Citation or discussion of a reference herein shall not be construed as an admission that such is prior art to the present invention.

SUMMARY

In one aspect, the present invention refers to a polypeptide selected from the group consisting of hfB3-292S (amino acids 1-764 or 26-764 of SEQ ID NO:2), hfB3-292SN480 (amino acids 1-480 or 26-480 of SEQ ID NO:2), and hfB3-292S/Fc (1-990 or 26-990 of SEQ ID NO:22).

In another aspect, the present invention refers to a nucleic acid comprising a nucleotide sequence encoding the polypeptide as disclosed herein.

In yet another aspect, the present invention refers to a viral vector comprising the nucleic acid as disclosed herein.

In a further aspect, the present invention refers to a pharmaceutical preparation comprising the polypeptide of as disclosed herein, the nucleic acid as disclosed herein, the viral vector as disclosed herein or any combination thereof.

In one aspect, the present invention refers to use of the polypeptide as disclosed herein, the nucleic acid as disclosed herein, the viral vector as disclosed herein, the pharmaceutical composition of as disclosed herein, or any combination thereof in the manufacture of a medicament for inhibiting complement activity, wherein the use comprises introduction or administration to a site of the complement activity the polypeptide as disclosed herein, the nucleic acid as disclosed herein, the viral vector as disclosed herein, the pharmaceutical composition as disclosed herein, or any combination thereof in an amount sufficient to inhibit the complement activity, wherein the polypeptide inhibits the complement activity.

In yet another aspect, the present invention refers to use of the polypeptide, the nucleic acid, the viral vector, the pharmaceutical composition or any combination thereof, as defined herein, in the manufacture of a medicament for treating a complement mediated disease in a patient.

In a further aspect, the present invention refers to a cell comprising the nucleic acid as disclosed herein, wherein the cell expresses the polypeptide.

In another aspect, the present invention refers to an *in vitro* method of producing a polypeptide comprising a complement factor B protein analog, the method comprising expressing in a cell the polypeptide of as disclosed herein; and purifying said polypeptide.

The disclosure describes polypeptides comprising a complement factor B analog. The disclosure also describes various complement factor B analogs. In some examples, a complement factor B analog comprises a mutation of a free cysteine amino acid. The disclosure also describes nucleic acids and viral vectors comprising a nucleotide sequence encoding polypeptides and complement factor B protein analogs of the invention. Some examples provide cells, wherein the cells comprise a nucleic acid encoding a complement factor B protein analog of the invention and wherein the cells express a complement factor B protein analog.

Additionally, the disclosure describes pharmaceutical preparations comprising a polypeptide or complement factor B protein analog as disclosed therein, a nucleic acid of the invention, a viral vector as disclosed herein or any combination thereof.

Also described in by the present disclosure are methods of treating a complement-mediated disease comprising administering to a patient a pharmaceutical preparation of the invention, a polypeptide of the invention, a complement factor B protein analog as disclosed herein, a nucleic acid as disclosed herein, a viral vector disclosed herein or any combination thereof.

The disclosure also describes methods of producing a polypeptide comprising a complement factor B protein analog, the method comprising: expressing in a cell a complement factor B protein analog of the invention and purifying said complement factor B protein analog.

Polypeptides disclosed herein, complement factor B analogs as disclosed herein, and nucleic acids and vectors encoding them, can be used to modulate a complement pathway and for the study and/or treatment of various conditions or diseases related to a complement pathway.

The disclosure is based on the findings that mutation or removal of a free cysteine (i) improves the yield of an active and/or properly folded complement factor B protein analog (*e.g.*, see Examples 9 and 10); (ii) enhances the thermostability of a complement factor B protein analog (*e.g.*, see Examples 13 and 14); and/or (iii) reduces aggregation of a complement factor B protein analog (*e.g.*, see Example 6).

This summary does not necessarily describe all features or necessary features of the invention. The invention may also reside in a sub-combination of the described features.

BRIEF DESCRIPTION OF THE FIGURES

For the purpose of illustrating the invention, there are depicted in the drawings certain embodiments of the invention. However, the invention is not limited to the precise arrangements and instrumentalities of embodiments depicted in the drawings.

Figure 1A depicts the classical and lectin complement pathways. The classical pathway is initiated through C1 while the lectin pathway is initiated through mannose binding lectin (MBL). C4bC2a is a protease that cleaves C3 to C3a and C3b and is termed the C3 convertase. Similarly, C4bC2aC3b cleaves C5 to C5a and C5b and is termed the C5 convertase. C3a, C4a, and C5a have inflammatory properties and attract phagocytotic cells. C5b6-9 forms the membrane attack complex (MAC), which creates membrane pores that kill infectious agents but can also damage host cells. MASP is mannan-binding lectin associated serine protease.

Figure 1B depicts the alternative complement pathway. This pathway is constitutively active at a low level through spontaneous cleavage of C3. In the presence of an appropriate surface, C3b binds to complement factor B (fB). This complex is then cleaved by complement factor D (fD) to yield C3bBb. Spontaneous dissociation ("decay") of this complex within minutes leads to its inactivation, whereas stabilization by properdin generates a complex that cleaves C3; that is, a C3 convertase. Several of the factors that attenuate the complement pathways do so by accelerating the decay of the C3 and C5 convertases. C3b participates in the C3 convertase to generate additional C3b thereby creating a positive feedback loop as shown by the large arrow. C3bBb is a C3 convertase. C3bBbC3b is a C5 convertase.

Figure 2 is a hfB3-292S expression construct. CMV-cytomegalovirus immediate early promoter; IRES-internal ribosomal entry site; Neo-neomycin phosphotransferase gene; SynPolyA-synthetic polyA; Amp-Ampicillin-resistant gene. SEQ ID NO:8 is the nucleotide sequence of the hfB3-292S expression construct shown in Figure 2.

Figure 3 shows Western blot analysis of raw cell culture supernatants containing either hfB3 or hfB3-292S protein after cell culture incubation for 72 hour (2 x 10^6 cells/mL). Lane 1, one µl of cell culture medium from naïve un-transfected 293 FreeStyle cells served as a negative control; Lane 2, one hundred ng of human wild type factor B (Quidel, Santa Clara,