR1-CDR1-FR2-CDR2-FR3) linked to the unique region at the junction of CDR3 and FR4 containing the BSD and human germ-line joining segment ("J-segment") sequences. *See* U.S. Patent Publication No. US 2005/0255552 A1. Alternatively, the human V-segment libraries can be generated by sequential cassette replacement in which only part of the murine V-segment is initially replaced by a library of human sequences. The identified human "cassettes" supporting antigen binding in the context of residual murine sequences are then recombined in a second library screen to generate completely human V-segments. *See* U.S. Patent Publication No. US 2006/0134098 A1. In each case, paired heavy and light chain CDR3-FR4 segments containing specificity determinants from the reference antibody are used to constrain the binding specificity so that antigen-binding Fab' fragments obtained from the library retain the epitope specificity of the starting antibody (*i.e.*, mAb 1379).

[0050] Additional maturational changes may be introduced in the CDR3 regions of each chain during library construction in order to identify antibodies with optimal binding kinetics.

[0051] The resulting humaneered antibodies have V-segment sequences derived from the human sequence libraries, retain the short BSD sequence from within the V_L and V_H chain CDR3 regions, and have human germline FR4 regions.

[0052] Cassette replacement was successfully used for the humaneering of mAb 1379. A number of Fab' fragments with high affinity for factor B were identified by this approach. Three humaneered Fab' fragments with higher affinity for factor B than the reference murine antibody (*i.e.*, mAb 1379) were identified.

2. Methods

2.1 Cloning of murine V-regions from the hybridoma producing mAb 1379

[0053] The murine V-regions were cloned from the hybridoma producing mAb 1379 as follows. First, hybridoma cells were cultured according to established procedures. The cells were then collected and messenger RNA ("mRNA") was extracted from the cell pellet by standard procedures known to one skilled in the art. First strand complementary DNA ("cDNA") was generated from the purified mRNA by primer extension with poly-deoxythymidine ("poly-dT") primer extension using reverse transcriptase, according to standard methods known to one skilled in the art. The first strand cDNA was then used as template for amplification of the antibody V-region sequences using degenerate primers according to standard procedures described in detail by Chardès, T., et al., "Efficient amplification and direct sequencing of mouse variable regions from any immunoglobulin gene family," *FEBS Lett.* 452(3):386-394 (1999), which is incorporated herein by reference. cDNA from the heavy chain variable region ("V_H") and the light chain variable region V-kappa ("V_κ") region was sequenced and checked for identity to amino-terminal peptide sequence data generated by Taligen. V-regions were cloned as Fab' fragments and expressed in *Escherichia coli* ("*E. coli*") from proprietary KaloBios expression vectors. The purified Fab' protein was shown to bind purified factor B protein in an enzyme-linked immunosorbent assay ("ELISA") performed according to standard methods.

2.2 Fab' purification

[0054] Fab' fragments were expressed in *E. coli* using proprietary KaloBios protein expression vectors. Bacteria were cultured at 37°C in 2X YT medium (16 g Bacto-tryptone, 10 g Bacto-yeast extract, and 5 g NaCl per liter of distilled, deionized water ("ddH_2O")) to an optical density of 0.6 absorbance units measured at a wavelength of 600 nm. Protein expression was induced using isopropyl-β-thiogalactopyranoside ("IPTG") for 3 hours at 33°C. The appropriate IPTG concentration to obtain optimal expression of the desired protein is determined empirically using methods known to one skilled in the art, and typically varies between 0.01 mM to 5.0 mM. Assembled Fab' fragments were obtained from periplasmic fractions and purified by affinity chromatography over Streptococcal Protein G columns (HiTrap™ Protein G HP columns; purchased from GE Healthcare, Piscataway, NJ) according to standard methods known to one skilled in the art. Fab' fragments were bound to the column in 20 mM sodium phosphate, pH=7.0, eluted in 0.1 M glycine at ~pH=2.0, and immediately adjusted to neutral pH (~7.0) with an appropriate volume of 1 M Tris-HCl at pH=9.0, all according to the manufacturer's instructions. The purified Fab' fragments were then dialyzed against phosphate-buffered saline ("PBS") at pH=7.4 (1X PBS = 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4; note that PBS lacks Ca^{2+} and Mg^{2+}).

2.3 Enzyme-linked immunosorbent assay ("ELISA")

[0055] Taligen provided 3 mg purified recombinant human factor B. Typically, 50 ng of purified recombinant factor B was adsorbed to the wells of a 96-well microtiter plate overnight at 4°C. The plate was blocked with a solution of 5% (w/v) powdered non-fat milk in PBST (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, and 0.1% (v/v) Tween-20™). Purified humaneered Fab' fragments or the reference Fab' ("TA10") were diluted in 1X PBS. Fifty microliters of antibody fragment were added to each well of the microtiter plate. After one hour at 33°C, the wells of the microtiter plate were rinsed three times with PBST. Next, fifty microliters of anti-human κ chain antibody conjugated to horseradish peroxidase ("HRP")(Sigma-Aldrich, St. Louis, MO) diluted to 0.1 ng/ml in PBST was added to each well, and the plate was incubated forty minutes at 33°C. The wells of the microtiter plate were then washed three times with PBST, once with 1X PBS. Then 100 μl TMB (3,3',5,5'-tetramethylbenzidene) substrate (Sigma) was added to each well, and the plate was incubated for approximately 5 minutes at room temperature (~25°C). Finally, the reactions were stopped by addition of 100 μl 0.2 N sulfuric acid (H_2SO_4) to each well. The plate was read in a spectrophotometer at a wavelength of 450 nm.

2.4 Colony lift binding assay

[0056] Humaneered Fab' fragment libraries were screened using nitrocellulose filters coated with recombinant human factor B, essentially as described in Example 5 of U.S. Patent

Publication No. US 2005/0255552 A1, which is incorporated herein by reference. *See also* U.S. Patent Publication No. US 2006/0134098 A1.

[0057] Briefly, antibody libraries were transformed into a suitable bacterial host, such as the *E. coli* strain TOP10. The transformed bacterial cells are plated onto plates containing 2X YT agar (16 g pancreatic digest of casein, 10 g yeast extract, 5 g NaCl, and 15 g agar per liter) (Difco™, Becton Dickinson, Franklin Lakes, NJ) and an appropriate selection agent (*i.e.*, an antibiotic selected based on the particular protein expression vector used to construct the library). Plating efficiency can be adjusted to produce discrete bacterial colonies while maximizing the number of colonies per plate. At optimal density, a 10 cm diameter plate would contain ~4000 colonies, a 15 cm diameter plate would contain ~10,000 colonies, and a 25 cm diameter plate would contain ~50,000 colonies.

[0058] Nitrocellulose filters of 8.2 cm diameter, 13.2 cm diameter, or 20 cm diameter (Whatman® Schleicher & Schuell® Protran® BA85 nitrocellulose filters) (Sigma Aldrich, St. Louis, MO) were pre-coated with antigen (*i.e.*, human factor B) in PBS at an empirically determined concentration (typically between 0.5 μg/ml and 20 μg/ml). The volume of coating solution varied depending on the filter size, with 4 ml used for the 8.2 cm diameter filters, 8 ml used for the 13.2 cm diameter filters, and 20 ml used for the 20 cm diameter filters. The filters were placed face down in the antigen-PBS solution for 2-3 hours at 33°C, with occasional agitation. The filters were then rinsed once with excess PBS and blocked with a 5% (w/v) solution of non-fat dry milk in PBS for 2 hours at 25°C with agitation. The filters were then drained, rinsed once in PBS + 0.1% Tween-20™ ("TBST") and twice in 2X YT liquid medium supplemented with selection agent (*i.e.*, an appropriate antibiotic) and transcription inducer (*i.e.*, IPTG). The filters were then drained and placed on 2X YT agar plates supplemented with the appropriate antibiotic and IPTG (the "expression plates").

[0059] Uncoated dry nitrocellulose filters of the appropriate size were placed facedown on the plates containing the *E. coli* library expressing the desired population of antibody fragments. Once the filters were visibly wet (~20-30 seconds), the filters were quickly lifted and placed colony side up onto a coated filter on an expression plate. The filters are marked to indicate the appropriate plate and orientation for ease of subsequent identification.

[0060] The expression plates covered with nitrocellulose filter "sandwiches" were placed at 33°C for 12-16 hours. During that time, the bacterial colonies expressed and secreted the antibody fragments, which then diffused through the first nitrocellulose filter containing the colony lifts onto the antigen-coated filter beneath. Antibody fragments capable of binding the target antigen (*i.e.*, human factor B) were retained on the antigen filter.

[0061] Antigen-bound antibody fragments were detected with immunological methods. Briefly, the filters containing antigen-bound antibody fragments were removed from the expression plates, washed 3 times for 5 minutes each in PBST, and blocked for 1.5 hours at 25°C in a solution of 5% (w/v) non-fat dry milk in PBST. The antigen-antibody fragment complexes retained on the filters were then incubated with an appropriate primary antibody (*e.g.*, goat anti-κ antibody conjugated to HRP, and the like), followed if necessary by an appropriate secondary antibody. Other standard immunological detection methods may be used, including biotin/streptavidin, as well as other detection methods, including various fluorescent labels. The filters were then washed 4 times for 10 minutes each in PBST, incubated in peroxidase substrate solution, and exposed to light-sensitive photographic film. Alternatively, various imaging systems can be used to visualize the positive colonies, such as the Typhoon (Amersham Biosciences, GE Healthcare, Piscataway, NJ) or the FX-Pro PhosphorImager (Biorad, Hercules, CA). The images on the film are then aligned to the appropriate plate, positive colonies (*i.e.*, those producing antibody fragments capable of binding the desired antigen (*e.g.*, human factor B)) were picked, inoculated into 2X YT medium plus selection agent, and further analyzed through subsequent rounds of CLBA using substantially the same procedures.

2.5 Affinity measurements

[0062] Binding kinetics of the Fab' fragments were analyzed using a FortéBio Octet biosensor (FortéBio, Inc., Menlo Park, CA). Recombinant human factor B was biotinylated with the EZ-link biotinylation system (Pierce Biotechnology, Rockford, IL) according to the manufacturer's instructions. The antigen was then coupled to neutravidin-coated sensors (FortéBio, Inc., Menlo Park, CA) according to the manufacturer's instructions. Fab' binding was then monitored in real time using bio-layer interferometry analysis and software provided by the manufacturer. Antigen binding affinities were calculated for the tested Fab' fragments based on the measured association ("K_{assoc}") and dissociation ("K_{dissoc}") constants. Preferably humaneered antibodies or antibody fragments with equilibrium dissociation constants the same or higher than that of the reference antibody (*i.e.*, mAb 1379) or antibody fragment (*i.e.*, TA10).

3. Results

3.1 Cloning and expression of V-regions from the hybridoma producing mAb 1379

3.1.1. V_H and V_κ chain amplification from first strand cDNA

[0063] Variable regions from the antibody light chain (κ isoform) and heavy chain were amplified from first strand cDNA using fifteen V_H and eighteen V_κ primer sets. Each V_H primer set contained one of fifteen degenerate forward primers specific for the known murine heavy chain families paired with an appropriate reverse primer specific for a constant domain from one of the four common murine isoforms of the γ heavy chain (*i.e.*, the murine γ_1 isoform). *See, e.g.*, Chardès et al., *FEBS Lett.* 452(3):386-394 (1999). Each V_κ primer set contained one of eighteen degenerate forward primers specific for the known murine κ families paired with a reverse primer specific for a constant domain from the κ isoform of the murine light chain. *See, e.g.*, Chardès et al., *FEBS Lett.* 452(3):386-394 (1999).

[0064] Two primer sets produced PCR products for the heavy chain, and two primer sets produced PCR products for the light chain. Although the degenerate forward primers were designed to hybridize to the relatively conserved signal sequences of each murine heavy and light chain family, not every primer pair amplifies the expected product because germline signal sequences vary. In addition, immunoglobulin loci frequently contain pseudogenes that can produce a product of the expected size yet do not encode the predicted open reading frame, as was the case with the product produced by the $V_\kappa10$ primer pair (see paragraph [0065] below). Figure 1 is an agarose gel stained with ethidium bromide to show double-stranded cDNA products amplified from first strand cDNA prepared from mRNA isolated from the hybridoma producing mAb 1379. Primer pairs $V_\kappa4$ (SEQ ID NO: 1 (forward primer) and SEQ ID NO: 2 (reverse primer)) and $V_\kappa10$ (SEQ ID NO: 3 (forward primer) and SEQ ID NO: 4 (reverse primer)) produced products of the expected size from the antibody light chain. Primer pairs V_H6 (SEQ ID NO: 5 (forward primer) and SEQ ID NO: 6 (reverse primer)) and V_H7 (SEQ ID NO: 7 (forward primer) and SEQ ID NO: 8 (reverse primer)) produced products of the expected size from the antibody heavy chain.

3.1.2. Murine V-region amino acid sequences

[0065] The V_H and V_κ cDNA clones obtained as described in paragraph [0063] and [0064] above were sequenced by standard methods to verify the correct products were obtained. The V-region sequences obtained are shown in Figure 2. CDR sequences are underlined. Two glutamine residues that differ from the murine germline sequence corresponding to the original mAb 1379 antibody are shown shaded grey. The products obtained with the V_H6 (SEQ ID NO: 10) and V_H7 (SEQ ID NO: 11) primer sets were identical in amino acid sequence. The $V_\kappa10$ product was amplified from a cDNA containing a rearrangement or frameshift that disrupted the protein open reading frame, and so is not shown. The $V_\kappa4$ (SEQ ID NO: 9) product contained the expected open reading frame. One of the selected murine V_H clones was then attached to a human IgG_1 C_H1-region, and the murine $V_\kappa4$ clone was attached to a human C_κ-region to make the reference Fab' (*i.e.*, TA10). The humaneered Fab' variants also comprised human constant region sequences.

3.1.3. Comparison of cloned V-region and amino-terminal amino acid sequences provided by Taligen

[0066] The amino-terminal amino acid sequences of mAb 1379 were then compared to the same portion of the cloned V_H and V_κ sequences. Figure 3 shows the aligned portions of the sequences, first from the V_H chain (top, compare "1379H" (SEQ ID NO: 31) to "TA-V_H6" (SEQ ID NO: 33)), then from the V_H and V_κ chain (bottom, compare "1379L" (SEQ ID NO: 32) to "TA-V_κ4" (SEQ ID NO: 34)). The amino-terminal sequences of mAb 1379 and the cloned sequences were identical apart from four residues (shown shaded grey). Those differences resulted from errors introduced during the Edman-degradation reaction used to obtain the amino-terminal peptide sequences of mAb 1379.

3.1.4. Confirmation of factor B binding activity of the cloned V-regions by ELISA

[0067] Next, the ability of the cloned V_H and V_κ sequences to bind factor B was assayed. The cloned V_H- and V_κ-regions were expressed in bacteria as Fab' fragments, purified, and tested for binding to factor B in a dilution ELISA. Figure 4 compares factor B binding of the cloned Fab' TA003 to that of a Fab' derived from mAb1379. As expected, both the cloned Fab' and murine Fab produced binding curves that were dependent on both antibody and antigen concentration.

3.2 Humaneering of mAb 1379 V-regions

3.2.1. Library construction and V-region cassettes

[0068] Epitope-focused libraries were constructed by linking human V-segment library sequences (isolated from spleen) to the unique CDR3-FR4 region containing the BSD and human germ-line J-segment sequences. These "full-length" libraries were used as a base for construction of "cassette" libraries in which only part of the murine V-segment is initially replaced by a library of human sequences. The cassettes for both V_H and V_κ chains were made by bridge PCR with overlapping common sequences within the FR2 region. In this way, "front-end" and "middle" human cassette libraries were constructed for human V_H1, V_H3, and $V_\kappa IV$ isotypes. Typically, approximately 10,000 unique Fab' clones are screened between the "front-end" and "middle" human cassette libraries to identify a pool of candidate antibody fragments that bind the desired antigen (*i.e.*, human factor B) with a binding affinity at least equal to or greater than the binding affinity of a reference antibody or antibody fragment (*i.e.*, mAb 1379 or TA10).

[0069] Human "front-end" and "middle" cassettes which supported binding to factor B were identified by colony-lift binding assay and ranked according to affinity in ELISA and FortéBio analysis. Colony-lift binding assays were performed as described above, essentially as in Example 5 of U.S. Patent Publication No. US 2005/0255552 A1, which is incorporated herein by reference. Pools of the highest affinity "cassettes" (with antigen-binding affinity preferably equal to or greater than TA10, the reference Fab' derived from mAb 1379) were then recombined via the common FR2 sequences in a second library screen to generate completely human V-segments.

[0070] After identification of a pool of high affinity, fully humaneered Fab' fragments, affinity maturation libraries were built. The common BSD sequences of a panel of humaneered Fab' clones were randomly mutated using degenerate PCR primers to generate libraries. These mutagenic libraries were screened by colony lift binding assay. The selected Fab' fragments were ranked for binding affinity with ELISA and FortéBio analysis. Mutations which supported equal or improved binding affinity for antigen compared to the TA10 reference Fab' fragment were identified.

[0071] In some cases, the humaneering process results in isolation of a pool of fully humaneered Fab' fragments with the same or very similar binding affinities for the target antigen. In such cases, the pool of Fab' fragments is sequenced and compared to the closest human germline V_H- and V_L- (*i.e.*, V_κ-) region sequences, and the humaneered antibody fragments with the highest degree of amino acid sequence identity to the human germline are selected for further analysis. The higher the degree of amino acid sequence identity to the human germline sequence, the less immunogenic a humaneered antibody or antibody fragment will be, and thus, the less likely it will be to provoke an immune or inflammatory response, or to increase an existing immune or inflammatory response. Because the humaneered variants of mAb 1379 may be used to treat conditions in which an immune or inflammatory response has already been triggered (*i.e.*, conditions in which activation of the alternative complement pathway plays a role, such as airway hyperresponsiveness and the like), it is essential that the immunogenicity of the humaneered variants be reduced as much as possible. Furthermore, because administration of proteins into the lung (*i.e.*, by inhalation, as contemplated herein) is more likely to induce an immune response than other routes of administration, it is even more important that the humaneered anti-factor B variants be minimally immunogenic.

[0072] Thus, it is desirable to isolate humaneered variants with the highest possible degree of amino acid sequence identity to the closest human germline sequences (for variants derived from mAb 1379, the closest human germline sequences are V_κIV-B3/J_κ2 (SEQ ID NO:

12) and V_H1-02/J_H4 (SEQ ID NO: 13)). Preferably, the humaneered variants have V_H- and V_κ-region amino acid sequences at least 80% identical to the closest human germline V_H- and V_κ-region amino acid sequences, more preferably at least 85% identical to the closest human germline V_H- and V_κ- region amino acid sequences, still more preferably at least 90% identical to the closest human germline V_H- and V_κ- region amino acid sequences, and even more preferably at least 95% identical to the closest human germline V_H- and V_κ- region amino acid sequences.

[0073] Preferably, a humaneered antibody variant will have a binding affinity equal to or greater than the reference antibody or antibody fragment, and would further comprise V_H- and V_κ-regions having amino acid sequences 80% identical to the closest human germline sequence, 85% identical to the closest human germline sequence, 90% identical to the closest human germline sequence, or 95% identical to the closest human germline sequence. It is not always possible to humaneer antibody or antibody fragment variants that share both those characteristics, however.

3.2.2. Binding affinity of Fab' fragments for human factor B using FortéBio Octet analysis

[0074] Fully humaneered Fab' fragments were isolated by colony lift binding assays and confirmed as factor B binders by ELISA. Humaneered Fab' fragments showing strong positive signals by ELISA were purified and further characterized in comparison to the reference Fab' fragment TA10, which has murine V-region sequences from mAb 1379. Kinetics of Fab' fragment binding to recombinant human factor B were analyzed with the FortéBio Octet system by bio-layer interferometry, providing real time label-free monitoring of protein-protein interactions. Representative kinetic analyses are shown in Figure 5. Measured association (K_{assoc}) and dissociation (K_{dissoc}) constants, and calculated equilibrium dissociation constants ($K_D = K_{dissoc}/K_{assoc}$) (*i.e.*, binding affinity), are shown in Table 1.

[0075] **Table 1**. Kinetic analysis of humaneered antibodies compared to a reference antibody.

TrackingID	**TA102-4**	**TA103-2**	**TA10 (Reference)**	**TA101-1**
Concentration (M)	1×10^{-7}	1×10^{-7}	1×10^{-7}	1×10^{-7}
K_{dissoc} (1/sec)	4.37×10^{-3}	2.80×10^{-3}	2.96×10^{-3}	2.33×10^{-3}
K_{dissoc} (error)	1.03×10^{-4}	1.29×10^{-4}	1.07×10^{-4}	1.27×10^{-4}
K_{assoc} (1/(M•sec))	8.10×10^{-5}	7.50×10^{-5}	4.52×10^{-5}	5.14×10^{-5}
K_D (M)	5.40×10^{-9}	3.73×10^{-9}	6.55×10^{-9}	4.53×10^{-9}

Clearly, all three humaneered antibody fragments have equilibrium dissociation constants equal to or better than the TA10 reference antibody fragment.

3.3 Sequence analysis of humaneered Fab' fragments

3.3.1. Alignment of reference and humaneered Fab' amino acid sequences

[0076] After kinetic characterization, the three humaneered antibody isolates were sequenced. Amino acid sequences derived from the V_κ- and V_H-region sequences of antibody isolates TA101-1 (SEQ ID NOS: 16 and 17), TA102-4 (SEQ ID NOS: 18 and 19), and TA103-2 (SEQ ID NOS: 20 and 21) were compared to the corresponding sequences from the reference antibody TA10 (SEQ ID NOS: 14 and 15) and from the closest human germline light and heavy chain variable domain genes ("V_κ-" and "V_H-gene") and joining segments ("J-segment") (human

V_κIV-B3/J_κ2 (SEQ ID NO: 12) and V_H1-02/J_H4 (SEQ ID NO: 13)). Aligned sequences are shown in Figure 6. The sequences CDR1, CDR2, and CDR3 are boxed and labeled accordingly. Amino acid residues that differ from the corresponding germline position (excluding the CDR3 BSD sequence) are shaded in grey. Affinity maturation changes to the CDR3 amino acid sequences of humaneered variants TA101-1, TA102-4, and TA103-2 are shaded in grey and shown in boldface type.

[0077] In certain embodiments, the V_H-region sequences of TA101-1 (SEQ ID NO: 35), TA102-4 (SEQ ID NO: 36), and TA103-2 (SEQ ID NO: 37) are modified to replace the amino-terminal glutamine (Q) residue of the humaneered anti-factor B variants with a glutamic acid (E) residue as found in the reference antibody (TA10) and the original mAb 1379. This change prevents cyclization of the glutamine (Q) residue and promotes a more uniform final product when manufacturing the humaneered variants. Although the closest human germline gene (V_H1-02/J_H4 (SEQ ID NO: 13)) also has a glutamine (Q) residue at its amino terminus, this conservative amino acid substitution likely has minimal impact on immunogenicity of the variants.

3.3.2. Percent identity to human germline sequences

[0078] Finally, the V_H-region and V_κ-region amino acid sequences derived from the TA101-1, TA102-4, and TA103-2 isolates and the TA10 reference antibody were compared to a single human germline antibody sequence across the V-region, excluding the CDR3 BSD sequences. Table 2 shows the percent amino acid identity to the germline sequence for each.

Clone	V_κ % identity (aligned to V_κIV)	V_H % identity (aligned to V_H1-02)	Total % identity across V-region (excluding CDR3)
TA10 reference	70.4%	84.9%	77.7%
TA101-1	96.2%	96.3%	96.25%
TA102-4	97.1%	96.3%	96.7%
TA103-2	95.3%	96.3%	95.8%

Clearly, the V_κ- and V_H-regions of all three humaneered Fab' fragments share high amino acid sequence identity to the human germline sequence, with percent identities of about 96% compared to about 78% for the reference Fab' fragment, TA10.

4. **Discussion**

[0079] Cassette replacement was used successfully for humaneering of mAB 1379. Partial V-region cassettes isolated from a human library were recombined to form the final engineered human V-regions for each of the heavy and light chains.

[0080] The amino acid sequences of the V-regions from the Fab' fragment clones are provided above. V-segment sequences were isolated by recombination of two V_H cassettes and two V_κ cassettes for each Fab' fragment (a "front-end" and a "middle" cassette for each of the V_H and V_κ polypeptides). Kinetic analysis using the ForteBio Octet biosensor identified three Fab' fragments (TA101-1, TA102-4, and TA103-2) with higher binding affinities than the reference Fab' fragment. This increased binding affinity resulted from an improved off-rate in the three humaneered variants (*i.e.*, TA101-1, TA102-4, and TA103-2) when compared to the reference molecule. Thus, it may also be desirable to screen for variants based upon increased off-rates (K_{dissoc}) and/or increased binding affinities, as well as % amino acid sequence identity between the humaneered V_H and V_κ polypeptides and the closest human germline V_H and V_κ sequences.

[0081] Each of the three Fab' fragment clones has a heavy chain variable region (V_H) with a high degree of amino acid sequence identity to the human V_H1-02 germ-line gene. The FR_H4 segment is provided by the human germ-line J_H4 sequence.

[0082] The light chain V-segments are closest to the V_κIV-B3 germline gene. The FR_L4 the same is provided by the human germ-line $J_\kappa2$ segment. The humaneered Fab' fragment V_H and V_L regions show greater than 96% amino acid sequence identity to the closest corresponding human germ-line sequence cassettes outside the unique CDR3 regions.

5. Formulations, compositions, and methods relating to certain embodiments of the invention

[0083] One aspect of the present invention generally relates to compositions and methods for selectively inhibiting activation of the alternative complement pathway in an animal that has, or is at risk of developing, a condition or disease in which activation of the alternative complement pathway contributes to the condition or disease, exacerbates at least one symptom of the condition or disease, or causes the condition or disease.

5.1 Methods relating to certain embodiments of the invention

[0084] Certain embodiments of the present invention related to methods of treating diseases or disorders in which activation of the alternative complement pathway plays a role. Such methods involve administering a humaneered variant of mAb 1379 as described above, such as TA101-1, TA102-4, and TA103-2, or antigen-binding fragments thereof, to an individual that has, or is at risk of developing, a disease in which activation of the alternative complement pathway plays a role. In one aspect, the humaneered antibody variants and antigen-binding fragments thereof are administered by a route selected from the group consisting of oral, nasal, topical, inhaled, intratracheal, transdermal, rectal and parenteral routes. In another aspect, the humaneered antibody variants and antigen-binding fragments thereof are administered with a pharmaceutically acceptable carrier selected from the group consisting of: a dry, dispersible powder; anhydrous ethanol; small capsules; liposomes; a nebulized spray; and an injectable excipient. In another aspect, the humaneered variants and antigen-binding fragments thereof are administered in a carrier or device selected from the group consisting of: anhydrous ethanol; a dry powder inhalation system; ultrasonic inhalation system; a pressurized metered dose inhaler; and a metered solution device. In another aspect, the humaneered antibody variants and antigen-binding fragments thereof are administered in an amount effective to treat the disease or disorder in which activation of the alternative complement pathway plays a role. In still other aspects, the humaneered antibody variants and antigen-binding fragments thereof are administered alone, or in combination with another agent selected from the group consisting of: corticosteroids, β-agonists (long or short acting), leukotriene modifiers, antihistamines, phosphodiesterase inhibitors, sodium cromoglycate, Nedocromil, theophylline, cytokine antagonists, cytokine receptor antagonists, anti-IgE, and inhibitors of T cell function.

[0085] Still other embodiments of the present invention relate to a method to reduce or prevent airway hyperresponsiveness (AHR) or airway inflammation in an individuaal. The method includes the step of administering a humaneered variant of mAb 1379 as described above, such as TA101-1, TA102-4, and TA103-2, or antigen-binding fragments thereof, to an individual that has, or is at risk of developing, airway hyperresponsiveness associated with inflammation or airway inflammation. In one aspect, the humaneered variant of mAb 1379 or antigen-binding fragment thereof is administered by a route selected from the group consisting of oral, nasal, topical, inhaled, intratracheal, transdermal, rectal and parenteral routes. In another aspect, the humaneered variant of mAb 1379 or antigen-binding fragment thereof is administered to the animal in an amount effective to measurably reduce airway hyperresponsiveness in the individual as compared to prior to administration of the antibody or antigen binding fragment. In another aspect, the humaneered variant of mAb 1379 or antigen-binding fragment thereof is administered to the individual in an amount effective to measurably reduce airway hyperresponsiveness in the individual as compared to a level of airway hyperresponsiveness in a population of individuals having inflammation wherein the antibody or antigen binding fragment was not administered. In another aspect, the humaneered variant of mAb 1379 or antigen-binding fragment thereof is administered with a pharmaceutically acceptable carrier selected from the group consisting of: a dry, dispersible powder; anhydrous ethanol; small capsules; liposomes; a nebulized spray; and an injectable excipient. In another aspect, the humaneered variant of mAb 1379 or antigen-binding fragment thereof is administered in a carrier or device selected from the group consisting of: anhydrous ethanol; a dry powder inhalation system; ultrasonic inhalation system; a pressurized metered dose inhaler; and a metered solution device.

[0086] In yet another aspect, the humaneered variant of mAb 1379 or antigen-binding fragment thereof is administered to an individual in conjunction with an agent selected from the group consisting of: corticosteroids, β-agonists (long or short acting), leukotriene modifiers, antihistamines, phosphodiesterase inhibitors, sodium cromoglycate, Nedocromil, theophylline, cytokine antagonists, cytokine receptor antagonists, anti-IgE, and inhibitors of T cell function. In yet another aspect, the airway hyperresponsiveness or airway inflammation is associated with a disease selected from the group consisting of asthma, chronic obstructive pulmonary disease

(COPD), allergic bronchopulmonary aspergillosis, hypersensitivity pneumonia, eosinophilic pneumonia, emphysema, bronchitis, allergic bronchitis bronchiectasis, cystic fibrosis, tuberculosis, hypersensitivity pneumonitis, occupational asthma, sarcoid, reactive airway disease syndrome, interstitial lung disease, hyper-eosinophilic syndrome, rhinitis, sinusitis, exercise-induced asthma, pollution-induced asthma, cough variant asthma, parasitic lung disease, respiratory syncytial virus (RSV) infection, parainfluenza virus (PIV) infection, rhinovirus (RV) infection and adenovirus infection. In one aspect, the airway hyperresponsiveness is associated with allergic inflammation. The method of the present invention can be administered, in a preferred embodiment, to mammals, and more preferably, to humans.

[0087] Another embodiment of the present invention relates to a method to reduce or prevent airway hyperresponsiveness (AHR) or airway inflammation in an individual. The method includes the step of administering a reagent that selectively inhibits the alternative complement pathway to an individual that has, or is at risk of developing, airway hyperresponsiveness associated with inflammation or airway inflammation. In certain aspects, that reagent is a humaneered variant of mAb 1379, such as TA101-1, TA102-4, and TA103-2, or antigen-binding fragments thereof.

5.2 Formulations or compositions relating to certain embodiments of the invention

[0088] Certain embodiments of the humaneered anti-factor B antibody variants of the present invention include a formulation or composition comprising an inhibitor of the alternative complement pathway and particularly, a selective inhibitor of the alternative complement pathway as described herein. The formulations or compositions can be used in any of the methods described herein and with any of the reagents described herein (*e.g.*, the humaneered factor B antibody variants TA101-1, TA102-4, and TA103-2 or antigen-binding fragments thereof as described herein). In one embodiment, the composition is useful for reducing or preventing airway hyperresponsiveness in an animal. In another embodiment, the composition is useful for reducing or preventing ischemia-reperfusion injury in an animal. In yet another embodiment, the composition is useful for treating or preventing a condition or disease by selective inhibition of the alternative complement pathway. The formulation comprises: (a) an inhibitor of the alternative complement pathway as described herein; and (b) a pharmaceutically acceptable carrier.

[0089] In one embodiment, the formulation or composition can include one or more additional agents, such as an anti-inflammatory agent suitable for reducing inflammation in an animal that has, or is at risk of developing, airway hyperresponsiveness, and particularly, airway hyperresponsiveness associated with inflammation. The anti-inflammatory agent can be any anti-inflammatory agent suitable for use in reducing inflammation in a patient that has an inflammatory condition associated with airway hyperresponsiveness, including, but not limited to: corticosteroids, (oral, inhaled and injected), β-agonists (long or short acting), leukotriene modifiers (inhibitors or receptor antagonists), cytokine or cytokine receptor antagonists, anti-IgE antibdoies, phosphodiesterase inhibitors, sodium cromoglycate, nedocrimal, theophylline, and inhibitors of T cell function. Particularly preferred anti-inflammatory agents for use in the present formulation include, corticosteroids, leukotriene modifiers, and cytokine or cytokine receptor antagonists.

[0090] In another embodiment, the formulation or composition can include one or more additional agents, such as an additional agent suitable for preventing or reducing ischemia-reperfusion injury in an animal. Such agents include, but are not limited to, anti-inflammatory agents; or inhibitors of oxidation and free radical damage.

[0091] In another embodiment, the formulation or composition can include one or more additional agents, such as an additional agent suitable for treatment of another disease or condition associated with activation of the alternative complement pathway.

[0092] According to the present invention, a "pharmaceutically acceptable carrier" includes pharmaceutically acceptable excipients and/or pharmaceutically acceptable delivery vehicles, which are suitable for use in the administration of a formulation or composition to a suitable *in vivo* site. A suitable *in vivo* site is preferably any site wherein the alternative complement pathway can be inhibited. In one preferred embodiment, when the patient has or is at risk of developing airway hyperresponsiveness and/or airway inflammation, a suitable *in vivo* site is preferably in the lung tissue or airways. Other preferred *in vivo* sites include other tissues or organs where conditions associated with the alternative complement pathway may be centered. In another preferred embodiment, a suitable *in vivo* site is any site where ischemia-reperfusion injury occurs, such as in the heart or pulmonary system, central nervous system, limbs or digits, internal organs (*e.g.*, lung, liver or intestine), or in any transplanted organ or tissue. Preferred pharmaceutically acceptable carriers are capable of maintaining an agent used in a formulation of the invention in a form that, upon arrival of the agent at the target site in a patient, the agent is capable of acting on its target (*e.g.*, a protein that is a component of the alternative complement pathway), preferably resulting in a therapeutic benefit to the patient.

[0093] Suitable excipients for use in the present invention include excipients or formularies that transport or help transport, but do not specifically target a composition to a cell or tissue (also referred to herein as non-targeting carriers). Examples of pharmaceutically acceptable excipients include, but are not limited to water, phosphate buffered saline ("PBS"), Ringer's solution, dextrose solution, serum-containing solutions, Hank's Balanced Salt Solution ("HBSS"), and other aqueous physiologically balanced solutions, oils, esters and glycols. Aqueous carriers can contain suitable auxiliary substances required to approximate the physiological conditions of the recipient, for example, by enhancing chemical stability and isotonicity. Suitable auxiliary substances include, for example, sodium acetate, sodium chloride, sodium lactate, potassium chloride, calcium chloride, and other substances used to produce phosphate buffer, Tris buffer, and bicarbonate buffer. Auxiliary substances can also include preservatives, such as thimerosal, *m*- or *o*-cresol, formalin and benzyl alcohol. Formulations of the present invention can be sterilized by conventional methods and/or lyophilized.

[0094] One type of pharmaceutically acceptable carrier includes a controlled-release formulation that is capable of slowly releasing a composition of the present invention into an animal. As used herein, a controlled-release formulation comprises an agent of the present invention in a controlled-release vehicle. Suitable controlled-release vehicles include, but are not limited to, biocompatible polymers, other polymeric matrices, capsules, microcapsules, microparticles, bolus preparations, osmotic pumps, diffusion devices, liposomes, lipospheres, and transdermal delivery systems. Other suitable carriers include any carrier that can be bound to or incorporated with the agent that extends that half-life of the agent to be delivered. Such a carrier can include any suitable protein carrier or even a fusion segment that extends the half-life of a protein when delivered *in vivo*. Suitable delivery vehicles have been previously described herein, and include, but are not limited to liposomes, viral vectors or other delivery vehicles, including ribozymes. Natural lipid-containing delivery vehicles include cells and cellular membranes. Artificial lipid-containing delivery vehicles include liposomes and micelles. As discussed above, a delivery vehicle of the present invention can be modified to target to a particular site in a patient, thereby targeting and making use of an inhibitory agent at that site. Suitable modifications include manipulating the chemical formula of the lipid portion of the delivery vehicle and/or introducing into the vehicle a targeting agent capable of specifically targeting a delivery vehicle to a preferred site, for example, a preferred cell type. Other suitable delivery vehicles include gold particles, poly-L-lysine/DNA-molecular conjugates, and artificial chromosomes.

[0095] In one embodiment, an agent useful in the present methods is administered in a formulation suitable for pulmonary or nasal delivery, and particularly, aerosol delivery, also referred to herein as an aerosolized formulation. Such a route of delivery is particularly useful in the method to prevent or inhibit AHR and/or airway inflammation in a patient, but can be used in other conditions when delivery to the lung or airways is desired. In addition, these formulations are particularly useful for the delivery of antibodies. Such a formulation generally includes a carrier, and preferably, a pharmaceutically acceptable carrier. Carriers that are particularly useful for aerosol delivery according to the present invention include, but are not limited to: anhydrous ethanol; dry, dispersible powders; small capsules (*e.g.*, microcapsules or microparticles); liposomes; injectable excipients; and nebulized sprays. Anhydrous ethanol for the delivery of proteins and peptides is described, for example, in Choi et al., *Proc. Nat'l Acad. Sci. USA* 98(20):11103-11107 (2001). Dry, dispersible powders suitable for aerosolized delivery of agents are described in detail, for example, in U.S. Patent No. 6,165,463, incorporated herein by reference in its entirety (*See also* products from Inhale Therapeutic Systems, Inc., now Nektar, and Quadrant Technology). Suitable liposomes for use in aerosols include any liposome, and particularly, any liposome that is sufficiently small to be delivered by aerosol in the method of the invention. Microcapsules and microparticles are known in the art. For example, Alliance Pharmaceutical Corporation has a particle engineering technology called PulmoSphere, in which microparticles are prepared by a proprietary spray-drying process and are designed to be both hollow and porous. A product by Ventolin consists of micronized albuterol (free base) particles suspended in a mixture of CFC-based propellants. Proventil HFA contains micronized albuterol sulfate and a small percentage of an ethanol co-solvent to solubilize the stabilizing oleic acid surfactant. Incorporation of drugs into liposomes has several advantages for aerosol delivery. Because liposomes are relatively insoluble, the retention time of some drugs in the lung can be prolonged for increased efficacy. Liposomes are also taken up primarily by phagocytic cells which make them particularly suitable for delivery of certain drugs. Devices for delivery of aerosolized formulations include, but are not limited to, pressurized metered dose inhalers ("MDI"), dry powder inhalers ("DPI"), metered solution devices ("MSI"), and ultrasonic inhalers, and include devices that are nebulizers and inhalers. Various agents can be used in formulations delivered by such devices as suspension aids and solubilizers that are particularly useful for the delivery of proteins (*e.g.*, oligolactic acid, acyl-amide acids, and mono-functionalized M-PEGS; *see, e.g.*, McKenzie and Oliver; 2000, Formulating Therapeutic Proteins and Peptides in Pressurized Metered Dose Inhalers For Pulmonary Delivery, 3M Health Care Ltd., Morley Street, Loughborough, Leicesteshire LE11 1EP, UK).

[0096] A pharmaceutically acceptable carrier which is capable of targeting is herein referred to as a "targeting delivery vehicle." Targeting delivery vehicles of the present invention are capable of delivering a formulation, including an inhibitory agent, to a target site in a patient. A "target site" refers to a site in a patient to which one desires to deliver a therapeutic formulation. For example, a target site can be any cell or tissue which is targeted by an antibody of the present invention, or by direct injection or delivery using liposomes, viral vectors or other delivery vehicles, including ribozymes. A delivery vehicle or antibody of the present invention can be modified to target a particular site in an animal, thereby targeting and making use of particular compound, antibody, protein, or nucleic acid molecule at that site. Suitable modifications include manipulating the chemical formula of the lipid portion of a delivery vehicle and/or introducing into the vehicle a compound capable of specifically targeting a delivery vehicle to a preferred site, for example, a preferred cell or tissue type. Specifically, targeting refers to causing a delivery vehicle to bind to a particular cell by the interaction of the compound in the vehicle to a molecule on the surface of the cell. Suitable targeting compounds include ligands capable of selectively (*i.e.*, specifically) binding another molecule at a particular site. Examples of such ligands include antibodies, antigens, receptors and receptor ligands. Particularly useful examples include any ligands associated with the complement pathway (*e.g.*, CR2, C3, C3d, C3dg, iC3b, C3b) or any ligands associated with the cell type, tissue type, or site in the animal to be treated. Manipulating the chemical formula of the lipid portion of the delivery vehicle can modulate the extracellular or intracellular targeting of the delivery vehicle. For example, a chemical can be added to the lipid formula of a liposome that alters the charge of the lipid bilayer of the liposome so that the liposome fuses with cells having particular charge characteristics.

[0097] One delivery vehicle useful for a variety of administration routes and agents is a liposome. A liposome is capable of remaining stable in an animal for a sufficient amount of time to deliver a nucleic acid molecule, or even a protein or antibody as described in the present invention, to a preferred site in the animal. According to the present invention, a liposome comprises a lipid composition that is capable of delivering a nucleic acid molecule, protein, or antibody as described in the present invention to a particular, or selected, site in an animal. A liposome according to the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents into a cell. Suitable liposomes for use with the present invention include any liposome. Preferred liposomes of the present invention include those liposomes typically used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes comprise liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Complexing a liposome with a nucleic acid molecule, protein or antibody of the present invention can be achieved using methods standard in the art.

[0098] In accordance with the present invention, determination of acceptable protocols to administer an agent, composition or formulation, including the route of administration and the effective amount of an agent to be administered to an animal, can be accomplished by those skilled in the art. An agent of the present invention can be administered *in vivo* or *ex vivo*. Suitable *in vivo* routes of administration can include, but are not limited to, oral, nasal, inhaled, topical, intratracheal, transdermal, rectal, and parenteral routes. Preferred parenteral routes can include, but are not limited to, subcutaneous, intradermal, intravenous, intramuscular, and intraperitoneal routes. Preferred topical routes include inhalation by aerosol (*i.e.*, spraying) or topical surface administration to the skin of an animal. Preferably, an agent is administered by nasal, inhaled, intratracheal, topical, or systemic routes (*e.g.*, intraperitoneal, intravenous). The term "*ex vivo*" refers to performing part of the administration step outside of the patient. Preferred routes of administration for antibodies include parenteral routes and aerosol/nasal/inhaled routes.

[0099] Intravenous, intraperitoneal, and intramuscular administrations can be performed using methods standard in the art. Aerosol (inhalation) delivery can be performed using methods standard in the art (*see, e.g.*, Stribling et al., *Proc. Nat'l Acad. Sci. USA* 189:11277-11281 (1992), which is incorporated herein by reference in its entirety). Carriers suitable for aerosol delivery are described above. Devices for delivery of aerosolized formulations include, but are not limited to, pressurized metered dose inhalers ("MDI"), dry powder inhalers ("DPI"), and metered solution devices ("MSI"), and include devices that are nebulizers and inhalers. Oral delivery can be performed by complexing a therapeutic composition of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Direct injection techniques are particularly useful for administering a recombinant nucleic acid molecule to a cell or tissue that is accessible by surgery, and particularly, on or near the surface of the body. Administration of a composition locally within the area of a target cell refers to injecting the composition centimeters and preferably, millimeters from the target cell or tissue.

[0100] A preferred single dose of an agent, including proteins, small molecules and antibodies, for use in any method described herein, comprises between about 0.01 μg/kg and about 10 mg/kg body weight of an animal. A more preferred single dose of an agent comprises between about 1 μg/kg and about 10 mg/kg body weight of an animal. An even more preferred single dose of an agent comprises between about 5 μg/kg and about 7 mg/kg body weight of an animal. An even more preferred single dose of an agent comprises between about 10 μg/kg and about 5 mg/kg body weight of an animal. A particularly preferred single dose of an agent comprises between about 0.01 mg/kg and about 1 mg/kg body weight of an animal, if the agent is delivered by aerosol. Another particularly preferred single dose of an agent comprises between about 1 mg/kg and about 10 mg/kg body weight of an animal, if the agent is delivered parenterally.

[0101] In one embodiment a suitable dose of an agent of the present invention for use in any method described herein is a dose effective to inhibit the expression or activity of at least one protein in the alternative complement pathway as described herein (*e.g.*, factor B, factor D or properdin), as compared to in the absence of the administration of the agent. Methods of measuring the expression or biological activity of a protein are known in the art and include, for example, Northern blotting, Western blotting, real time RT-PCR, and the like. In another embodiment, a suitable dose of an agent of the present invention is a dose that measurably inhibits the alternative complement pathway of the invention. Activation of complement and inhibition thereof can be measured using techniques/assays that are well-known in the art. For example, one can perform an *in vitro* analysis of C3 deposition on zymosan A particles as described in the examples of co-pending U.S Patent Publication No. US-2005/0260198 A1, which is incorporated herein by reference. One can also test the ability of the agent to inhibit lysis of unsensitized erythrocytes by human serum. Extrapolation of *in vitro* results to *in vivo* dosages based on these assays is within the ability of those of skill in the art.

[0102] In humans, it known in the art that, using conventional methods for aerosol delivery, only about 10% of the delivered solution typically enters the deep airways, even using an inhaler. If the aerosolized delivery is by direct inhalation, one may assume a dosage of about 10% of that administered by nebulization methods. Finally, one of skill in the art will readily be capable of converting a mouse dosage to a human dosage using alometric scaling. Essentially, a scale of dosage from mouse to human is based on the clearance ratio of a compound and the body surface of the mouse. The conversion for mg/kg is one twelfth of the "no observed adverse event level" ("NOEL") to obtain the concentration for human dosage. This calculation assumes that the elimination between mouse and human is the same, which is believed to be the case for antibodies.

[0103] Accordingly, a preferred single dose of an antibody comprises between about 1 ng/kg and about less than 1 mg/kg body weight of an animal. A more preferred single dose of an antibody comprises between about 20 ng/kg and about 600 μg/kg body weight of the animal. An even more preferred single dose of an antibody, particularly when the antibody formulation is delivered by nebulization, comprises between about 20 ng/kg and about 600 μg/kg body weight of the animal, and more preferably, between about 20 ng/kg and about 500 μg/kg, and more preferably, between about 20 ng/kg and about 400 μg/kg, and more preferably, between about 20 ng/kg and about 300 μg/kg, and more preferably, between about 20 ng/kg and about 200 μg/kg, and more preferably, between about 20 ng/kg and about 100 μg/kg, and more preferably, between about 20 ng/kg and about 50 μg/kg body weight of the animal.

[0104] Another preferred single dose of an antibody, particularly when the antibody formulation is delivered by nebulization, comprises between about 200 ng/kg and about 600 μg/kg body weight of the animal, and more preferably, between about 200 ng/kg and about 500 μg/kg, and more preferably, between about 200 ng/kg and about 400 μg/kg, and more preferably, between about 200 ng/kg and about 300 μg/kg, and more preferably, between about 200 ng/kg and about 200 μg/kg, and more preferably, between about 200 ng/kg and about 100 μg/kg, and more preferably, between about 200 ng/kg and about 50 μg/kg body weight of the animal.

[0105] Another preferred single dose of an antibody, particularly when the antibody formulation is delivered by direct inhalation from an inhaler, comprises between about 2 ng/kg and about 100 μg/kg body weight of the animal, and more preferably, between about 2 ng/kg and about 50 μg/kg, and more preferably, between about 2 ng/kg and about 10 μg/kg, and more preferably, between about 2 ng/kg and about 5 μg/kg, and more preferably, between about 2 ng/kg and about 1 μg/kg, and more preferably, between about 2 ng/kg and about 0.5 μg/kg, and more preferably, between about 2 ng/kg and about 0.25 μg/kg, and more preferably, between about 2 ng/kg and about 0.1 μg/kg body weight of the animal.

[0106] In another embodiment, the antibody is administered at a dose of less than about 500 μg antibody per milliliter of formulation, and preferably, less than about 250 μg antibody per milliliter of formulation, and more preferably, less than about 100 μg antibody per milliliter of formulation, and more preferably, less than about 50 μg antibody per milliliter of formulation, and more preferably, less than about 40 μg antibody per milliliter of formulation, and more preferably, less than about 30 μg antibody per milliliter of formulation, and more preferably, less than about 20 μg antibody per milliliter of formulation, and more preferably, less than about 10 μg antibody per milliliter of formulation, and even more preferably, between about 5 μg antibody and about 10 μg antibody per milliliter of formulation.

[0107] With more particular regard to the method of reducing or preventing airway hyperresponsiveness and/or airway inflammation or a condition or disease related thereto, a suitable single dose of an inhibitory agent to administer to an animal is a dose that is capable of reducing or preventing airway hyperresponsiveness and/or airway inflammation, or reducing at least one other symptom of a disease to be treated (*e.g.*, asthma), in an animal when administered one or more times over a suitable time period. When the patient has or is at risk of developing AHR, a suitable single dose of an agent comprises a dose that improves AHR by a doubling dose of a provoking agent or improves the static respiratory function of an animal.

[0108] According to the method of the present invention, an effective amount of an agent that inhibits AHR to administer to an animal comprises an amount that is capable of reducing airway hyperresponsiveness (AHR) or airway inflammation without being toxic to the animal. An amount that is toxic to an animal comprises any amount that causes damage to the structure or function of an animal (*i.e.*, poisonous).

[0109] In one embodiment of the present invention, in an animal that has AHR, an effective amount of an agent to administer to an animal is an amount that measurably reduces AHR in the animal as compared to prior to administration of the agent. In another embodiment, an effective amount of an agent to administer to an animal is an amount that measurably reduces AHR in the animal as compared to a level of airway AHR in a population of animals with inflammation that is associated with AHR wherein the agent was not administered. The agent is preferably capable of reducing AHR in an animal, even when the agent is administered after the onset of the physical symptoms of AHR (*i.e.*, after acute onset AHR). Most preferably, an effective amount of the agent is an amount that reduces the symptoms of AHR to the point where AHR is no longer detected in the patient. In another embodiment, an effective amount of the agent is an amount that prevents, or substantially inhibits the onset of AHR when the agent is administered prior to exposure of the patient to an AHR-provoking stimulus, such as an allergen, in a manner sufficient to induce AHR in the absence of the agent.

[0110] One of skill in the art will be able to determine that the number of doses of an agent to be administered to an animal is dependent upon the extent of the airway hyperresponsiveness and the underlying condition of which AHR is a symptom, and the response of an individual patient to the treatment. In addition, the clinician will be able to determine the appropriate timing for delivery of the agent in a manner effective to reduce AHR in the animal.

Preferably, the agent is delivered within 48 hours prior to exposure of the patient to an amount of an AHR provoking stimulus effective to induce AHR, and more preferably, within 36 hours, and more preferably within 24 hours, and more preferably within 12 hours, and more preferably within 6 hours, 5 hours, 4 hours, 3 hours, 2 hours, or 1 hour prior to exposure of the patient to an amount of AHR provoking stimulus effective to induce AHR. In one embodiment, the agent is administered as soon as it is recognized (*i.e.*, immediately) by the patient or clinician that the patient has been exposed or is about to be exposed to an AHR provoking stimulus, and especially an AHR provoking stimulus to which the patient is sensitized (*i.e.*, an allergen). In another embodiment, the agent is administered upon the first sign of development of AHR (*i.e.*, acute onset AHR), and preferably, within at least 2 hours of the development of symptoms of AHR, and more preferably, within at least 1 hour, and more preferably within at least 30 minutes, and more preferably within at least 10 minutes, and more preferably within at least 5 minutes of development of symptoms of AHR. Symptoms of AHR and methods for measuring or detecting such symptoms have been described in detail above. Preferably, such administrations are given until signs of reduction of AHR appear, and then as needed until the symptoms of AHR are gone.

[0111] With particular regard to the method of inhibiting or preventing ischemia-reperfusion injury, an effective amount of an agent, and particularly an anti-factor B antibody or antigen binding fragment thereof (or antigen binding polypeptide) to administer to an animal is an amount that measurably inhibits histological damage, including oxidative damage or cell death, in the animal as compared to in the absence of administration of the agent. In the case of renal ischemia-reperfusion injury, an effective amount of an agent to administer to an animal is an amount that measurably inhibits increases in serum urea nitrogen or measurably decreases histologic injury to the tissues of the kidney of the animal as compared to in the absence of administration of the agent. A suitable single dose of an inhibitory agent to administer to an animal is a dose that is capable of reducing or preventing at least one symptom, type of injury, or resulting damage, from ischemia-reperfusion injury in an animal when administered one or more times over a suitable time period. Suitable doses of antibodies, including for various routes of administration, are described in detail above. In one aspect, an effective amount of an agent that inhibits ischemia-reperfusion injury to administer to an animal comprises an amount that is capable of inhibiting at least one symptom or damage caused by ischemia-reperfusion injury without being toxic to the animal.

[0112] Any of the methods of the present invention can be used in any animal, and particularly, in any animal of the vertebrate class Mammalia (*i.e.*, mammals), including, without limitation, primates, rodents, livestock and domestic pets. Preferred mammals to treat with the methods of the present invention are humans.

CLAIMS

We claim:

1. A humaneered anti-factor B antibody or antigen-binding fragment thereof derived from murine monoclonal antibody 1379 ("mAb 1379") that selectively binds to factor B within the third short consensus repeat ("SCR") domain and prevents formation of the C3bBb complex, wherein the humaneered antibody or antigen-binding fragment thereof has an equilibrium dissociation constant ("K_D") between about 1.0×10^{-8} M and about 1.0×10^{-10} M.

2. The humaneered anti-factor B antibody or antigen binding fragment thereof of claim 1, wherein the K_D is between about 1.0×10^{-9} M and about 9.0×10^{-9} M.

3. The humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 1, wherein the K_D is between about 3.0×10^{-9} M and about 7.0×10^{-9} M.

4. The humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 1, wherein the K_D is about 3.7×10^{-9} M or less.

5. The humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 1, wherein the K_D is about 4.5×10^{-9} M or less.

6. The humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 1, wherein the K_D is about 5.4×10^{-9} M or less.

7. The humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 1, wherein the K_D is about 6.5×10^{-9} M or less.

8. The humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 1, comprising a V_κ-region polypeptide selected from the group consisting of SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, and SEQ ID NO: 20, and a V_H-region polypeptide selected

from the group consisting of SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 21.

9. The humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 8, comprising a V_κ-region polypeptide of SEQ ID NO: 14 and a V_H-region polypeptide of SEQ ID NO: 15.

10. The humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 8, comprising a V_κ-region polypeptide of SEQ ID NO: 16 and a V_H-region polypeptide of SEQ ID NO: 17.

11. The humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 8, comprising a V_κ-region polypeptide of SEQ ID NO: 18 and a V_H-region polypeptide of SEQ ID NO: 19.

12. The humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 8, comprising a V_κ-region polypeptide of SEQ ID NO: 20 and a V_H-region polypeptide of SEQ ID NO: 21.

13. The humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 1, wherein the antigen-binding fragment is selected from the group consisting of Fab', $(Fab')_2$, Fv, scFv, and diabodies.

14. The humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 13, wherein the fragment is a Fab'.

15. The humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 1, wherein the V_κ-region of the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a binding specificity determinant ("BSD") derived from the CDR3-

FR4 region selected from the group consisting of SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, and SEQ ID NO: 28 and the V_H-region of the humaneered anti-factor B antibody or antigen-binding fragment thereof comprises a BSD derived from the CDR3-FR4 region selected from the group consisting of SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, and SEQ ID NO: 29.

16. The humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 15, comprising a V_κ-region BSD polypeptide of SEQ ID NO: 22 and a V_H-region BSD polypeptide of SEQ ID NO: 23.

17. The humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 15, comprising a V_κ-region BSD polypeptide of SEQ ID NO: 24 and a V_H-region BSD polypeptide of SEQ ID NO: 25.

18. The humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 15, comprising a V_κ-region BSD polypeptide of SEQ ID NO: 26 and a V_H-region BSD polypeptide of SEQ ID NO: 27.

19. The humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 15, comprising a V_κ-region BSD polypeptide of SEQ ID NO: 28 and a V_H-region BSD polypeptide of SEQ ID NO: 29.

20. The humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 15, wherein the antigen-binding fragment is selected from the group consisting of Fab', $(Fab')_2$, Fv, scFv, and diabodies.

21. The humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 20, wherein the fragment is a Fab'.

22. A method of treating a disease or disorder in which activation of the alternative complement pathway plays a role, comprising administering the humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 1 to an individual that has, or is at risk of developing, said disease or disorder.

23. The method of claim 22, wherein the disease or disorder is airway hyperresponsiveness ("AHR") or airway inflammation.

24. The method of claim 23, wherein the humaneered anti-factor B antibody or antigen-binding fragment thereof is administered to the individual in an amount effective to measurably reduce AHR or airway inflammation in the animal as compared to before administration of the antibody or antigen-binding fragment thereof.

25. The method of claim 24, wherein said AHR or airway inflammation is associated with a disease selected from the group consisting of asthma, chronic obstructive pulmonary disease ("COPD"), allergic bronchopulmonary aspergillosis, hypersensitivity pneumonia, eosinophilic pneumonia, emphysema, bronchitis, allergic bronchitis bronchiecstasis, cyctic fibrosis, tuberculosis, hypersensitivity pneumonitis, occupational asthma, sarcoid, reactive airway disease syndrome, interstitial lung disease, hyper-eosinophilic syndrome, rhinitis, sinusitis, exercise-induced asthma, pollution-induced asthma, cough variant asthma, parasitic lung disease, respiratory syncytial virus ("RSV") infection, parainfluenza virus ("PIV") infection, rhinovirus ("RV") infection, and adenovirus infection.

26. The method of claim 24, wherein the AHR or airway inflammation is associated with allergic inflammation.

27. The method of claim 24, wherein the AHR or airway inflammation is associated with asthma.

28. The method of claim 24, wherein the AHR or airway inflammation is associated with COPD.

29. A method of selectively inhibiting activation of the alternative complement pathway in an individual that has, or is at risk of developing, a condition or disease in which activation of the alternative complement pathway contributes to the condition or disease, exacerbates at least one symptom of the condition or disease, or causes the condition or disease, comprising administering the humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 1 to an individual in need thereof.

30. A pharmaceutical composition comprising an effective amount of the humaneered anti-factor B antibody or antigen-binding fragment thereof of claim 1, and a pharmaceutically acceptable carrier.

Figure 1.

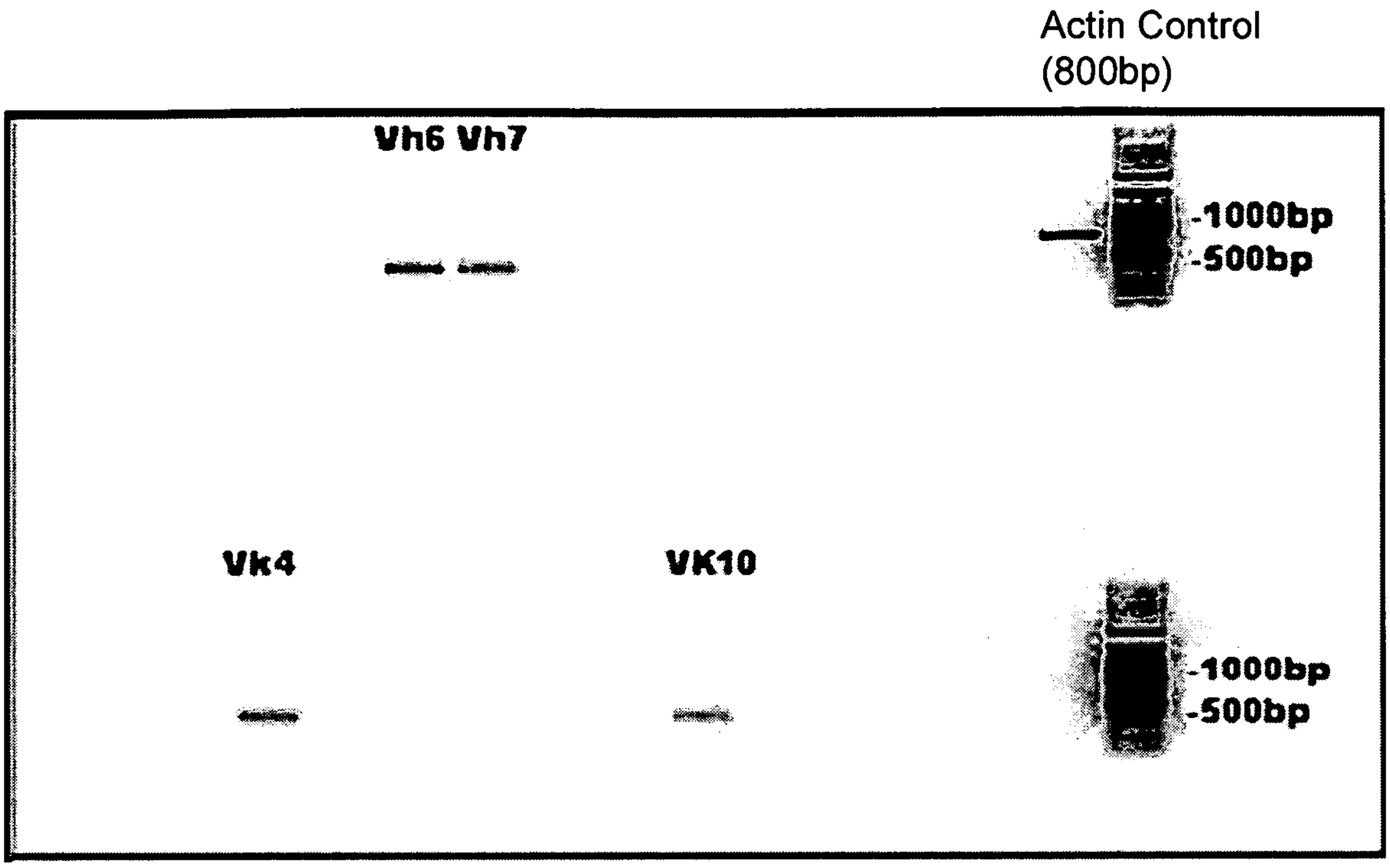

Actin Control
(800bp)
Vh6 Vh7
-1000bp
-500bp
Vk4
VK10
-1000bp
-500bp

Figure 2.

TA-V_H6	1	EVQLQQSGPELVKPGASVKIPCKASGYTFTDYNMDWVKQSHGKSLEWIGD	SEQ ID NO:10
TA-V_H7	1	EVQLQQSGPELVKPGASVKIPCKASGYTFTDYNMDWVKQSHGKSLEWIGD	SEQ ID NO:11
TA-V_H6	51	INPNNGGTIYNQKFKGKATLTVDKSSSTAYMELRSLTSEDTAVYYCARGY	SEQ ID NO:10
TA-V_H7	51	INPNNGGTIYNQKFKGKATLTVDKSSSTAYMELRSLTSEDTAVYYCARGY	SEQ ID NO:11
TA-V_H6	101	YSNSAWFAYWGQGTLVTVSA	SEQ ID NO:10
TA-V_H7	101	YSNSAWFAYWGQGTLVTVSA	SEQ ID NO:11

TA-V_κ4	1	DIVMSQSPSSLAVSAGEKVTMSCKSSQSLLNSRTRKNYLAWYQQKPGQSP	SEQ ID NO:9
TA-V_κ4	51	KLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCKQSYNL	
TA-V_κ4	101	PWTFGGGTKLEIKR	

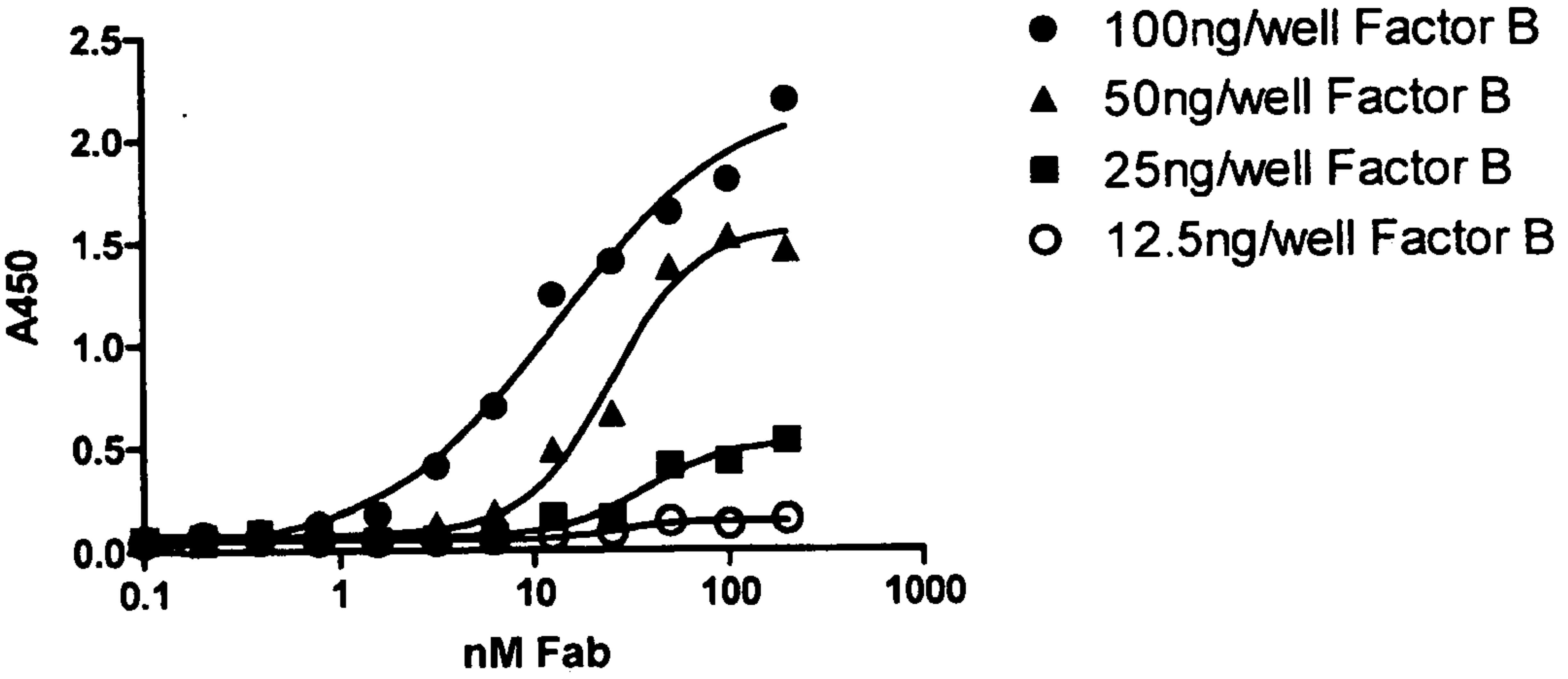
Figure 3.

1379H	E V Q - - Q S G P E L V K P G A S V K I P	SEQ ID NO:31
TA-V_H6	E V Q L Q Q S G P E L V K P G A S V K I P	SEQ ID NO:33

1379L	D I V M S Q S P S S L A V S A G E K V T M S S K K	SEQ ID NO:32
TA-$V_κ$4	D I V M S Q S P S S L A V S A G E K V T M S C K S	SEQ ID NO:34

Figure 4.

ELISA: Chimeric TA003 binding to Factor B



ELISA: Murine Fab (1379) binding to Factor B

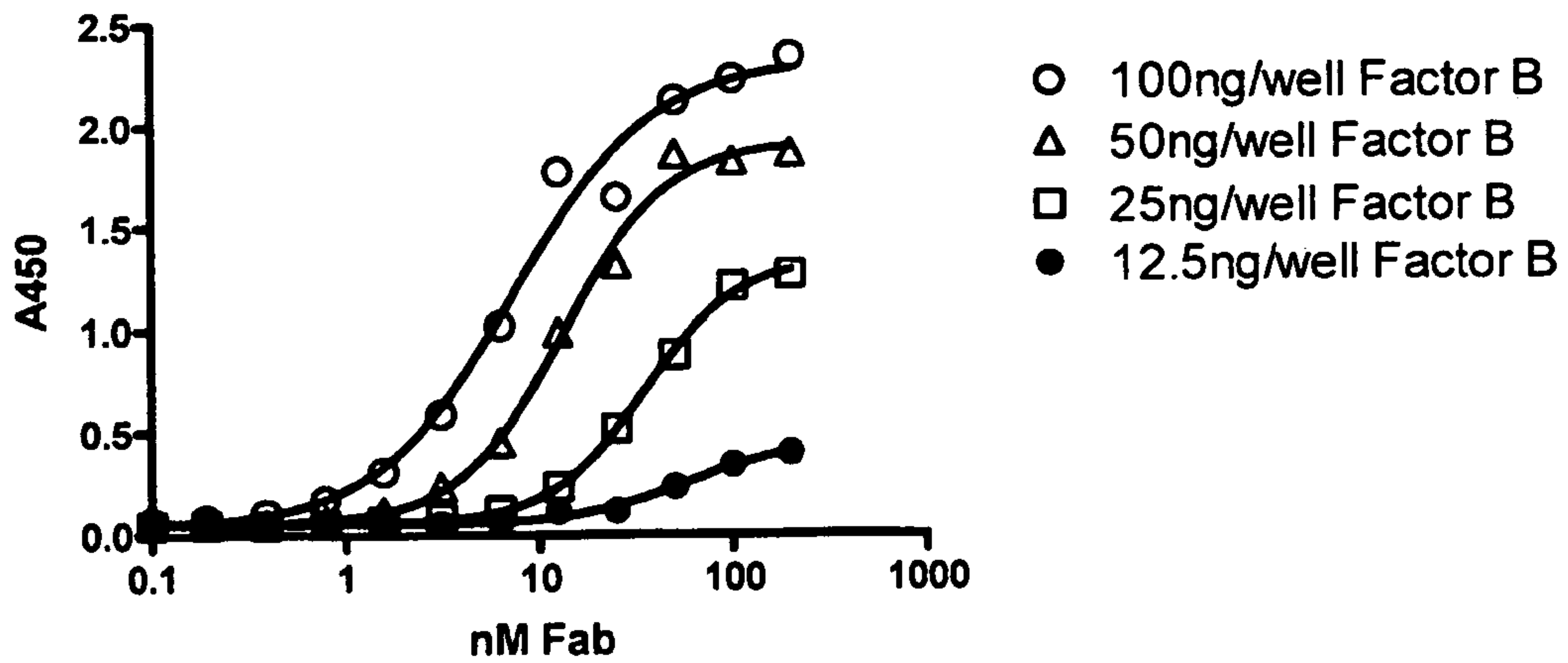


Figure 5.

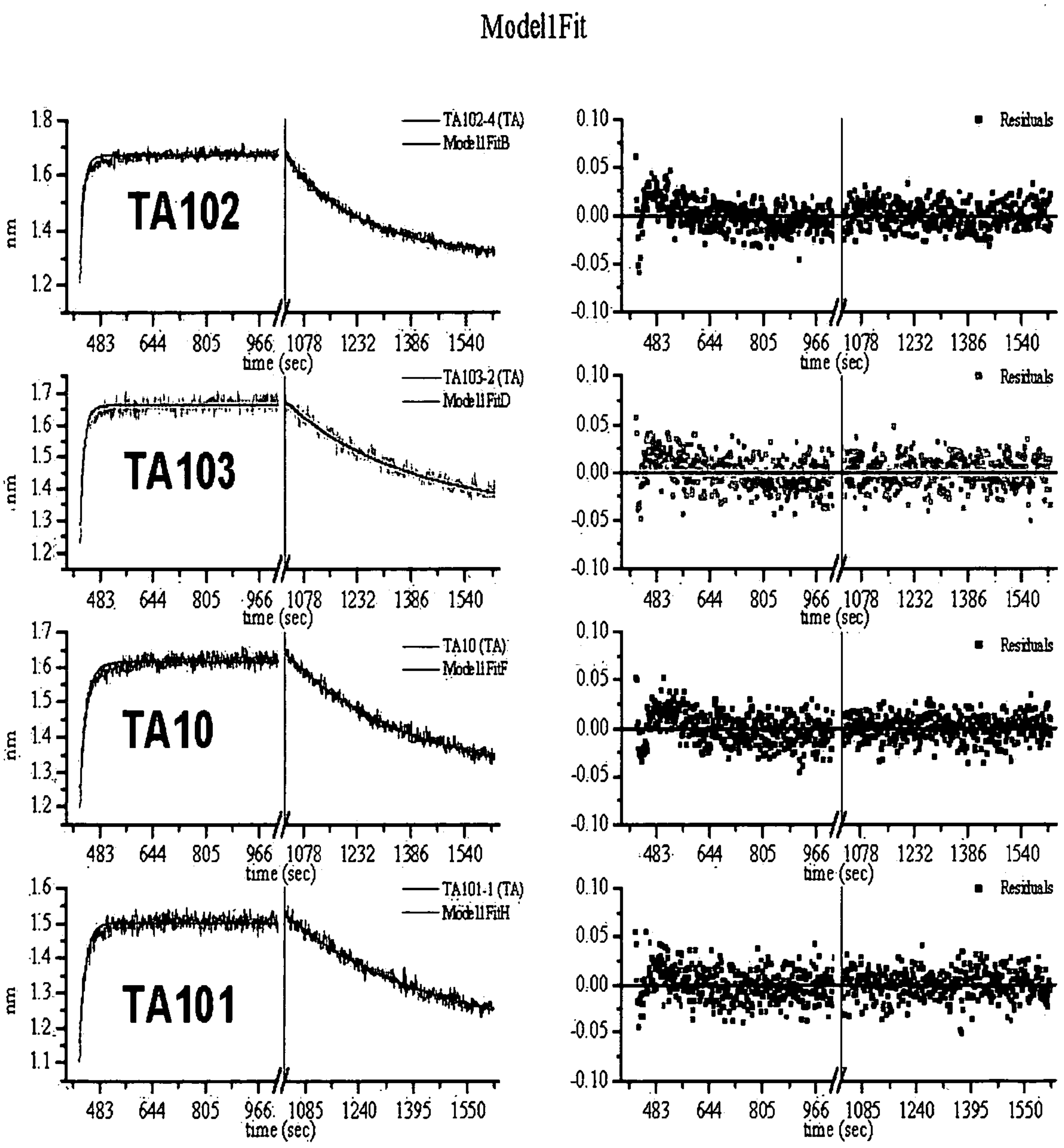

Model1Fit
TA102
TA103
TA10
TA101
TA102-4 (TA)
Model1FitB
TA103-2 (TA)
Model1FitD
TA10 (TA)
Model1FitF
TA101-1 (TA)
Model1FitH
Residuals
nm
time (sec)

Figure 6.

V_H Alignment

1-02/J_H4	1-QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVT	SEQ ID NO:13
TA10	1-EVQLQQSGPELVKPGASVKIPCKASGYTFTDYNMDWVKQSHGKSLEWIGDINPNNGGTIYNQKFKGKAT	SEQ ID NO:15
TA101-1	1-QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYYMIWVRQAPGQGLEWMGWINPNSGGTKYAQKFQGRVT	SEQ ID NO:17
TA102-4	1-QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYYMIWVRQAPGQGLEWMGWINPNSGGTKYAQKFQGRVT	SEQ ID NO:19
TA103-2	1-QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYYMIWVRQAPGQGLEWMGWINPNSGGTKYAQKFQGRVT	SEQ ID NO:21

(CDR1: GYYMH / DYNMD / DYYMI; CDR2: WINPNSGGTNYAQKFQG …)

1-02/J_H4	70-MTRDTSISTAYMELSRLRSDDTAVYYCAR YFDYWGQGTLVTVSS	SEQ ID NO:13
TA10	70-LTVDKSSSTAYMELRSLTSEDTAVYYCARGYYSNSAWFAYWGQGTLVTVSS	SEQ ID NO:15
TA101-1	70-MTRDTSISTAYMELSRLRSDDTAVYYCARGYYANSAWFAYWGQGTLVTVSS	SEQ ID NO:17
TA102-4	70-MTRDTSISTAYMELSRLRSDDTAVYYCARGYYANSAWFAYWGQGTLVTVSS	SEQ ID NO:19
TA103-2	70-MTRDTSISTAYMELSRLRSDDTAVYYCARGYYANSAWFAYWGQGTLVTVSS	SEQ ID NO:21

(CDR3)

V_κ Alignment

V_κIV B3/J_κ2	1-DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPD	SEQ ID NO:12
TA10	1-DIVMSQSPSSLAVSAGEKVTMSCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIYWASTRESGVPD	SEQ ID NO:14
TA101-1	1-DIVMTQSPDSLAVSLGERATINCKSSQSLLNSSTSKNYLAWYQQKPGQPPKLLIYWASTRESGVPD	SEQ ID NO:16
TA102-4	1-DIVMTQSPDSLAVSLGERATINCKSSQSVLNSRNKKNYLAWYQQKPGQPPKLLIYWASTRESGVPD	SEQ ID NO:18
TA103-2	1-DIVMTQSPDSLAVSLGERATINCKSSQSLLNSRTSKNYLAWYQQKPGQPPKLLIYWASTRESGVPD	SEQ ID NO:20

(CDR1, CDR2)

VKIV B3/Jk2	67-RFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPWTFGQGTKLEIK	SEQ ID NO:12
TA10	67-RFTGSGSGTDFTLTISSVQAEDLAVYYCKQSYNLPWTFGQGTKLEIK	SEQ ID NO:14
TA101-1	67-RFSGSGSGTDFTLTISSLQAEDVAVYYCKQVYNLPWTFGQGTKLEIK	SEQ ID NO:16
TA102-4	67-RFSGSGSGTDFTLTISSLQAEDVAVYYCKQVYNLPWTFGQGTKLEIK	SEQ ID NO:18
TA103-2	67-RFSGSGSGTDFTLTISSLQAEDVAVYYCKQVYNLPWTFGQGTKLEIK	SEQ ID NO:20

(CDR3)