



US009295713B2

(12) **United States Patent**
Smith et al.

(10) **Patent No.:** **US 9,295,713 B2**
(45) **Date of Patent:** **Mar. 29, 2016**

(54) **TREATMENT OF CHRONIC NEPHROPATHIES USING SOLUBLE COMPLEMENT RECEPTOR TYPE I (sCR1)**

(75) Inventors: **Richard J. H. Smith**, Iowa City, IA (US); **Yuzhou Zhang**, Coralville, IA (US); **Henry C. Marsh**, Reading, MA (US)

(73) Assignees: **CELLEX THERAPEUTICS, INC.**, Needham, MA (US); **UNIVERSITY OF IOWA RESEARCH FOUNDATION**, Iowa City, IA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 639 days.

(21) Appl. No.: **13/233,916**

(22) Filed: **Sep. 15, 2011**

(65) **Prior Publication Data**

US 2012/0071413 A1 Mar. 22, 2012

Related U.S. Application Data

(60) Provisional application No. 61/383,004, filed on Sep. 15, 2010.

(51) **Int. Cl.**
A61K 38/00 (2006.01)
A61P 13/12 (2006.01)
A61K 38/17 (2006.01)

(52) **U.S. Cl.**
CPC **A61K 38/1725** (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,252,216 A	10/1993	Folena-Wasserman et al.
5,456,909 A	10/1995	Marsh et al.
5,840,858 A	11/1998	Smith et al.
5,981,481 A	11/1999	Fearon et al.
6,193,979 B1	2/2001	Rittershaus et al.
6,316,604 B1	11/2001	Fearon et al.
6,458,360 B1	10/2002	Fearon et al.
6,713,606 B1	3/2004	Smith et al.
2009/0118163 A1	5/2009	Gronski

FOREIGN PATENT DOCUMENTS

WO WO 89/09220 A1 10/1989

OTHER PUBLICATIONS

Servais et al ("Primary glomerulonephritis with isolated C3 deposits: a new entity with shared common genetic risk factors with haemolytic uraemic syndrome", *J Med Genet* (2007), vol. 44:193-199).*

Mache et al. ("Complement Inhibitor Eculizumab in Atypical Hemolytic Uremic Syndrome", *Clin J Am Soc Nephrol*, vol. 4:1312-1316, published May 2009).*

Zimmerman et al. (*Critical Care Medicine*: Sep. 2000, vol. 28 (9) Abstract).*

Makrides et al. (*Therapeutic Inhibition of the Complement System, Pharmacological Reviews*; vol. 50 (1), 1998).*

Abrera-Abeleda et al., "Variations in the complement regulatory genes factor H (CFH) and factor H related 5 (CFHR5) are associated with membranoproliferative glomerulonephritis type II (dense deposit disease)", *J. Med. Genet.*, 43: 582-589 (2006).

Appel et al., "Membranoproliferative glomerulonephritis type II (Dense Deposit Disease): an update", *J. Am. Soc. Nephrol.*, 16: 1392-1403 (2005).

Ault, B.H., "Factor H and the pathogenesis of renal diseases", *Pediatr. Nephrol.*, 14(10-11): 1045-1053 (2000).

Barbiano di Belgiojoso et al., "The prognostic value of some clinical and histological parameters in membranoproliferative glomerulonephritis (MPGN): report of 112 cases", *Nephron.*, 19(5): 250-258 (1977).

Cameron et al., "Idiopathic mesangiocapillary glomerulonephritis. Comparison of types I and II in children and adults and long-term prognosis", *Am. J. Med.*, 74(2): 175-192 (1983).

Delvaeye et al., "Thrombomodulin mutations in atypical hemolytic-uremic syndrome", *N. Engl. J. Med.*, 361: 345-357 (2009).

Fakhouri et al., "Treatment with human complement factor H rapidly reverses renal complement deposition in factor H-deficient mice", *Kidney International*, 78: 279-286 (2010).

Fearon, D.T., "Identification of the membrane glycoprotein that is the C3b receptor of the human erythrocyte, polymorphonuclear leukocyte, B lymphocyte, and monocyte", *J. Exp. Med.*, 152(1): 20 (1980).

Fearon et al., "Regulation of the amplification C3 convertase of human complement by an inhibitory protein isolated from human erythrocyte membrane", *Proc. Natl. Acad. Sci. USA*, 76(11): 5867-5871 (1979).

Fremaux-Bacchi et al., "Mutations in complement C3 predispose to development of atypical hemolytic uremic syndrome", *Blood*, 112(13): 4948-4952 (2008).

Goicoechea de Jorge et al., "Gain-of-function mutations in complement factor B are associated with atypical hemolytic uremic syndrome", *Proc. Natl. Acad. Sci., USA*, 104(1): 240-245 (2007).

Habib et al., "Dense deposit disease: a variant of membranoproliferative glomerulonephritis", *Kidney Int.*, 7(4): 204-215 (1975).

Habib et al., "Glomerular lesions in the transplanted kidney in children", *Am. J. Kidney Disease*, 10(3): 198-207 (1987).

Hogasen et al., "Hereditary porcine membranoproliferative glomerulonephritis type II is caused by factor H deficiency", *J. Clin. Invest.*, 95(3): 1054-1061 (1995).

Iida et al., "Complement receptor is an inhibitor of the complement cascade", *J. Exp. Med.*, 153: 1138 (1981).

Jozsi et al., "Attachment of the soluble complement regulator factor H to cell and tissue surfaces: relevance for pathology", *Histol. Histopathol.*, 19(1): 251-258 (2004).

Moore et al., "Association of factor H autoantibodies with deletions of CFHR1, CFHR3, CFHR4, and with mutations in CFH, CFI, CD46, and C3 in patients with atypical hemolytic uremic syndrome", *Blood*, 115(2): 379-387 (2009).

(Continued)

Primary Examiner — James H Alstrum Acevedo

Assistant Examiner — Tara Martinez

(74) *Attorney, Agent, or Firm* — Leon R. Yankwich; David G. O'Brien; Yankwich & Associates, P.C.

(57) **ABSTRACT**

A method is disclosed for treating nephropathies involving undesired alternative pathway complement activation by administration of a complement inhibitory protein such as soluble complement receptor type I (sCR1).

19 Claims, 6 Drawing Sheets

(56)

References Cited

OTHER PUBLICATIONS

Noris et al., "Atypical hemolytic-uremic syndrome", *N. Engl. J. Med.*, 361(17): 1676-1687 (2009).

Orth et al., "The nephrotic syndrome", *New Engl. J. Med.*, 338(17): 1202-1211 (1998).

Pickering et al., "Uncontrolled C3 activation causes membranoproliferative glomerulonephritis in mice deficient in complement factor H", *Nat. Genet.*, 31(4): 424-428 (2002).

Pruitt et al., "The effect of soluble complement receptor type 1 on hyperacute allograft rejection", *J. Surg. Res.*, 50(4): 350-355 (1991).

Pruitt et al., "The effect of soluble complement receptor type 1 on hyperacute xenograft rejection", *Transplantation*, 52 (5): 868-873 (1991).

Repik et al., "A transgenic mouse model for studying the clearance of blood-borne pathogens via human complement receptor 1 (CR1)", *Clinical and Experimental Immunology*, 140(2): 230-240 (2005).

Scesney et al., "A soluble deletion mutant of the human complement receptor type 1, which lacks the C4b binding site, is a selective

inhibitor of the alternative complement pathway", *Eur. J. Immunol.*, 26(8): 1729-1735 (1996).

Schwartz et al., "Complement analysis in children with idiopathic membranoproliferative glomerulonephritis: a long-term follow-up", *Pediatr. Allergy Immunol.*, 12(3): 166-172 (2001).

Seed, "An LFA-3 cDNA encodes a phospholipid-linked membrane protein homologous to its receptor CD2", *Nature*, 329: 840-842 (1987).

Swainson et al., "Mesangiocapillary glomerulonephritis: a long-term study of 40 cases", *J. Pathol.*, 141(4): 449-468 (1983).

Weisman et al., "Soluble human complement receptor type 1: in vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis", *Science*, 249(4965): 146-151 (1990).

Wilson et al., "Characterization of human T lymphocytes that express the C3b receptor", *J. Immunol.*, 131(2): 684-689 (1983).

Yeh et al., "Recombinant soluble human complement receptor type 1 inhibits inflammation in the reversed passive arthus reaction in rats", *J. Immunol.*, 146(1): 250-256 (1991).

* cited by examiner

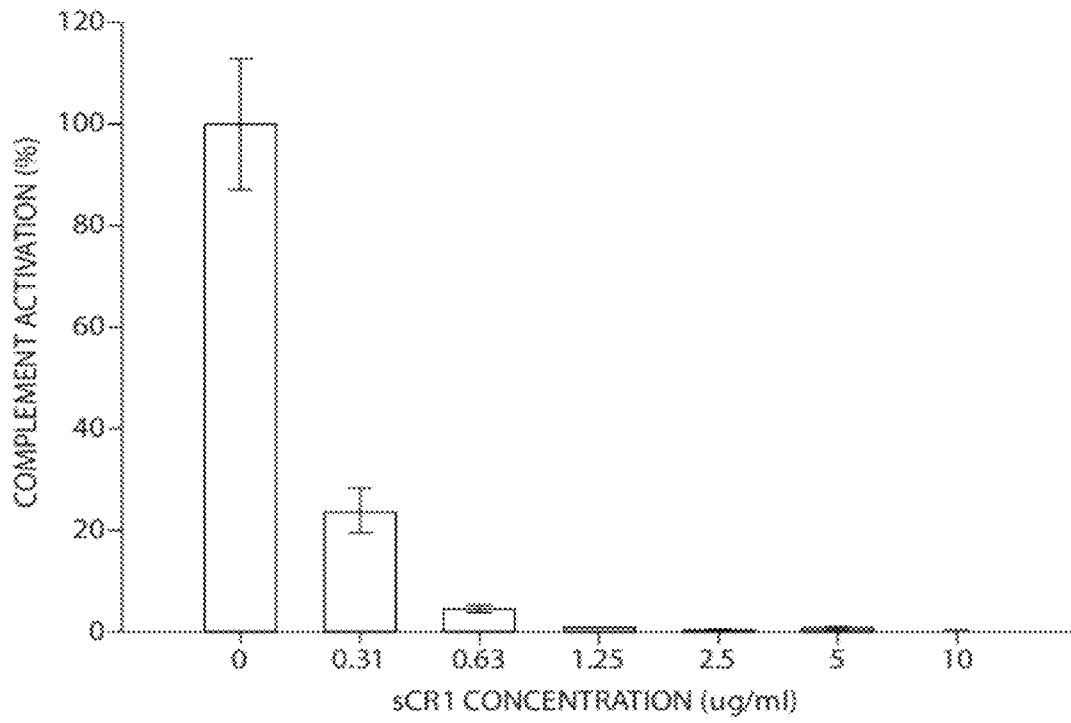


Fig. 1

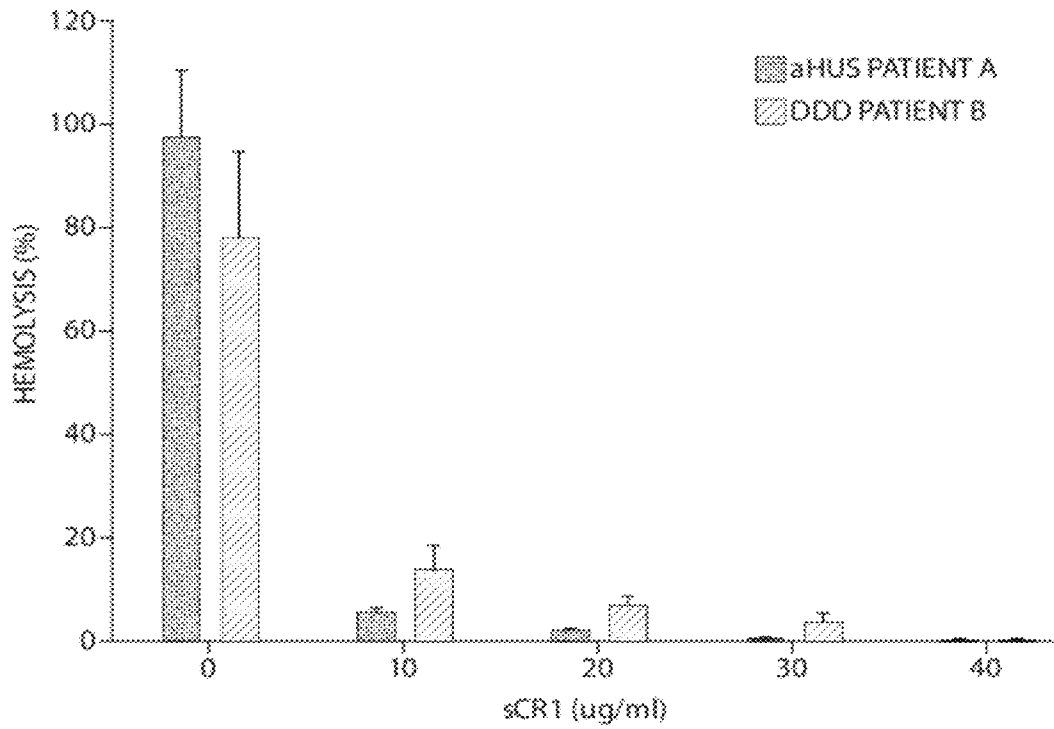


Fig. 2

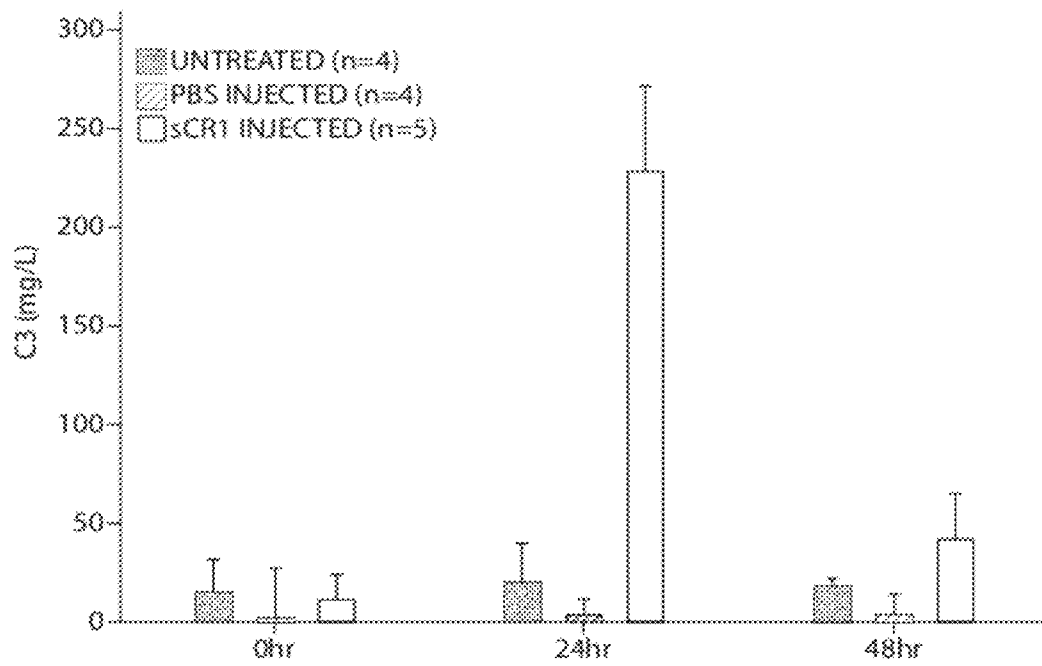


Fig. 3

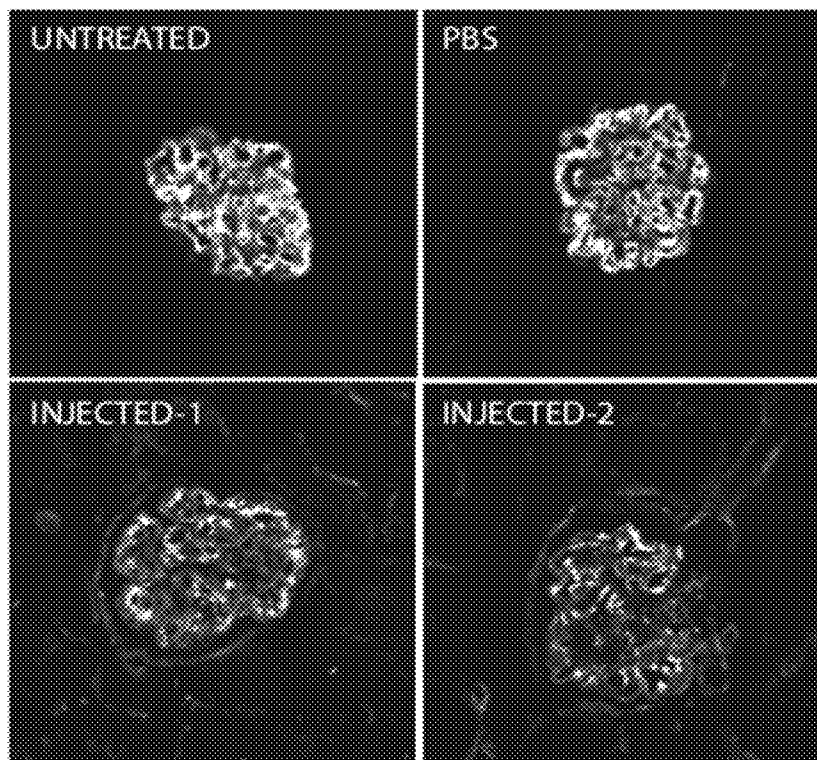


Fig. 4

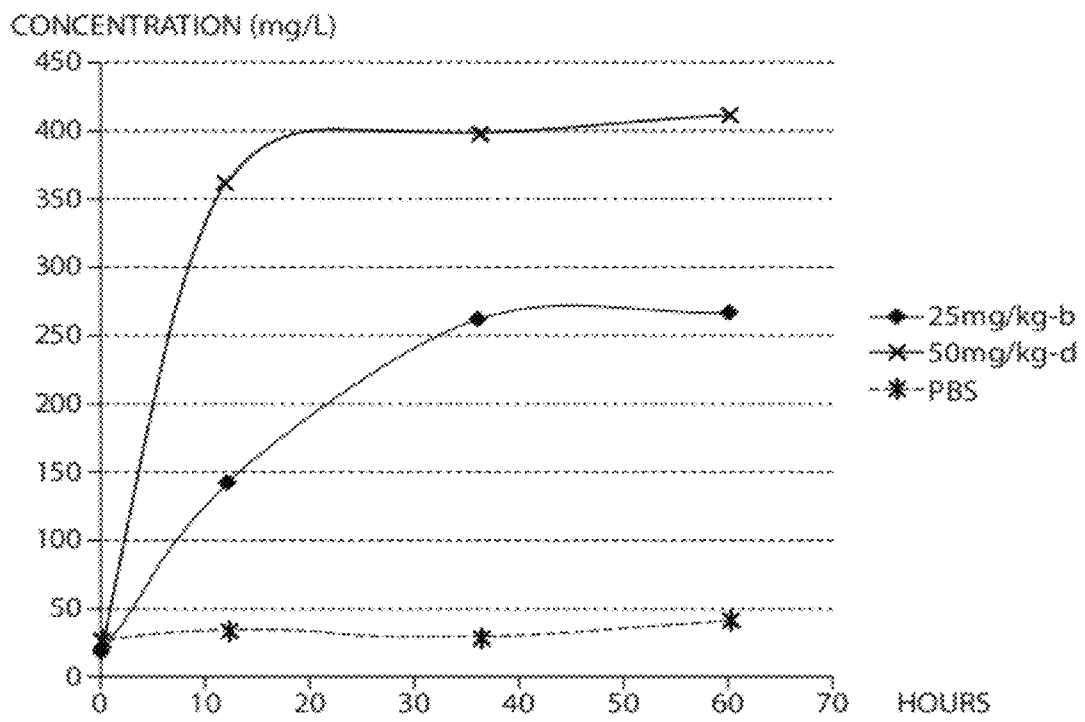
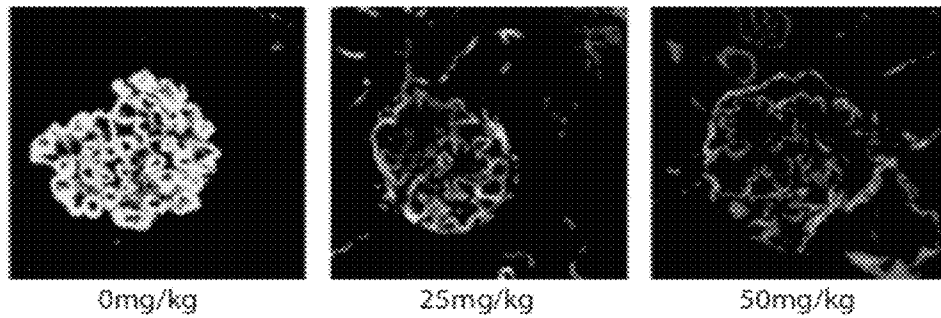


Fig. 5



0mg/kg

25mg/kg

50mg/kg

Fig. 6

**TREATMENT OF CHRONIC
NEPHROPATHIES USING SOLUBLE
COMPLEMENT RECEPTOR TYPE I (sCR1)**

CROSS-REFERENCE TO PRIORITY
APPLICATIONS

This application claims priority to U.S. Provisional Appln. No. 61/383,004 filed Sep. 15, 2010, the contents of which are incorporated herein.

This invention was made with government support under grant DK074409 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates to pharmaceutical compositions for treatment of diseases associated with dysregulation of the alternative pathway complement activation which ultimately harms kidney function, in particular atypical hemolytic uremic syndrome (aHUS) and dense deposit disease (DDD, also known as membrano-proliferative glomerulonephritis type II or MPGN2), as well as a recently described syndrome referred to as glomerulonephritis with isolated C3 deposits (GN-C3) or C3 glomerulopathy (C3G). Specifically, the invention relates to the use of pharmaceutical compositions comprising a soluble complement receptor type I (sCR1) to treat such diseases.

BACKGROUND OF THE INVENTION

The complement system comprises more than 40 different proteins directly or indirectly mediating attack and elimination of microbes, foreign particles and altered self cells via three different pathways of activation: classical pathway, alternative pathway, and lectin pathway (see, *The Complement System*, 2nd revised edition, Rother et al. (eds); Springer Verlag, 1998). The complement system is a major component of innate immunity and is a central host defense against infection. Activation of the complement cascade via the classical pathway, involving antigen-antibody complexes, by the lectin pathway, or by the alternative pathway, involving the recognition of certain cell wall polysaccharides, mediates a range of activities including lysis of microorganisms, chemotaxis, opsonization, stimulation of vascular and other smooth muscle cells, degranulation of mast cells, increased permeability of small blood vessels, directed migration of leukocytes, and activation of B lymphocytes and macrophages. The membrane attack complex (MAC) is the final product of the activated complement cascade. It is a lytic multi-protein complex that is lethal to pathogens and, at sublytic levels, causes the release of cytokines and growth factors such as beta-FGF and VEGF from nucleated cells (e.g., smooth muscle cells, endothelial cells).

Several human diseases are characterized by an unwanted activation of the complement cascade via one or more of these activation pathways, which is reflected by elevated levels of typical activation markers such as downstream components of the complement cascade, e.g., cleavage products of the complement system and inhibitor-protease complexes. Proteolytic cleavage of C3 by specific C3 convertases plays a major role in complement activation. C3 convertases generate forms of C3b, which represent a potential component of new C3 convertase molecules, thereby stimulating the cascade.

The protection of self-cells and tissue is normally tightly regulated by specific complement regulatory proteins or inhibitors, existing in the fluid-phase (soluble form) and/or in

membrane-bound forms. The membrane-bound complement regulatory proteins include complement receptor type I (CR1 or CD35), which binds C3b and C4b, disassembles C3 convertases and permits C3b/C4b degradation by factor I; decay accelerating factor (DAF or CD55), which binds C3b and disassembles C3/C5 convertase; and membrane co-factor protein (MCP or CD46), which binds C3b and C4b to permit their degradation by factor I). In addition to the membrane-anchored complement regulatory proteins, the soluble regulatory protein Factor H acts as a potent protective factor for cells by attachment to the polyanionic surface of self cells, where it increases complement inhibitory potential (Jozsi et al., *Histol. Histopathol.*, 19:251-8 (2004)). This protective activity of Factor H is mainly achieved by its efficient reduction of the lifetime of the alternative C3 convertase C3bBb by (1) binding to the covalently bound C3b and displacing Bb (decay acceleration), and (2) catalyzing the permanent inactivation of C3b via proteolytic cleavage by the serine proteinase factor I (co-factor activity: generation of, e.g., iC3b, C3c). (*The Complement System*, 2nd revised edition, Rother et al. (eds); Springer Verlag, 1998; pp. 28, 34-7.) The activity of Factor H as co-factor for factor I in the outer phase of the surface layer (approx. 20-140 nm) is facilitated by binding of Factor H to surface-located proteoglycans by means of the C-terminal short consensus repeat (Jozsi et al. (2004), supra). The protective potential of Factor H limits locally the progression of the complement cascade. This is of particular importance for cells that express a low number of the membrane-anchored complement regulators, or for tissues which completely lack such complement regulatory proteins, such as the kidney glomerular basement membrane (Hogasen et al., *J. Clin. Invest.*, 95:1054-61 (1995)).

A significant reduction or absence of functional Factor H protein, i.e., due to reduced or eliminated Factor H expression, or mutation of the Factor H gene leading to production of mutant Factor H that is non-functional or has reduced functionality, has been demonstrated as one possible cause in diseases such as atypical hemolytic uremic syndrome (aHUS), dense deposit disease (DDD, also known as membranoproliferative glomerulonephritis type II or MPGN2), and glomerulonephritis with isolated C3 deposits (GN-C3, also sometimes referred to as C3 glomerulopathy, or C3G). These diseases ultimately harm kidney function. Since the glomerular membrane lacks endogenous complement regulatory membrane proteins, continuous cleavage of C3 occurs at this site, resulting in deposition of complement activation products, resulting in C3 convertase-mediated damage of the glomerular basement membranes and of epithelial tubules and endothelial cells, membrane thickening via deposition of extracellular matrix and/or components of the complement system (e.g., C3 cleavage products) and of antibodies, and, consequently, in defective filtration (proteinuria).

Dense deposit disease (DDD), also termed membranoproliferative glomerulonephritis type II or MPGN2, is a rare disease which is characterized by complement-containing dense deposits within the basement membrane of the glomerular capillary wall, followed by capillary wall thickening, mesangial cell proliferation and glomerular fibrosis (Ault, *Pediatr. Nephrol.*, 14:1045-53(2000)). Besides DDD, there are two other types of membranoproliferative glomerulonephritis, i.e., types I and III (MPGN1 and MPGN3, respectively). The membranoproliferative glomerulonephritides are diseases of diverse and often obscure etiology that account for 4% and 7% of primary renal causes of nephrotic syndrome in children and adults, respectively (Orth et al., *New Engl. J. Med.*, 338:1202-1211 (1998)). Membranoproliferative glomerulonephritis (MPGN) types I and III are variants of immune

complex-mediated disease; MPGN type II, in contrast, has no known association with immune complexes (Appel et al., "Membranoproliferative glomerulonephritis type II (Dense Deposit Disease): an update," *J. Am. Soc. Nephrol.*, 16:1392-1403 (2005)).

DDD accounts for less than 20% of cases of MPGN in children and only a fractional percentage of cases in adults (Orth et al., 1998, supra; Habib et al., *Kidney Int.*, 7:204-15 (1975); Habib et al., *Am. J. Kidney Dis.*, 10:198-207 (1987)). Both sexes are affected equally, with the diagnosis usually made in children between the ages of 5-15 years who present with non-specific findings like hematuria, proteinuria, acute nephritic syndrome or nephrotic syndrome (Appel et al., 2005, supra). More than 80% of patients with DDD are also positive for serum C3 nephritic factor (C3NeF), an autoantibody directed against C3bBb, the convertase of the alternative pathway of the complement cascade (Schwartz et al., *Pediatr. Allergy Immunol.*, 12:166-172 (2001)). C3NeF is found in up to one-half of persons with MPGN types I and III and also in healthy individuals, making the electron microscopic demonstration of dense deposits in the glomerular basement membrane (GBM) necessary for a definitive diagnosis of DDD (Appel et al., 2005, supra). This morphological hallmark is characteristic of DDD and is the reason "dense deposit disease" or "DDD" have become the more common terms for this MPGN.

C3NeF autoantibodies persists throughout the disease course in more than 50% of patients with DDD (Schwartz et al., 2001). Its presence is typically associated with evidence of complement activation, such as a reduction in CH50, decrease in C3, increase in C3dg/C3d, and persistently high levels of activation of the alternative pathway of the complement cascade. In DDD, C3NeF binds to C3bBb (or to the assembled convertase) to prolong the half-life of this enzyme, resulting in persistent C3 consumption that overwhelms the normal regulatory mechanisms to control levels of C3bBb and complement activation (Appel et al., 2005, supra). Most DDD patients do not have disease-causing mutations in Factor H, however, several alleles of both Factor H and the complement Factor H-related 5 gene (CFHR5) are preferentially associated with DDD (Abrera-Abeleda, M. A., et al., *Journal of Medical Genetics*, 43:582-589 (2006)).

Spontaneous remissions of DDD are uncommon (Habib et al., 1975, supra; Habib et al., 1987, supra; Cameron et al., *Am. J. Med.*, 74:175-192 (1983)). The more common outcome is chronic deterioration of renal function leading to end-stage renal disease (ESRD) in about half of patients within 10 years of diagnosis (Barbiano di Belgiojoso et al., *Nephron.*, 19:250-258 (1977)); Swainson et al., *J. Pathol.*, 141:449-468 (1983)). In some patients, rapid fluctuations in proteinuria occur with episodes of acute renal deterioration in the absence of obvious triggering events; in other patients, the disease remains stable for years despite persistent proteinuria.

Atypical hemolytic-uremic syndrome (aHUS) consists of the triad of microangiopathic hemolytic anemia, thrombocytopenia, and renal failure. aHUS, although rare, is a severe disease with death rates up to 25% in the acute phase and 50% developing end-stage renal disease (Noris, M., et al., *N. Engl. J. Med.*, 361:1676-1687 (2009)).

Research has linked atypical haemolytic-uremic syndrome to uncontrolled activation of the complement system. Approximately half of the patients with aHUS have mutations in CFH, CFI and MCP, encoding the complement regulatory proteins complement factor H, factor I and membrane cofactor protein, respectively (www.FH-HUS.org) (Noris, M., et al., 2009, supra). Gain-of-function mutations in key proteins of the alternative pathway cascade, complement factor B

(CFB) and C3 have also been reported (Goicoechea de Jorge, E., et al., *Proc. Natl. Acad. Sci. USA*, 104:240-245 (2007); Fremeaux-Bacchi, V. et al., *Blood*, 112:4948-4952 (2008)). More recently, mutations in the gene encoding thrombomodulin (THBD), a membrane-bound glycoprotein with anticoagulant properties that modulates complement activation on cell surfaces, have also been associated with aHUS (Delvaeye, M., et al., *N. Engl. J. Med.*, 361:345-357 (2009)). Finally, aHUS associated with anti-CFH autoantibodies has been described in sporadic forms mostly in association with deficiency of factor H related proteins 1 and 3 (Moore, I., et al., *Blood*, 115:379-387 (2009)).

In vitro functional tests with recombinant or plasma-purified CFH, MCP, CFI and THBD all documented that aHUS-associated mutations impair the capacity of regulatory proteins to control the activity of the alternative pathway of complement on endothelial cell surface (Noris, M., et al., 2009, supra). On the other hand, gain of function mutations in CFB and C3 resulted in hyperfunctional components of the C3 convertase that caused complement deposition on cell surface in vitro (Goicoechea de Jorge, E., et al., 2007, supra; Fremeaux-Bacchi, V. et al., 2008, supra). These findings indicate that aHUS is a disease of excessive complement activation on endothelial cells, which eventually results in renal microvascular thrombosis.

Factor H replacement therapy, inter alia, has been proposed for aHUS and DDD patients (see, e.g., US Pat. Publication 2009-0118163), however difficulties arise where the normal levels of a non-functional mutant Factor H are underlying the disease. It was not previously known whether addressing the continuous activation of complement via the alternative pathway would be a viable therapy, and a persistent need for new therapeutic approaches is evident.

SUMMARY OF THE INVENTION

The present invention relates to the use of a soluble complement receptor type I protein for the therapeutic treatment of nephropathies, in particular atypical hemolytic uremic syndrome (aHUS), dense deposit disease (DDD, also known as membranoproliferative glomerulonephritis type II or MPGN2), and glomerulonephritis with isolated C3 deposits (GN-C3, also sometimes referred to as C3 glomerulopathy, or C3G).

Thus, in one aspect, the present invention provides a new pharmaceutical composition for the treatment of aHUS, DDD or GN-C3 comprising an amount of a soluble CR1 protein, effective to inhibit complement and a pharmaceutically acceptable vehicle.

In another aspect, the present invention relates to the use of a soluble complement receptor type I (sCR1) polypeptide in the treatment of a nephropathy characterized by undesired alternative pathway complement activation.

In preferred aspects of the invention, the sCR1 polypeptide used in the methods herein is selected from a fragment of human CR1 comprising at least short consensus repeats 8-11; a fragment of human CR1 comprising at least short consensus repeats 15-18; a soluble CR1 polypeptide comprising human CR1 short consensus repeats 8-11 and 15-18; a fragment of human CR1 comprising long homologous repeat B; a fragment of human CR1 comprising long homologous repeat C; a fragment of human CR1 comprising long homologous repeats B and C; a fragment of human CR1 comprising long homologous repeats B, C and D; a fragment of human CR1 comprising at least long homologous repeats A and B; a fragment of human CR1 comprising long homologous repeats A, B and C; a fragment of human CR1 comprising

long homologous repeats A, B, C and D; a fragment of human CR1 comprising the extracellular domain of CR1; a fragment of human CR1 comprising the extracellular domain of CR1 and having the N-terminal LHR A deleted (sCR1[desLHR-A]); a soluble CR1 polypeptide having modified glycosylation to improve serum half-life in vivo; a soluble CR1 polypeptide having glycosylation modified to exhibit sialyl Lewis X moieties (sCR1-sLe^x); a soluble CR1 construct having two or more CR1 polypeptide moieties linked to a carrier molecule; and combinations thereof.

In another aspect of the invention, the sCR1 polypeptide or fragment thereof used in the methods disclosed herein exhibits a complement regulatory activity selected from the group consisting of: (i) the ability to bind C3b; (ii) the ability to bind C4b; (iii) the abilities to bind C3b and to bind C4b; (iv) factor I cofactor activity; (v) the ability to inhibit classical C3 convertase activity; (vi) the ability to inhibit alternative C3 convertase activity; (vii) the ability to inhibit classical C5 convertase activity; (viii) the ability to inhibit alternative C5 convertase activity; (ix) the ability to inhibit neutrophil oxidative burst; (x) the ability to inhibit complement-mediated hemolysis; (xi) the ability to inhibit C3a production; and (xii) the ability to inhibit C5a production. In yet another aspect of the invention, the sCR1 polypeptide or fragment thereof exhibits combinations of the above activities.

In another aspect, the sCR1 polypeptide or fragment thereof used in the methods disclosed herein exhibits ability to inhibit complement activation via both the classical activation pathway and the alternative activation pathway.

Another aspect of the invention relates to the use of a soluble complement receptor type I (sCR1) polypeptide in the treatment of a nephropathy in a mammal, including humans, characterized by undesired alternative pathway complement activation.

In yet another aspect of the invention, the nephropathy characterized by undesired alternative pathway complement activation results in C3 deposition in kidney tissue.

In one aspect of the invention, the use of the sCR1 polypeptide in the treatment of a nephropathy described herein results in a reduction of further C3 deposition in kidney tissue and/or at least partially reverses existing C3 deposition and reduces further C3 deposition in kidney tissue.

In yet another aspect of the invention, the use of a soluble complement receptor type I (sCR1) polypeptide in the treatment of a nephropathy characterized by undesired alternative pathway complement activation reduces kidney damage, reduces further kidney damage, and/or at least partially reverses existing kidney damage.

In another aspect of the invention, the use of a soluble complement receptor type I (sCR1) polypeptide in the treatment of a nephropathy characterized by undesired alternative pathway complement activation reduces deterioration in renal function and/or improves renal function. In one aspect of the invention, the improved renal function is indicated by one or more of i) reduced proteinuria, ii) reduced serum creatinine, and/or iii) improved glomerular filtration rate.

In another aspect of the invention, the use of a soluble complement receptor type I (sCR1) polypeptide in the treatment of a nephropathy characterized by undesired alternative pathway complement activation increases serum levels of C3.

Another aspect of the invention relates to a method for treating DDD comprising administration of an amount of a soluble CR1 protein effective to inhibit alternative pathway complement activation to a mammalian subject suffering from DDD. Another aspect of the invention relates to a method for treating aHUS comprising administration of an amount of a soluble CR1 protein effective to inhibit alterna-

tive pathway complement activation to a mammalian subject suffering from aHUS. Another aspect of the invention relates to a method for treating GN-C3 comprising administration of an amount of a soluble CR1 protein effective to inhibit alternative pathway complement activation to a mammalian subject suffering from GN-C3.

Another aspect of the invention relates to a method for treating DDD comprising systemic administration of an amount of a soluble CR1 protein effective to inhibit complement activity to a mammalian subject suffering from DDD. In this aspect, administration of the soluble CR1 protein may be intravenous (IV), subcutaneous (SC), intramuscular (IM), intra-arterial, intraperitoneal (IP), intrathecal, pulmonary, or oral. Another aspect of the invention relates to a method for treating aHUS comprising systemic administration of an amount of a soluble CR1 protein effective to inhibit complement activity to a mammalian subject suffering from aHUS. In this aspect, administration of the soluble CR1 protein may be intravenous (IV), subcutaneous (SC), intramuscular (IM), intra-arterial, intraperitoneal (IP), intrathecal, pulmonary, or oral.

Yet another aspect of the invention relates to a method for treating GN-C3 comprising systemic administration of an amount of a soluble CR1 protein effective to inhibit complement activity to a mammalian subject suffering from GN-C3. In this aspect, administration of the soluble CR1 protein may be intravenous (IV), subcutaneous (SC), intramuscular (IM), intra-arterial, intraperitoneal (IP), intrathecal, pulmonary, or oral.

Pharmaceutical compositions for use in treating DDD, GN-C3 or aHUS comprising a soluble complement receptor type I and a pharmaceutically acceptable diluent, carrier or excipient are also contemplated. Use of a soluble complement receptor type I in the manufacture of a medicament for the treatment of DDD, GN-C3 or aHUS is also contemplated.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph showing the dose-dependent inhibition of alternative pathway (AP) complement activation by sCR1.

FIG. 2 is a graph showing the results of in vitro hemolytic assays in a patient with aHUS and a patient with DDD, showing that sCR1 is a potent inhibitor of C3 convertase activity in both patients, and even in the presence of C3NeF in the DDD patient.

FIG. 3 is a graph showing the results of an in vivo study of C3 levels in Cfh^{-/-} mice injected with a single dose of sCR1 at 50 mg/kg. C3 levels in sCR1-injected mice significantly increased after 24 hours.

FIG. 4 are histopathologic slides comparing C3 deposition in the kidneys at 48 hours in a Cfh^{-/-} test animal treated with a single dose of sCR1 (Injected-1 and Injected-2) vs. the negative controls (Untreated and PBS).

FIG. 5 is a graph showing the results of an in vivo study of C3 concentrations in Cfh^{-/-}_{tg-CR1} mice injected with 3 doses of sCR1 at 0, 24, and 48 hours at a dose of 25 mg/kg and 50 mg/kg. C3 concentration was measured at 0, 12, 36, and 60 hours.

FIG. 6 are histopathologic slides comparing C3 deposition in the kidneys in Cfh^{-/-}_{tg-CR1} mouse test animals treated with a single dose of sCR1 (25 mg/kg and 50 mg/kg) vs. the negative control (0 mg/kg) at 60 hours post-injection.

DETAILED DESCRIPTION

The present invention is based on the important and surprising discovery that administration of a complement inhibi-

tory protein, in particular soluble CR1, is effective in inhibiting alternative pathway complement activity in patients with chronic nephropathies/glomerulopathies, in particular atypical hemolytic uremic syndrome (aHUS), dense deposit disease (DDD, also known as membranoproliferative glomerulonephritis type II or MPGN2), and glomerulonephritis with isolated C3 deposits (GN-C3, also sometimes referred to as C3 glomerulopathy or C3G).

In order that the invention may be more fully understood, the following terms are defined.

The term “nephropathy” or “nephrosis” as used herein refers to damage to or disease or disorder of the kidney, including diseases/disorders associated with undesired alternative pathway complement activation and/or deposition of complement activation products in kidney tissue, including atypical hemolytic uremic syndrome (aHUS) and/or dense deposit disease (DDD) and/or glomerulonephritis with isolated C3 deposits (GN-C3).

The term “complement inhibitory protein” as used herein refers to any of the complement regulatory proteins that have a negative regulatory activity on complement activation. Complement inhibitory proteins useful in the present invention include, specifically, soluble complement receptor type I (sCR1), C4-binding protein (C4-BP), decay accelerating factor (DAF), membrane cofactor protein (MCP), and Factor H.

As used herein, the terms “soluble complement receptor type I”, “soluble CR1 polypeptides” or “soluble CR1” or “sCR1” will be used to refer to portions of full-length human CR1 protein which, in contrast to the native CR1 proteins, are not expressed on the cell surface as transmembrane proteins but nevertheless exhibit a complement regulatory activity such as C3b binding, C4b binding, the ability to inhibit the classical complement activation pathway and/or the alternative complement activation pathway, and/or the lectin complement activation pathway, etc. In particular, CR1 polypeptides which substantially lack a transmembrane region, or, preferably, which comprise all or part of the extracellular domain of CR1 and retain a complement regulatory activity, are soluble CR1 polypeptides. In a preferred embodiment, the soluble CR1 polypeptides useful in the present invention are secreted by a cell in which they are expressed. Suitable soluble CR1 polypeptides and preparations are described in detail, e.g., in U.S. Pat. No. 5,981,481; U.S. Pat. No. 5,456,909; and U.S. Pat. No. 6,193,979, which are incorporated herein by reference. Soluble CR1 polypeptides having at least one C3b/C4b binding site intact are preferred, as such molecules have the ability to block complement activation via the classical activation pathway and the alternative activation pathway both. Reference to specific complement inhibitory proteins includes fragments of such proteins produced by truncation or splicing-out of unwanted polypeptide segments, so long as complement regulatory activity is retained. Derivatives made by one or more amino acid substitutions or linking to other structures such as carrier proteins or immunoglobulin constant regions are also contemplated, again so long as complement inhibitory activity is retained. In particular, soluble CR1 polypeptides having at least one of the two C3b/C4b binding sites (specifically, short consensus repeats (SCRs) 8-11 and 15-18) intact are preferred, because such molecules will retain the ability to block complement activation via the alternative complement pathway.

Special mention is made of a soluble CR1 polypeptide having glycosylation modified to exhibit sialyl Lewis X moieties (sCR1-sLe^x), as described in U.S. Pat. No. 6,193,979; novel glycoform preparations of soluble CR1 having an increased in vivo half-life described in U.S. Pat. No. 5,456,909; and soluble constructs having two or more CR1 moieties

linked to a carrier molecule, e.g., an sCR1-F(ab)₂ fusion, as described in U.S. Pat. No. 6,458,360. Also contemplated are soluble CR1 polypeptides having at least one of the C3b or C4b binding sites intact covalently linked to lipopeptides to facilitate localization on cell surfaces, as disclosed in U.S. Pat. No. 6,713,606. More preferably, the method of the invention utilizes a polypeptide comprising the extracellular domain of mature human CR1 (SEQ ID NO:1).

As used herein, the terms “treatment” or “treating” refers to any regimen that alleviates one or more symptoms of a disease or disorder, that inhibits progression of a disease or disorder, that arrests progression or reverses progression (causes regression) of a disease or disorder, or that prevents onset of a disease or disorder. Treatment includes prophylaxis and includes but does not require cure of a disease or disorder.

As used herein, the terms “disease” and “disorder” have the meaning generally known and understood in the art and comprise any abnormal condition in the function or well being of a host individual. A diagnosis of a particular disease or disorder, such as atypical hemolytic uremic syndrome (aHUS) and/or dense deposit disease (DDD) and/or glomerulonephritis with isolated C3 deposits (GN-C3) by a healthcare professional may be made by direct examination and/or consideration of results of one or more diagnostic tests.

A composition or method described herein as “comprising” one or more named elements or steps is open-ended, meaning that the named elements or steps are essential, but other elements or steps may be added within the scope of the composition or method. To avoid prolixity, it is also understood that any composition or method described as “comprising” (or “comprises”) one or more named elements or steps also describes the corresponding, more limited, composition or method “consisting essentially of” (or “consists essentially of”) the same named elements or steps, meaning that the composition or method includes the named essential elements or steps and may also include additional elements or steps that do not materially affect the basic and novel characteristic(s) of the composition or method. It is also understood that any composition or method described herein as “comprising” or “consisting essentially of” one or more named elements or steps also describes the corresponding, more limited, and close-ended composition or method “consisting of” (or “consists of”) the named elements or steps to the exclusion of any other unnamed element or step. In any composition or method disclosed herein, known or disclosed equivalents of any named essential element or step may be substituted for that element or step.

The definitions of other terms used herein are those understood and used by persons skilled in the art and/or will be evident to persons skilled in the art from their usage in the text.

The method of this invention can be practiced by using any soluble complement receptor type I polypeptide which is effective to block alternate pathway complement activation. Such complement inhibitory proteins include, for example, soluble complement receptor type I (sCR1) of SEQ ID NO:1, i.e., comprising the extracellular domain of human CR1, or fragments of CR1 that retain complement inhibiting properties, such as the ability to inhibit complement activation, to bind C3b, or to bind both C3b and C4b, or factor I co-factor activity. Preferably, the complement inhibitory protein used in the methods described herein is a soluble (non-membrane-bound) form of human CR1 comprising at least long homologous repeats (LHRs) B and/or C, preferably both LHRs B and C, more preferably long homologous repeats A, B, and C or A, B, C, and D, and most preferably substantially the entire extracellular domain of human CR1 or the molecule sCR1

[desLHR-A], which is the extracellular domain of CR1 including the LHRs BCD but omitting the N-terminal LHR A (see, Scesney, S. M. et al, *Eur. J. Immunol.*, 26:1729-35 (1996)). Suitable soluble CR1 polypeptides and preparations are described in detail, e.g., in U.S. Pat. No. 5,981,481; U.S. Pat. No. 5,456,909; and U.S. Pat. No. 6,193,979. Modified sCR1 molecules having, for example, a modified glycosylation, e.g., to improve serum half-life, such as those described in U.S. Pat. No. 5,456,909 may also be used. Soluble CR1 polypeptides having glycosylation modified to exhibit sialyl Lewis X moieties (designated sCR1-sLe^x), as described in U.S. Pat. No. 6,193,979, may also be used. And soluble constructs having two or more CR1 moieties linked to a carrier molecule, e.g., an sCR1-F(ab)2 fusion, as described in U.S. Pat. No. 6,458,360, may also be used.

As discussed more fully below, it has been demonstrated herein that administration of sCR1 alleviates the effects of undesirable alternative pathway complement activation, in particular in nephropathic diseases such as atypical hemolytic uremic syndrome (aHUS), dense deposit disease (DDD), or glomerulonephritis with isolated C3 deposits (GN-C3). We have thus discovered that administration of a complement inhibitory protein to a subject in a relevant aHUS or MPGN2 model reduces and/or ameliorates the pathogenesis of massive activation of the alternative pathway and terminal complement cascade, with subsequent deposition of complement activation products (iC3b, C3c, C3d, sMAC) in the glomerular basement membrane. The effects of sCR1 in nephropathic diseases has been demonstrated in vivo, which demonstrates an important aspect previously unknown, namely, whether sCR1 could be delivered to affect C3 deposition at particular tissues lacking complement regulatory proteins, such as kidney glomerular basement membrane, whether the regulatory activity of sCR1 could persist for a meaningful period in vivo to alleviate the effects of unregulated complement activation and such outward indicators as C3 deposition in kidney tissues, and whether administration of sCR1 could be effective at a dosage level that would make sCR1 a realistic candidate as a therapeutic.

It has also now been demonstrated that sCR1 can effectively compete with C3Nef autoantibodies and counterbalance C3Nef-mediated complement activation that occurs in about 85% of DDD patients.

The human C3b/C4b receptor, termed complement receptor type I (CR1) or CD35, is naturally present on the membranes of erythrocytes, monocytes/macrophages, granulocytes, B cells, some T cells, splenic follicular dendritic cells, and glomerular podocytes. (Fearon, 1980, *J. Exp. Med.*, 152: 20; Wilson, J. G., et al., 1983, *J. Immunol.*, 131: 684). CR1 specifically binds C3b, C4b, iC3b and iC4b.

CR1 can inhibit the classical and alternative pathway C3/C5 convertases and act as a cofactor for the cleavage of C3b and C4b by factor I, indicating that CR1 also has complement regulatory functions in addition to serving as a receptor. (Fearon, D. T., 1979, *Proc. Natl. Acad. Sci. U.S.A.*, 76: 5867; Iida, K. I. and Nussenzweig, V., 1981, *J. Exp. Med.*, 153: 1138.) In the alternative pathway of complement activation, the bimolecular complex C3bBb is a C3 protease (convertase). CR1 can bind to C3b thereby promoting the dissociation of fragment Bb from the complex. In the alternative pathway of complement activation, the tri-molecular complex C3bC3bBb is a C5 protease (convertase). CR1 can bind to C3bC3b thereby promoting the dissociation of fragment Bb from the complex. Furthermore, binding of C3b to CR1 renders C3b susceptible to irreversible proteolytic inactivation by factor I, resulting in the production of inactivated derivatives of C3b (namely, iC3b, C3d and C3dg). In the

classical pathway of complement activation, the bimolecular complex C4bC2a is the C3 convertase. CR1 binds to C4b thereby promoting the dissociation of C2a from the complex. In the classical pathway of complement activation, the complex C3bC4bC2a is the C5 convertase. CR1 binds to C4b and/or C3b thereby promoting the dissociation of C2a from the complex. The binding renders C4b and/or C3b susceptible to irreversible proteolytic inactivation by factor I. Finally, the lectin pathway (also called the mannose binding lectin or MBL pathway) feeds into the classical pathway upstream of the C3 convertase. Thus, CR1 inhibits lectin pathway activation through its inhibitory activities on the classical pathway at the C3 and C5 activation steps.

Factor H has some of the same properties exhibited by CR1 but is not effective to block both activation pathways. Factor H has decay accelerating activity and factor I co-factor activity in the alternative pathway only. In addition, the activity of Factor H is restricted to non-activating surfaces. This is an important distinction with respect to CR1, which is active both on activating and non-activating surfaces and is therefore more suitable for use under conditions of an ongoing disease. Activating surfaces would include, e.g., the presence of necrotic and inflamed tissue.

Several soluble (non-membrane bound) fragments of CR1 have been generated via recombinant DNA procedures by eliminating the transmembrane and cytoplasmic regions from the DNAs being expressed. See, e.g., Fearon et al., Intl. Patent Publ. WO 89/09220, Oct. 5, 1989. The soluble CR1 fragments are functionally active, i.e., retaining the ability to bind C3b and/or C4b, inhibiting complement activation, and demonstrating factor I co-factor activity, depending upon the native CR1 regions the CR1 fragments contain. Such constructs inhibit in vitro the consequences of complement activation such as neutrophil oxidative burst, complement mediated hemolysis, C3a and C5a production, and the production of C5b-9 (MAC). A soluble construct, sCR1/pBSCR1c, also has demonstrated in vivo activity in a reversed passive Arthus reaction (Yeh et al., 1991, *J. Immunol.*, 146:250), suppressed post-ischemic myocardial inflammation and necrosis (Weisman et al., 1990, *Science*, 249: 146-151) and extended survival rates following transplantation (Pruitt et al., 1991, *J. Surg. Res.*, 50: 350; Pruitt et al., 1991, *Transplantation*, 52: 868).

The complete cDNA coding sequence and amino acid sequence of the human CR1 protein is described in U.S. Pat. No. 5,981,481, which is incorporated herein by reference. The isolation of the full-length CR1 gene, expression and purification of the full-length protein and active fragments thereof, and demonstration of activity in the full-length protein and fragments derived from the full-length protein, are described in U.S. Pat. No. 5,981,481.

The complement inhibitory proteins such as sCR1 that are useful in the methods of the present invention are advantageously produced in quantity using recombinant DNA technology to express the protein in a host cell, such as bacterial cells, mammalian cells, or even plant cells. For the complement inhibitory proteins contemplated herein, mammalian host cells, such as Chinese Hamster ovary (CHO) cells, African Green Monkey kidney (COS) cells, or human cells, retina-derived cells (e.g., PER.C6 cells) being preferred. Yeast expression, *E. coli* expression, baculovirus expression, and plant expression are also contemplated, where non-mammalian glycosylation patterns do not have a significant impact on biological function or pharmacokinetics. Other expression systems for the production of recombinant proteins will also be useful for the production of complement receptor type I polypeptides contemplated herein. The isolated gene encod-

ing the desired protein can be inserted into an appropriate cloning vector. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to, plasmids or modified viruses. The vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as pBR322, pUC or CDM8 plasmids (Seed, 1987, *Nature*, 329: 840-842) or derivatives of those well-known vectors. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc.

Recombinant cells producing a preferred form of sCR1 are deposited with the American Type Culture Collection, Rockville, Md. (accession no. CRL 10052). The deposited cells are a Chinese Hamster ovary cell line DUX B11 carrying plasmid pBSCR1c/pTCSgpt clone 35.6, encoding the extracellular domain of human CR1. Such sCR1 polypeptide in purified form is produced under the product designation TP10 and also by the designation CDX-1135 by Celldex Therapeutics, Inc. (Needham, Mass.).

After expression in a host cell, the soluble CR1 molecules may be isolated and purified by standard methods including chromatography (e.g., ion exchange, affinity, and sizing column chromatography, high pressure liquid chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Preferred purification methods are described in U.S. Pat. No. 6,316,604, U.S. Pat. No. 5,252,216, and U.S. Pat. No. 5,840,858, which are incorporated herein by reference.

Soluble CR1 proteins are therapeutically useful in the modulation of complement-mediated diseases, that is, diseases or conditions characterized by inappropriate or undesired complement activation. A soluble CR1 protein or fragment which can bind C3b and/or retains the ability to inhibit the alternative or classical C3 or C5 convertases, and/or retains factor I cofactor activity, can be used in the methods and uses disclosed herein. In the present invention, we have demonstrated that soluble CR1 can be used to ameliorate or inhibit undesirable complement activity in the pathogenesis of nephropathies caused by DDD and/or aHUS.

In the method of the invention, a soluble CR1 polypeptide is administered to a subject who suffers from aHUS, DDD, and/or GN-C3 in order to attenuate complement activation and its role in the pathogenesis in persistent reduction in serum C3 and deposition of complement activation products, resulting in C3 convertase-mediated damage of the glomerular basement membranes and of epithelial tubules and endothelial cells, membrane thickening via deposition of extracellular matrix and/or components of the complement system (e.g., C3 cleavage products) and of antibodies, and, consequently, in defective filtration (proteinuria).

In a method of treating DDD, aHUS, or GN-C3 according to the invention, a therapeutically active amount of a soluble complement receptor type I polypeptide is administered to a mammalian subject in need of such treatment. The preferred subject is a human. The amount administered should be sufficient to inhibit complement activation and/or restore normal alternative pathway regulation. The determination of a therapeutically effective dose is within the capability of practitioners in this art, however, as an example, in embodiments of the method described herein utilizing systemic administration of sCR1 for the treatment of DDD, an effective human dose will be in the range of 0.1-150 mg/kg; preferably 1-100 mg/kg, more preferably 3-75 mg/kg, most preferably 5-60 mg/kg patient body weight (e.g., 5 mg/kg, 10 mg/kg, 25 mg/kg, 50 mg/kg, etc.). The route of administration may affect the recommended dose. Repeated systemic doses are

contemplated in order to maintain an effective level, e.g., to attenuate or inhibit complement activation in a patient's system, depending on the mode of administration adopted.

Soluble CR1 may be used in combination or alternating with the administration of other therapeutics prescribed for DDD and/or aHUS and/or GN-C3.

For administration, the sCR1 or other therapeutic protein may be formulated into an appropriate pharmaceutical composition. Such a composition typically contains a therapeutically active amount of the sCR1 or other protein and a pharmaceutically acceptable excipient or carrier such as saline, buffered saline, salt solutions (e.g., BSS®), phosphate buffers, dextrose, or sterile water. Compositions may also comprise specific stabilizing agents such as sugars, including mannose and mannitol.

Various delivery systems are known and can be used for delivery of complement inhibitory proteins such as sCR1 polypeptides in accordance with this invention, e.g., encapsulation in liposomes, microparticles, or microcapsules. Suitable modes of administration include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intrathecal, or epidural injection, and oral or pulmonary delivery.

Pharmaceutical compositions containing one or more complement inhibitory proteins for use in the present invention may be formulated in accordance with routine procedures as a pharmaceutical composition for systemic administration to an individual suffering from DDD and/or aHUS and/or GN-C3. Typically compositions for systemic administration are solutions in sterile aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of injection. Generally, the ingredients will be supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent in activity units. Where the composition is to be administered by injection, an ampoule of sterile water for injection or saline may be provided so that the ingredients may be mixed prior to administration.

A pharmaceutical pack comprising one or more containers filled with one or more of the ingredients of the pharmaceutical composition is also contemplated.

The following examples illustrate the methods of the present invention. They are provided by way of illustration and not for purposes of limitation.

EXAMPLE 1

Recombinant soluble complement receptor type I (sCR1) consisting of the extracellular portion of human CR1, produced in CHO cells, was used in the following experiments. The sCR1 was obtained from Celldex Therapeutics, Inc. (Needham, Mass.).

Complement Activity Assay

Alternative pathway (AP) complement activity was evaluated in the fluid phase using the Wieslab complement AP assay kit (Wieslab AB, Lund, Sweden). This method combines principles of the hemolytic assay for complement activation with the use of labeled antibodies specific for a neoantigen produced as a result of complement activation. The amount of neoantigen generated is proportional to the functional activity of the alternative pathway.

Twenty (20) microliters of pooled normal serum (Innovative research, Cat #IPLA-CSER, Novo, Mich.) was diluted in 340 μ l of diluents (Wieslab complement AP assay kit;

Wieslab AB, Lund, Sweden) containing specific blockers to ensure that only the alternative pathway is activated. Soluble CR1 polypeptide (TP10, Celldex Therapeutics, Inc., Needham, Mass.) was added to a final concentration of 10 $\mu\text{g/ml}$, 5 $\mu\text{g/ml}$, 2.5 $\mu\text{g/ml}$, 1.25 $\mu\text{g/ml}$, 0.63 $\mu\text{g/ml}$, 0.31 $\mu\text{g/ml}$ or 0 $\mu\text{g/ml}$. The mixture was then incubated on ice for 15 minutes; thereafter, each diluted serum was transferred in 100 microliter aliquots to microtiter wells. Activation was initiated during incubation of diluted serum in microtiter wells coated with specific complement activators of the alternative pathway, i.e., LPS (lipopolysaccharides). The wells were washed with the provided buffer and C5b-9 (MAC) was detected using the provided phosphatase-labeled antibody to the neoantigen that is exposed during MAC formation.

Data showed that sCR1 strongly inhibits fluid phase activation of the alternative pathway in a dose-dependent manner (see, FIG. 1).

Hemolytic Assay

The sheep erythrocyte lysis assay measures complement-mediated lysis of sheep erythrocytes secondary to activation of the alternative pathway on a cell surface. Sheep erythrocytes generally act as non-activators of complement-mediated lysis in human serum. A small number of C3b molecules spontaneously generated through alternative pathway tick-over are deposited on the surface of sheep erythrocytes. In normal human serum, factor H binds to C3b molecules through N-terminal domains and to sheep erythrocytes through C-terminal domains. These interactions protect sheep erythrocytes from complement and no lysis is observed.

Hemolysis was observed 30 minutes after mixing 20 microliters of patient A serum (FIG. 2, aHUS, dark blue) and sheep erythrocytes (50 μl , $1 \times 10^8/\text{ml}$) in the presence of $\text{Mg}^{++}/\text{EGTA}$ (AP activation possible) at 37° C. In parallel tests, various amounts of sCR1 (to final concentrations 0 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$, 20 $\mu\text{g/ml}$, 30 $\mu\text{g/ml}$, 40 $\mu\text{g/ml}$) were added to the same amount serum (20 μl) from patient A before adding sheep erythrocytes (50 μl , $1 \times 10^8/\text{ml}$) and incubating for 15 minutes on ice. Hemolysis was greatly reduced by the addition of sCR1 (see, FIG. 2).

Patient B has dense deposit disease (DDD) and very strong C3NeF activity, which causes uncontrolled alternative pathway activation with massive C3 consumption. As a consequence, alternative pathway complement factors are totally consumed. To test whether sCR1 can prevent C3NeF from stabilizing C3 convertase, 10 μl of patient B's serum were added to 10 μl of sheep erythrocytes ($1 \times 10^9/\text{ml}$) coated with pre-formed C3 convertase. Pre-formed C3 convertase was allowed to decay at 30° C. (water bath) for 20 minutes. The pre-formed C3 convertase was made by adding normal human serum to sheep erythrocytes and incubating first at room temperature (water bath) for 8 minutes and then on ice for 40 minutes. Sheep RBCs were lysed in the prolonged presence of C3 convertase. Hemolysis was assayed by adding rat serum (1:5 diluted in GVB-EDTA buffer) as a source of C3-9 (FIG. 2, DDD, light blue). In parallel tests, various amounts of sCR1 (to final concentrations 0 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$, 20 $\mu\text{g/ml}$, 30 $\mu\text{g/ml}$, 40 $\mu\text{g/ml}$) were added to the patient serum before mixing with sheep erythrocytes and incubating on ice for 15 minutes. Data showed that sCR1 suppressed C3NeF activity in a dosage-dependent manner (see, FIG. 2). Repetition of this experiment with sera from ten DDD patients showed similar results.

The results of the in vitro hemolytic assays in a patient with aHUS and the patients with DDD show that sCR1 is a potent inhibitor of C3 convertase activity, even in the presence of C3NeF.

EXAMPLE 2

Cfh^{-/-} In Vivo Mouse Study

Complement factor H (CFH) deficiencies have been associated with dense deposit disease (DDD) and aHUS (Fakhouri et al., *Kidney International*, 78:279-286 (2010)). Gene-targeted CFH-deficient mice (Cfh^{-/-}) spontaneously develop low plasma C3 levels and deposition of C3 along the murine glomerular basement membrane, analogous to human dense deposit disease (Pickering, M C, et al., *Nat. Genet.*, 31:424-428 (2002)). Accordingly, Cfh^{-/-} mice were selected as an animal model for this experiment.

Five Cfh^{-/-} mice, a gift from Drs. Matthew Pickering and Marina Botto of the Imperial College London, were injected with sCR1 at a dose of 50 mg/kg (tail vein injection). As controls, one littermate was injected with the same amount of PBS and another one littermate was left untreated. Sera were collected by tail bleeding at 0, 24 and 48 hours. Serum C3 levels were measured using the mouse complement C3 kit (Kamiya Biomedical, Seattle, Wash.). C3 levels in sCR1-injected mice dramatically increased at 24 hours (rising close to the low end of normal reference values, ~300-1500 mg/L); however, C3 levels dropped to near pre-injection state by 48 hours in all injected mice (see, FIG. 3).

Kidneys were harvested at the time of euthanasia (48 hours) and imbedded in tissue-freezing medium (Triangle Biomedical Sciences, Durham, N.C.). Blocks were cut to a thickness of 5 micron and C3 deposition was assayed with FITC-conjugated C3 antibody (MP Biomedicals, Solon, Ohio).

C3 deposition was decreased in all sCR1-injected mice (see, FIG. 4). C3 immunofluorescence was decreased at 48 hours after a single dose of sCR1. By 48 hours, alternative pathway activation was again robust as evidenced by a decrease in C3 levels (see, FIG. 3). The decrease in C3 immunofluorescence reflects the transient control of C3 convertase activity over the 24 hour period following sCR1 injection.

The experiment was repeated, using Cfh^{-/-}_{tg-CR1} mice, a gift from Dr. Richard Quigg of the University of Michigan Medical Center (i.e., factor H-knock-out mice transgenic for human CR1). These mice are identical to the Cfh^{-/-} mouse described above however they have been crossed with a mouse transgenic for human CR1 (Repik, A. et al., *Clinical and Experimental Immunology*, 140:230-240 (2005)). Four mice were injected (intraperitoneally) with sCR1 at 0, 24, and 48 hours (3 injections per mouse) at doses of either 25 mg/kg (2 mice) or 50mg/kg (2 mice). As controls, two additional mice were injected with the same volume of PBS. C3 levels were measured at 0, 12, 36, and 60 hours. Because the Cfh^{-/-}_{tg-CR1} mouse expresses human CR1, it does not develop an immune response against sCR1 and is suitable for longer studies that employ multiple doses of sCR1. The results are shown in FIG. 5. C3 levels in sCR1-injected mice showed a dramatic and sustained increase (again, rising close to the low end of normal reference values, ~300-1500 mg/L).

Kidneys were harvested at the time of euthanasia (60 hours) and imbedded in tissue-freezing medium (Triangle Biomedical Sciences, Durham, N.C.). Blocks were cut to a thickness of 5 micron and C3 deposition was assayed with FITC-conjugated C3 antibody (MP Biomedicals, Solon, Ohio). The results are shown in FIG. 6.

C3 deposition was decreased in all sCR1-injected Cfh^{-/-}_{tg-CR1} mice at both concentrations (see, FIG. 5). C3 immunofluorescence was significantly decreased at 60 hours after the three-dose regimen sCR1 at 50 mg/kg. As seen in FIG. 6, the three-dose regimen, leading to sustained levels of

sCR1 through the end of the experiment (see, FIG. 5), led to a remarkable decrease in C3 deposition on kidney sections at the end of the experiment. These results indicate that susceptible kidney tissues in DDD can be protected by systemic administration of sCR1.

These data indicate a treatment for the rare complement-mediated diseases of DDD (MPGN2) and/or aHUS and/or GN-C3 to alleviate undesired complement activity in the short term, and to improve or protect renal function in the long term.

Following the foregoing description, additional therapeutic formulations containing other embodiments of the complement regulatory protein sCR1 may readily be tested,

prepared and used for the treatment of DDD (MPGN2) and/or aHUS and/or GN-C3. Additional embodiments of the invention and alternative methods adapted to a particular composition and mode of delivery will be evident from studying the foregoing description. All such embodiments and obvious alternatives are intended to be within the scope of this invention, as defined by the claims that follow.

Publications referred to above are hereby incorporated by reference.

A preferred soluble complement receptor type I polypeptide for use according to the present disclosure has the amino acid sequence:

(SEQ ID NO: 1)

```

Gln Cys Asn Ala Pro Glu Trp Leu Pro Phe Ala Arg Pro Thr Asn Leu
1          5          10          15
Thr Asp Glu Phe Glu Phe Pro Ile Gly Thr Tyr Leu Asn Tyr Glu Cys
20          25          30
Arg Pro Gly Tyr Ser Gly Arg Pro Phe Ser Ile Ile Cys Leu Lys Asn
35          40          45
Ser Val Trp Thr Gly Ala Lys Asp Arg Cys Arg Arg Lys Ser Cys Arg
50          55          60
Asn Pro Pro Asp Pro Val Asn Gly Met Val His Val Ile Lys Gly Ile
65          70          75          80
Gln Phe Gly Ser Gln Ile Lys Tyr Ser Cys Thr Lys Gly Tyr Arg Leu
85          90          95
Ile Gly Ser Ser Ser Ala Thr Cys Ile Ile Ser Gly Asp Thr Val Ile
100         105         110
Trp Asp Asn Glu Thr Pro Ile Cys Asp Arg Ile Pro Cys Gly Leu Pro
115         120         125
Pro Thr Ile Thr Asn Gly Asp Phe Ile Ser Thr Asn Arg Glu Asn Phe
130         135         140
His Tyr Gly Ser Val Val Thr Tyr Arg Cys Asn Pro Gly Ser Gly Gly
145         150         155         160
Arg Lys Val Phe Glu Leu Val Gly Glu Pro Ser Ile Tyr Cys Thr Ser
165         170         175
Asn Asp Asp Gln Val Gly Ile Trp Ser Gly Pro Ala Pro Gln Cys Ile
180         185         190
Ile Pro Asn Lys Cys Thr Pro Pro Asn Val Glu Asn Gly Ile Leu Val
195         200         205
Ser Asp Asn Arg Ser Leu Phe Ser Leu Asn Glu Val Val Glu Phe Arg
210         215         220
Cys Gln Pro Gly Phe Val Met Lys Gly Pro Arg Arg Val Lys Cys Gln
225         230         235         240
Ala Leu Asn Lys Trp Glu Pro Glu Leu Pro Ser Cys Ser Arg Val Cys
245         250         255
Gln Pro Pro Pro Asp Val Leu His Ala Glu Arg Thr Gln Arg Asp Lys
260         265         270
Asp Asn Phe Ser Pro Gly Gln Glu Val Phe Tyr Ser Cys Glu Pro Gly
275         280         285
Tyr Asp Leu Arg Gly Ala Ala Ser Met Arg Cys Thr Pro Gln Gly Asp
290         295         300
Trp Ser Pro Ala Ala Pro Thr Cys Glu Val Lys Ser Cys Asp Asp Phe
305         310         315         320
Met Gly Gln Leu Leu Asn Gly Arg Val Leu Phe Pro Val Asn Leu Gln
325         330         335
    
```


-continued

Leu Gly Ala Lys Val Asp Phe Val Cys Asp Glu Gly Phe Gln Leu Lys
 340 345 350
 Gly Ser Ser Ala Ser Tyr Cys Val Leu Ala Gly Met Glu Ser Leu Trp
 355 360
 Asn Ser Ser Val Pro Val Cys Glu Gln Ile Phe Cys Pro Ser Pro Pro
 370 375 380
 Val Ile Pro Asn Gly Arg His Thr Gly Lys Pro Leu Glu Val Phe Pro
 385 390 395 400
 Phe Gly Lys Ala Val Asn Tyr Thr Cys Asp Pro His Pro Asp Arg Gly
 405 410 415
 Thr Ser Phe Asp Leu Ile Gly Glu Ser Thr Ile Arg Cys Thr Ser Asp
 420 425 430
 Pro Gln Gly Asn Gly Val Trp Ser Ser Pro Ala Pro Arg Cys Gly Ile
 435 440 445
 Leu Gly His Cys Gln Ala Pro Asp His Phe Leu Phe Ala Lys Leu Lys
 450 455 460
 Thr Gln Thr Asn Ala Ser Asp Phe Pro Ile Gly Thr Ser Leu Lys Tyr
 465 470 475 480
 Glu Cys Arg Pro Glu Tyr Tyr Gly Arg Pro Phe Ser Ile Thr Cys Leu
 485 490 495
 Asp Asn Leu Val Trp Ser Ser Pro Lys Asp Val Cys Lys Arg Lys Ser
 500 505 510
 Cys Lys Thr Pro Pro Asp Pro Val Asn Gly Met Val His Val Ile Thr
 515 520 525
 Asp Ile Gln Val Gly Ser Arg Ile Asn Tyr Ser Cys Thr Thr Gly His
 530 535 540
 Arg Leu Ile Gly His Ser Ser Ala Glu Cys Ile Leu Ser Gly Asn Ala
 545 550 555 560
 Ala His Trp Ser Thr Lys Pro Pro Ile Cys Gln Arg Ile Pro Cys Gly
 565 570 575
 Leu Pro Pro Thr Ile Ala Asn Gly Asp Phe Ile Ser Thr Asn Arg Glu
 580 585 590
 Asn Phe His Tyr Gly Ser Val Val Thr Tyr Arg Cys Asn Pro Gly Ser
 595 600 605
 Gly Gly Arg Lys Val Phe Glu Leu Val Gly Glu Pro Ser Ile Tyr Cys
 610 615 620
 Thr Ser Asn Asp Asp Gln Val Gly Ile Trp Ser Gly Pro Ala Pro Gln
 625 630 635 640
 Cys Ile Ile Pro Asn Lys Cys Thr Pro Pro Asn Val Glu Asn Gly Ile
 645 650 655
 Leu Val Ser Asp Asn Arg Ser Leu Phe Ser Leu Asn Glu Val Val Glu
 660 665 670
 Phe Arg Cys Gln Pro Gly Phe Val Met Lys Gly Pro Arg Arg Val Lys
 675 680 685
 Cys Gln Ala Leu Asn Lys Trp Glu Pro Glu Leu Pro Ser Cys Ser Arg
 690 695 700
 Val Cys Gln Pro Pro Pro Asp Val Leu His Ala Glu Arg Thr Gln Arg
 705 710 715 720
 Asp Lys Asp Asn Phe Ser Pro Gly Gln Glu Val Phe Tyr Ser Cys Glu
 725 730 735
 Pro Gly Tyr Asp Leu Arg Gly Ala Ala Ser Met Arg Cys Thr Pro Gln
 740 745 750
 Gly Asp Trp Ser Pro Ala Ala Pro Thr Cys Glu Val Lys Ser Cys Asp
 755 760 765

-continued

Asp Phe Met Gly Gln Leu Leu Asn Gly Arg Val Leu Phe Pro Val Asn
 770 775 780
 Leu Gln Leu Gly Ala Lys Val Asp Phe Val Cys Asp Glu Gly Phe Gln
 785 790 795 800
 Leu Lys Gly Ser Ser Ala Ser Tyr Cys Val Leu Ala Gly Met Glu Ser
 805 810 815
 Leu Trp Asn Ser Ser Val Pro Val Cys Glu Gln Ile Phe Cys Pro Ser
 820 825 830
 Pro Pro Val Ile Pro Asn Gly Arg His Thr Gly Lys Pro Leu Glu Val
 835 840 845
 Phe Pro Phe Gly Lys Ala Val Asn Tyr Thr Cys Asp Pro His Pro Asp
 850 855 860
 Arg Gly Thr Ser Phe Asp Leu Ile Gly Glu Ser Thr Ile Arg Cys Thr
 865 870 875 880
 Ser Asp Pro Gln Gly Asn Gly Val Trp Ser Ser Pro Ala Pro Arg Cys
 885 890 895
 Gly Ile Leu Gly His Cys Gln Ala Pro Asp His Phe Leu Phe Ala Lys
 900 905 910
 Leu Lys Thr Gln Thr Asn Ala Ser Asp Phe Pro Ile Gly Thr Ser Leu
 915 920 925
 Lys Tyr Glu Cys Arg Pro Glu Tyr Tyr Gly Arg Pro Phe Ser Ile Thr
 930 935 940
 Cys Leu Asp Asn Leu Val Trp Ser Ser Pro Lys Asp Val Cys Lys Arg
 945 950 955 960
 Lys Ser Cys Lys Thr Pro Pro Asp Pro Val Asn Gly Met Val His Val
 965 970 975
 Ile Thr Asp Ile Gln Val Gly Ser Arg Ile Asn Tyr Ser Cys Thr Thr
 980 985 990
 Gly His Arg Leu Ile Gly His Ser Ser Ala Glu Cys Ile Leu Ser Gly
 995 1000 1005
 Asn Thr Ala His Trp Ser Thr Lys Pro Pro Ile Cys Gln Arg Ile
 1010 1015 1020
 Pro Cys Gly Leu Pro Pro Thr Ile Ala Asn Gly Asp Phe Ile Ser
 1025 1030 1035
 Thr Asn Arg Glu Asn Phe His Tyr Gly Ser Val Val Thr Tyr Arg
 1040 1045 1050
 Cys Asn Leu Gly Ser Arg Gly Arg Lys Val Phe Glu Leu Val Gly
 1055 1060 1065
 Glu Pro Ser Ile Tyr Cys Thr Ser Asn Asp Asp Gln Val Gly Ile
 1070 1075 1080
 Trp Ser Gly Pro Ala Pro Gln Cys Ile Ile Pro Asn Lys Cys Thr
 1085 1090 1095
 Pro Pro Asn Val Glu Asn Gly Ile Leu Val Ser Asp Asn Arg Ser
 1100 1105 1110
 Leu Phe Ser Leu Asn Glu Val Val Glu Phe Arg Cys Gln Pro Gly
 1115 1120 1125
 Phe Val Met Lys Gly Pro Arg Arg Val Lys Cys Gln Ala Leu Asn
 1130 1135 1140
 Lys Trp Glu Pro Glu Leu Pro Ser Cys Ser Arg Val Cys Gln Pro
 1145 1150 1155
 Pro Pro Glu Ile Leu His Gly Glu His Thr Pro Ser His Gln Asp
 1160 1165 1170
 Asn Phe Ser Pro Gly Gln Glu Val Phe Tyr Ser Cys Glu Pro Gly
 1175 1180 1185

-continued

Tyr Asp Leu Arg Gly Ala Ala Ser Leu His Cys Thr Pro Gln Gly
 1190 1195 1200
 Asp Trp Ser Pro Glu Ala Pro Arg Cys Ala Val Lys Ser Cys Asp
 1205 1210 1215
 Asp Phe Leu Gly Gln Leu Pro His Gly Arg Val Leu Phe Pro Leu
 1220 1225 1230
 Asn Leu Gln Leu Gly Ala Lys Val Ser Phe Val Cys Asp Glu Gly
 1235 1240 1245
 Phe Arg Leu Lys Gly Ser Ser Val Ser His Cys Val Leu Val Gly
 1250 1255 1260
 Met Arg Ser Leu Trp Asn Asn Ser Val Pro Val Cys Glu His Ile
 1265 1270 1275
 Phe Cys Pro Asn Pro Pro Ala Ile Leu Asn Gly Arg His Thr Gly
 1280 1285 1290
 Thr Pro Ser Gly Asp Ile Pro Tyr Gly Lys Glu Ile Ser Tyr Thr
 1295 1300 1305
 Cys Asp Pro His Pro Asp Arg Gly Met Thr Phe Asn Leu Ile Gly
 1310 1315 1320
 Glu Ser Thr Ile Arg Cys Thr Ser Asp Pro His Gly Asn Gly Val
 1325 1330 1335
 Trp Ser Ser Pro Ala Pro Arg Cys Glu Leu Ser Val Arg Ala Gly
 1340 1345 1350
 His Cys Lys Thr Pro Glu Gln Phe Pro Phe Ala Ser Pro Thr Ile
 1355 1360 1365
 Pro Ile Asn Asp Phe Glu Phe Pro Val Gly Thr Ser Leu Asn Tyr
 1370 1375 1380
 Glu Cys Arg Pro Gly Tyr Phe Gly Lys Met Phe Ser Ile Ser Cys
 1385 1390 1395
 Leu Glu Asn Leu Val Trp Ser Ser Val Glu Asp Asn Cys Arg Arg
 1400 1405 1410
 Lys Ser Cys Gly Pro Pro Pro Glu Pro Phe Asn Gly Met Val His
 1415 1420 1425
 Ile Asn Thr Asp Thr Gln Phe Gly Ser Thr Val Asn Tyr Ser Cys
 1430 1435 1440
 Asn Glu Gly Phe Arg Leu Ile Gly Ser Pro Ser Thr Thr Cys Leu
 1445 1450 1455
 Val Ser Gly Asn Asn Val Thr Trp Asp Lys Lys Ala Pro Ile Cys
 1460 1465 1470
 Glu Ile Ile Ser Cys Glu Pro Pro Pro Thr Ile Ser Asn Gly Asp
 1475 1480 1485
 Phe Tyr Ser Asn Asn Arg Thr Ser Phe His Asn Gly Thr Val Val
 1490 1495 1500
 Thr Tyr Gln Cys His Thr Gly Pro Asp Gly Glu Gln Leu Phe Glu
 1505 1510 1515
 Leu Val Gly Glu Arg Ser Ile Tyr Cys Thr Ser Lys Asp Asp Gln
 1520 1525 1530
 Val Gly Val Trp Ser Ser Pro Pro Pro Arg Cys Ile Ser Thr Asn
 1535 1540 1545
 Lys Cys Thr Ala Pro Glu Val Glu Asn Ala Ile Arg Val Pro Gly
 1550 1555 1560
 Asn Arg Ser Phe Phe Ser Leu Thr Glu Ile Ile Arg Phe Arg Cys
 1565 1570 1575
 Gln Pro Gly Phe Val Met Val Gly Ser His Thr Val Gln Cys Gln
 1580 1585 1590

-continued

Thr Asn Gly Arg Trp Gly Pro Lys Leu Pro His Cys Ser Arg Val
 1595 1600 1605
 Cys Gln Pro Pro Pro Glu Ile Leu His Gly Glu His Thr Leu Ser
 1610 1615 1620
 His Gln Asp Asn Phe Ser Pro Gly Gln Glu Val Phe Tyr Ser Cys
 1625 1630 1635
 Glu Pro Ser Tyr Asp Leu Arg Gly Ala Ala Ser Leu His Cys Thr
 1640 1645 1650
 Pro Gln Gly Asp Trp Ser Pro Glu Ala Pro Arg Cys Thr Val Lys
 1655 1660 1665
 Ser Cys Asp Asp Phe Leu Gly Gln Leu Pro His Gly Arg Val Leu
 1670 1675 1680
 Leu Pro Leu Asn Leu Gln Leu Gly Ala Lys Val Ser Phe Val Cys
 1685 1690 1695
 Asp Glu Gly Phe Arg Leu Lys Gly Arg Ser Ala Ser His Cys Val
 1700 1705 1710
 Leu Ala Gly Met Lys Ala Leu Trp Asn Ser Ser Val Pro Val Cys
 1715 1720 1725
 Glu Gln Ile Phe Cys Pro Asn Pro Pro Ala Ile Leu Asn Gly Arg
 1730 1735 1740
 His Thr Gly Thr Pro Phe Gly Asp Ile Pro Tyr Gly Lys Glu Ile
 1745 1750 1755
 Ser Tyr Ala Cys Asp Thr His Pro Asp Arg Gly Met Thr Phe Asn
 1760 1765 1770
 Leu Ile Gly Glu Ser Ser Ile Arg Cys Thr Ser Asp Pro Gln Gly
 1775 1780 1785
 Asn Gly Val Trp Ser Ser Pro Ala Pro Arg Cys Glu Leu Ser Val
 1790 1795 1800
 Pro Ala Ala Cys Pro His Pro Pro Lys Ile Gln Asn Gly His Tyr
 1805 1810 1815
 Ile Gly Gly His Val Ser Leu Tyr Leu Pro Gly Met Thr Ile Ser
 1820 1825 1830
 Tyr Ile Cys Asp Pro Gly Tyr Leu Leu Val Gly Lys Gly Phe Ile
 1835 1840 1845
 Phe Cys Thr Asp Gln Gly Ile Trp Ser Gln Leu Asp His Tyr Cys
 1850 1855 1860
 Lys Glu Val Asn Cys Ser Phe Pro Leu Phe Met Asn Gly Ile Ser
 1865 1870 1875
 Lys Glu Leu Glu Met Lys Lys Val Tyr His Tyr Gly Asp Tyr Val
 1880 1885 1890
 Thr Leu Lys Cys Glu Asp Gly Tyr Thr Leu Glu Gly Ser Pro Trp
 1895 1900 1905
 Ser Gln Cys Gln Ala Asp Asp Arg Trp Asp Pro Pro Leu Ala Lys
 1910 1915 1920
 Cys Thr Ser Arg Ala His Asp Ala
 1925 1930

 SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 1

<210> SEQ ID NO 1

<211> LENGTH: 1931

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

-continued

```

<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(1931)
<223> OTHER INFORMATION: soluble CR1 polypeptide

<400> SEQUENCE: 1

Gln Cys Asn Ala Pro Glu Trp Leu Pro Phe Ala Arg Pro Thr Asn Leu
 1             5             10             15

Thr Asp Glu Phe Glu Phe Pro Ile Gly Thr Tyr Leu Asn Tyr Glu Cys
      20             25             30

Arg Pro Gly Tyr Ser Gly Arg Pro Phe Ser Ile Ile Cys Leu Lys Asn
      35             40             45

Ser Val Trp Thr Gly Ala Lys Asp Arg Cys Arg Arg Lys Ser Cys Arg
      50             55             60

Asn Pro Pro Asp Pro Val Asn Gly Met Val His Val Ile Lys Gly Ile
      65             70             75             80

Gln Phe Gly Ser Gln Ile Lys Tyr Ser Cys Thr Lys Gly Tyr Arg Leu
      85             90             95

Ile Gly Ser Ser Ser Ala Thr Cys Ile Ile Ser Gly Asp Thr Val Ile
      100            105            110

Trp Asp Asn Glu Thr Pro Ile Cys Asp Arg Ile Pro Cys Gly Leu Pro
      115            120            125

Pro Thr Ile Thr Asn Gly Asp Phe Ile Ser Thr Asn Arg Glu Asn Phe
      130            135            140

His Tyr Gly Ser Val Val Thr Tyr Arg Cys Asn Pro Gly Ser Gly Gly
      145            150            155            160

Arg Lys Val Phe Glu Leu Val Gly Glu Pro Ser Ile Tyr Cys Thr Ser
      165            170            175

Asn Asp Asp Gln Val Gly Ile Trp Ser Gly Pro Ala Pro Gln Cys Ile
      180            185            190

Ile Pro Asn Lys Cys Thr Pro Pro Asn Val Glu Asn Gly Ile Leu Val
      195            200            205

Ser Asp Asn Arg Ser Leu Phe Ser Leu Asn Glu Val Val Glu Phe Arg
      210            215            220

Cys Gln Pro Gly Phe Val Met Lys Gly Pro Arg Arg Val Lys Cys Gln
      225            230            235            240

Ala Leu Asn Lys Trp Glu Pro Glu Leu Pro Ser Cys Ser Arg Val Cys
      245            250            255

Gln Pro Pro Pro Asp Val Leu His Ala Glu Arg Thr Gln Arg Asp Lys
      260            265            270

Asp Asn Phe Ser Pro Gly Gln Glu Val Phe Tyr Ser Cys Glu Pro Gly
      275            280            285

Tyr Asp Leu Arg Gly Ala Ala Ser Met Arg Cys Thr Pro Gln Gly Asp
      290            295            300

Trp Ser Pro Ala Ala Pro Thr Cys Glu Val Lys Ser Cys Asp Asp Phe
      305            310            315            320

Met Gly Gln Leu Leu Asn Gly Arg Val Leu Phe Pro Val Asn Leu Gln
      325            330            335

Leu Gly Ala Lys Val Asp Phe Val Cys Asp Glu Gly Phe Gln Leu Lys
      340            345            350

Gly Ser Ser Ala Ser Tyr Cys Val Leu Ala Gly Met Glu Ser Leu Trp
      355            360            365

Asn Ser Ser Val Pro Val Cys Glu Gln Ile Phe Cys Pro Ser Pro Pro
      370            375            380

```

-continued

Val Ile Pro Asn Gly Arg His Thr Gly Lys Pro Leu Glu Val Phe Pro
 385 390 395 400
 Phe Gly Lys Ala Val Asn Tyr Thr Cys Asp Pro His Pro Asp Arg Gly
 405 410 415
 Thr Ser Phe Asp Leu Ile Gly Glu Ser Thr Ile Arg Cys Thr Ser Asp
 420 425 430
 Pro Gln Gly Asn Gly Val Trp Ser Ser Pro Ala Pro Arg Cys Gly Ile
 435 440 445
 Leu Gly His Cys Gln Ala Pro Asp His Phe Leu Phe Ala Lys Leu Lys
 450 455 460
 Thr Gln Thr Asn Ala Ser Asp Phe Pro Ile Gly Thr Ser Leu Lys Tyr
 465 470 475 480
 Glu Cys Arg Pro Glu Tyr Tyr Gly Arg Pro Phe Ser Ile Thr Cys Leu
 485 490 495
 Asp Asn Leu Val Trp Ser Ser Pro Lys Asp Val Cys Lys Arg Lys Ser
 500 505 510
 Cys Lys Thr Pro Pro Asp Pro Val Asn Gly Met Val His Val Ile Thr
 515 520 525
 Asp Ile Gln Val Gly Ser Arg Ile Asn Tyr Ser Cys Thr Thr Gly His
 530 535 540
 Arg Leu Ile Gly His Ser Ser Ala Glu Cys Ile Leu Ser Gly Asn Ala
 545 550 555 560
 Ala His Trp Ser Thr Lys Pro Pro Ile Cys Gln Arg Ile Pro Cys Gly
 565 570 575
 Leu Pro Pro Thr Ile Ala Asn Gly Asp Phe Ile Ser Thr Asn Arg Glu
 580 585 590
 Asn Phe His Tyr Gly Ser Val Val Thr Tyr Arg Cys Asn Pro Gly Ser
 595 600 605
 Gly Gly Arg Lys Val Phe Glu Leu Val Gly Glu Pro Ser Ile Tyr Cys
 610 615 620
 Thr Ser Asn Asp Asp Gln Val Gly Ile Trp Ser Gly Pro Ala Pro Gln
 625 630 635 640
 Cys Ile Ile Pro Asn Lys Cys Thr Pro Pro Asn Val Glu Asn Gly Ile
 645 650 655
 Leu Val Ser Asp Asn Arg Ser Leu Phe Ser Leu Asn Glu Val Val Glu
 660 665 670
 Phe Arg Cys Gln Pro Gly Phe Val Met Lys Gly Pro Arg Arg Val Lys
 675 680 685
 Cys Gln Ala Leu Asn Lys Trp Glu Pro Glu Leu Pro Ser Cys Ser Arg
 690 695 700
 Val Cys Gln Pro Pro Pro Asp Val Leu His Ala Glu Arg Thr Gln Arg
 705 710 715 720
 Asp Lys Asp Asn Phe Ser Pro Gly Gln Glu Val Phe Tyr Ser Cys Glu
 725 730 735
 Pro Gly Tyr Asp Leu Arg Gly Ala Ala Ser Met Arg Cys Thr Pro Gln
 740 745 750
 Gly Asp Trp Ser Pro Ala Ala Pro Thr Cys Glu Val Lys Ser Cys Asp
 755 760 765
 Asp Phe Met Gly Gln Leu Leu Asn Gly Arg Val Leu Phe Pro Val Asn
 770 775 780
 Leu Gln Leu Gly Ala Lys Val Asp Phe Val Cys Asp Glu Gly Phe Gln
 785 790 795 800
 Leu Lys Gly Ser Ser Ala Ser Tyr Cys Val Leu Ala Gly Met Glu Ser

-continued

805					810					815					
Leu	Trp	Asn	Ser	Ser	Val	Pro	Val	Cys	Glu	Gln	Ile	Phe	Cys	Pro	Ser
		820						825					830		
Pro	Pro	Val	Ile	Pro	Asn	Gly	Arg	His	Thr	Gly	Lys	Pro	Leu	Glu	Val
		835					840					845			
Phe	Pro	Phe	Gly	Lys	Ala	Val	Asn	Tyr	Thr	Cys	Asp	Pro	His	Pro	Asp
	850					855					860				
Arg	Gly	Thr	Ser	Phe	Asp	Leu	Ile	Gly	Glu	Ser	Thr	Ile	Arg	Cys	Thr
865					870					875					880
Ser	Asp	Pro	Gln	Gly	Asn	Gly	Val	Trp	Ser	Ser	Pro	Ala	Pro	Arg	Cys
			885						890						895
Gly	Ile	Leu	Gly	His	Cys	Gln	Ala	Pro	Asp	His	Phe	Leu	Phe	Ala	Lys
		900							905					910	
Leu	Lys	Thr	Gln	Thr	Asn	Ala	Ser	Asp	Phe	Pro	Ile	Gly	Thr	Ser	Leu
		915					920						925		
Lys	Tyr	Glu	Cys	Arg	Pro	Glu	Tyr	Tyr	Gly	Arg	Pro	Phe	Ser	Ile	Thr
	930					935					940				
Cys	Leu	Asp	Asn	Leu	Val	Trp	Ser	Ser	Pro	Lys	Asp	Val	Cys	Lys	Arg
945					950					955					960
Lys	Ser	Cys	Lys	Thr	Pro	Pro	Asp	Pro	Val	Asn	Gly	Met	Val	His	Val
			965						970						975
Ile	Thr	Asp	Ile	Gln	Val	Gly	Ser	Arg	Ile	Asn	Tyr	Ser	Cys	Thr	Thr
		980						985						990	
Gly	His	Arg	Leu	Ile	Gly	His	Ser	Ser	Ala	Glu	Cys	Ile	Leu	Ser	Gly
		995					1000						1005		
Asn	Thr	Ala	His	Trp	Ser	Thr	Lys	Pro	Pro	Ile	Cys	Gln	Arg	Ile	
	1010						1015					1020			
Pro	Cys	Gly	Leu	Pro	Pro	Thr	Ile	Ala	Asn	Gly	Asp	Phe	Ile	Ser	
	1025					1030						1035			
Thr	Asn	Arg	Glu	Asn	Phe	His	Tyr	Gly	Ser	Val	Val	Thr	Tyr	Arg	
	1040					1045						1050			
Cys	Asn	Leu	Gly	Ser	Arg	Gly	Arg	Lys	Val	Phe	Glu	Leu	Val	Gly	
	1055					1060						1065			
Glu	Pro	Ser	Ile	Tyr	Cys	Thr	Ser	Asn	Asp	Asp	Gln	Val	Gly	Ile	
	1070					1075						1080			
Trp	Ser	Gly	Pro	Ala	Pro	Gln	Cys	Ile	Ile	Pro	Asn	Lys	Cys	Thr	
	1085					1090						1095			
Pro	Pro	Asn	Val	Glu	Asn	Gly	Ile	Leu	Val	Ser	Asp	Asn	Arg	Ser	
	1100					1105						1110			
Leu	Phe	Ser	Leu	Asn	Glu	Val	Val	Glu	Phe	Arg	Cys	Gln	Pro	Gly	
	1115					1120						1125			
Phe	Val	Met	Lys	Gly	Pro	Arg	Arg	Val	Lys	Cys	Gln	Ala	Leu	Asn	
	1130					1135						1140			
Lys	Trp	Glu	Pro	Glu	Leu	Pro	Ser	Cys	Ser	Arg	Val	Cys	Gln	Pro	
	1145					1150						1155			
Pro	Pro	Glu	Ile	Leu	His	Gly	Glu	His	Thr	Pro	Ser	His	Gln	Asp	
	1160					1165						1170			
Asn	Phe	Ser	Pro	Gly	Gln	Glu	Val	Phe	Tyr	Ser	Cys	Glu	Pro	Gly	
	1175					1180						1185			
Tyr	Asp	Leu	Arg	Gly	Ala	Ala	Ser	Leu	His	Cys	Thr	Pro	Gln	Gly	
	1190					1195						1200			
Asp	Trp	Ser	Pro	Glu	Ala	Pro	Arg	Cys	Ala	Val	Lys	Ser	Cys	Asp	
	1205					1210						1215			

-continued

Asp Phe	Leu Gly	Gln Leu	Pro	His Gly	Arg Val	Leu	Phe Pro	Leu		
1220			1225			1230				
Asn Leu	Gln Leu	Gly Ala	Lys	Val Ser	Phe Val	Cys	Asp Glu	Gly		
1235			1240			1245				
Phe Arg	Leu Lys	Gly Ser	Ser	Val Ser	His Cys	Val	Leu Val	Gly		
1250			1255			1260				
Met Arg	Ser Leu	Trp Asn	Asn	Ser Val	Pro Val	Cys	Glu His	Ile		
1265			1270			1275				
Phe Cys	Pro Asn	Pro Pro	Ala	Ile Leu	Asn Gly	Arg	His Thr	Gly		
1280			1285			1290				
Thr Pro	Ser Gly	Asp Ile	Pro	Tyr Gly	Lys Glu	Ile	Ser Tyr	Thr		
1295			1300			1305				
Cys Asp	Pro His	Pro Asp	Arg	Gly Met	Thr Phe	Asn	Leu Ile	Gly		
1310			1315			1320				
Glu Ser	Thr Ile	Arg Cys	Thr	Ser Asp	Pro His	Gly	Asn Gly	Val		
1325			1330			1335				
Trp Ser	Ser Pro	Ala Pro	Arg	Cys Glu	Leu Ser	Val	Arg Ala	Gly		
1340			1345			1350				
His Cys	Lys Thr	Pro Glu	Gln	Phe Pro	Phe Ala	Ser	Pro Thr	Ile		
1355			1360			1365				
Pro Ile	Asn Asp	Phe Glu	Phe	Pro Val	Gly Thr	Ser	Leu Asn	Tyr		
1370			1375			1380				
Glu Cys	Arg Pro	Gly Tyr	Phe	Gly Lys	Met Phe	Ser	Ile Ser	Cys		
1385			1390			1395				
Leu Glu	Asn Leu	Val Trp	Ser	Ser Val	Glu Asp	Asn	Cys Arg	Arg		
1400			1405			1410				
Lys Ser	Cys Gly	Pro Pro	Pro	Glu Pro	Phe Asn	Gly	Met Val	His		
1415			1420			1425				
Ile Asn	Thr Asp	Thr Gln	Phe	Gly Ser	Thr Val	Asn	Tyr Ser	Cys		
1430			1435			1440				
Asn Glu	Gly Phe	Arg Leu	Ile	Gly Ser	Pro Ser	Thr	Thr Cys	Leu		
1445			1450			1455				
Val Ser	Gly Asn	Asn Val	Thr	Trp Asp	Lys Lys	Ala	Pro Ile	Cys		
1460			1465			1470				
Glu Ile	Ile Ser	Cys Glu	Pro	Pro Pro	Thr Ile	Ser	Asn Gly	Asp		
1475			1480			1485				
Phe Tyr	Ser Asn	Asn Arg	Thr	Ser Phe	His Asn	Gly	Thr Val	Val		
1490			1495			1500				
Thr Tyr	Gln Cys	His Thr	Gly	Pro Asp	Gly Glu	Gln	Leu Phe	Glu		
1505			1510			1515				
Leu Val	Gly Glu	Arg Ser	Ile	Tyr Cys	Thr Ser	Lys	Asp Asp	Gln		
1520			1525			1530				
Val Gly	Val Trp	Ser Ser	Pro	Pro Pro	Arg Cys	Ile	Ser Thr	Asn		
1535			1540			1545				
Lys Cys	Thr Ala	Pro Glu	Val	Glu Asn	Ala Ile	Arg	Val Pro	Gly		
1550			1555			1560				
Asn Arg	Ser Phe	Phe Ser	Leu	Thr Glu	Ile Ile	Arg	Phe Arg	Cys		
1565			1570			1575				
Gln Pro	Gly Phe	Val Met	Val	Gly Ser	His Thr	Val	Gln Cys	Gln		
1580			1585			1590				
Thr Asn	Gly Arg	Trp Gly	Pro	Lys Leu	Pro His	Cys	Ser Arg	Val		
1595			1600			1605				

-continued

Cys 1610	Gln	Pro	Pro	Pro	Glu	Ile 1615	Leu	His	Gly	Glu	His 1620	Thr	Leu	Ser
His 1625	Gln	Asp	Asn	Phe	Ser	Pro 1630	Gly	Gln	Glu	Val	Phe 1635	Tyr	Ser	Cys
Glu 1640	Pro	Ser	Tyr	Asp	Leu	Arg 1645	Gly	Ala	Ala	Ser	Leu 1650	His	Cys	Thr
Pro 1655	Gln	Gly	Asp	Trp	Ser	Pro 1660	Glu	Ala	Pro	Arg	Cys 1665	Thr	Val	Lys
Ser 1670	Cys	Asp	Asp	Phe	Leu	Gly 1675	Gln	Leu	Pro	His	Gly 1680	Arg	Val	Leu
Leu 1685	Pro	Leu	Asn	Leu	Gln	Leu 1690	Gly	Ala	Lys	Val	Ser 1695	Phe	Val	Cys
Asp 1700	Glu	Gly	Phe	Arg	Leu	Lys 1705	Gly	Arg	Ser	Ala	Ser 1710	His	Cys	Val
Leu 1715	Ala	Gly	Met	Lys	Ala	Leu 1720	Trp	Asn	Ser	Ser	Val 1725	Pro	Val	Cys
Glu 1730	Gln	Ile	Phe	Cys	Pro	Asn 1735	Pro	Pro	Ala	Ile	Leu 1740	Asn	Gly	Arg
His 1745	Thr	Gly	Thr	Pro	Phe	Gly 1750	Asp	Ile	Pro	Tyr	Gly 1755	Lys	Glu	Ile
Ser 1760	Tyr	Ala	Cys	Asp	Thr	His 1765	Pro	Asp	Arg	Gly	Met 1770	Thr	Phe	Asn
Leu 1775	Ile	Gly	Glu	Ser	Ser	Ile 1780	Arg	Cys	Thr	Ser	Asp 1785	Pro	Gln	Gly
Asn 1790	Gly	Val	Trp	Ser	Ser	Pro 1795	Ala	Pro	Arg	Cys	Glu 1800	Leu	Ser	Val
Pro 1805	Ala	Ala	Cys	Pro	His	Pro 1810	Pro	Lys	Ile	Gln	Asn 1815	Gly	His	Tyr
Ile 1820	Gly	Gly	His	Val	Ser	Leu 1825	Tyr	Leu	Pro	Gly	Met 1830	Thr	Ile	Ser
Tyr 1835	Ile	Cys	Asp	Pro	Gly	Tyr 1840	Leu	Leu	Val	Gly	Lys 1845	Gly	Phe	Ile
Phe 1850	Cys	Thr	Asp	Gln	Gly	Ile 1855	Trp	Ser	Gln	Leu	Asp 1860	His	Tyr	Cys
Lys 1865	Glu	Val	Asn	Cys	Ser	Phe 1870	Pro	Leu	Phe	Met	Asn 1875	Gly	Ile	Ser
Lys 1880	Glu	Leu	Glu	Met	Lys	Lys 1885	Val	Tyr	His	Tyr	Gly 1890	Asp	Tyr	Val
Thr 1895	Leu	Lys	Cys	Glu	Asp	Gly 1900	Tyr	Thr	Leu	Glu	Gly 1905	Ser	Pro	Trp
Ser 1910	Gln	Cys	Gln	Ala	Asp	Asp 1915	Arg	Trp	Asp	Pro	Pro 1920	Leu	Ala	Lys
Cys 1925	Thr	Ser	Arg	Ala	His	Asp 1930	Ala							

What is claimed is:

1. A method for treating dense deposit disease (DDD) and/or glomerulonephritis with isolated C3 deposits (GN-C3) in a mammalian subject comprising administering to a mammalian subject in need of treatment an effective amount of a soluble complement receptor type I (sCR1) polypeptide.

2. The method according to claim 1, wherein said soluble CR1 polypeptide is selected from the group consisting of:

- a fragment of human CR1 comprising at least short consensus repeats 15-18;
- a soluble CR1 polypeptide comprising human CR1 short consensus repeats 8-11 and 15-18;
- a fragment of human CR1 comprising long homologous repeat B;
- a fragment of human CR1 comprising long homologous repeat C;
- a fragment of human CR1 comprising long homologous repeats B and C;

35

a fragment of human CR1 comprising long homologous repeats B, C and D;

a fragment of human CR1 comprising at least long homologous repeats A and B;

a fragment of human CR1 comprising long homologous repeats A, B and C;

a fragment of human CR1 comprising long homologous repeats A, B, C and D;

a fragment of human CR1 comprising the extracellular domain of CR1;

a fragment of human CR1 comprising the extracellular domain of CR1 and having the N-terminal LHR A deleted (sCR1[desLHR-A]);

a soluble CR1 polypeptide having modified glycosylation to improve serum half-life in vivo;

a soluble CR1 polypeptide having glycosylation modified to exhibit sialyl Lewis X moieties (sCR1-sLe^x);

a soluble CR1 construct having two or more CR1 polypeptide moieties linked to a carrier molecule; and combinations thereof.

3. The method according to claim 2, wherein said soluble CR1 polypeptide is selected from the group consisting of:

- a fragment of human CR1 comprising the extracellular domain of CR1;
- a soluble CR1 polypeptide having modified glycosylation to improve serum half-life in vivo;
- a soluble CR1 polypeptide having glycosylation modified to exhibit sialyl Lewis X moieties (sCR1-sLe^x); and combinations thereof.

4. The method according to claim 2, wherein said soluble CR1 polypeptide exhibits a complement regulatory activity selected from the group consisting of:

- (i) the ability to bind C3b;
- (ii) the ability to bind C4b;
- (iii) the abilities to bind C3b and to bind C4b;
- (iv) factor I cofactor activity;
- (v) the ability to inhibit classical C3 convertase activity;
- (vi) the ability to inhibit alternative C3 convertase activity;
- (vii) the ability to inhibit classical C5 convertase activity;
- (viii) the ability to inhibit alternative C5 convertase activity;
- (ix) the ability to inhibit neutrophil oxidative burst;
- (x) the ability to inhibit complement-mediated hemolysis;
- (xi) the ability to inhibit C3a production; and
- (xii) the ability to inhibit C5a production.

5. The method according to claim 1, wherein said soluble CR1 polypeptide exhibits the ability to inhibit complement activation via both the classical activation pathway and the alternative activation pathway.

36

6. The method according to claim 4, wherein said mammalian subject is a human.

7. The method according to claim 6, wherein the administration of said soluble CR1 polypeptide reduces further C3 deposition in kidney tissue.

8. The method according to claim 6, wherein the administration of said soluble CR1 polypeptide at least partially reverses existing C3 deposition in kidney tissue.

9. The method according to claim 6, wherein the administration of said soluble CR1 polypeptide reduces kidney damage.

10. The method according to claim 9, wherein the administration of said soluble CR1 polypeptide reduces further kidney damage.

11. The method according to claim 9 wherein the administration of said soluble CR1 polypeptide at least partially reverses existing kidney damage.

12. The method according to claim 6, wherein the administration of said soluble CR1 polypeptide reduces deterioration in renal function.

13. The method according to claim 6, wherein the administration of said soluble CR1 polypeptide improves renal function.

14. The method according to claim 13, wherein the administration of said soluble CR1 polypeptide improves renal function as indicated by one or more of i) reduced proteinuria, ii) reduced serum creatinine, and/or iii) improved glomerular filtration rate.

15. The method according to claim 6, wherein the administration of said soluble CR1 polypeptide increases serum levels of C3.

16. The method according to claim 6, wherein said human subject suffers from dense deposit disease (DDD).

17. The method according to claim 6, wherein said human subject suffers from primary glomerulonephritis with isolated C3 deposits (GN-C3).

18. The method according to claim 1, wherein said polypeptide is administered by an intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intrathecal, epidural, oral or pulmonary route.

19. A method for treating dense deposit disease (DDD) and/or glomerulonephritis with isolated C3 deposits (GN-C3) in a mammalian subject comprising administering to a mammalian subject in need of treatment an effective amount of a soluble complement receptor type I (sCR1) polypeptide comprising the extracellular domain of mature human CR1 (SEQ ID NO: 1).

* * * * *