CLAIMS

1. Use of a composition that inhibits activation of the complement alternative pathway in the manufacture of a medicament for treating a subject having a complement-mediated hemolytic disorder affecting blood cells,

wherein the composition inhibits activation of complement component C3 (C3) and opsonization of red blood cells by fragments of C3.

2. Use of a composition that inhibits activation of the complement alternative pathway in the manufacture of a medicament for treating complement-mediated hemolysis in a subject,

wherein the composition maintains normal serum levels of lactate dehydrogenase and hemoglobin.

3. The use of claim 1 or 2, wherein the composition selectively inhibits the complement alternative pathway.

4. The use of claim 2, wherein a terminal complement inhibitor is to be administered to the subject.

5. The use of any one of claims 1-4, wherein the subject has paroxysmal nocturnal hemoglobinuria (PNH) and the subject's red blood cells are opsonized by fragments of C3 in the absence of the composition.

6. The use of any one of claims 1-5, wherein the composition that inhibits the activity of the complement alternative pathway comprises a fusion protein comprising:

a complement receptor 2 (CR2) protein or biologically active fragment thereof; and

a factor H (fH) protein or biologically active fragment thereof.

7. The use of claim 6, wherein the fusion protein comprises the first four amino-terminal short consensus repeat (SCR) domains of CR2 fused to the first five amino-terminal SCR domains of fH.

8. The use of any one of claims 1-7, wherein the individual has previously been treated with a terminal complement inhibitor.

9. The use of claim 8, wherein the individual was not responsive, partially responsive, or has progressed on the treatment of the terminal complement inhibitor.

10. The use of any one of claims 1-9, wherein the terminal complement inhibitor inhibits cleavage of complement protein C5 (C5).

11. The use of claim 10, wherein the terminal complement inhibitor is a humanized anti-C5 antibody or antigen-binding fragment thereof.

12. The use of claim 11, wherein the terminal complement inhibitor is eculizumab.

13. The use of any one of claims 1-12, wherein the subject has one or more of the following characteristics:

a. the subject exhibits signs or symptoms continued loss of red blood cells by ongoing or intermittent intravascular hemolysis and/or extravascular hemolysis;
b. the subject has red blood cells opsonized by fragments of C3;
c. the subject requires periodic blood transfusions;
d. the subject has low normal or below normal levels of hemoglobin;
e. the subject has low normal or below normal levels of platelets;
f. the subject has high normal or above normal reticulocytes;
g. the subject has high normal or above normal bilirubin; or
h. the subject has iron overload or is at risk of iron overload.

14 The use of claim 13, wherein the subject requires periodic blood transfusions.

15. The use of claim 13, wherein the subject is thereby rendered transfusion independent.

16. The use of claim 13, wherein the subject has below normal levels of hemoglobin.

17. The use of any one of claims 1-16, wherein the composition increases the survival of red blood cells in the subject.

18. The use of any one of claims 1-17, wherein the complement-mediated hemolytic disorder is sickle cell anemia.

TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA, HEMOLYTIC ANEMIAS AND DISEASE STATES INVOLVING INTRAVASCULAR AND EXTRAVASCULAR HEMOLYSIS

RELATED APPLICATION

[0001] This application claims priority benefit of U.S. Provisional Patent Application Serial No. 61/280,567, filed on November 5, 2009~~, the content of which is incorporated herein by reference in its entirety~~.

FIELD OF THE INVENTION

[0002] The present invention relates to methods and materials for treatment of subjects suffering disorders that involve abnormal lysis of red blood cells, including intravascular and extravascular hemolysis, utilizing inhibitors of the complement alternative pathway and complement component C3 fragment activity. More particularly, the present invention relates to methods and materials for treatment of subjects with paroxysmal nocturnal hemoglobinuria, aHUS, hemolytic anemias and other disorders that involve complement-mediated hemolysis, and which may involve an extravascular component which is not adequately treated by terminal inhibitors of complement, such as inhibitors of C5 activity.

BACKGROUND OF THE INVENTION

[0003] Complement-mediated hemolytic anemia is a significant health problem, and contributes to numerous disorders of the red blood cells, such as paroxysmal nocturnal hemoglobinuria (PNH). PNH is a hematological disorder characterized by the clonal expansion of one or a few hematopoietic stem cells which are incapable of glycosylphosphatidylinositol (GPI)-anchor biosynthesis, due to an acquired somatic mutation in the phosphatidylinositol glycan class A (*PIG-A*) gene. Affected progeny cells are deficient in all GPI-anchored surface proteins, including complement regulators CD55 and CD59. Thus, PNH red blood cells (RBCs) are vulnerable to activated complement, and particularly to the membrane attack complex (MAC), resulting in chronic intravascular hemolysis with recurrent exacerbations. Other complement-mediated disorders that affect red blood cells include atypical hemolytic uremic syndrome (aHUS); chronic hemolytic anemia; antibody-mediated autoimmune hemolytic anemia; anemia caused by hemoglobinopathies such as

sickle cell disease; anemia caused by infection such as malaria; anemia due to transfusion reaction; and cold agglutinin disease (CAD).

[0004] Treatment with eculizumab, a monoclonal antibody (Mab) directed against complement component 5 (C5), has been partially effective in PNH and other hematological disorders. However, a significant subpopulation of patients with PNH exhibit suboptimal hematological response to treatment with anti-C5 Mab. In this subpopulation, little improvement of anemia is observed, and some still require blood transfusion, with continuing signs of persistent hemolysis (reticulocytosis, elevated unconjugated bilirubin). Risitano and Rotoli, Biologics, 2:205-222 (2008). The recurrence may be characterized as "breakthroughs" where hemolytic activity may persist despite treatment with terminal complement inhibitors. Hill et al., Blood, 106:2559-65 (2005). For these subjects, a need remains for additional methods and materials for the effective treatment of PNH.

[0005] Complement inhibitors are known in the art, and a new class of targeted complement inhibitors has been developed, which allows treatment in a fashion which results in high localized concentrations of inhibitor at the tissue sites where complement is activated, while minimizing potentially adverse systemic effects. This class of inhibitors includes, for example, TT30 (SEQ ID NO:3), TT31 and TT32. TT30 is an immunomodulatory compound which inhibits the complement alternative pathway. TT30 comprises a complement alternative pathway inhibitory portion of Factor H protein, which is targeted to sites of complement activation and inflammation through fusion with a portion of the complement receptor 2 protein (CR2 or CD21) which is known to bind to tissue/cell-fixed fragments of the complement component 3 (C3). TT31 is similar to TT30, but contains an additional copy of the complement alternative pathway inhibitory portion of Factor H protein. TT32 comprises a complement inhibitory portion of complement receptor 1 (CR1), targeted through fusion with the same portion of the CR2 protein. CR1 is known to be a broader inhibitor of complement than is Factor H. TT32 will therefore inhibit not only the complement alternative pathway, but will locally inhibit both the classic and lectin pathways of complement as well. Suitable targeted inhibitors are described in Gilkeson et al., US Patent Publication 2008/0221011~~, the disclosure of which is hereby specifically incorporated herein by reference~~.

[0022] In preferred embodiments, the inhibitor of the complement alternative pathway may comprise a fusion of the CR2 protein, or a fragment comprising at least the first two amino terminal short consensus repeat (SCR) domains of CR2, fused to factor H (FH) protein, or a biologically active fragment thereof. The biologically active fragment of FH protein may comprise at least the first four amino terminal SCR domains of FH. One such complement inhibitor is TT30 (SEQ ID NO:3), a fusion protein that comprises the first four amino-terminal short consensus repeat (SCR) domains of CR2 fused to the first five amino-terminal SCR domains of FH. Another such complement inhibitor comprises the first two amino terminal SCR domains of CR2 fused to the first four amino-terminal SCR domains of FH. The linkage can be at either end of the domains, such that the fusion protein may be described, proceeding from amino to carboxy termini, as either CR2-FH or FH-CR2.

[0023] In other preferred embodiments, the inhibitor of the complement alternative pathway may comprise a fusion of the CR2 protein, or a targeting fragment thereof comprising at least the first two amino terminal short consensus repeat (SCR) domains of CR2, fused to a complement inhibitor selected from the group consisting of Crry, DAF, MCP, complement factor I, compstatin or CR1, or biologically active fragments thereof. In other preferred embodiments, the inhibitory portion of the fusion protein may comprise an antibody to a factor selected from the group consisting of Factor B, Factor D or properdin; or an antigen-binding fragment thereof. As mentioned above, although not direct inhibitors of the complement alternative pathway, inhibitors of MASP-1 may effectively reduce the amount of C3b present, thereby at least partially inhibiting the amplification loop of the complement alternative pathway. Accordingly, inhibitos of MASP1 protein, such as antibodies to MASP1 and the endogenous MASP1 inhibitor MAP1 may be useful in certain embodiments of the present invention.

[0024] In a preferred embodiment, the inhibitor of the complement alternative pathway comprises a fusion of the CR2 protein, or a targeting fragment thereof comprising at least the first two amino terminal short consensus repeat (SCR) domains of CR2, fused to an antibody to Factor B within the third SCR domain and prevents formation of the C3bBb complex, or an antigen-binding fragment thereof. Suitable antibodies are described, for example, in Holers et al., US Patent Publication 2005/0260198 and in Emblen et al., US Patent Publication 2008/0299114. ~~The disclosure of these documents is hereby specifically incorporated herein by reference.~~ The linkage can be at either end of the domains, such that

protein (CR2 or CD21) which is known to bind to tissue/cell-fixed fragments of the complement component 3 (C3). TT31 is similar to TT30, but contains an additional copy of the complement alternative pathway inhibitory portion of Factor H protein. TT32 comprises a complement inhibitory portion of complement receptor 1 (CR1), targeted through fusion with the same portion of the CR2 protein. CR1 is known to be a broader inhibitor of complement than is Factor H. TT32 will therefore inhibit not only the complement alternative pathway, but will locally inhibit both the classic and lectin pathways of complement as well. Other suitable targeted inhibitors are described in Gilkeson et al., US Patent Publication 2008/0221011~~, the disclosure of which is hereby specifically incorporated herein by reference~~.

[0079] The present invention provides methods and compositions for the treatment of subjects having any of a number of complement-mediated disease states which affect red blood cells. Among these are subjects having paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS); chronic hemolytic anemia; antibody-mediated autoimmune hemolytic anemia; anemia caused by hemoglobinopathies such as sickle cell disease; anemia caused by infection such as malaria; anemia due to transfusion reaction; and cold agglutinin disease (CAD). In particular embodiments, the present invention provides methods and compositions for the treatment of subjects with hemolytic anemia, which may be caused by the above disease states, or who exhibit at least one characteristic, which characteristics may be symptoms of residual anemia and/or complement-mediated extravascular hemolysis and/or incomplete control of intravascular hemolysis:

a. the subject exhibits signs or symptoms continued loss of red blood cells by ongoing or intermittent intravascular hemolysis and/or extravascular hemolysis;
b. the subject has red blood cells opsonized by fragments of C3;
c. the subject requires periodic blood transfusions;
d. the subject has low normal or below normal levels of hemoglobin;
e. the subject has low normal or below normal levels of platelets;
f. the subject has high normal or above normal reticulocytes;
g. the subject has high normal or above normal bilirubin; or
h. the subject has iron overload or is at risk of iron overload.

37:1111-22 (2000). Accordingly, the present invention may include methods for assessing the binding avidity and affinity of a targeting moiety; mutating or modifying the targeting moiety and assessing the effects of such mutation or modification, in order to obtain targeting moieties with improved targeting characteristics, e.g., binding avidity.

[0126] Although certain sections herein discuss CR2-targeted constructs, it is understood that the same applies to other targeting moieties described herein. The different targeting moieties are described herein in more detail.

CR2 and CR2 Fragments.

[0127] The use of complement receptor 2 (CR2), or functional fragments thereof, to target complement modulators to tissue which exhibit or express C3, or fragments of C3 to which the CR2 is able to bind, including C3b, iC3b, C3d and C3dg, is described in US 2008/0267980, Tomlinson and Holers~~, the disclosure of which is hereby incorporated herein by reference~~. Such CR2 molecules, and functional fragements thereof, can be used in the present invention as the targeting moiety. In particularly preferred embodiments, the first two N-terminal short concensus repeat domains (SCRs), comprising an active binding site for C3dg, can be used in the present invention as the targeting moiety. The present inventors have found that, while red blood cells are normally privileged from complement attack, the complement-mediated blood disorders treatable by the present invention may lead to abnormal coating of the RBC surface with C3 and/or C3 fragments, rendering the affected RBCs vulnerable to complement attack and opsonization.

[0128] Human complement receptor 2, also referred to as CD21 (CR2/CD21)(SEQ ID NO:1 and SEQ ID NO:2), is a ~145 kD transmembrane protein of the C3 binding protein family comprising 15 or 16 short consensus repeat (SCR) domains, structural units characteristic of such proteins. CR2 is expressed on mature B cells and follicular dendritic cells, and plays an important role in humoral immunity. J. Hannan et al., Biochem. Soc. Trans. (2002) 30:983-989; K.A. Young et al., J. Biol. Chem. (2007) 282(50):36614-36625. CR2 protein does not bind intact C3 protein, but binds its breakdown products, including the C3b, iC3b, and C3d cleavage fragments, via a binding site located within the first two amino-terminal short consensus repeats ("SCRs 1-2") of the CR2 protein. Consequently, the SCR1-2 domain of CR2 discriminates between cleaved (i.e., activated) forms of C3 and intact circulating C3. As a targeting group, SCRs 1-2 of CR2 are therefore able to discriminate between circulating C3

the first two N-terminal SCR domains of CR2, including for example at least any of the first 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 SCR domains of CR2.

[0132] Cleavage of C3 results initially in the generation and deposition of C3b on the activating cell surface. The C3b fragment is involved in the generation of enzymatic complexes that amplify the complement cascade. On a cell surface, C3b is rapidly converted to inactive iC3b, particularly when deposited on a host surface containing regulators of complement activation (i.e., most host tissue). Even in the absence of membrane-bound complement regulators, substantial levels of iC3b are formed because of the action of serum factor H and serum factor I. iC3b is subsequently digested to the membrane-bound fragments C3dg and then C3d by factor I and other proteases and cofactors, but this process is relatively slow. Thus, the C3 ligands for CR2 are relatively long lived once they are generated and are present in high concentrations at sites of complement activation.

Antibodies to C3 and C3 Fragments. ***Antibodies to C3 and C3 Fragments.***

[0133] In place of a targeting moiety derived from CR2, the targeting moiety may comprise antibodies which bind to C3 or C3 fragments such as C3b, iC3b, C3d and C3dg, or an antigen-binding fragment thereof. Antibodies that bind to C3 and to cleavage fragments C3b and C3d, are known. For example, see US Patent 6,572,856, Taylor; Tosic et al., J. Immunological Methods, 120:241-249 (1989); Sokoloff et al., Cancer Immunology and Immunotherapy, 49:551-562 (2000); Mastellos et al., Molecular Immunology, 40:1213-1221 (2004); Dilillo et al., Molecular Immunology, 43:1010-1019 (2006); Campagne, US 2009/0081211; Etemad-Gilbertson et al., US 2009/0175875; Aguado et al., J. Clin. Invest., 76:1418-1426 (1985). ~~The disclosure of these documents is hereby incorporated herein by reference.~~ Such antibodies, and functional fragments thereof, may be useful in the present invention as the targeting moiety for directing therapeutic fragments to tissue experiencing activated complement activity, and thus expressing C3 or its fragments. Functional fragments of antibodies may include, for example, single-chain variable fragments (scFvs) which preferably comprise of V_H and V_L domains, optionally joined by a flexible peptide linker. Such scFvs usually retain the specificity of the parental antibody and bind the target antigen in a monovalent manner. Conversion of antibodies to scFvs is well known process (see Nat. Biotechnol. 23:1126 (2005); Biomol Eng. 24:201 (2007); J Immunol Methods., 168:149 (1994); Arch Virol. 148:497 (2003). ScFvs can be constructed by either de novo gene synthesis, overlap extension polymerase chain reaction (PCR) or sequential ligation of

the individual heavy (V_H) and light (V_L) chain variable gene segments. Sequential cloning of the individual V_H and V_L genes into a vector containing the synthetic linker sequence (e.g., $(Gly_4Ser)_3$) can be performed as well. The sequence can be either V_H–linker-V_L or V_L–linker-V_H.

[0134] In particularly preferred embodiments, the targeting moiety may comprise an antibody which binds to C3d, such as those produced by: (1) hybridoma cell line 3d-9a/25, deposited on May 26, 2010, and designated as ATCC Patent Deposit PTA-10998; (2) hybridoma cell line 3d-8b/2, deposited on May 26, 2010, and designated as ATCC Patent Deposit PTA-10999; (3) hybridoma cell line 3d-29/5/2, deposited on May 26, 2010, and designated as ATCC Patent Deposit PTA-11000; (4) hybridoma cell line 3d-10/14/1, deposited on June 2, 2010, and designated as ATCC Patent Deposit PTA-11010; (5) hybridoma cell line 3d-11/14, deposited on June 2, 2010, and designated as ATCC Patent Deposit PTA-11011; (6) hybridoma cell line 3d-15A9, deposited on June 2, 2010, and designated as ATCC Patent Deposit PTA-11012; (7) hybridoma cell line 3d-3/28/4, deposited on June 9, 2010, and designated as ATCC Patent Deposit PTA-11025; (8) hybridoma cell line 3d-16/3/3, deposited on June 9, 2010, and designated as ATCC Patent Deposit PTA-11026; and (9) hybridoma cell line 3d-31/A6/9, deposited on June 9, 2010, and designated as ATCC Patent Deposit PTA-11027. Those antibodies are described in more detail in the US Provisional Patent Application Serial No. 61/357,499, filed on June 22, 2010, entitled "Antibodies to the C3d Fragment of Complement Component 3 ," ~~which is incorporated herein by reference in its entirety~~. The complete nucleotide and amino acid sequences of the monoclonal antibodies produced by those hybridoma cell lines can easily be determined with standard methods such as, for example, the polymerase chain reaction (PCR) and automated sequencing.

Conversion of anti-C3d monoclonal antibodies into single-chain variable fragments

[0135] (scFvs). Conversion of antibodies to scFvs is well known process (see Nat. Biotechnol. 23:1126 (2005); Biomol Eng. 24:201 (2007); J Immunol Methods., 168:149 (1994); Arch Virol. 148:497 (2003). ScFvs can be constructed by either de novo gene synthesis, overlap extension polymerase chain reaction (PCR) or sequential ligation of the individual heavy (V_H) and light (V_L) chain variable gene segments. Sequential cloning of the individual V_H and V_L genes into a vector containing the synthetic linker sequence (e.g.,

INHIBITION OF COMPLEMENT ALTERNATIVE PATHWAY

[0141] The compositions described herein in some embodiments comprise an active agent moiety that significantly or selectively inhibits the complement alternative pathway. By "significantly inhibiting the complement alternative pathway" it is meant that the active agent inhibits the complement alternative pathway with a potency or percent inhibition that measures at least 10%, preferably 20%, 30% or 40%, and more preferably at least about 50%, 60%, 80% or 90% of alternative complement activity in the absence of said active agent moiety. By "selectively inhibits the complement alternative pathway" it is meant that the active agent inhibits the complement alternative pathway with a potency or percent inhibition which measures at least two, three, four, five or ten times greater than that of the classical or lectin complement pathways. More preferably, the active agent will selectively inhibit the complement alternative pathway at a potency or percent inhibition of at least one, two or three orders of magnitude greater than that the classical or lectin complement pathways.

[0142] In preferred embodiments, active agent moiety comprises an inhibitor of the complement alternative pathway, or a biologically active fragment thereof, selected from the group consisting of Factor H protein, Crry, Decay Accelerating Factor (DAF), MCP, CR1, or biologically active fragments thereof. In some embodiments, the active moiety is an inhibitor of the amplification loop of the alternative complement pathway and will prevent the formation and activity of the amplification C3 convertase, as well as the downstream C5 convertase. In some embodiments, the active moiety will be able to inactivate the initiation C3 convertase once it is formed on the red blood cell surface, and may partially prevent formation of the initiation C3 convertase in the fluid phase.

[0143] In a particularly preferred embodiment, the active agent moiety comprises Factor H protein or a biologically active fragment thereof, which retains the ability to inhibit the complement alternative pathway. Suitable Factor H protein an biologically active fragments thereof include the first four N-terminal short consensus sequences (SCRs of Factor H, and are described in US 2008/0221011, Gilkeson et al~~, the disclosure of which is hereby specifically incorporated herein by reference~~.

[0144] Other complement inhibitors, and fragments thereof, which may be useful as the active agent moiety in the present invention are described, for example, in Tomlinson and Holers, US 2008/0267980. Suitable complement inhibitors, for example, may include

complement receptor 1 (CR1); MCP; Crry; or DAF. ~~The disclosure of Tomlinson and Holers is hereby specifically incorporated herein by reference.~~ Other complement inhibitors, which may be useful as the active agent moiety include compstatin, see Janssen et al., J. Biol. Chem. 282:29241-7 (2007). In preferred embodiments, the complement inhibitor is an inhibitor of the amplification loop of the alternative complement pathway.

[0145] In certain embodiments, the active moiety comprises two or more complement inhibitors, for example, two or more biologically active fragments of factor H as in TT31; or an active fragment of factor H in combination with an active fragment of complement receptor 1, MCP; Crry; complement factor I; CD59 or DAF. In such embodiments, the active moiety may be provided in multiple forms, for example, when the active moiety comprises a combination of Factor H and CD59, the composition may include two or more of the following: CR2-FH-CD59; CR2-CD59-FH; antiC3d-FH-CD59 and antiC3d-CD59-FH.

[0146] As used herein, the term "biologically active" fragment of a complement inhibitor refers a fragment of the complement inhibitor which retains some or all of the inhibitory activity of the full-length complement inhibitory protein. For example, "biologically active" fragments of factor H include, but are not limited to, factor H fragments comprising SCRs 1-4, SCRs 1-5, SCRs 1-8, SCRs 1-18, SCRs 19-20, or any homolog of a naturally-occurring factor H or fragment thereof, as described in detail below. In certain embodiments, the biologically active fragment of factor H has one or more of the following properties: (1) binding to C-reactive protein (CRP), (2) binding to C3b, (3) binding to heparin, (4) binding to sialic acid, (5) binding to endothelial cell surfaces, (6) binding to cellular integrin receptor, (7) binding to pathogens, (8) C3b co-factor activity, (9) C3b decay-acceleration activity, and (10) inhibiting the alternative complement pathway.

[0147] It is contemplated that variants and modifications of the complement inhibitors described above may be used as the active agent moiety in certain embodiments of the present invention. For example, through deletion analysis, it may be possible to identify smaller fragments of some of the above complement inhibitors, which comprise the minimal sequence elements required for complement inhibition. In other embodiments, the complement inhibitors, or the minimal sequence elements thereof required for complement inhibition, may be modified in order to increase half-life, stability or potency of the active agent moiety as an inhibitor. For example, the active agent moiety may comprise the complement inhibitor, or active fragment thereof, tethered to a protein or non-protein scaffold

Such DNA viruses include adenoviruses (preferably Ad2 or Ad5 based vectors), herpes viruses (preferably herpes simplex virus based vectors), and parvoviruses (preferably "defective" or non-autonomous parvovirus based vectors, more preferably adeno-associated virus based vectors, most preferably AAV-2 based vectors). See, e.g., Ali et al. (1994) Gene Therapy 1:367-384; U.S. Pat. No. 4,797,368~~, incorporated herein by reference,~~ and U.S. Pat. No. 5,139,941.

[0159] Retroviruses from which the retroviral plasmid vectors hereinabove mentioned may be derived include, but are not limited to, Moloney Mouse Leukemia Virus, spleen necrosis virus, retroviruses such as Rotis Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus. In one embodiment, the retroviral plasmid vector is derived from Moloney Mouse Leukemia Virus.

[0160] Adenoviruses have the advantage that they have a broad host range, can infect quiescent or terminally differentiated cells, such as neurons or hepatocytes, and appear essentially non-oncogenic. See, e.g., Ali et al. (1994), supra, p. 367. Adenoviruses do not appear to integrate into the host genome. Because they exist extrachromosomally, the risk of insertional mutagenesis is greatly reduced. Ali et al. (1994), supra, p. 373.

[0161] Adeno-associated viruses exhibit similar advantages as adenoviral-based vectors. However, AAVs exhibit site-specific integration on human chromosome 19 (Ali et al. (1994), supra, p. 377).

[0162] The gene therapy vectors may include one or more promoters. In some embodiments, the vector has a promoter that drives expression in multiple cell types. In some embodiments, the vector has a promoter that drives expression in specific cell types (such as cells of retina or cells in the kidney). Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CVM) promoter described in Miller et al. (1989) Biotechniques 7(9):980-990, or any other promoter (e.g., cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and .beta.-actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

instruction(s) on methods of using the composition, such as uses described herein. The kits described herein may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for performing any methods described herein.

[0171] The compositions and formulations of the present invention are useful for the treatment of conditions associated with complement activation, preferably those which involve the complement alternative pathway, which is largely unaffected by terminal complement inhibitors [e.g., inhibitors of steps of the complement pathway subsequent to the activation of C3].

[0172] In the foregoing specification and in the examples below, the invention has been described with specific embodiments thereof. However, it will be evident to those skilled in the art that various modifications and changes may be made thereto without departing from the broader scope of the invention.

~~**[0173]** All publications that are cited herein are hereby specifically incorporated herein by reference into the disclosure for the teachings for which they are cited.~~

EXAMPLES

EXAMPLE 1. Demonstration of Possible Mechanism of Protection of Red Blood Cells from Hemolytic Lysis in Paroxysmal Nocturnal Hemoglobinuria (PNH).

[0174] This example uses TT30 (SEQ ID NO:3) and PNH as an example to demonstrate a possible mechanism under which TT30 can protect red blood cells from hemolytic lysis.

[0175] PNH RBCs lack the ability to control C3 convertase via CD55 and C5 convertase-mediated formation of the membrane attack complex (MAC) via CD59. In the absence of local complement alternative pathway (CAP) control (see Figure 20), spontaneous tickover generates fluid-phase C3(H_2O) that associates with factor B (fB) and properdin (P) in the presence of factor D (fD) to form the fluid-phase *initiation* C3 convertase, which cleaves C3 to release C3a and deposit covalently-bound C3b on the RBC surface. Association of C3b with fB and P in the presence of fD generates the surface-bound *amplifying* C3 convertase. The CAP amplification loop generates additional C3b and C3 convertase, and by adding C3b to C3 convertase, forms surface-bound C5 convertase. C5 convertase cleaves C5 to release

TABLE 1A

IC_{10} and IC_{90} Values for TT30 Inhibition of CAP-mediated Hemolysis of $CD59^-$ PNH RBCs in an In Vitro Hemolysis Assay

Time (hr)	IC_{10} (μg/mL)	IC_{90} (μg/mL)
1	4.3	87.9
3	5.9	57.5
6	11.4	30.3
24	16.5	55.9

IC_{10} and IC_{90} values calculated using data illustrated in Figure 32.

[0203] In addition, when C3frag+ PNH RBCs from PNH patients on eculizumab were utilized, TT30 did not allow further C3frag accumulation and TT30 could be demonstrated to be bound to the surface of these protected PNH RBCs. These results suggest that modulation of the CAP with TT30 is more effective than inhibition of C5 with eculizumab at preventing hemolysis of PNH RBCs, and that, unlike eculizumab, TT30 also inhibits the C3 activation and deposition on surviving PNH RBCs. It is thus hypothesized that the in vitro hemolysis of RBCs from PNH patients on eculizumab despite the presence of protective concentrations of eculizumab may be related to increased susceptibility to lysis of C3frag+ RBCs. An alternative explanation might be that, in addition to being coated with C3frag, the PNH RBCs become coated by C3 and C5 convertases and that some small amount of C5 is released by eculizumab and cleaved by the C5 convertases, leading to MAC formation and lysis.

EXAMPLE 3: Biological Mechanism of Residual Anemia in PNH Subjects Treated with Anti-C5 Antibodies.

[0204] The following experiment was conducted to investigate the biological mechanisms underlying residual anemia in some PNH subjects on eculizumab, looking for a possible role of the early steps of the complement cascade. The results have been published in Risitano et al., Blood, 113:4094-4100 (2009)~~, the disclosure of which is hereby incorporated herein by reference~~.

[0205] A total of 56 PNH subjects were examined. Of these, 41 had previously received eculizumab treatment; and 15 did not receive eculizumab treatment. All subjects receiving eculizamab were treated according to a standard procedure: (900 mg every 14±2 days, after a loading phase of 600 mg every 7±1 days for 4 doses). Thirteen subjects were analyzed before

Claims

1. A CR2-FH molecule for treating a subject having a complement-mediated hemolytic disorder affecting blood cells in combination with a terminal complement inhibitor, said CR2-FH molecule comprising:

a) a CR2 portion comprising a CR2 or a fragment thereof; and
b) a FH portion comprising a FH or a fragment thereof;

wherein said CR2 portion of the CR2-FH molecule is capable of binding to a CR2 ligand, and wherein said FH portion of the CR2-FH molecule is capable of inhibiting complement activation of the alternative pathway.

2. The CR2-FH molecule of claim 1, wherein:

(i) said CR2 portion comprises the first two amino-terminal short consensus repeat (SCR) domains of CR2; or
(ii) said FH portion comprises the first four amino-terminal SCR domains of FH.

3. The CR2-FH molecule of claim 1, wherein said CR2 portion comprises the first four amino-terminal SCR domains of CR2.

4. The CR2-FH molecule of claim 1, wherein said FH portion comprises the first five amino-terminal SCR domains of FH.

5. The CR2-FH molecule of claim 1 or 2, wherein said CR2-FH molecule comprises the first two amino-terminal SCR domains of CR2 and said FH portion comprises the first four amino-terminal SCR domains of FH.

6. The CR2-FH molecule of claim 1 or 2, wherein said CR2-FH molecule comprises the first four amino-terminal SCR domains of CR2 and said FH portion comprises the first five amino-terminal SCR domains of FH.

7. The CR2-FH molecule of claim 1 or 2, wherein said CR2 portion comprises amino acids 23 to 271 of SEQ ID NO: 1.

8. The CR2-FH molecule of claim 1 or 2, wherein said CR2-FH molecule comprises the amino acid sequence of SEQ ID NO: 3.

9. The CR2-FH molecule of any one of claims 1 to 8, wherein said CR2-FH molecule comprises two or more FH portions, wherein each of said two or more FH portions comprises a FH or a fragment thereof that is capable of inhibiting complement activation of the alternative pathway.

10. The CR2-FH molecule of claim 9, wherein said CR2 portion and the FH portion is fused with a linker.

11. The CR2-FH molecule of any one of claims 1 to 10, wherein said subject was not responsive, partially responsive, initially responsive but experiencing recurrence, or has progressed on the treatment of the terminal complement inhibitor.

12. The CR2-FH molecule of any one of claims 1 to 11, wherein said hemolytic disorder results in a symptom selected from the group consisting of:

(a) continued loss of red blood cells by ongoing or intermittent intravascular hemolysis and/or extravascular hemolysis;
(b) red blood cells opsonized by fragments of C3;
(c) a need for periodic blood transfusions;
(d) low normal or below normal levels of hemoglobin;
(e) low normal or below normal levels of platelets;
(f) high normal or above normal reticulocytes;
(g) high normal or above normal bilirubin;
(h) iron overload or a risk of iron overload;
(i) residual anemia;
(j) complement-mediated extravascular hemolysis (EVH); and
(k) incomplete control of intravascular hemolysis (IVH).

13. The CR2-FH molecule of claim 12, wherein said complement-mediated hemolytic disorder results in:

(i) a need for periodic blood transfusions; or
(ii) below normal levels of hemoglobin.

14. The CR2-FH molecule of any one of claims 1 to 13, wherein:

(i) the CR2-FH molecule increases the survival of red blood cells in the subject;
(ii) the CR2-FH molecule prevents or reduces opsonization of C3 fragments on red blood cells (RBCs) in said subject;
(iii) the CR2-FH molecule protects RBCs from hemolytic lysis in said subject; or
(iv) said subject is rendered transfusion independent after administering the CR2-FH molecule.

15. The CR2-FH molecule of claim 14, wherein the CR2-FH molecule protects RBCs from extravascular hemolysis (EVH).

16. The CR2-FH molecule of any one of claims 1 to 15, wherein said complement-mediated hemolytic disorder is selected from the group consisting of: paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), chronic hemolytic anemia, antibody-mediated autoimmune hemolytic anemia, anemia caused by hemoglobinopathies, sickle cell disease, anemia caused by infection, malaria, anemia due to transfusion reaction, and cold agglutinin disease (CAD).

17. The CR2-FH molecule of claim 16, wherein the complement-mediated hemolytic disorder is paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), or sickle cell anemia.

18. The CR2-FH molecule of any one of claims 1 to 17, wherein said CR2-FH molecule is formulated for intravenous, intraperitoneal, intraocular, intra-arterial, intrapulmonary, oral, inhalation, intravesicular, intramuscular, intra-tracheal, subcutaneous, intraocular, intrathecal, transdermal, transpleural, intraarterial, topical, inhalational, mucosal, subcutaneous, transdermal, gastrointestinal, intraarticular, intracistemal, intraventricular, rectal, vaginal, intracranial, intraurethral, intrahepatic, or intratumoral administration.

19. The CR2-FH molecule of claim 18, wherein said CR2-FH molecule is formulated for intravenous administration.

20. The CR2-FH molecule of any one of claims 1 to 19, wherein said subject is administered said CR2-FH molecule and said terminal complement inhibitor simultaneously or sequentially in either order.

21. The CR2-FH molecule of claim 20, wherein said subject is administered said terminal complement inhibitor prior to administration of said CR2-FH molecule.

22. The CR2-FH molecule of claim 21, wherein said subject is administered said CR2-FH molecule 1 day to 8 weeks after administration of said terminal complement inhibitor.

23. The CR2-FH molecule of claim 21, wherein said subject is administered said CR2-FH molecule at least 1 week after the administration of said terminal complement inhibitor.

24. The CR2-FH molecule of claim 21, wherein said subject is administered said CR2-FH molecule at least 2 weeks after the administration of said terminal complement inhibitor

25. The CR2-FH molecule of claim 20, wherein said subject is administered said CR2-FH molecule prior to administration of said terminal complement inhibitor.

26. The CR2-FH molecule of claim 25, wherein said subject is administered said terminal complement inhibitor 1 day to 8 weeks after administration of said CR2-FH molecule.

27. The CR2-FH molecule of claim 25, wherein said subject is administered said terminal complement inhibitor at least 1 week after the administration of said CR2-FH molecule.

28. The CR2-FH molecule of claim 25, wherein said subject is administered said terminal complement inhibitor at least 2 week after the administration of said CR2-FH molecule.

29. The CR2-FH molecule of any one of claims 1 to 28, wherein:

(i) the terminal complement inhibitor inhibits cleavage of complement protein C5 (C5); or

(ii) the terminal complement inhibitor inhibits the formation of the Membrane Attack Complex (MAC).

30. The CR2-FH molecule of any one of claims 1 to 29, wherein the terminal complement inhibitor is a humanized anti-C5 antibody or antigen-binding fragment thereof.

31. The CR2-FH molecule of any one of claims 1 to 30, wherein the terminal complement inhibitor is eculizumab or pexelizumab.

32. Use of CR2-FH molecule for the preparation of a medicament for treating a subject having a complement-mediated hemolytic disorder affecting blood cells, wherein the medicament is to be administered in combination with a terminal complement inhibitor, said CR2-FH molecule comprising:

b) a CR2 portion comprising a CR2 or a fragment thereof; and
b) a FH portion comprising a FH or a fragment thereof;

wherein said CR2 portion of the CR2-FH molecule is capable of binding to a CR2 ligand, and wherein said FH portion of the CR2-FH molecule is capable of inhibiting complement activation of the alternative pathway.

33. The use of claim 32, wherein:

(ii) said CR2 portion comprises the first two amino-terminal short consensus repeat (SCR) domains of CR2; or
(ii) said FH portion comprises the first four amino-terminal SCR domains of FH.

34. The use of claim 32, wherein said CR2 portion comprises the first four amino-terminal SCR domains of CR2.

35. The use of claim 32, wherein said FH portion comprises the first five amino-terminal SCR domains of FH.

36. The use of claim 32 or 33, wherein said CR2-FH molecule comprises the first two amino-terminal SCR domains of CR2 and said FH portion comprises the first four amino-terminal SCR domains of FH.

37. The use of claim 32 or 33, wherein said CR2-FH molecule comprises the first four amino-terminal SCR domains of CR2 and said FH portion comprises the first five amino-terminal SCR domains of FH.

38. The use of claim 32 or 33, wherein said CR2 portion comprises amino acids 23 to 271 of SEQ ID NO: 1.

39. The use of claim 32 or 33, wherein said CR2-FH molecule comprises the amino acid sequence of SEQ ID NO: 3.

40. The use of any one of claims 32 to 39, wherein said CR2-FH molecule comprises two or more FH portions, wherein each of said two or more FH portions comprises a FH or a fragment thereof that is capable of inhibiting complement activation of the alternative pathway.

41. The use of claim 40, wherein said CR2 portion and the FH portion is fused with a linker.

42. The use of any one of claims 32 to 41, wherein said subject was not responsive, partially responsive, initially responsive but experiencing recurrence, or has progressed on the treatment of the terminal complement inhibitor.

43. The use of any one of claims 32 to 42, wherein said hemolytic disorder results in a symptom selected from the group consisting of:

(a) continued loss of red blood cells by ongoing or intermittent intravascular hemolysis and/or extravascular hemolysis;
(b) red blood cells opsonized by fragments of C3;
(c) a need for periodic blood transfusions;
(d) low normal or below normal levels of hemoglobin;
(e) low normal or below normal levels of platelets;
(f) high normal or above normal reticulocytes;
(g) high normal or above normal bilirubin;
(h) iron overload or a risk of iron overload;
(i) residual anemia;
(j) complement-mediated extravascular hemolysis (EVH); and
(k) incomplete control of intravascular hemolysis (IVH).

44. The use of claim 43, wherein said complement-mediated hemolytic disorder results in:

(i) a need for periodic blood transfusions; or
(ii) below normal levels of hemoglobin.

45. The use of any one of claims 32 to 44, wherein:

(i) the CR2-FH molecule increases the survival of red blood cells in the subject;
(ii) the CR2-FH molecule prevents or reduces opsonization of C3 fragments on red blood cells (RBCs) in said subject;
(iii) the CR2-FH molecule protects RBCs from hemolytic lysis in said subject; or
(iv) said subject is rendered transfusion independent after administering the CR2-FH molecule.

46. The use of claim 45, wherein the CR2-FH molecule protects RBCs from extravascular hemolysis (EVH).

47. The use of any one of claims 32 to 46, wherein said complement-mediated hemolytic disorder is selected from the group consisting of: paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), chronic hemolytic anemia, antibody-mediated autoimmune hemolytic anemia, anemia caused by hemoglobinopathies, sickle cell disease, anemia caused by infection, malaria, anemia due to transfusion reaction, and cold agglutinin disease (CAD).

48. The use of claim 47, wherein the complement-mediated hemolytic disorder is paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), or sickle cell anemia.

49. The use of any one of claims 32 to 48, wherein said CR2-FH molecule is formulated for intravenous, intraperitoneal, intraocular, intra-arterial, intrapulmonary, oral, inhalation, intravesicular, intramuscular, intra-tracheal, subcutaneous, intraocular, intrathecal, transdermal, transpleural, intraarterial, topical, inhalational, mucosal, subcutaneous, transdermal, gastrointestinal, intraarticular, intracistemal, intraventricular, rectal, vaginal, intracranial, intraurethral, intrahepatic, or intratumoral administration.

50. The use of claim 49, wherein said CR2-FH molecule is formulated for intravenous administration.

51. The use of any one of claims 32 to 50 wherein said subject is to be administered said CR2-FH molecule and said terminal complement inhibitor simultaneously or sequentially in either order.

52. The use of claim 51, wherein said subject is to be administered said terminal complement inhibitor prior to administration of said CR2-FH molecule.

53. The use of claim 52, wherein said subject is to be administered said CR2-FH molecule 1 day to 8 weeks after administration of said terminal complement inhibitor.

54. The use of claim 52, wherein said subject is to be administered said CR2-FH molecule at least 1 week after the administration of said terminal complement inhibitor.

55. The use of claim 52, wherein said subject is to be administered said CR2-FH molecule at least 2 weeks after the administration of said terminal complement inhibitor

56. The use of claim 51, wherein said subject is to be administered said CR2-FH molecule prior to administration of said terminal complement inhibitor.

57. The use of claim 56, wherein said subject is to be administered said terminal complement inhibitor 1 day to 8 weeks after administration of said CR2-FH molecule.

58. The use of claim 56, wherein said subject is to be administered said terminal complement inhibitor at least 1 week after the administration of said CR2-FH molecule.

59. The use of claim 56, wherein said subject is to be administered said terminal complement inhibitor at least 2 week after the administration of said CR2-FH molecule.

60. The use of any one of claims 32 to 59, wherein:

(i) the terminal complement inhibitor inhibits cleavage of complement protein C5 (C5); or

(ii) the terminal complement inhibitor inhibits the formation of the Membrane Attack Complex (MAC).

61. The use of any one of claims 32 to 60, wherein the terminal complement inhibitor is a humanized anti-C5 antibody or antigen-binding fragment thereof.

62. The use of any one of claims 32 to 61, wherein the terminal complement inhibitor is eculizumab or pexelizumab.