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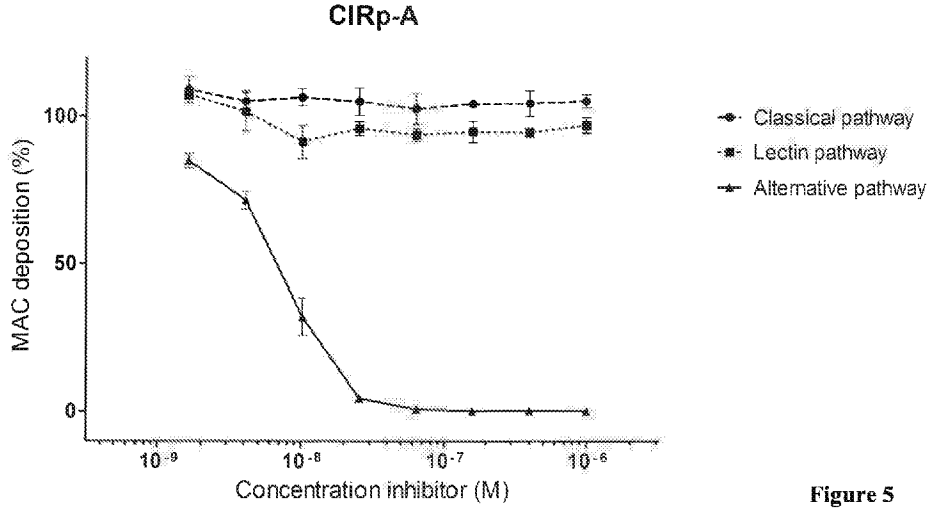

Figure 5

(57) Abstract: The present invention relates to an isolated polypeptide comprising or consisting of: (a) the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5 or 6; or (b) the amino acid sequence of (a) with a signal sequence and/or a purification tag sequence; or (c) a variant amino acid sequence having at least 60% sequence identity to (a) or (b); or (d) an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% sequence identity to (a) or (b); or (e) an active fragment of (a), (b), (c) or (d) that is at least 40, 50, 60, 65, 70 or 75 amino acids in length. The present invention also relates to a polynucleotide encoding a polypeptide of the invention, an expression vector or host cell comprising a polynucleotide of the invention. The invention also provides a composition or pharmaceutical composition comprising an isolated polypeptide, a polynucleotide, a vector or host cell of the invention, and uses thereof.

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POLYPEPTIDES AND USES THEREOF

This invention relates to polypeptides, and in particular to polypeptides that are capable of inhibiting the activity or activation of the complement system. The present invention also relates to nucleic acids that encode the polypeptides and to uses of the polypeptides.

The complement system helps or "complements" the ability of antibodies and phagocytic cells to clear pathogens from an organism. It forms part of the innate immune system. The complement system consists of over 30 proteins found in the blood and on cell membranes, those in solution are generally synthesized by the liver, and normally circulate as inactive precursors (pro-proteins). When stimulated by one of several triggers, proteases in the system cleave specific proteins to initiate an amplifying cascade of further cleavages. The end-result of this activation cascade is massive amplification of the response and activation of the cell-killing membrane attack complex, recruitment of white blood cells and release of pro-inflammatory mediators. Complement proteins account for about 5% of the globulin fraction of blood serum and can serve as opsonins.

The complement system may be activated mainly by three different biochemical pathways: the classical complement pathway, the alternative complement pathway, and the lectin pathway. The non-specific protease dependent pathway may also play a more limited role in complement activation.

The classical complement pathway is typically activated by antigen-antibody complexes, pentraxins, or apoptotic cells binding to the complement system protein C1q. Activation of the classical complement pathway is involved in tissue damage resulting from deposition of autoantibodies and immune complexes, which may occur in autoimmune diseases such as systemic lupus erythematosus, myasthenia gravis and Goodpasture's syndrome. Classical pathway activation is responsible for tissue injury in hyperacute xenograft rejection triggered by the direct binding of preformed host antibodies to the graft endothelium. Inappropriate complement activation is also an important mediator of ischemia and reperfusion injury occurring, for example, in stroke and myocardial infarction and after major surgery.

The lectin complement pathway is typically activated by microbial saccharides via the mannose-binding lectin or by ficolin in response to pathogens.

The alternative pathway is typically activated by surfaces of pathogens that have neutral or positive charge characteristics and do not express or contain complement inhibitors. However, under normal conditions a low level of alternative pathway activation occurs spontaneously in the blood, and there is therefore a low level of attack on the endothelial lining of vessels. If not sufficiently regulated by the resident complement regulators on these cells, this may lead to inappropriate and uncontrolled attack on human cells.

Activation of the complement system has many protective functions in immunity, both as a first line defence mechanism against pathogens and as a facilitator of acquired immunity. On the other hand, complement activation is a major cause of tissue injury in many pathological conditions.

Down-regulation of complement activation has been demonstrated to be effective in treating several disease indications in animal models and in ex vivo studies, including systemic lupus erythematosus and glomerulonephritis, arthritis, rheumatoid arthritis, cardiopulmonary bypass and hemodialysis, hypercute rejection in organ transplantation, myocardial infarction, reperfusion injury, and adult respiratory distress syndrome. In addition, other inflammatory conditions and autoimmune/immune complex diseases are also closely associated with complement activation, including thermal injury, severe asthma, anaphylactic shock, bowel inflammation, urticaria, angioedema, vasculitis, multiple sclerosis, myasthenia gravis, membranoproliferative glomerulonephritis, Sjogren's syndrome, renal disease, sepsis, paroxysmal nocturnal hemoglobinuria, psoriasis, transplant rejection, cancer, stroke, age-related macular degeneration, atypical haemolytic uremic syndrome, haemolytic uremic syndrome, Crohn’s disease, Alzheimer’s disease, cerebrovascular insufficiency, small vessel disease, frontotemporal disorder, Parkinson’s disease, multiple sclerosis, amyotrophic lateral sclerosis, ischemia reperfusion injury, glaucoma and dense deposit disease aka membranoproliferative glomerulonephritis type II.

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The first complement specific drug to obtain market approval was eculizumab, an antibody against the complement component C5. However, because there are a wide range of diseases related to complement activation there is an increasing need for treatments that can reduce or prevent activity or activation of the complement system. It may also be valuable to find therapies that are able to block or reduce activity of only some of the pathways, for example, only one or two of the three activation pathways (classical pathway, lectin pathway or alternative pathway) rather than all three. It is also advantageous to provide treatments that can reduce or prevent activity at specific points in the activation pathways. Inhibitors that inhibit points earlier in the complement activation pathway, for example, may be less broad acting and therefore better able to target specific diseases.

It is the aim of the present invention to provide novel polypeptides that can be used for the treatment of diseases or disorders that relate to inappropriate activation of one or more of the complement pathways, and may also be referred to as complement associated disorders.

In a first aspect, the present invention provides an isolated polypeptide comprising or consisting of:

(a) the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5 or 6; or

(b) the amino acid sequence of (a) with a signal sequence and/or a purification tag sequence; or

(c) a variant amino acid sequence having at least 60% sequence identity to (a) or (b); or

(d) an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% sequence identity to (a) or (b); or

(e) an active fragment of (a), (b), (c) or (d) that is at least 40, 50, 60, 65, 70 or 75 amino acids in length.

The polypeptides of SEQ ID NOs: 7 and 8 fall into (b) above and comprise the sequence of SEQ ID NO: 1 with a signal peptide and a His purification tag (SEQ ID NO 7) or just with a His purification tag (SEQ ID NO 8).

The polypeptides of SEQ ID NOs: 9 and 10 fall into (b) above and comprise the sequence of SEQ ID NO: 3 with a signal peptide and a His purification tag (SEQ ID NO 10) or just with a His purification tag (SEQ ID NO 9).

The polypeptide of the present invention may be a polypeptide comprising or consisting of the amino acid sequence shown in SEQ ID NO: 1, 2, 3, 4, 5 or 6 with a 6 histidine tag at the 5' or 3' end or another purification tag at the 5' or 3' end.

The polypeptide may comprise the amino acid sequence of SEQ ID NO: 1, 2, 3, 4, 5 or 6 with additional amino acids at one and/or both ends. For example, a purification tag or other sequence may be added at the N-terminal end and/or at the C-terminal end. If a tag is included the sequence may include a cleavage site to facilitate removal of the tag. The polypeptide may comprise or consist of a fusion protein wherein the fusion protein comprises a sequence according to (a), (b), (c), (d) or (e) fused to one or more further polypeptides. The further polypeptides may be, for example, one or more active or inactive domains of a protein, one or more active or inactive full-length proteins and/or one or more active or inactive protein fragments. The further polypeptide may be an antibody or part thereof. The antibody or part thereof may act to target the polypeptide of the invention to a particular location.

The polypeptide may comprise an active fragment of a polypeptide having the sequence of SEQ ID NO 1, 2, 3, 4, 5 or 6. The active fragment may comprise or consist of at least at least 40, at least 42, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70 or at least 75 contiguous amino acids of the sequence set out in SEQ ID NO: 1, 2, 3, 4, 5 or 6, or a sequence of 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more sequence identity thereto.

Preferably an active fragment according to the invention displays at least 50%, 60%, 70%, 75%, 80%, 85%, 90% or more of the activity, in relation to the complement system, of the polypeptide from which it is derived. That is, for example, the fragment has least 50% of the inhibitory activity with respect to complement activation as the polypeptide from which it is derived. An active fragment according to the invention may display at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% or more of the activity, in relation to the complement system in a particular tissue, of the polypeptide from

which it is derived. An active fragment may have lower activity, for example about 20%, 30%, 40% or 50% of the inhibitory activity with respect to complement activation in a particular tissue compared to the polypeptide from which it is derived, if it is also specific or targeted to the particular tissue.

If the polypeptide of the invention includes a signal and/or tag sequence one or more of these may be removed in order to release the active form of the polypeptide.

The invention also provides for a variant amino acid sequence having at least 60% sequence identity to SEQ ID NO: 1, 2, 3, 4, 5 or 6, the variant amino acid sequence may have at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or more sequence identity to SEQ ID NO: 1, 2, 3, 4, 5, or 6. The polypeptide of SEQ ID NO 1 of the invention was first isolated from the tick *Rhipicephalus pulchellus*, and the invention provides for variants thereof including homologues derived from other tick species which retain at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more sequence identity with SEQ ID NO: 1, 2, 3, 4, 5 or 6. Homologues may include paralogues and orthologues of the sequence of SEQ ID NO: 2, 3, 4, 5 or 6 including, for example polypeptides from other tick species including *Rhipicephalus appendiculatus, Rhipicephalus sanguineus, Rhipicephalus bursa, Amblyomma americanum, Amblyomma cajennense, Amblyomma hebraeum, Boophilus microplus, Boophilus annulatus, Boophilus decoloratus, Dermacentor reticulatus, Dermacentor andersoni, Dermacentor marginatus, Dermacentor variabilis, Haemaphysalis inermis, Haemaphysalis leachii, Haemaphysalis punctata, Hyalomma anatolicum anatolicum, Hyalomma dromedarii, Hyalomma marginatum marginatum, Ixodes ricinus, Ixodes persulcatus, Ixodes scapularis, Ixodes hexagonus, Argas persicus, Argas reflexus, Ornithodoros erraticus, Ornithodoros moubata moubata, Ornithodoros moubata porcinus,* and *Ornithodoros savignyi*.

Amino acid identity may be calculated using any suitable algorithm. For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (for example used on its default settings) (Devereux et al (1984) Nucleic Acids Research 12, 387-395). The PILEUP and BLAST algorithms can be used to calculate homology or line up sequences (such as identifying equivalent or corresponding sequences (typically on their default settings), for example as described

in Altschul S. F. (1993) J MoI Evol 36:290-300; Altschul, S, F et al (1990) J MoI Biol 215:403-10.

Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul et al, supra). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) Proc. Natl. Acad. ScL USA 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90: 5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two polynucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1 , more preferably less than about 0.01, and most preferably less than about 0.001.

The variant sequences typically differ by at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50 or more mutations (which may be substitutions, deletions or insertions of amino acids). For example, from 1 to 50, 2 to 40, 3 to 30 or 5 to 20

amino acid substitutions, deletions or insertions may be made. The substitutions are preferably conservative substitutions, for example according to the table below. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other.

Aliphatic	Non-Polar	G A P
		I L V
	Polar – uncharged	C S T M
		N Q
	Polar - charged	D E
		K R
Aromatic		H F W Y

The polypeptides of the invention may also be provided as a fusion protein comprising a polypeptide of the invention genetically or chemically fused to another peptide. The purpose of the other peptide may be any purpose, including, to aid detection, expression, separation or purification of the protein. Alternatively the protein may be fused to a peptide such as an Fc peptide to increase the circulating half life of the protein. Examples of other fusion partners include beta-galactosidase, glutathione-S-transferase, or luciferase.

The polypeptides of the invention may be chemically modified, e.g. post-translationally modified. For example, they may be glycosylated, pegylated, phosphorylated or comprise modified amino acid residues. They may be modified by the addition of histidine residues to assist their purification or by the addition of a signal sequence to promote insertion into the cell membrane. Such modified polypeptides fall within the scope of the term "polypeptide" used herein.

Polypeptides of the invention may be in a substantially isolated form. It will be understood that the polypeptide may be mixed with carriers or diluents which will not interfere with the intended purpose of the polypeptide and the polypeptide will still be regarded as substantially isolated. A polypeptide for use in the invention may also be in a substantially purified form, in which case it will generally comprise the polypeptide in a preparation in which more than 50%, e.g. more than 60%, 70%, 75%, 80%, 85%, 90%, 95% or 99%, by weight of the polypeptide in the preparation is a polypeptide of the invention.

Polypeptides of the invention may be made synthetically or be recombinantly produced. For example, a recombinant polypeptide may be produced by transfecting mammalian, fungal, bacterial, plant or insect cells in culture with an expression vector comprising a nucleotide sequence encoding the polypeptide operably linked to suitable control sequences, culturing the cells, extracting and purifying the polypeptide of the invention produced by the cells. The amino acid sequence of polypeptides for use in the invention may be modified to include non-naturally occurring amino acids or to increase the stability of the compound. When the polypeptides are produced by synthetic means, such amino acids may be introduced during production. The polypeptides may also be modified following either synthetic or recombinant production.

Polypeptides of the invention may also be produced using D-amino acids A number of side chain modifications are known in the art and may be made to the side chains of the polypeptides of the invention providing the activity of the polypeptide is retained.

Preferably a polypeptide, or composition, of the invention has the ability to inhibit or reduce the activity or activation of the complement system. By "inhibit" it is meant that the polypeptide is able to inhibit or reduce the activation or activity of one or more of the alternative, classical or lectin pathways of complement activation. Preferably the polypeptide, or composition of the invention, is able to inhibit the alternative pathway of the complement system. Preferably the polypeptide or composition of the invention has less, or no significant, effect on the lectin and/or classical pathways of complement activation. The ability of a polypeptide or composition to reduce the effect of a complement pathway may be determined by any standard haemolytic or other suitable assays known in the art, such as, for example, those described in the Examples and in Giclas et al (1994) or an enzyme immunoassay for the qualitative determination of functional classical, MBL and/or alternative complement pathways in human serum such as the assay described in Seelen *et al.* Journal of Immunological Methods Volume 296, Issues 1–2, January 2005, Pages 187–198, which is also known as a Wieslab® assay.

Preferably, the presence of a polypeptide or composition of the invention reduces red blood cell lysis in a suitable assay or demonstrates a reduction in complement activation in a suitable assay by at least 20% compared to the same assay in the absence of a polypeptide or composition of the present invention. A complement inhibitor molecule, polypeptide or composition of the present invention may, more preferably, reduce complement activation in a suitable assay by at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98% or 100%.

The polypeptide may reduce the total activity of the complement system by more than 10%, more than 20%, more than 30 %, more than 40%, more than 50%, more than 60%, more than 70%, more than 80% more than 90%, more than 95%, more than 98%, more than 99% or the polypeptide may reduce the activity of the complement by 100%.

The polypeptide may reduce the activity of the alternative pathway of the complement system by more than 10%, more than 20%, more than 30 %, more than 40%, more than 50%, more than 60%, more than 70%, more than 80% more than 90%, more than 95%, more than 98%, more than 99% or the polypeptide may reduce the activity of the alternative pathway of the complement system by 100%.

The polypeptide may reduce the total activity of the complement system in one or more specific tissues by more than 10%, more than 20%, more than 30 %, more than 40%, more than 50%, more than 60%, more than 70%, more than 80% more than 90%, more than 95%, more than 98%, more than 99% or the polypeptide may reduce the activity of the complement system in one or more specific tissues by 100%.

The polypeptide may reduce the activity of the alternative pathways of the complement system in one or more specific tissues by more than 10%, more than 20%, more than 30 %, more than 40%, more than 50%, more than 60%, more than 70%, more than 80% more than 90%, more than 95%, more than 98%, more than 99% or the polypeptide may reduce the activity of the alternative pathway of the complement system in one or more specific tissues by 100%.

The polypeptide or composition of the invention may act in the complement pathway by binding to properdin and may prevent or reduce activation or prolongation of activation of the alternative pathway by binding to properdin.

According to a further aspect, the invention provides a polynucleotide encoding a polypeptide of the invention. The polynucleotide may be DNA or RNA.

The invention may further provide a vector, for example an expression vector, comprising a polynucleotide of the invention. Such expression vectors are routinely constructed in the art of molecular biology and may for example involve the use of plasmid DNA and appropriate initiators, promoters, enhancers and other elements, such as for example polyadenylation signals, which may be necessary and which are positioned in the correct orientation in order to allow for protein expression. The coding sequences may also be selected to provide a preferred codon usage suitable for the host organism to be used. Other suitable vectors would be apparent to persons skilled in the art.

Preferably, a polynucleotide for use in the invention in a vector is operably linked to a control sequence which is capable of providing for the expression of the coding sequence by the host cell, i.e. the vector is an expression vector. The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A regulatory sequence, such as a promoter, "operably linked" to a coding sequence is positioned in such a way that expression of the coding sequence is achieved under conditions compatible with the regulatory sequence.

The vectors may be for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. The vector is typically adapted to be used in vivo. The vector may be a gene therapy vector, for example an adenovirus vector, a lentivirus vector or a CRISPR vector.

Promoters and other expression regulation signals may be selected to be compatible with the host cell for which expression is designed. Mammalian promoters, such as [beta]-actin promoters, may be used. Tissue-specific promoters are especially

preferred. Viral promoters may also be used, for example the Moloney murine leukaemia virus long terminal repeat (MMLV LTR), the Rous sarcoma virus (RSV) LTR promoter, the SV40 promoter, the human cytomegalovirus (CMV) IE promoter, adenovirus promoters, HSV promoters (such as the HSV IE promoters), or HPV promoters, particularly the HPV upstream regulatory region (URR). Viral promoters are readily available in the art.

The vector may further include sequences flanking the polynucleotide giving rise to polynucleotides which comprise sequences homologous to eukaryotic genomic sequences, preferably mammalian genomic sequences. This will allow the introduction of the polynucleotides of the invention into the genome of eukaryotic cells by homologous recombination. In particular, a plasmid vector comprising the expression cassette flanked by viral sequences can be used to prepare a viral vector suitable for delivering the polynucleotides of the invention to a mammalian cell. Other examples of suitable viral vectors include herpes simplex viral vectors and retroviruses, including lentiviruses, adenoviruses, adeno-associated viruses and HPV viruses. Gene transfer techniques using these viruses are known to those skilled in the art. Retrovirus vectors for example may be used to stably integrate the polynucleotide giving rise to the polynucleotide into the host genome. Replication-defective adenovirus vectors by contrast remain episomal and therefore allow transient expression.

The invention may further provide a host cell comprising the polynucleotide and/or the vector of the invention. The host cell may be a cell of the subject to be treated.

According to a further aspect the invention provides a composition comprising one or more isolated polypeptides of the invention. The composition of the present invention may be formulated for use *in vitro*. The composition of the present invention may be formulated for use *in vivo*, for example in humans. The composition of the present invention may be formulated for use in animals, for example, mammals, horses, cattle, pigs, sheep, goats, dogs, cats, rodents, fish, reptiles or birds. The composition of the present invention may be a pharmaceutical composition.

According to a yet further aspect the invention provides a pharmaceutical composition comprising one or more of (i) an isolated polypeptide of the invention; (ii) a

polynucleotide of the invention; (iii) a vector or the invention; and (iv) a host cell of the invention. The pharmaceutical composition may comprise further ingredients, for example, one or more pharmaceutically acceptable excipient or carrier.

The pharmaceutical composition of the present invention may comprise one or more further active ingredients in addition to the one or more isolated polypeptides of the invention. The pharmaceutical composition may comprise one or more further active ingredients that modulate the immune system.

The polypeptide, polynucleotide or composition of the invention may be intended to be administered by enteral or parenteral routes such as via oral, buccal, anal, pulmonary, intravenous, subcutaneous, intra-arterial, intramuscular, intraperitoneal, intraarticular, topical, inhalation, intraocular or other appropriate administration routes.

The polypeptide, polynucleotide or composition of the invention may be intended to be administered in a variety of dosage forms. It may be administered orally (e.g. as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules), parenterally, subcutaneously, intravenously, intramuscularly, intraocularly, intranasally, transdermally, topically or by infusion techniques.

Typically the polypeptide, polynucleotide or composition of the invention is formulated for use with a pharmaceutically acceptable carrier or diluent and this may be carried out using routine methods in the pharmaceutical art. The pharmaceutical carrier or diluent may be, for example, an isotonic solution. For example, solid oral forms may contain, together with the active compound, diluents, e.g. lactose, dextrose, saccharose, cellulose, corn starch or potato starch; lubricants, e.g. silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; binding agents; e.g. starches, arabic gums, gelatin, methylcellulose, carboxymethylcellulose or polyvinyl pyrrolidone; disaggregating agents, e.g. starch, alginic acid, alginates or sodium starch glycolate; effervescing mixtures; dyestuffs; sweeteners; wetting agents, such as lecithin, polysorbates, laurylsulphates; and, in general, non-toxic and pharmacologically inactive substances used in pharmaceutical formulations. Such pharmaceutical preparations may be manufactured in known manner, for example, by means of mixing, granulating, tabletting, sugar-coating, or film coating processes.

Liquid dispersions for oral administration may be syrups, emulsions and suspensions. The syrups may contain as carriers, for example, saccharose or saccharose with glycerine and/or mannitol and/or sorbitol.

Suspensions and emulsions may contain as carrier, for example a natural gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose, or polyvinyl alcohol. The suspensions or solutions for intramuscular injections may contain, together with the active compound, a pharmaceutically acceptable carrier, e.g. sterile water, olive oil, ethyl oleate, glycols, e.g. propylene glycol, and if desired, a suitable amount of lidocaine hydrochloride.

Solutions for intravenous administration or infusions may contain as carrier, for example, sterile water or preferably they may be in the form of sterile, aqueous, isotonic saline solutions.

For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1% to 2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10% to 95% of active ingredient, preferably 25% to 70%. Where the pharmaceutical composition is lyophilised, the lyophilised material may be reconstituted prior to administration, e.g. a suspension. Reconstitution is preferably effected in buffer.

Capsules, tablets and pills for oral administration to a patient may be provided with an enteric coating comprising, for example, Eudragit "S", Eudragit "L", cellulose acetate, cellulose acetate phthalate or hydroxypropylmethyl cellulose. Pharmaceutical compositions suitable for delivery by needleless injection, for example, transdermally, may also be used. The compositions according to the invention may be presented in all dosage forms normally used for topical application, in particular in the form of aqueous, aqueous-alcoholic or, oily solutions, of dispersions of the lotion or serum type, of anhydrous or lipophilic gels, of emulsions

of liquid or semi-solid consistency of the milk type, obtained by dispersing a fatty phase in an aqueous phase (O/W) or vice versa (W/O), or of suspensions or emulsions of soft, semi-solid consistency of the cream or gel type, or alternatively of microemulsions, of microcapsules, of microparticles or of vesicular dispersions to the ionic and/or nonionic type. These compositions are prepared according to standard methods. They may also be used for the scalp in the form of aqueous, alcoholic or aqueous-alcoholic solutions, or in the form of creams, gels, emulsions or foams or alternatively in the form of aerosol compositions also containing a propellant agent under pressure.

The amounts of the different constituents of the compositions according to the invention are those traditionally used in the fields in question.

Preferably a therapeutically effective amount of a polypeptide, polynucleotide or composition of the invention is administered or intended to be administered. The dose may be determined according to various parameters, especially according to the polypeptide, polynucleotide or composition used; the age, weight and condition of the patient to be treated; the route of administration; and the required regimen. Again, a physician will be able to determine the required route of administration and dosage for any particular patient. A typical daily dose is from about 0.001 to 50mg per kg, preferably from about 0.0lmg/kg to 10mg/kg of body weight, according to the activity of the polypeptide, the age, weight and conditions of the subject to be treated, the type and severity of the disease and the frequency and route of administration. Preferably, daily dosage levels are from 0.5mg to 2g. Lower dosages may be used for topical administration.

According to a further aspect, the invention provides a composition comprising a polypeptide of the invention; a polynucleotide of the invention; a vector of the invention; or a host cell of the invention; for use in medicine.

According to a further aspect, the invention provides a composition comprising a polypeptide of the invention; a polynucleotide of the invention; a vector of the invention; or a host cell of the invention; for use in the treatment of a disease or a condition mediated by complement.

The treatment may be therapeutic or prophylactic.

According to a another aspect, the invention provides a method for the treatment of a disease or a condition mediated by complement comprising administering to a subject in need thereof an effective amount of a polypeptide of the invention; a polynucleotide of the invention; a vector of the invention; or a host cell of the invention.

According to a another aspect, the invention provides use of a polypeptide of the invention; a polynucleotide of the invention; a vector of the invention; a host cell of the invention; or a composition of the invention in the preparation of a medicament for the treatment of a disease or a condition mediated by complement.

A disease or disorder mediated by complement which may treatable or preventable by the polypeptide, polynucleotide or composition of the present invention may be any disease or disorder that results from, or results in, activation, or increased activation, of the complement system in a subject. The polypeptide, polynucleotide, composition or pharmaceutical composition of the present invention may be useful in preventing activation of the complement system so that it is kept at a normal level in an individual who is at risk of abnormal activation of the complement system or any disease or disorder related to abnormal activation of the complement system. The polypeptide, polynucleotide, composition or pharmaceutical composition of the present invention may be particularly useful in preventing activation of the alternative pathway with a less significant effect on the classical and/or lectin pathway.

The polypeptide, polynucleotide, composition or pharmaceutical composition of the present invention may be useful in reducing the activity of the complement system in a subject that has an abnormally active complement system. The polypeptide, polynucleotide, composition or pharmaceutical composition of the present invention may be useful in reducing activation of the classical pathway, alternative and/or the lectin pathway, preferably the alternative pathway. This may be useful in treatment of conditions where uncontrolled or inappropriate activation of the alternative pathway may occur including conditions such as age related macular degeneration, Alzheimer's, haemolytic uremic syndrome, atypical haemolytic uremic syndrome, asthma and chronic obstructive pulmonary disease.

The polypeptide, polynucleotide or composition of the present invention may be for use in reducing activity of one or more of the complement pathways or inhibiting activation of one or more of the complement pathways. In one embodiment, the polypeptide, polynucleotide or composition of the present invention may be for use in reducing activity of the alternative pathway of the complement system, and has little of no effect on the classical or lectin pathways.

The polypeptide, polynucleotide or composition of the present invention may be for use in the treatment of a disease or disorder associated with increased activity in one or more of the complement pathways, preferably the alternative pathway.

The polypeptide, polynucleotide or composition of the present invention may be for use in the treatment of a disease or disorder associated with inappropriate activation of one or more the complement pathways, such as the alternative pathway.

A disease or disorder mediated by complement may include an inflammatory disease, ischemia, reperfusion injury, an autoimmune disease, an infection, an infection disease, transplant rejection, an ocular disease or a cancer.

A disease or disorder mediated by complement may also include a disease or disorder selected from systemic lupus erythematosus and glomerulonephritis, arthritis, rheumatoid arthritis, cardiopulmonary bypass and hemodialysis, hypercute rejection in organ transplantation, myocardial infarction, reperfusion injury, and adult respiratory distress syndrome. In addition, other inflammatory conditions and autoimmune/immune complex diseases are also closely associated with complement activation, including thermal injury, severe asthma, anaphylactic shock, bowel inflammation, urticaria, angioedema, vasculitis, multiple sclerosis, myasthenia gravis, membranoproliferative glomerulonephritis, Sjogren's syndrome, renal disease, sepsis, paroxysmal nocturnal hemoglobinuria, psoriasis, transplant rejection, cancer, stroke, age-related macular degeneration, atypical haemolytic uremic syndrome, haemolytic uremic syndrome, Crohn’s disease, Alzheimer’s disease, cerebrovascular insufficiency, small vessel disease, frontotemporal disorder, Parkinson’s disease, multiple sclerosis, amyotrophic lateral sclerosis, ischemia reperfusion injury, glaucoma and dense deposit disease aka membranoproliferative glomerulonephritis type II.

Polypeptides and compositions of the present invention may be particularly useful in the treatment or prevention of acute rejection, nerve disorders mediated by antibody mediated complement activation (e.g. myasthenia gravis, Guillain-Barre syndrome, Miller-Fisher syndrome, neuromyelitis optica) and anti-phospholipid syndrome.

Polypeptides and compositions of the present invention may be particularly useful in the treatment or prevention of age related macular degeneration, Alzheimer's disease, atypical haemolytic uremic syndrome, haemolytic uremic syndrome, cerebrovascular insufficiency, small vessel disease, frontotemporal disorder, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, glaucoma, asthma and chronic obstructive pulmonary disease.

Polypeptides and compositions of the present invention may be particularly useful in the treatment or prevention of glomerulonephritis, arthritis, rheumatoid arthritis, ischemia reperfusion injury, age-related macular degeneration, paroxysmal nocturnal hemoglobinuria, atypical haemolytic uremic syndrome, transplant rejection, inflammation and systemic lupus.

The present invention further provides a method of reducing the activity or activation of the classical complement pathway, alternative complement pathway and/or the lectin complement pathway in a subject, preferably the alternative pathway, the method comprising the step of administering an effective amount of a polypeptide, polynucleotide or composition of the invention.

The polypeptide, polynucleotide or composition of the invention may be advantageous because a unit dose of the polypeptide can reduce the activity of the alternative pathway, preferably to about 5, 10, 15, 20 or more times greater extent than the classical and/or lectin complement pathways. In an embodiment the polypeptide, polynucleotide or composition of the invention may be advantageous because a unit dose of the polypeptide can reduce the activity of the alternative pathway, whilst have little or no effect on the classical and/or lectin complement pathways. Therefore, the polypeptides, polynucleotides or compositions of the invention may be used to reduce the activity of the alternative pathway without having a significant effect on the classical and/or lectin complement pathways. This aspect may be particularly useful

where uncontrolled or inappropriate activation of the alternative pathway may occur including conditions such as age related macular degeneration, Alzheimer's, haemolytic uraemic syndrome, atypical haemolytic uraemic syndrome. At a suitable dosage the activity of the alternative pathway may be greatly reduced while the activity of the classical and lectin complement pathways is only slightly reduced or not reduced at all.

The present invention further provides a method of treating a disease or disorder associated with abnormal and/or increased activity of the complement pathway in a subject, wherein the method comprises administering to the subject an effective amount of a polypeptide, polynucleotide or composition of the invention.

The present invention further provides the use of a polypeptide, polynucleotide or composition of the invention in an in vitro method. For example, the polypeptide of the present invention may be used for investigating the complement pathways. The polypeptide of the present invention may be used to raise antibodies that specifically bind to the polypeptide of the invention. An isolated nucleic acid of the invention may be used as a polynucleotide probe in an *in vitro* method.

The polypeptide of the present invention or the polynucleotide of the present invention may be used in a diagnostic assay to test the activation of the complement system. For example the polypeptide or the isolated nucleic acid of the present invention may be used in a diagnostic assay to distinguish activation of the classical pathway or the lectin pathway from activation of the alternative pathway.

The present invention provides a method of providing a polypeptide of the invention, the method comprising expressing the polypeptide in suitable cell.

The cell may be a bacterial cell, a yeast cell, an insect cell or a mammalian cell. The cell may be a Drosophila S2 cell.

The polypeptide may be synthesised chemically *in vitro*.

The skilled man will appreciate that preferred features of any one embodiment and/or aspect and/or claim of the invention may be applied to all other embodiments and/or aspects and/or claims of the invention.

There now follows by way of example only a detailed description of the present invention with reference to the accompanying drawings, in which;

Figure 1 - shows an overview of the classical pathway, lectin pathway and alternative pathway of the complement system.

Figure 2A – shows the amino acid sequence of CIRp-A derived from *Rhipicephalus pulchellus* (SEQ ID NO 1), SEQ ID NO: 7 shows CIRp-A with a signal sequence and 6 His tag, and SEQ ID NO: 8 shows CIRp-A with a 6 His tag. SEQ ID NOs 2, 3, 4, 5 and 6 are homologues of SEQ ID NO: 1 (CIRp-A) and all have no signal sequence or 6 His tag. SEQ ID NO: 9 shows SEQ ID NO: 3 with a his Tag and SEQ ID NO: 10 shows SEQ ID NO: 3 with a His tag and signal peptide. The homologues are derived from *Rhipicephalus appendiculatus* (SEQ ID NOs 2 and 6), *Rhipicephalus microplus* (SEQ ID NO 3) and *Rhipicephalus pulchellus* (SEQ ID NOs 4 and 5).

Figure 2B – shows the structure of CIRp-A derived from *Rhipicephalus pulchellus*.

Figure 3 shows a Clustal Omega multiple sequence alignment of SEQ ID Nos 1 to 6.

Figure 4 shows a Percentage Identity Matrix - created by Clustal Omega of SEQ ID NOs 1 to 6.

Figure 5 shows the results of complement inhibition assays using SEQ ID NO: 8 (CIRp-A) purified from *D. melanogaster* S2 cells. A Wieslab assay (Eurodiagnostica, Sweden) was carried out with titrations of CIRp-A showing that CIRp-A inhibits the alternative pathway but not the classical and lectin pathways at the concentrations tested. Values were normalised for serum only (100% activity) and no serum (0% activity). N=3 and error bars represent s.e.m.

Figure 6 shows C3a levels in the supernatants of the Wieslab assay at 1 μM inhibitor depicted in Figure 5. Levels were measured with a Microvue C3a kit (Quidel, USA). CIRp-A (polypeptide of SEQ ID NO: 8) can be seen to significantly inhibit C3a formation through the alternative pathway - $p < 0.0001$ by unpaired two-tailed t test, with PBS as a reference. N=3 and error bars represent s.e.m.

Figure 7 shows C3b deposition in an ELISA based assay for Classical and Alternative pathway inhibition. 96 well plates were coated with IgM (classical pathway) or LPS (alternative pathway). Diluted normal human serum was added in the presence or absence of the CIRp-A inhibitor (SEQ ID NO: 8). Plates were incubated for 1 hour at 37°C and C3b deposition was detected using an anti-C3 antibody coupled to HRP (Amsbio, UK). N = 3 and error bars indicate s.e.m. Values are normalised for no serum samples (0% activity) and no additive (100% activity)

Figure 8 demonstrates that the CIRp-A polypeptide (SEQ ID NO: 8) binds properdin. Figure 8 shows the results of a western blot with a polyclonal antibody against properdin and shows that CIRp-A binds properdin, while empty beads (-ve control) do not bind properdin. CIRp-A was coupled to NHS-activated magnetic beads (Thermo Scientific, UK) following the manufacturer's instructions. The beads were incubated with normal human serum followed by three wash steps. Bound proteins were eluted by heating the beads in non-reducing SDS-loading buffer and resolved on a SDS-PAGE gel. Proteins were transferred to a PVDF membrane which was blotted for properdin using polyclonal anti-properdin antibodies (Comptech, USA). Pure properdin (Comptech, USA) was loaded as positive control.

Figure 9 shows the inhibition of the alternative pathway by CIRp-A and a homolog thereof (SEQ ID NO 3 + His tag = SEQ ID NO 9), as determined by ELISA measuring MAC deposition. Supernatants of transfected cells were used. Only CIRp-A (SEQ ID NO: 8) and SEQ ID NO 9 show significant reduction in MAC formation. However other experiments undertaken by the inventors suggest that the other homologs may be active against pig serum. 96

well plates were coated with LPS and diluted normal human serum was added in the presence or absence of inhibitor. Plates were incubated for 1 hour at 37 C and MAC deposition was detected using an anti-C5b-9 monoclonal antibody (Abcam, UK). N = 3 and error bars indicate s.e.m. Values are normalised for no serum samples (0% activity) and no additive (100% activity).

Figure 10 shows that the CIRp-A polypeptide (SEQ ID NO 8) potently inhibits serum from humans and pigs in an alternative pathway haemolytic assay using rabbit red blood cells. Final serum dilutions used were 1/8 for both. N = 3 and error bars indicate s.e.m. Values are normalised for no serum samples (0% lysis) and no additive (100% lysis).

MATERIALS AND METHODS

Sequence and expression of polypeptides

Polypeptides of SEQ ID NO 1 to 6 were expressed with a his-Tag from pExpres2-2 vectors in *Drosophila melanogaster* S2 cells (ExpreS2ion Biotechnologies, Denmark). All polypeptides were expressed with the signal sequence MKLCILLAVVAFVGLSLG which was cleaved during expression in *Drosophila melanogaster* S2 cells to provide the peptides shown in SEQ ID NOs 1-6 with a His-Tag – exemplified by SEQ ID Nos: 8, 9 10). The proteins were stably expressed according to the manufacturer's protocol (ExpreS2ion Biotechnologies, Denmark). Activity of homologs was tested using supernatants 7 days after transfection. Spent medium was cleared by centrifugation and tested for anti-complement activity using complement inhibition assays

CIRp-A fused to an N-terminal His6-tag (His6- CIRp-A) was expressed from a pExpres2-2 vector in *Drosophila melanogaster* S2 cells (ExpreS2ion Biotechnologies, Denmark). The generation of a stable cell line and expression of His6-CIRp-A were done according to the manufacturer's protocol (ExpreS2ion Biotechnologies, Denmark). Cell cultures were cleared by centrifugation and His6- CIRp-A was purified from the supernatant using a complete His-Tag Purification column (Roche) , followed by a gel filtration step.

Wieslab assay

Complement inhibiting activity of CIRp-A for all three pathways (classical, lectin and alternative) was determined using the Complement System Screen WIESLAB (Euro Diagnostica, Sweden), according to the manufacturer's protocol with the following modifications. A dilution series of CIRp-A (SEQ ID NO: 8) was added to the wells, prior to the addition of 100 µL diluted normal human serum using dilution reagents and dilution factors provided by the manufacturer. Values were normalised for serum only (100% activity) and no serum (0% activity).

C3a detection

C3a levels were determined in supernatants from the Wieslab assays in the presence of 1 µM inhibitor using a MicroVue C3a kit (Quidel), per the manufacturer's instructions. Supernatants from the Wieslab assay were diluted 40-fold (classical and lectin pathways) and 500-fold (alternative pathway) with diluent provided with the kit. Supernatants from Wieslab assays in the presence of 10 mM EDTA or 1 µM non-inhibiting protein were used as positive and negative controls respectively.

C3b and MAC deposition assay

MaxiSorp 96 well plates were coated with 2 µg/ml IgM (Bio-Rad) (classical pathway) or 1.8 mg/ml LPS from *Salmonella typhosa* (Sigma-Aldrich) (alternative pathway) in 100mM Na_2CO_3/$NaHCO_3$ pH 9.6 overnight at room temperature. Plates were blocked with 1% BSA. Diluted normal human serum (1/100 in 142 mM NaCl, 50 mM sodium 5,5-diethylbarbiturate, 0.5 mM $MgCl_2$, 2 mM $CaCl_2$, and 0.1% gelatin; pH 7.5 for classical pathway and 1/10 in 142 mM NaCl, 50 mM sodium 5,5-diethylbarbiturate, 10 mM EGTA, 5 mM MgCl2, and 0.1% gelatin; pH7.5 for alternative pathway) in the presence or absence of the CIRp-A inhibitor (SEQ ID NO: 8). Plates were incubated for 1 hour at 37°C and C3b deposition was detected using an anti-C3 antibody coupled to HRP (Amsbio, UK) and MAC deposition using an anti C5b-9 mouse monoclonal antibody followed by an anti-mouse HRP conjugated antibody (Promega). 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (Sigma-Aldrich) was used as substrate. The reaction was stopped by addition of an equal volume of 1% SDS and the absorption was measured at 405 nm. Measurements were subtracted with buffer-only samples and normalized for serum-only samples (100% activity).

Pulldown assay

CIRp-A was coupled to Pierce NHS-activated magnetic beads (Thermo Scientific), per the manufacturer's instructions. 20 µl beads was incubated with normal human serum in the presence of 10 mM EDTA, for 30 min at room temperature. Beads were washed three times with 1 ml PBS supplemented with 0.005% Tween-20 and once with 100 µl PBS. Bound proteins were recovered by boiling the beads in non-reducing SDS-PAGE loading buffer, before separation on SDS-PAGE gel. Proteins were transferred to a PVDF membrane which was blotted for properdin using polyclonal anti-properdin antibodies (Comptech, USA), followed by anti-goat HRP antibodies (Promega).

Haemolysis assays

Rabbit red blood cells (TCS Biosciences) were washed with PBS and resuspended in 142 mM NaCl, 50 mM sodium 5,5-diethylbarbiturate, 0.1% gelatin, 8 mM EGTA, and 0.5 mM $MgCl_2$, pH 7.35 (GVB Mg/EGTA). 50 µl cells (2×10^8 cells/ml) was mixed with an equal volume of 4-fold diluted human or pig serum in GVB Mg/EGTA in the presence or absence of inhibitor and incubated at 37 °C for 1 h with shaking. 100 µl of PBS was added, and non-lysed cells were pelleted by centrifugation. 100 µl of the supernatant was transferred to a clean plate, and haemolysis was quantified at A_{405}. Cells with GVB Mg/EGTA instead of serum were used as a blank, and cells with serum only were used for normalization (100% activity).

RESULTS

Complement inhibition assay for CIRp-A

The results provided herein demonstrate the specificity of CIRp-A (SEQ ID NO: 1) and homologues thereof for inhibiting the alternative complement pathway.

The results of the complement inhibition assay shown in Figure 5 illustrate the effect of CIRp-A on the Classical Pathway (CP), Lectin Pathway (LP) and Alternative Pathway (AP). The assay is performed at increasing concentrations of CIRp-A and the differences in the level of MAC deposition across this concentration gradient reveal the ability of CIRp-A to inhibit the different pathways. Since the levels of MAC formation for the CP and LP remain high at all CIRp-A concentrations tested whilst those for the AP rapidly decrease as CIRp-A is added this demonstrates that CIRp-A specifically inhibits the AP.

Activation of complement results in the cleavage of the protein C3 to form two fragments C3a and C3b. Figure 6 shows the effect of CIRp-A on C3a levels whilst Figure 7 shows the effect on C3b levels upon activation of each of the three pathways. Taking Figures 6 and 7 together they demonstrate that CIRp-A inhibits solely the AP and acts to decrease the level of C3 cleavage so reducing levels of both C3a and C3b.

Figure 8 shows the results of an assay in which recombinant CIRp-A was coupled to magnetic beads and used to specifically pull down properdin from human serum. In the negative control lane empty beads were used. In the CIRp-A lane beads covered in CIRp-A were used. Beads were incubated with serum, then washed and bound proteins were eluted by boiling in sample buffer prior to separation on SDS-PAGE gel. The only serum protein retained on the CIRp-A coated beads and not on the empty beads was properdin, as shown by western blot using an antibody against properdin. The third lane (labelled properdin) contains purified properdin to confirm the specificity of the antibody used to detect properdin. The strength of signal and its position on the gel demonstrate that CIRp-A specifically binds to properdin in human serum.

The results shown in Figure 9 confirm the activity of the related sequences on the Alternative Pathway (AP) to those presented in Figure 5, in this figure the assay is repeated using supernatants from insect cells expressing CIRp-A (positive control), an unrelated protein (negative control) and homologues of CIRp-A. The data shows that like CIRp-A, SEQ ID NO 3 inhibits the AP.

The results in Figure 10 demonstrates that CIRp-A can inhibit the AP in species other than human, namely in pig serum. The alternative pathway of human and pigs was activated in the presence of rabbit red blood cells and increasing concentrations of CIRp-A and the level of activation was determined by measuring lysis of the red blood cells. CIRp-A inhibits lysis of red blood cells through the AP activation of both human and pig serum.

CLAIMS

1. An isolated polypeptide comprising or consisting of:

(a) the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5 or 6; or

(b) the amino acid sequence of (a) with a signal sequence and/or a purification tag sequence; or

(c) a variant amino acid sequence having at least 60% sequence identity to (a) or (b); or

(d) an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% sequence identity to (a) or (b); or

(e) an active fragment of (a), (b), (c) or (d) that is at least 40, 50, 60, 65, 70 or 75 amino acids in length.

2. The isolated polypeptide of claim 1 consisting of the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5 or 6 with additional amino acids at one or both ends.

3. The isolated polypeptide according to claim 1 or 2 wherein the polypeptide consists of a fusion protein comprising a sequence according to (a), (b), (c), (d) or (e) fused to another peptide.

4. The isolated polypeptide according to any preceding claims wherein the polypeptide has the ability to inhibit or reduce the activity or activation of the complement system.

5. The isolated polypeptide according to any preceding claims wherein the polypeptide is able to inhibit or reduce the activation or activity of the alternative pathway of complement.

6. The isolated polypeptide according to claim 4 or 5 wherein the polypeptide reduces complement activation in a suitable assay by at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98% or 100%.

7. The isolated polypeptide according to any of claims 4 to 6 wherein the polypeptide reduces the total activity of the complement system by more than 10%, more than 20%, more than 30 %, more than 40%, more than 50%, more than 60%, more than 70%, more than 80% more than 90%, more than 95%, more than 98% or by more than 99%.

8. The polypeptide of any preceding claim where the polypeptide binds to or prevents the activity of properdin.

9. A polynucleotide encoding a polypeptide of any preceding claim.

10. An expression vector, comprising a polynucleotide according to claim 9.

11. A host cell comprising the polynucleotide of claim 9 and/or the vector of claim 10.

12. A composition comprising one or more isolated polypeptides according to any of claims 1 to 8.

13. A pharmaceutical composition comprising one or more of (i) an isolated polypeptide of any of claims 1 to 8; (ii) a polynucleotide of claim 9; (iii) a vector of claim 10; and (iv) a host cell of claim 11 and a pharmaceutically acceptable excipient or carrier.

14. The pharmaceutical composition of claim 13 further comprising one or more further active.

15. A pharmaceutical composition according to claim 13 or 14 for use in medicine.

16. A pharmaceutical composition according to claim 13 or 14 for use in inhibiting or reducing the activation or activity of complement.

17. A pharmaceutical composition according to claim 16 for use in inhibiting or reducing activation or activity of the alternative pathway of complement.

18. A pharmaceutical composition according to claim 16 or 17 for use in the prophylactic or therapeutic treatment of a disease or a condition mediated by complement.

19. A pharmaceutical composition according to claim 16, 17 or 18 for use in the prophylactic or therapeutic treatment or prevention of age related macular degeneration, Alzheimer's disease, haemolytic uremic syndrome, atypical haemolytic uremic syndrome, cerebrovascular insufficiency, small vessel disease, frontotemporal disorder, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, glaucoma, asthma and chronic obstructive pulmonary disorder.

20. A method of treating a disease or disorder associated with abnormal and/or increased activity of the complement pathway in a subject, wherein the method comprises administering to the subject an effective amount of a polypeptide, polynucleotide or composition according to preceding claim.

21. The method of claim 20 wherein the complement pathway is the alternative complement pathway.

22. The method of claim 20 or 21 wherein the disorder is selected from the group consisting of age related macular degeneration, Alzheimer's disease, haemolytic uremic syndrome, atypical haemolytic uremic syndrome, cerebrovascular insufficiency, small vessel disease, frontotemporal disorder, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, glaucoma, asthma and chronic obstructive pulmonary disorder.

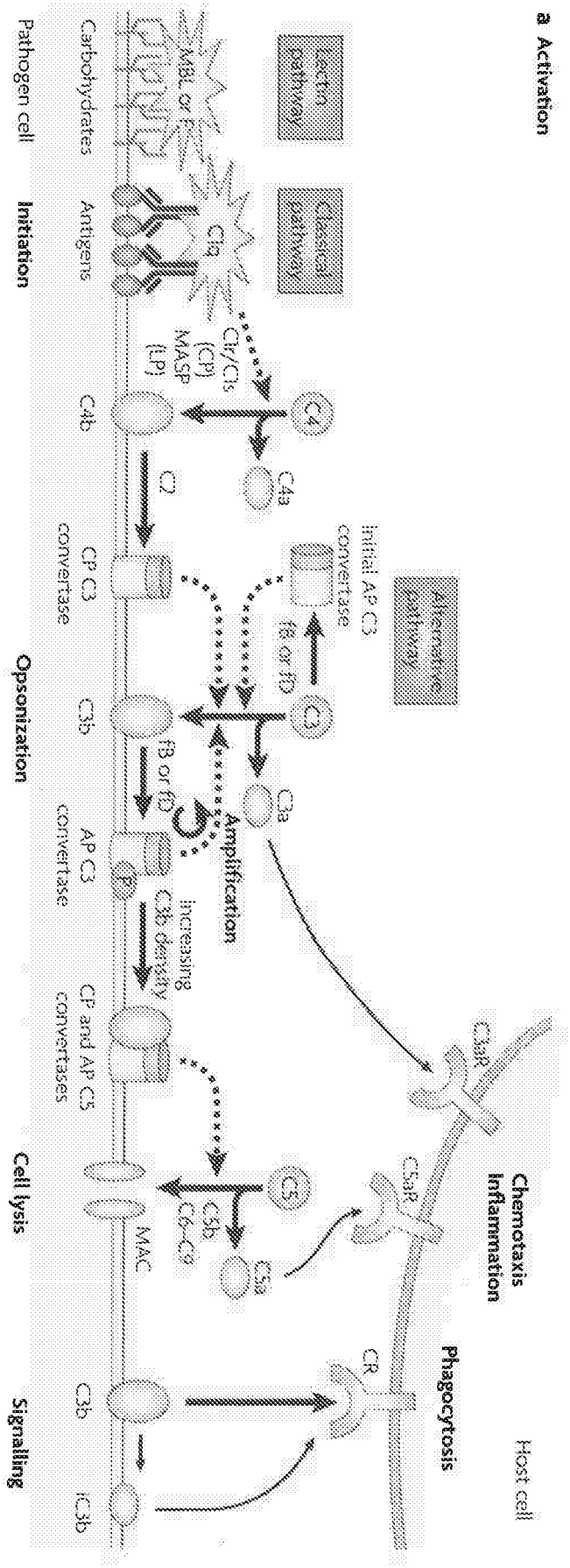
a Activation
Lectin pathway
Classical pathway
Alternative pathway
MBL or Ficolins
Clq
Clr/Cls (CP) MASP (LP)
Carbohydrates
Antigens
Pathogen cell
Initiation
C4
C4a
C4b
C2
CP C3 convertase
Initial AP C3 convertase
fB or fD
C3
C3a
C3b
Opsonization
Amplification
P
AP C3 convertase
increasing C3b density
CP and AP C5 convertases
C5
C5b
C6-C9
C5a
MAC
Cell lysis
Chemotaxis Inflammation
C5aR
CR
Phagocytosis
Host cell
iC3b
Signalling

Figure 1

SEQ ID NO: 1
EDQETDFSSTDGAELIAKEPEVYPIDQFMNNTEIWVFNTTQPDPPNCKKDKSKSMTQTATSFVRSHVK
NGNIIEENLVGNFTYFNDKEKVYDGIYISGESSGVYAEHLYYVSEDKKCGLFQVFAHVNDKTTIWRDV
RVSGRPEEGVPLELNCTKEFDEYVKLVNATSKSPYTSECQ

SEQ ID NO: 2
NEQDSEIYRFDQFMNTDDYIWVFNTTQEGPKECEKDKKHNMTNDKIIFVRSHQEETKIVNETIIGDF
FHYSDNKSVYDGIYISGDKREVHAEHLYYSSEDMICGLVQVFARQTDAWTELRVRGRRSYKSLDEVC
RTQYEKYVEAIKHTKTSTSPYRDDCQ

SEQ ID NO: 3
QDSEIDHTDETTSSTAEDVEDYAIDKFMNTSLEIWVLNTTQQNPHNCQKDLPYNMTGNTTFFFRSHE
EGHNITNESLAGHFEKFAGTEKVYNQIALSGGSSHVHAEFLFYATKNLTCGLVRVFAFVDGYNVMWD
DLRVKGRPTNLSDIKECTEEFNKYVDAMITKNWTSPYNASCK

SEQ ID NO: 4
NDWNPESFAIDEFMNTTDDIWVLNTTQQNPQACKKDKKHNITENGIYFFRSHKENGQIKTQTLFGEF
IHFSEEEKVNNRISISDESSGVHAEHLYYSSEDKKCGLVQVFAKDQNVWTELRVRGHPNYGSLDAGC
RREYEAYVKEIKGKKNSTSPYSDDCQIKV

SEQ ID NO: 5
EDQETDFSSTDGAELIAKEPEVYPIDQFMNNTEIWVFNTTQPDPPNCKKDKSKSMTQTATSFVRSHV
KNGNIIEENLVGNFTYFNDKEKVYDGIYISGESSGVYAEHLYYVSEDKKCGLVQVFAKDQNVWTELR
VRGHPNYGSLDAECRREYEAYVKEIKGKKNSTSPYSDDCQIKV

SEQ ID NO: 6
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FANFYDKPDGVYDRIDITGDKTGVYEEALAYASKENTCGVVGVWAFDGETTVVWRELRVRNRPNDATK
VDEMCKKKFDDYVQVVNKSWTSPYNEKCK

SEQ ID NO: 7 (Seq ID No: 1 + signal peptide and His tag)
MKLCILLAVVAFVGLSLGHHHHHHAGEDQETDFSSTDGAELIAKEPEVYPIDQFMNNTEIWVFNTT
QPDPPNCKKDKSKSMTQTATSFVRSHVKNGNIIEENLVGNFTYFNDKEKVYDGIYISGESSGVYAEHL
YYVSEDKKCGLFQVFAHVNDKTTIWRDVRVSGRPEEGVPLELNCTKEFDEYVKLVNATSKSPYTSECQ

SEQ ID NO: 8 (Seq ID No: 1 + His tag)
HHHHHHAGEDQETDFSSTDGAELIAKEPEVYPIDQFMNNTEIWVFNTTQPDPPNCKKDKSKSMTQ
TATSFVRSHVKNGNIIEENLVGNFTYFNDKEKVYDGIYISGESSGVYAEHLYYVSEDKKCGLFQV
FAHVNDKTTIWRDVRVSGRPEEGVPLELNCTKEFDEYVKLVNATSKSPYTSECQ

SEQ ID NO: 9 (Seq ID No: 3 + His tag)
HHHHHHAGQDSEIDHTDETTSSTAEDVEDYAIDKFMNTSLEIWVLNTTQQNPHNCQKDLPYNMTGNT
TFFFRSHEEGHNITNESLAGHFEKFAGTEKVYNQIALSGGSSHVHAEFLFYATKNLTCGLVRVFAFV
DGYNVMWDDLRVKGRPTNLSDIKECTEEFNKYVDAMITKNWTSPYNASCK

SEQ ID NO: 10 (Seq ID No: 3 + signal peptide and His tag)
MKLCILLAVVAFVGLSLGHHHHHHAG
QDSEIDHTDETTSSTAEDVEDYAIDKFMNTSLEIWVLNTTQQNPHNCQKDLPYNMTGNTTFFFRSHE
EGHNITNESLAGHFEKFAGTEKVYNQIALSGGSSHVHAEFLFYATKNLTCGLVRVFAFVDGYNVMWD
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Figure 2A

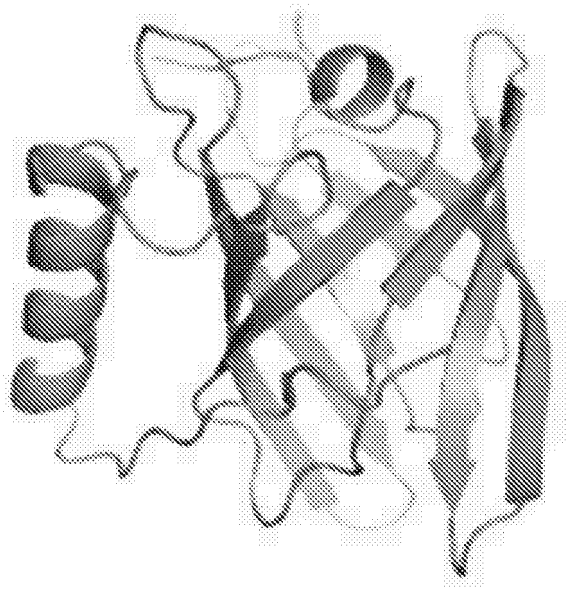

Figure 2B

```
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Seq_ID3          1 --QDSEIDHTDETTSSTAEDVEDYAIDKPMNTSLEIWVLNTTQQNPHNCQKDLPYNMTGNT 59
Seq_ID2          1 ----------------------NEQDSEIYRPDQPMNTDDYIWVFNTTQEGPKECEKDKKHNMTNDK 45
Seq_ID4          1 ----------------NDWNPESFAIDEPMNTTDDIWVLNTTQQNPQACKKDKKHNITENG 45
Seq_ID1          1 EDQETDFSST-DGAELIAKEPEVYPIDQPMNNT-EIWVFNTTQPDPPNCKKDKSKSMTQTA 58
Seq_ID5          1 EDQETDFSST-DGAELIAKEPEVYPIDQPMNNT-EIWVFNTTQPDPPNCKKDKSKSMTQTA 59

Seq_ID6         48 TFFTRSHEEQDQIHEQELVGKPANFYDKPDGVYDRIDITGDKTGVYEEALAYASKENTCGV 108
Seq_ID3         60 TFFFRSHEEGHNITNRSLAGHPEKFAG-TEKVYNQIALSGGSSHVHAEFLFYATKNLTCGL 119
Seq_ID2         46 IIFVRSHQEETKIVNETIIGDPFHYSD-NKSVYDGIYISGDKREVHAEHLYYSSEDMICGL 105
Seq_ID4         46 IYFFRSHKENGQIKTQTLFGEFIHPSE-EEKVNNRISISDESSGVHAEHLYYSSEDKKCGL 105
Seq_ID1         60 TSFVRSHVKNGNIIEENLVGNFTYFND-KEKVYDGIYISGESSGVYAEHLYYVSEDKKCGL 119
Seq_ID5         60 TSFVRSHVKNGNIIEENLVGNFTYFND-KEKVYDQIYISQESSGVYAEHLYYVSEDKKCGL 119

Seq_ID6        109 VGVWAFDGETTVVWRELRVRNRPNDATKVDEMCKKKFDDYVQVVNK--SWTSPYNEKCK--- 165
Seq_ID3        120 VRVFAFVDGYNVMWDDLRVKGRPTNL-SDIKECTEEFNKYVDAMIT-KNWTSPYNASCK--- 176
Seq_ID2        106 VQVFAR---QTDAWTELRVRGRRSYK-SLDEVCRTQYEKYVEAIKHTKTSTSPYRDDCQ--- 160
Seq_ID4        106 VQVFAK---DQNVWTELRVRGHPNYG-SLDAGCRREYEAYVKEIKGKKNSTSPYSDDCQIKV 163
Seq_ID1        120 FQVFAHVNDKTTIWRDVRVSGRPEEGVPLELNCTKEFDEYVKLVNA--TSKSPYTSECQ--- 176
Seq_ID5        120 VQVFAK---DQNVWTELRVRGHPNYG-SLDAECRREYEAYVKEIKGKKNSTSPYSDDCQIKV 177
```

Figure 3

1: Seq_ID6	100.00	43.56	41.77	41.77	45.40	46.54
2: Seq_ID3	43.56	100.00	42.77	47.17	43.35	45.03
3: Seq_ID2	41.77	42.77	100.00	56.25	47.77	55.97
4: Seq_ID4	41.77	47.17	56.25	100.00	50.96	70.99
5: Seq_ID1	45.40	43.35	47.77	50.96	100.00	81.98
6: Seq_ID5	46.54	45.03	55.97	70.99	81.98	100.00

Figure 4

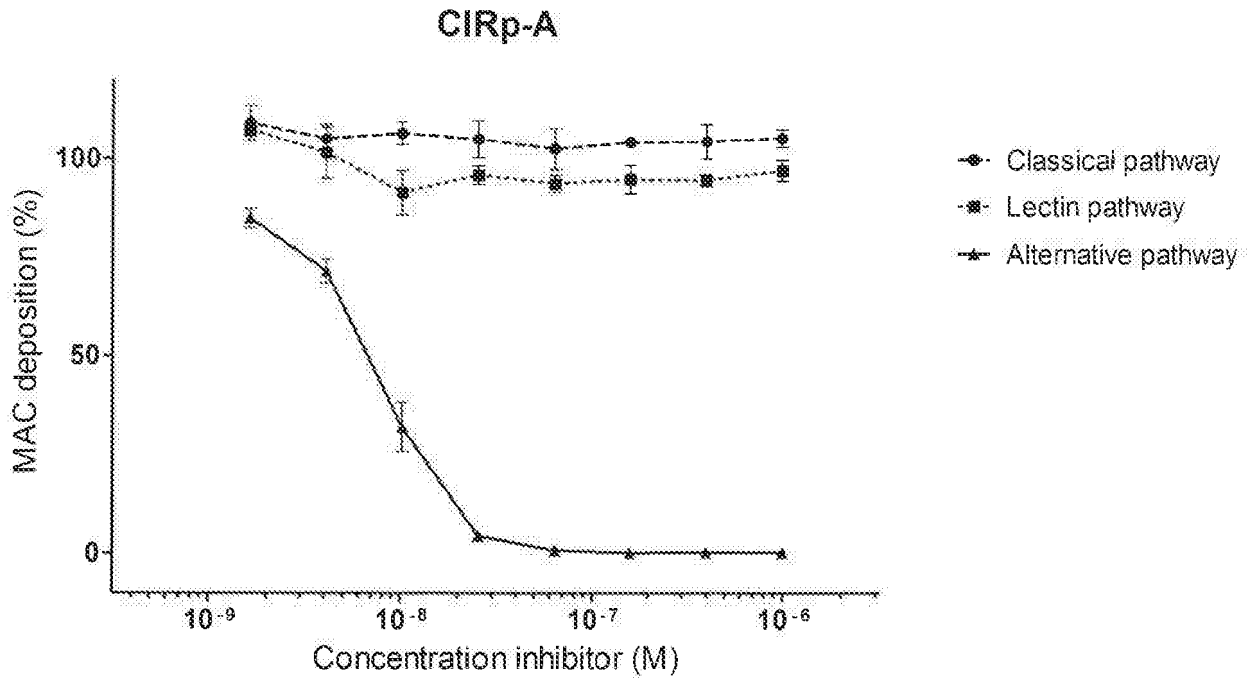

Figure 5

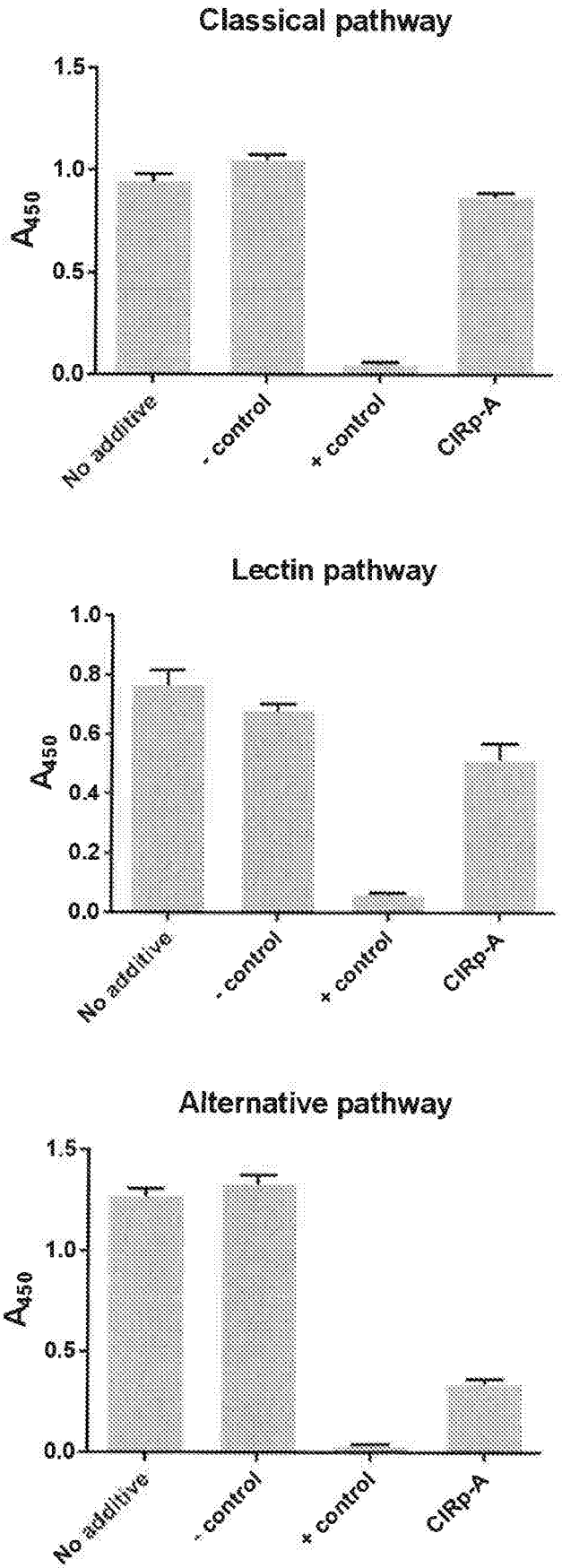
Classical pathway
1.5
1.0
0.5
0.0
A450
No additive
- control
+ control
ClRp-A
Lectin pathway
1.0
0.8
0.6
0.4
0.2
0.0
A450
No additive
- control
+ control
ClRp-A
Alternative pathway
1.5
1.0
0.5
0.0
A450
No additive
- control
+ control
ClRp-A

Figure 6

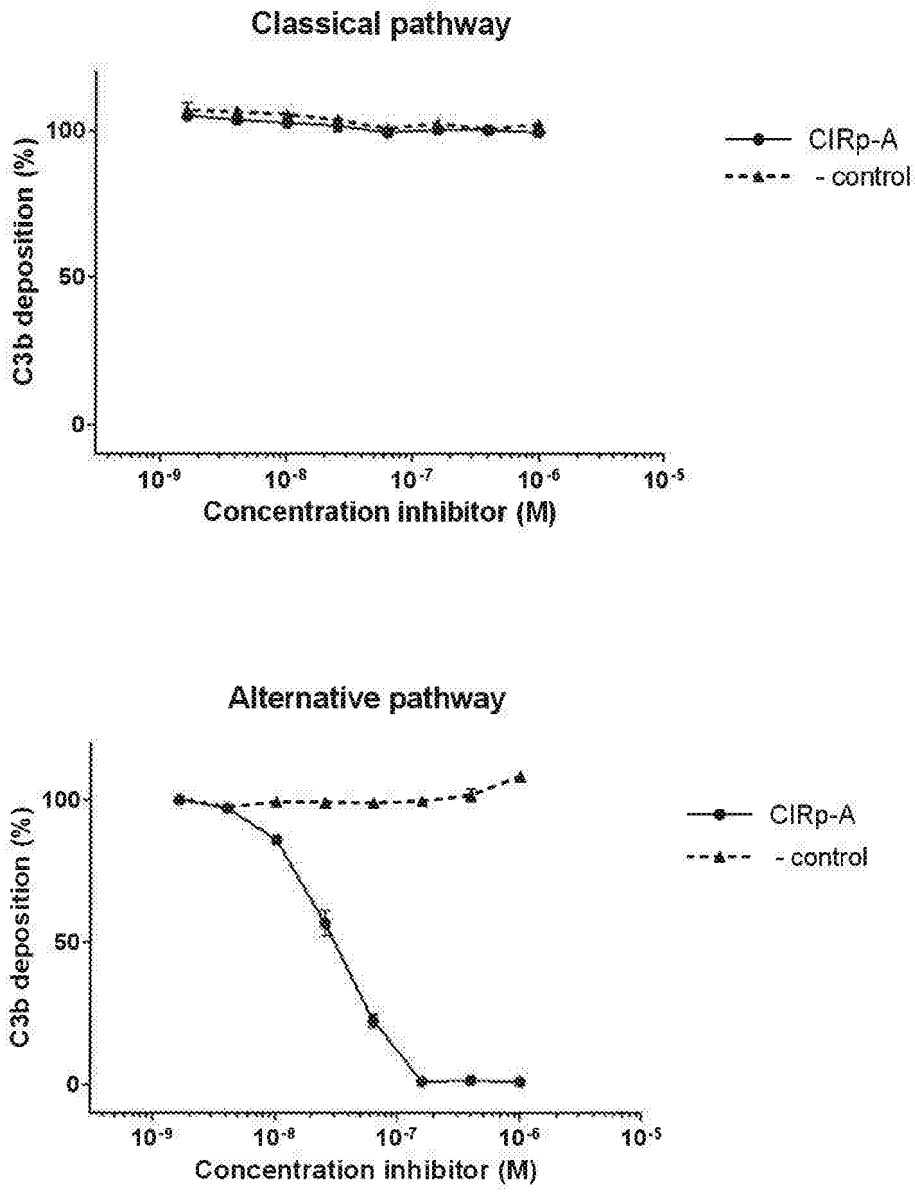
Classical pathway
C3b deposition (%)
100
50
0
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
Concentration inhibitor (M)
ClRp-A
- control
Alternative pathway
C3b deposition (%)
100
50
0
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
Concentration inhibitor (M)
ClRp-A
- control

Figure 7

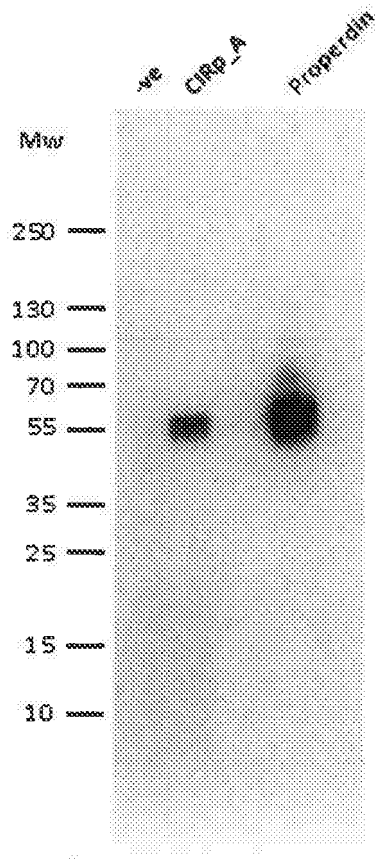

Figure 8

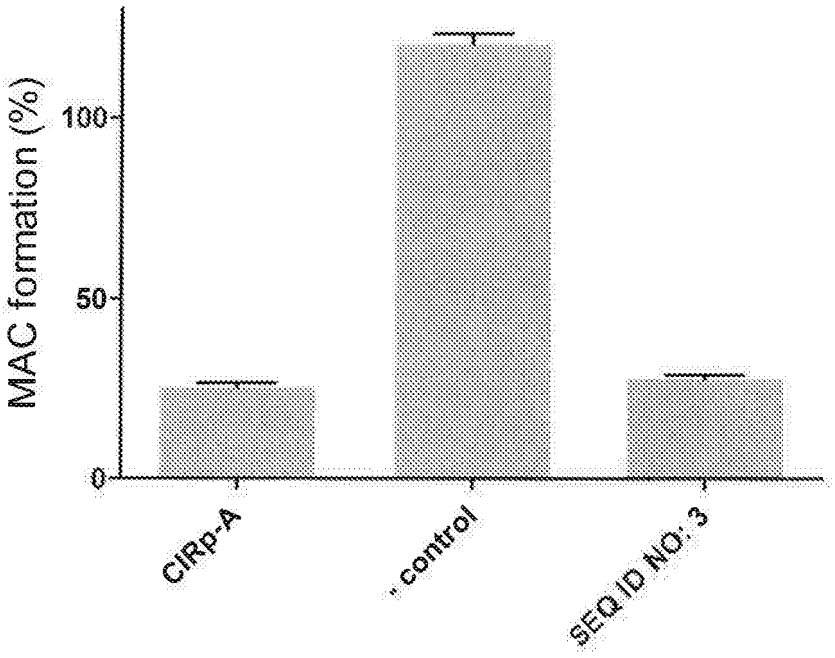

Figure 9

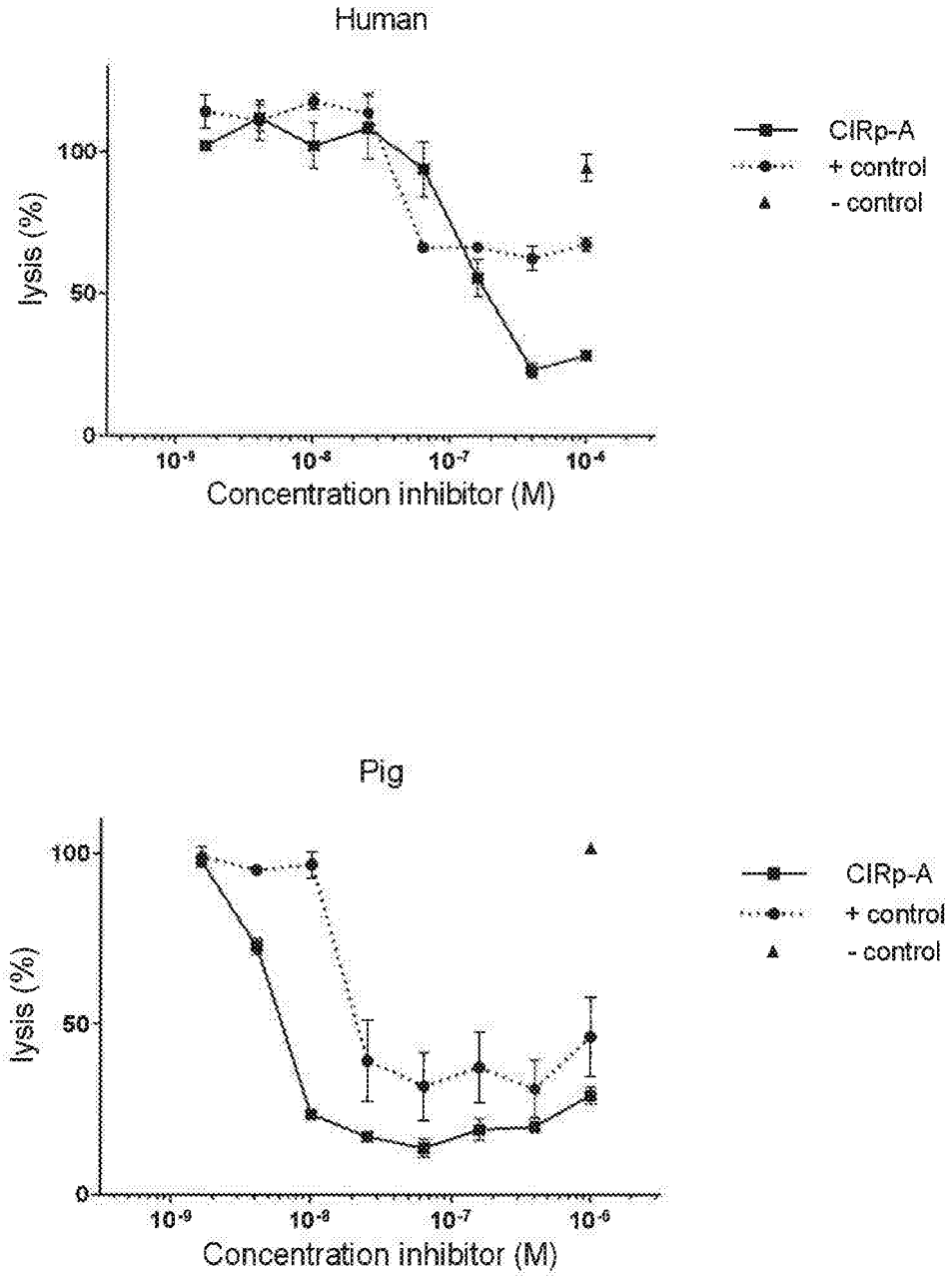
Human
lysis (%)
100
50
0
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
Concentration inhibitor (M)
CIRp-A
+ control
- control
Pig
lysis (%)
100
50
0
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
Concentration inhibitor (M)
CIRp-A
+ control
- control

Figure 10

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2018/052180

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/17 C07K14/435
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Anonymous: "Lipocalin - Rhipicephalus appendiculatus (Brown ear tick)", , 11 May 2016 (2016-05-11), XP55518683, Retrieved from the Internet: URL:https://www.uniprot.org/uniprot/A0A131YSA4 [retrieved on 2018-10-24] the whole document	1,3-13
X	WO 2015/185945 A2 (ISIS INNOVATION [GB]) 10 December 2015 (2015-12-10) abstract claims 1-29 figures 1/18-18/18	1-22

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
25 October 2018	08/11/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Keller, Yves

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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2018/052180

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 055 715 A1 (UNIV BRUXELLES [BE]) 6 May 2009 (2009-05-06) abstract paragraph [0007] - paragraph [0024] paragraph [0057] - paragraph [0070] paragraph [0081] - paragraph [0085] claims 1-19	1-22
X	NAYLENE C. S. SILVA ET AL: "Saliva of Rhipicephalus (Boophilus) microplus (Acari: Ixodidae) inhibits classical and alternative complement pathways", PARASITES & VECTORS, vol. 9, no. 1, 11 August 2016 (2016-08-11), XP55518694, DOI: 10.1186/s13071-016-1726-8 abstract page 2, column 2, paragraph 2 - page 3, column 1, paragraph 1 page 4, column 1, paragraph 4 - page 5, column 1 page 10, column 2, paragraph 3 - page 12, column 2, paragraph 1	1-22
X	MATTHIJS M JORE ET AL: "Structural basis for therapeutic inhibition of complement C5", NAT. STRUCT. MOL. BIOL., vol. 23, no. 5, 28 April 2016 (2016-04-28), pages 378-386, XP55398065, New York ISSN: 1545-9993, DOI: 10.1038/nsmb.3196 abstract examples 1-7 Discussion	1-22
T	ROVERSI ET AL: "The Structure of OMCI, a Novel Lipocalin Inhibitor of the Complement System", JOURNAL OF MOLECULAR BIOLOGY, ACADEMIC PRESS, UNITED KINGDOM, vol. 369, no. 3, 11 May 2007 (2007-05-11), pages 784-793, XP022077570, ISSN: 0022-2836, DOI: 10.1016/J.JMB.2007.03.064 the whole document	1-22

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2018/052180

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