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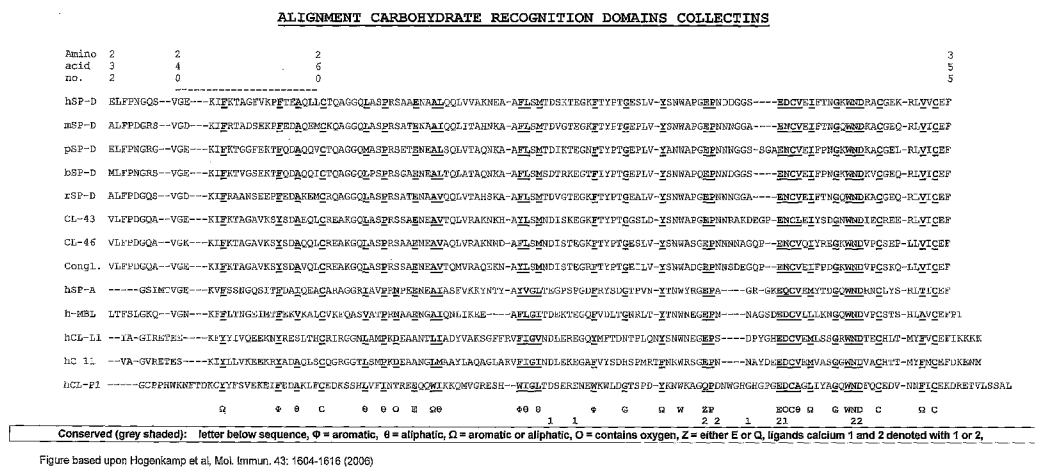

Figure based upon Hogenkamp et al, Mol. Immun. 43: 1604-1616 (2006)

(57) **Abstract:** The present invention relates to modified carbohydrate recognition domains (CRD's), in particular to CRD's derived from collectins, wherein a new glycosylation site is introduced. There is provided such modified CRD's, polypeptides and proteins comprising such modified CRD's and a method for the preparation of such CRD's, polypeptides and proteins. The modified CRD's, polypeptides and proteins are effective in the treatment and prophylaxis of many conditions, in particular infections by Influenza A viruses, and inflammatory conditions.

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Improved carbohydrate recognition domains

The present invention relates to a polypeptide comprising a modified carbohydrate recognition domain, derived from a collectin carbohydrate recognition domain.

Collectins are a family of collagenous calcium-dependent defence lectins in animals and belong to the C-type lectin superfamily.

Eight collectins have been identified so far (in different species): mannan-binding lectin (MBL; also known as mannose-binding protein; MBP), surfactant protein A (SP-A), surfactant protein D (SP-D), collectin liver 1 (CL-L1), collectin placenta 1 (CL-P1), conglutinin, collectin-43 (CL-43) and collectin-46 (CL-46) and collectin 11.

As part of the innate immune system, collectins have a key role in the first line of defence against invading micro-organisms, as demonstrated with genetically manipulated mice made deficient in MBL, SP-A or SP-D, which show increased susceptibility to bacterial and viral infections.

Collectins interact with glycoconjugates and/or lipid moieties present on the surface of a great variety of micro-organisms and allergens, and with receptors on host cells. Through these interactions, the collectins play an important role in innate host defence and various defence functions have been reported to date and include a role in agglutination, complement activation, opsonation and activation of phagocytosis, inhibition of microbial growth and modulation of inflammatory responses.

The polypeptide chains of collectins consist of four regions: a cysteine-rich N-terminal domain, a collagen-like region, an alpha-helical coiled-coil neck domain and a C-terminal lectin or carbohydrate-recognition domain (hereinafter also referred to as CRD). These polypeptide chains can form trimers that may assemble into larger oligomers. Collectins, their structure and functions are reviewed by van de Wetering and co-authors (*Eur. J. Biochem.* **271**, 1229-1249 (2004)), which review is included herein by reference.

In particular, collectins have the property to bind to unique patterns of carbohydrates, a property mediated by their CRDs. The actual carbohydrate binding site can be found in a shallow groove in the CRD.

The CRDs of collectins are compactly folded protein modules of about 115-130 amino acid residues and have been reported to be located at the C-terminus of the protein. Comparison of the CRD domains of soluble collectins has revealed that 22 amino acids are conserved within this domain (reviewed by van de Wetering, 2004).

The collectins that form multimers of trimeric subunits can be divided into two groups. MBL and SP-A form octadecamers of six trimeric subunits, with their overall structure resembling a bouquet of flowers, whereas SP-D and the bovine proteins conglutinin and CL-46 are assembled into dodecamers of four trimeric subunits and form a cruciform-like structure. In addition, SP-D can form even higher-order multimers, so-called 'fuzzy balls' with a mass of several million kDa.

Collectins, belonging to the C-type lectins, require a broad saccharide specificity in order to recognise (carbohydrates present on) a variety of cell surfaces. This broad specificity is thought to be achieved by the fact that the CRD's have a very open trough-like binding pocket.

The site appears to select its ligands mainly on the basis of the positioning of two vicinal hydroxyl groups, which form two co-ordination bonds with ligated calcium, four hydrogen bonds with calcium ligands and a single apolar Van der Waals contact (reviewed by van de Wetering, 2004).

Despite similarities between collectins these proteins can show differences in saccharide selectivity. These differences in ligand specificity are likely influenced by the distribution of non-conserved residues that are located near the ligand-binding pocket of the CRD (Drickamer, K. "Engineering galactose-binding activity into a C-type mannose-binding protein." Nature 360.6400 (1992): 183-86; Hoppe, H. J., P. N. Barlow, and K. B. M. Reid. "A parallel three stranded alpha-helical bundle at the nucleation site of collagen triple-helix formation." FEBS Lett. 344.2-3 (1994): 191-95.)

Collectins can posttranslationally be modified with for example O- and/or N-linked oligosaccharides in the collagen region.

For example, while SP-A is thought to bind to, for example, Influenza A virus (hereinafter also referred to as IAV) through interaction between sialic acid residues on a carbohydrate moiety located in its CRD and presumably a sialic acid receptor present on the hemagglutinin (hereinafter also referred to as HA), SP-D from various species appears to bind to IAV through interaction between

the CRD of SP-D and oligosaccharide moieties located on the HA of IAV.

The anti-IAV activity of SP-D, and especially that of the higher order multimeric form, is in general much greater than the activity of SP-A, suggesting qualitative differences in the mechanism by which a collectin binds to a micro-organism.

Recently it was found by the inventors that, like SP-A, porcine SP-D can contain a sialylated oligosaccharide moiety in its CRD, that might also be involved in binding to, for example, a micro-organism.

Collectins, and in particular their CRD's, have been subject to various types of research and have been proposed for various uses.

For example, US 2004/0047873 discloses a protein framework that allows active polypeptides like ligands or antigens to be displayed at increased concentration. The lectin-binding domains of collectins are replaced by a polypeptide of interest and the polypeptide can be multimerised by the framework of the collectin. As a result a greater number on the polypeptide of interest can be displayed.

US 6 190 886 disclose polypeptides comprising a collectin neck region that are able to trimerize. Such polypeptides may comprise additional amino acids that form a protein domain or which may be modified for attachment of a non-peptide moiety such as an oligosaccharide, or might comprise a carbohydrate binding domain such as a lectin.

Although collectins, and in particular the CRD's thereof, play an important role in the defence against a wide variety of micro-organisms, they have limited specificity and binding capacity to for example different types of virus like IAV.

To the knowledge of the inventor there has been no disclosure in the art of polypeptides comprising CRD's, or recombinant proteins like modified collectins comprising CRD's with improved activity towards for example their role in the (innate) immune system.

The above mentioned problem can be solved by providing a polypeptide comprising a modified carbohydrate recognition domain, derived from a collectin carbohydrate recognition domain, wherein the modification comprises introduction of at least one glycosylation site in the said collectin carbohydrate recognition domain, the said glycosylation site not being present in the said collectin carbohydrate domain before the said introduction.

The term "polypeptide" refers to a chain of covalently attached amino acids that are joined by peptide bonds. Polypeptide chains

typically fold into a compact, stable form that is part of a final protein. Such protein can either comprise one or more equal or different polypeptides. When, within the context of the current invention, the term "polypeptide" is used, also proteins and/or chimeras comprising such polypeptide are to be understood, such as for example HEADS (i.e. a neck/CRD trimer comprising a small part of a collagen, as described for example by Hakansson, K., et al. (Crystal structure of the trimeric alpha-helical coiled-coil and the three lectin domains of human lung surfactant protein D." Structure 7.3 (1999): 255-64.).

The term "carbohydrate recognition domain or CRD" is known in the art and refers to any domain that, for example in a folded protein, is capable of binding unique patterns of carbohydrates, that, for example, are found on the surface of a wide variety of micro-organisms.

In particular, the term "carbohydrate recognition domain" refers to C-type lectin domains (Crouch, E. C. "Collectins and pulmonary host defence." Am.J.Respir.Cell Mol.Biol. 19.2 (1998): 177-201; Holmskov, U., et al. "Collectins: collagenous C-type lectins of the innate immune defence system." Immunol.Today 15.2 (1994): 67-74; Thiel, S. and K. B. M. Reid. "Structures and functions associated with the group of mammalian lectins containing collagen-like sequences." FEBS Lett. 250.1 (1989): 78-84.).

The term "collectin" is known in the art, and refers to any protein comprising a collagen-like domain and a CRD, more particular a C-type lectin domain, typically assembled as trimers or higher order oligomers, and which can bind to unique patterns of carbohydrates, found on the surface of a wide variety of micro-organisms (reviewed by van de Wetering, 2004).

The term "modified carbohydrate recognition domain" denotes a CRD that is changed in form or character in comparison to the (natural) CRD it is derived from. In particular, "modified carbohydrate recognition domain" denotes the presence of a difference in the amino acid sequence of the modified CRD in comparison to the CRD it is derived from.

The term "glycosylation site" is known in the art and refers to a site within a polypeptide which is susceptible to forming a bond with a carbohydrate, or carbohydrate-chain, for example by post-translational modification processes occurring in eukaryotes or archaea, or by (bio)chemical treatment in the presence of enzymes.

For example, an oligosaccharide chain can be attached via N-glycosylation by an oligosaccharyl transferase to an asparagine occurring in a glycosylation site that consists of the triplet sequence Asn-X-Ser or Asn-X-Thr, where X could be any amino acid except Proline. This sequence is known as a glycosylation sequon, and the carbohydrates that can be N-linked comprise high-mannose oligosaccharides and complex oligosaccharides.

Alternatively, carbohydrates can be attached by O-linked glycosylation.

Without being bound by any theory, the inventors believe that by the introduction of at least one glycosylation site within a collectin carbohydrate recognition domain, a polypeptide is provided that comprises at least one introduced glycosylation site which is positioned within the CRD as such that for example thereby binding to for example micro-organisms is improved.

According to a further embodiment, their is provided a polypeptide according to the invention further comprising a replacement of a glycosylation site present in the said collectin carbohydrate recognition domain by a non-glycosylation site.

It has been advantageously found that when an original glycosylation site is present in a collectin CRD, replacement of said original glycosylation site by a non-glycosylation site, and introduction of a glycosylation site not being present in the collectin CRD before the introduction further improves the use and/or activity of the polypeptide, for example in binding to micro-organisms.

For example, in the case of porcine SP-D, replacement of the glycosylation site present at Asn323 (according to van Eijk, M., et al. "Porcine surfactant protein D is N-glycosylated in its carbohydrate recognition domain and is assembled into differently charged oligomers." Am.J.Respir.Cell Mol.Biol. 26.6 (2002): 739-47), and introduction of a glycosylation site in the collectin CRD at a site previously not containing a glycosylation site, provides a polypeptide comprising a glycosylation at a site previously not being a glycosylation site, and a non-glycosylation site previously being a glycosylation site.

Thus, as exemplified above, a polypeptide can be provided wherein a glycosylation site in the CRD has been relocated relative to the glycosylation site that might be present in the collectin CRD.

It has been found that, depending on the desired properties of the polypeptide or protein, such relocation can dramatically improve the activity and/or use of the polypeptide, for example improved binding to micro-organisms, as will be exemplified in the examples below.

As will be understood by the person skilled in the art, it can be determined by straightforward experimentation, for example as discussed in the examples, whether the replacement of a glycosylation site in the CRD of the collectin further improves the use and/or function of the polypeptide according to the invention.

In a further embodiment, there is provided a polypeptide according to the invention, wherein said modified carbohydrate recognition domain comprises at least 2 glycosylation sites.

Surprisingly, it has been found that the presence of at least 2 glycosylation sites in the CRD might further improve the use and/or activity of the polypeptide. For example, the presence of at least 2 glycosylation-sites in the CRD might increase binding to different and more types, species and the like of different micro-organisms, for example, different strains of Influenza A viruses.

It will be clear for a person in the art that depending on the desired properties of the polypeptide, one, two, three, four, five, or more glycosylation sites can be introduced in a CRD.

In a preferred embodiment there is provided a polypeptide according to the invention, wherein said introduced glycosylation site has the amino acid sequence Asn-X-Ser or Asn-X-Thr, wherein X can be any amino acid except proline.

As will be understood by the person skilled in the art, Asn is the common abbreviation for the amino acid asparagine, Ser is the common abbreviation for the amino acid serine, and Thr is the common abbreviation for the amino acid threonine.

Within the preferred glycosylation sites of the invention, X can be any amino acid except proline. For example, X can be chosen from the group consisting of (between brackets 3 letter code followed by 1 letter code) glycine (Gly;G), alanine (Ala;A), valine (Val;V), leucine (Leu;L), isoleucine (Ile;I), methionine (Met;M), phenylalanine (Phe;F), tryptophan (Trp;W), serine (Ser;S), threonine (Thr;T), cysteine (Cys;C), tyrosine (Tyr;Y), asparagine (Asn;N), glutamine (Gln;Q), aspartic acid (Asp;D), glutamic acid (Glu;E), lysine (Lys;K), arginine (Arg;R), and histidine (His;H).

It was found that the preferred glycosylation sites according to the invention are those glycosylation sites that are susceptible to N-linked glycosylation, i.e. wherein glycosylation (i.e. addition of saccharides) occurs to the amide nitrogen of an asparagine side chain.

The term "N-linked glycosylation" is known in the art. In brief, N-linked glycosylation occurs in eukaryotes and widely in archaea, but rarely in prokaryotes, although prokaryotes might also be capable of glycosylation after introduction of enzymes required for glycosylation into these prokaryotes.

In typical cases, for N-linked oligosaccharides, a saccharide-precursor is first added to the asparagine in a polypeptide chain of a protein. The structure of this precursor can for example consist of 3 glucose, 9 mannose, and 2 N-acetylglucosamine molecules.

There are two major types of N-linked saccharides: high-mannose oligosaccharides, and complex oligosaccharides. High-mannose relates to in essence two N-acetylglucosamines with many mannose residues, whereas complex oligosaccharides can contain almost any number of other types of saccharides, and can include the original N-acetylglucosamines.

Proteins can be glycosylated by these types of saccharides and on different parts of the protein. During glycosylation, residues can be cleaved of, be added or be modified (for example, elongated with a variety of different monosaccharides including galactose, N-acetylglucosamine, N-acetylgalactosamine, fucose and sialic acid).

In a preferred embodiment, there is provided a polypeptide according to the invention, wherein said introduced glycosylation site is glycosylated with a carbohydrate. Such carbohydrate can be any carbohydrate/saccharide, as discussed above. The polypeptide can be glycosylated by means of an enzyme, for example present in a organism wherein the polypeptide is expressed, for example human embryonic kidney 293E cells, or for example by treatment of a purified polypeptide or protein in the presence of enzymes required for glycosylation, as discussed above, or by any chemical means.

It has been found that glycosylation of the introduced glycosylation site in the CRD of the polypeptide is required for improving the use and/or activity of the polypeptide, for example for increasing binding of the polypeptide to particular micro-organisms, for example pathogens, for example influenza A viruses.

As will be appreciated by the person skilled in the art, depending on the desired properties of the proteins, for example with respect to the micro-organisms whereto the polypeptide or protein should bind, or with respect to the desired strength of binding (as will be exemplified in the examples below), it might be required to glycosylate the introduced glycosylation site with the same, different or various types of carbohydrates.

In a preferred embodiment the said carbohydrate, that is or will be used for glycosylating the introduced glycosylation site in the CRD, comprises at least one sialic acid residue, preferably at least one terminal sialic acid residue.

It has been found that the presence of sialic acid residues on the carbohydrate that is attached to the polypeptide at the introduced glycosylation site of the collectin CRD, can dramatically improve the use and/or activity of the polypeptide, as will be exemplified in the examples below.

For example, glycosylation of a newly introduced glycosylation site in the CRD of the human collectin Surfactant Protein D, and subsequent sialylation, can dramatically improve binding of the polypeptide, for example as part of a mature protein, to various influenza A viruses.

Without being bound by theory, it is the inventors believe that this improved activity is related to binding of the sialic acid residue to, for example, the sialic acid receptor(s) present on a micro-organism, for example present on or within a hemagglutinin, a viral spike glycoprotein present on the surface of virus particles.

Thus, the introduction of a new glycosylation site within a collectin CRD, for example a surfactant protein D collectin, might provide for a new and versatile additional, for example improved, binding mechanism to micro-organisms.

For example, in the case of surfactant protein D, in addition to a mechanism of binding to influenza A virus involving SP-D binding to N-linked high-mannose carbohydrates present on a hemagglutinin on the virus, and the other IAV envelope glycoprotein, neuraminidase, the introduction of a glycosylation site in the CRD might allow for improved use and/or activity of the polypeptide by allowing improved binding to the micro-organism, possibly by binding to sialic acid receptors by means of the sialic acid residues present on a carbohydrate linked to the introduced glycosylation site in the polypeptide according to the invention.

It has been found that in particular sialic acid residues present on carbohydrates that are linked to a glycosylation site that has been introduced in the CRD of a collectin, can dramatically improve activity and/or use of the polypeptide.

It has been found that these carbohydrates can be highly heterogeneous, i.e. varying and different carbohydrates might be present.

Preferably, the carbohydrate is fully sialylated with sialic acid residues, preferably terminally linked sialic acid residues.

In a preferred embodiment a sialic acid residue is linked to the carbohydrate by alpha(2,3)-linkage or alpha(2,6)-linkage, or a mixture thereof.

It has been found that a sialic acid that is linked to the carbohydrate, more particular to a penultimate galactose residue, by either alpha(2,3) or alpha(2,6)-linkage, is in particular favourable in a polypeptide according to the invention.

It has been found that depending on the desired use of the polypeptide, for example for binding to influenza A virus isolated from human, birds, equine or porcine, sialic acids linked to the carbohydrate by alpha(2,3)-linkage or alpha(2,6)-linkage might be preferred.

For example, human influenza A virus appears to preferentially bind to a alpha(2,6)-linkage, those isolated from birds and equines appears preferentially to bind to alpha(2,3)-linkages, while porcine influenza A viruses appear to recognise both linkages of sialic acid residues in the polypeptides according to the invention.

According to a preferred embodiment there is provided a polypeptide according to the invention wherein said polypeptide is in the form of a trimer.

It has been found that when the introduced glycosylation site in the CRD of a collectin is present in a polypeptide that can multimerize, preferably trimerize (i.e. forming a folded protein structure comprising three polypeptides), the activity of the resulting multimer, preferably trimer, can, for example in comparison to a single polypeptide, be dramatically improved.

It is believed that the formation of a multimer, preferably a trimer, might enable improved target recognition (for example virus recognition), but also allows simultaneous and multivalent interactions with the target, thereby improving efficient binding to said target.

The person skilled in the art knows properties that are required within a polypeptide to allow for multimerization, and can for example comprise the presence of a collagen-like region characterised by repetitive triplet Gly-Xaa-Yaa sequences (wherein Xaa and Yaa can be any amino acid) which can make three polypeptides trimerize into a collagen triple helix (resulting in one collagen trimeric subunit), as has for example be shown for surfactant protein A.

The process of trimerization is suggested to be triggered by the neck region of collectins (Hoppe, H. J., P. N. Barlow, and K. B. M. Reid. "A parallel three stranded alpha-helical bundle at the nucleation site of collagen triple-helix formation." FEBS Lett. 344.2-3 (1994): 191-95.).

The polypeptides that multimerize can be identical or different, for example with respect to the presence of introduced glycosylation sites in the CRD. For example, a trimer can be formed comprising 1,2 or 3 identical polypeptides.

The collectin CRD can be derived from any organism comprising such CRD, but in a preferred embodiment, the polypeptide according to the invention comprises a collectin carbohydrate recognition domain derived from a collectin, originally from any of the group consisting of rodents, human, porcine and bird.

It has been found that in particular the use and/or activity of a polypeptide comprising a glycosylation site introduced in a CRD derived from the said group is preferable. These polypeptides according to the invention have broad applicability, for example in research, and various suitable methods for manipulating, expressing, isolating, modifying, assaying, and the like are readily available.

The collectin CRD can be derived from any protein/polypeptide comprising such CRD, but preferably, the collectin carbohydrate recognition domain is derived from any of the group consisting of mannan-binding lectin (MBL), surfactant protein A (SP-A), surfactant protein D (SP-D), collectin liver 1 (CL-L1), collectin placenta 1 (CL-P1), conglutinin, collectin-43 (CL-43) and collectin-46 (CL-46) and collectin 11.

The polypeptides comprising a glycosylation site introduced in a CRD derived from any of the group consisting of mannan-binding lectin (MBL), surfactant protein A (SP-A), surfactant protein D (SP-D), collectin liver 1 (CL-L1), collectin placenta 1 (CL-P1), conglutinin, collectin-43 (CL-43) and collectin-46 (CL-46) and

collectin 11 have broad applicability, for example in research, and various suitable methods for manipulating, expressing, isolating, modifying, assaying, and the like are readily available (Molecular Cloning: A Laboratory Manual Second Edition. Sambrook, J., Fritsch, E. and Maniatis,T. Cold Spring Harbor Press, Cold Spring Harbor, NY; Handbook of Molecular and Cellular Methods in Biology and Medicine. ed. Kaufman,P., Wu,W. and Kim,D. CRC Press, Boca Raton , FL (1995)).

DNA, RNA and/or cDNA sequences of these and other collectins are publicly available, for example from the NCBI database (www.ncbi.nlm.nih.gov), for example for (with between brackets the genebank accession number) human SP-D (X65018), porcine SP-D (AF132496), bovine SP-D (X75911), rat SP-D (M81231), mouse SP-D (AF047742), human SP-A (P07714), human MBP (X15422), bovine CL-43 (NM_001002237), bovine CL-46 (AF509590), bovine conglutinin (X71774), human CL-L1 (AB002631), human CL-P1 (AB005145), and human collectin11 (BC000078), which sequences are included herein by reference.

Preferably, the glycosylation site is introduced in said collectin carbohydrate recognition domain comprising any one of the amino acid sequences shown in figure 1.

The sequences of these and other collectin CRD's are publicly available, for example from the NCBI database (www.ncbi.nlm.nih.gov), as described above.

It has been found that in particular the introduction of at least one glycosylation site in a collectin carbohydrate recognition domain comprising an amino acid sequence as shown in figure 1 provides a polypeptide according to the invention with improved activity within the context of the invention.

The person skilled in the art understands that any CRD comprising conserved amino acids, as shown in bold and underlined in figure 1, is to be understood as a suitable candidate for introducing a glycosylation site according to the invention. These conserved amino acids are known in the art and are, for example, reviewed by Drickamer (Drickamer, K. "C-type lectin-like domains." Curr.Opin.Struct.Biol. 9.5 (1999): 585-90.)

As will be understood by the person skilled in the art, small deviations from the shown amino acid sequences are allowed without leaving the scope of the invention. Such allowable homologues show at least 55%, more preferably at least 75%, even more preferable at least 95% amino acid identity when compared to a amino acid sequence shown in figure 1, under the provision that the obtained polypeptide

according to the invention shows activity within the context according to the invention (such as described below and shown in the examples).

Thus, a polypeptide according to the invention can, besides the introduction of a glycosylation site as explained above, show variations, modifications, substitutions, replacements, deletions and/or additions of one or more amino acids within the sequences shown in figure 1, without leaving the scope of the invention, under the provision that the obtained polypeptide can be used and/or shows activity within the context of the invention.

In a preferred embodiment, the polypeptide according to the invention is a polypeptide wherein said introduced glycosylation site is introduced at a position between amino acid 240 and 260, preferably between 246 and 253, and/or between 267 and 298, preferably between 272 and 290, and/or between 304 and 331, preferably between 308 and 330, and/or between 331 and 344, preferably between 333 and 340 of an amino acid sequence of said collectin carbohydrate recognition domain, wherein the numbering of the amino acids is in accordance with the amino acid numbering of human SP-D in figure 1, and/or as described for SP-D (Lu, J., A. C. Willis, and K. B. M. Reid. "Purification, characterization and cDNA cloning of human lung surfactant protein D." Biochem.J. 284.Pt 3 (1992): 795-802.).

It is to be understood that when herein reference is made to positions within the amino acid sequence for a particular CRD, for example a position between amino acid 240 en 260 (including 240 and 260) in mouse SP-D, reference is made to the amino acid numbering as shown for hSP-D in figure 1. Corresponding positions in other CRD's, for example mouse SP-D or others shown or not shown, can easily be derived by alignment of the CRD with human SP-D, determining the positions of the amino acids from 240 to 260 in human SP-D, as shown in the figure 1 (VGEKIFKTAGFVKPFTEAQLL), and based on the alignment determine the corresponding positions in the other CRD, for example mouse SP-D (VGDKIFRTADSEKPFEDAQEM). For example, amino acids between position 350 and 355 in h-MBL are LAVCEF, and amino acids between position 232 and 240 in hC 11 are VAGVRETE. The inventors have found that an introduced glycosylation site at a such position as described above and shown in Figure 1 shows particular good activity within the context of the current invention, and for example as described in the examples.

For example, such polypeptide can show improved binding to various micro-organisms, like influenza A viruses, and can, like above, be suitably used in the treatment of infections with such micro-organisms.

It has been found that introduction of a glycosylation site at a position between the said amino acids, might provide a polypeptide according to the invention that is glycosylated and/or sialylated at the introduced glycosylation site, and wherein an attached carbohydrate is suitably positioned within the polypeptide, or for example within the formed trimer, allows for improved use and/or activity within the context of the invention.

It is therefore to be understood by the person skilled in the art that any introduced glycosylation site in a collectin CRD, or a homologue thereof, that provides for a polypeptide according to the invention and wherein an attached carbohydrate is suitably positioned within the polypeptide, or for example within a trimer, as can be shown by improved use and/or activity of the polypeptide within the context of the invention, is within the scope of the current invention.

For example, a polypeptide according to the invention can be provided, wherein, for example by insertion of amino acids, the introduced glycosylation site becomes positioned within the polypeptide at a position that provides a polypeptide according to the invention with improved use and/or activity.

Without being bound by theory, it is believed that such introduced glycosylation might be favourably situated at a position within a polypeptide according to the invention, for example in a region located at the exterior of the CRD which results in well-exposed N-linked oligosaccharides and thereby improve use and/or activity within the context of the invention.

Such regions and other have for example recently been described for SP-A (Head, J. F., et al. "Crystal structure of trimeric carbohydrate recognition and neck domains of surfactant protein A." J.Biol.Chem. 278.44 (2003): 43254-60.).

In a preferred embodiment there is provided a polypeptide according to the invention, wherein the polypeptide comprises a N-terminal region comprising cysteine residues and/or a collagen-like region characterised by repetitive Gly-Xaa-Yaa, wherein Xaa and Yaa can be any amino acid, sequences and/or a neck-region.

The terms "collagen-like region characterised by repetitive Gly-Xaa-Yaa" and "neck-region" are known to a person skilled in the art, and are for example reviewed by van de Wetering (2004).

Typically, a "collagen-like region characterised by repetitive triplet Gly-Xaa-Yaa sequences" refers to a region which can make three polypeptides trimerize into a collagen triple helix (resulting in one collagen trimeric subunit).

Typically, a neck-region is a short alpha-helical coiled-coil domain which initiates trimerization of three monomers due to a heptad repeat of hydrophobic residues, resulting in strong hydrophobic interactions between the polypeptide chains in this domain.

It has been found that a polypeptide according to the invention, wherein the polypeptide comprises a N-terminal region comprising cysteine residues and/or a collagen-like region characterised by repetitive Gly-Xaa-Yaa sequences and/or a neck-region can show improved use and/or activity within the context of the invention.

In particular polypeptides comprising a N-terminal region comprising cysteine residues and a collagen-like region characterised by repetitive Gly-Xaa-Yaa sequences and a neck-region can show improved use and/or activity within the context of the invention.

Polypeptides, or multimers, like trimers, comprising polypeptides according to the invention can show improved binding to various micro-organisms, in particular to influenza A viruses.

Such polypeptides according to the invention can therefore suitably be used in for example the treatment of infections with such micro-organisms, or inflammation.

As will be understood by a person skilled in the art, within the context of the invention treatment also includes prevention of the occurrence of a disease or inhibiting the development of a disease, for example as a consequence of an infection, for example by a influenza A virus.

In a preferred embodiment there is provided a polypeptide according to one of the previous claims, wherein said polypeptide is a non-natural polypeptide.

As will be understood by the person skilled in the art, multimers, preferably trimers, comprising at least one non-natural polypeptide, are also within the scope of the invention.

The term "non-natural polypeptide" relates to a polypeptide that does not normally occur in nature. For example, such polypeptide does not normally occur in more than 70% of individual animals of a particular species (including human beings), i.e. does not occur in 7 out of 10 randomly selected individual animals of a particular species, and, obviously, wherein said non-natural polypeptide according to the invention is not introduced in the animal by for example recombinant techniques.

It has been found that now for the first time there can be provided non-natural polypeptides according to the invention with improved use and/or activity, in comparison to the natural occurring polypeptides, within the context of the invention.

In a preferred embodiment, there is provided a polypeptide according to the invention for use in the treatment of an animal body, preferably a human body.

The polypeptides according to the invention can suitably be used in the treatment of an animal body, preferably a human body, and can show improved activity in the treatment of, for example, infection with various micro-organisms, for example pathogens, in particular with influenza viruses, more particular influenza A viruses.

As will be understood by a person skilled in the art, within the context of the invention treatment also includes prevention of the occurrence of a disease or inhibiting the development of a disease, for example as a consequence of an infection, for example by influenza A virus.

Additionally, polypeptides according to the invention can be provided, that can be useful in the treatment and/or prevention of bird flu and the like.

According to another aspect of the invention, there is provided a nucleic acid comprising a sequence of nucleotides encoding a polypeptide according to anyone of the previous claims.

It has been found that such nucleic acid can favourably be used to provide for increased quantities of a polypeptide according to the invention. Such nucleic acid can be expressed in well-known prokaryotic of eukaryotic expression systems, such as bacteria, yeast or human cell lines.

Although it is not necessary that expressed polypeptide according to the invention is glycosylated and/or sialylated by the

host organism, it can be envisaged that glycosylation and sialylation by the host is preferable.

In addition, the nucleic acid can also be introduced, by methods known in the art, in an organism, like pig or bird, in order to provide the animal protection against infection.

According to another aspect of the invention there is provided the use of a polypeptide according to the invention for the manufacture of a medicament for the prophylactic use or treatment of infections, cancer, inflammatory disease, allergy, eczema, COPD, asthma, cystic fibrosis, pneumonia, disorders associated with chemotherapy, such as infections, diseases associated with human immunodeficiency virus(HIV), diseases related with congenital or acquired immunodeficiency, multiple sclerosis, rheumatoid arthritis; autoimmune neutropenia, Crohn's disease, Coeliac disease, Asthma, Septic shock syndrome, Chronic fatigue syndrome, Psoriasis, Toxic shock syndrome, Diabetes, Sinusitis, hayfever, and the others involving a role the polypeptides according to the invention.

It has been found that a polypeptide can be favourably used in the manufacture of a medicament for the prevention or treatment of the conditions described above, in particular infection and inflammatory conditions.

Such medicament can further comprise usual compounds like stabilisers, preservatives and the like. The medicament can be in any suitable administration form like a spray, a fluid, a powder and the like.

It has been found that such medicament comprising a polypeptide according to the invention, for example in the form of a trimer, can be useful in the treatment of infection, in particular in the treatment of infection by influenza A virus.

In another aspect the invention relates to the use of a polypeptide according to the invention for binding a hemagglutinin or a micro-organism comprising a hemagglutin.

It has been found that polypeptides according to the invention can show improved binding to hemagglutinin or micro-organisms comprising a hemagglutin.

The person skilled in the art knows the term "hemagglutin". Typically, hemagglutin is described as an antigenic glycoprotein found on the surface of the influenza viruses and is responsible for binding the virus to the cell that is being infected.

According to another aspect of the invention there is provided a method for obtaining a polypeptide according to the invention, wherein said method comprises the step of introducing a glycosylation site at a non-glycosylation site in a collectin carbohydrate recognition domain.

Suitable methods are known to the person skilled in the art, and include, for example, site-directed mutagenesis, recombinant-DNA-techniques and the like.

According to a preferred embodiment of the method according to the invention, the method comprises the step of introducing a non-glycosylation site at a glycosylation site in a collectin carbohydrate recognition domain.

As above, suitable methods are known to the person skilled in the art, and include, for example, site-directed mutagenesis, recombinant-DNA-techniques and the like.

According to a preferred embodiment of the method according to the invention, said introducing comprises substitution, deletion and/or addition of at least one amino acid residue in said collectin carbohydrate recognition domain.

As above, suitable methods are known to the person skilled in the art, and include, for example, site-directed mutagenesis, recombinant-DNA-techniques and the like.

According to a preferred embodiment of the method according to the invention, the method comprises the step of expressing a nucleic acid according encoding a polypeptide according to the invention in a host organism under conditions that the polypeptide is formed and isolating the said polypeptide.

Suitable hosts can be determined by straight-forward experimentation, and include, for example, prokaryotic and eukaryotic hosts like bacteria, yeast cells and human cells.

Preferably, but not necessarily, the host is capable of glycosylating and/or sialylating the introduced glycosylation site in the polypeptide according to the invention, for example human embryonal kidney 293E cells.

Methods for isolating the formed polypeptide according to the invention are known to the person skilled in the art, and can include, for example, affinity chromatography, (SDS-page) gel electrophoresis and the like, for example as described by van Eijk (van Eijk, M., et al. "Porcine surfactant protein D is N-glycosylated in its carbohydrate recognition domain and is assembled into

differently charged oligomers." Am.J.Respir.Cell Mol.Biol. 26.6 (2002): 739-47.).

As will be understood by the person skilled in the art, isolating refers to any relative enrichment of the polypeptide according to the invention in comparison to a stadium prior to isolating, for example by increasing the percentage of the polypeptide relative to other proteins present in a solution, or concentrating the polypeptide according to the invention.

According to a preferred embodiment of the method according to the invention, the said nucleic acid is expressed in a cell capable of N-glycosylation of proteins, preferably capable of both N-glycosylation and sialylation.

Expressing a nucleic acid encoding a polypeptide according to the prevention in a cell capable of N-glycosylation of proteins, and/or capable of sialylation can advantageously be applied to provide for a polypeptide according to the invention, or a multimer according to the invention, that is provided with a suitable carbohydrate and sialic acid residues at the introduced glycosylation site, thus rendering it unnecessary to glycosylate and/or sialylate by other (bio)chemical means.

Polypeptides according to the invention, including multimers or proteins comprising such polypeptide will show improved use and/or activity like, but not limited to the prophylactic use or treatment of infections, cancer, inflammatory disease, allergy, eczema, COPD, asthma, cystic fibrosis, pneumonia, disorders associated with chemotherapy, such as infections, diseases associated with human immunodeficiency virus(HIV), diseases related with congenital or acquired immunodeficiency, multiple sclerosis, rheumatoid arthritis; autoimmune neutropenia, Crohn's disease, Coeliac disease, Asthma, Septic shock syndrome, Chronic fatigue syndrome, Psoriasis, Toxic shock syndrome, Diabetes, Sinusitis, hayfever. Other uses and/or activities involving a role for the polypeptides according to the invention include, but are not limited to, binding to carbohydrate-binding domains in proteins, or with proteins present in or on a micro-organisms, improving agglutination, complement activation, opsonization and activation of phagocytosis, inhibition of microbial growth and modulation of inflammatory responses.

The current invention will be further exemplified by the examples below. It is to be understood that the given examples do not impose any particular limitation with respect to embodiments of the

invention. Various embodiments can be envisaged without any further inventive thought.

Figures:

Figure 1 shows an alignment of carbohydrate recognition domains of various collectins. The CRD's of hSP-D (SEQ ID NO 1), mSP-D (SEQ ID NO 2), pSP-D (SEQ ID NO 3), bSP-D (SEQ ID NO 4), rSP-D (SEQ ID NO 5), CL-43 (SEQ ID NO 6), CL-46 (SEQ ID NO 7), conglutinin (SEQ ID NO 8), hSP-A (SEQ ID NO 9), h-MBL (SEQ ID NO 10), hCL-L1 (SEQ ID NO 11), hC 11 (SEQ ID NO 12) and hCL-P1 (SEQ ID NO 13). The CRD's are aligned based on conserved amino acid residues found in CRD's. Numbering of amino acid residues is based on human SP-D. Abbreviations of the amino acids is as described. Conserved amino acids for all CRD's are shown in bold and underlined. A position between amino acid 240 (V) and 260 (L) in human SP-D is indicated with the dashed line (---), as explained in the text, and corresponding positions in other CRD's can be easily derived by alignment of the CRD, and as indicate in figure 1. h=human, m=mouse, p=porcine, r=rat, and b=bovine.

Examples:

Example 1

Construct for full-length porcine SP-D was prepared as follows: Full-length sequence of porcine SP-D was obtained via standard PCR on cDNAs generated by Reverse transcriptase-PCR on porcine lung total RNA using the following primers: forward, 5'-gcgtctcggatccgcagaaatgaagacctattcccag-3'; reverse, 5'-gcggccgcgtgaggccagtttcgtac-3'. A PCR fragment of 1.3 kB was agarose gel purified and cloned into pCR4-TOPO by T/A cloning according to the manufacturers instructions (Invitrogen Corporation, Carlsbad, California, USA).

The inserts were released by restriction digestion and cloned into pABC expression vectors having different signal sequences. After amplification in TOP10 E'Coli, purified expression clones were transfected into HEK293-EBNA cells using polyethyleneimine (PEI). After five days medium was harvested and used for purification of secreted recombinant porcine SP-D by mannan affinity chromatography (calcium-dependent binding) and gel filtration (size exclusion)

according to van Eijk (van Eijk, M., et al. "Porcine surfactant protein D is N-glycosylated in its carbohydrate recognition domain and is assembled into differently charged oligomers." Am.J.Respir.Cell Mol.Biol. 26.6 (2002): 739-47.)

Example 2

Site-directed mutagenesis

Site-directed mutagenesis of porcine SP-D cDNA in pCR4-TOPO was performed using the Quikchange II site-directed mutagenesis kit according to the manufacturers instructions (Stratagene, La Jolla, CA 92037, USA), and with the primers described below. Entire sequence and desired mutations were checked by DNA sequencing and cloned into pABC expression vectors having different signal sequences. After amplification in TOP10 E'Coli, purified expression clones were transfected into HEK293-EBNA cells using polyethyleneimine (PEI). After five days medium was harvested and used for purification of secreted recombinant protein. Proteins were purified by sugar affinity chromatography (calcium-dependent binding) and gel filtration (size exclusion)as above. Fully assembled porcine SP-D was obtained with an approximate molecular size of more than 1 000 kD.

The primers used were:

1. knockout Nsugar porcine SP-D

Forward primer (SEQ ID NO 14):
GAC TGA CAT CAA GAC GGA GGG CCA GTT CAC CTA CCC CAC GGG GGA G
(5'-> 3' sequence)

Reverse primer (SEQ ID NO 15):
C TCC CCC GTG GGG TAG GTG AAC TGG CCC TCC GTC TTG ATG TCA GTC
(5'-> 3' sequence)

The polypeptide obtained does not comprise a glycosylation site in the modified CRD. The polypeptide is referred to as PP-0. PP-0 is not a polypeptide according to the invention.

2. knockin Nsugar porcine SP-D

Forward primer (SEQ ID NO 16):
GAG AAC GAG GCC TTG AGC AAC CTG ACC ACA GCT CAG AAT AAG GC

(5'-> 3' sequence)

Reverse primer (SEQ ID NO 17):
GC CTT ATT CTG AGC TGT GGT CAG GTT GCT CAA GGC CTC GTT CTC
(5'-> 3' sequence)

changing the amino acids ...MASPRSETENEALSQLVTAQNKAA... (amino acid residues 268 to 291 in the CRD of pSPD (SEQ ID NO 3) to ...MASPRSETENEALSNLTTAQNKAA...(SEQ ID NO 18).
The polypeptide obtained comprises two glycosylation sites in the modified CRD. The polypeptide is referred to as PP-2. PP-2 is a polypeptide according to the invention.

3. A third polypeptide was obtained by performing site-directed mutagenesis, firstly with the primers

Forward primer (SEQ ID NO 14):
GAC TGA CAT CAA GAC GGA GGG CCA GTT CAC CTA CCC CAC GGG GGA G
(5'-> 3' sequence)

Reverse primer (SEQ ID NO 15):
C TCC CCC GTG GGG TAG GTG AAC TGG CCC TCC GTC TTG ATG TCA GTC
(5'-> 3' sequence), followed by site-directed mutagenesis of the obtained sequence with the primers

Forward primer (SEQ ID NO 16)
GAG AAC GAG GCC TTG AGC AAC CTG ACC ACA GCT CAG AAT AAG GC
(5'-> 3' sequence)

Reverse primer (SEQ ID NO 17):
GC CTT ATT CTG AGC TGT GGT CAG GTT GCT CAA GGC CTC GTT CTC
(5'-> 3' sequence)

The polypeptide obtained comprises one glycosylation site in a modified carbohydrate recognition domain, derived from a collectin carbohydrate recognition domain of porcine SP-D. This glycosylation site was not present in the original porcine SP-D. The polypeptide is referred to as PP-1. PP-1 is a polypeptide according to the invention.

Example 3

Deglycosylation and sialic acid modification

N-Glycanase

N-deglycosylated CRD can be obtained by treatment with recombinant N-glycanase (from Flavobacterium meningosepticum; Glyko Inc., Novato,CA) as described previously by van Eijk (van Eijk, M., et al. "Porcine surfactant protein D is N-glycosylated in its carbohydrate recognition domain and is assembled into differently charged oligomers." Am.J.Respir.Cell Mol.Biol. 26.6 (2002): 739-47.) and purified by affinity chromatography on mannansepharose according to the procedure described above, but without gelfiltration chromatography. N-deglycosylation was verified by Western blot analysis using previously published methods described therein. The presence or absence of glycoconjugates was determined by digoxigenin (DIG) Glycan staining (Roche Diagnostics GmbH, Mannheim, Germany), in accordance with the instructions of the manufacturer.

Example 4

Sialidase

Desialylated CRDs can be obtained by treatment of the polypeptide with a non-specific recombinant sialidase (from Arthrobacter ureafaciens; Glyko Inc.) which releases terminal alpha(2,3/6/8/9)-linked sialic acids from complex carbohydrates. Purified polypeptide(60 microgram/200 microliter), dissolved in 50 mM sodium phosphate pH 6.0, was mixed with 100 mU sialidase and incubated at 37°C for 1 h. Sham-treated polypeptide was obtained by incubation in the absence of sialidase. After incubation polypeptides were purified by mannan affinity chromatography as described above. Efficiency of desialylation was determined, after SDS-PAGE and transfer to nitrocellulose, by detection with a DIG Glycan Differentiation kit (Roche Diagnostics GmbH), described below ('Sialic Acid Linkage Analysis').

Example 5

Sialyltransferase

Resialylation of the polypeptide (10 microgram/50 microliter) was performed in 20 mM Tris-HCl (pH 7.4), 150 mM NaCl and 5 mM EDTA, in the presence of 1.6 mU of either alpha2,3-(N)-sialyltransferase or

alpha2,6-(N)-sialyltransferase (37) and 1.5 mM CMP-N-acetylneuraminic acid (Calbiochem Biochemicals, San Diego, CA). The mixture was incubated for 2 h at 37°C, after which alpha(2,3)-resialylated pSP-D (pSP-Dα2,3TF) or alpha(2,6)-resialylated pSP-D (pSP-Dalpha2,6TF) were purified by mannan affinity chromatography as above. Sialic acid linkages present on resialylated polypeptides were determined by DIG-conjugated detection as described in 'Sialic Acid Linkage Analysis'.

Example 6

Sialic acid linkage analysis

Presence and linkage patterns of terminally linked sialic acids on the polypeptides were analyzed by reducing SDS-PAGE and Western blotting, followed by detection with digoxigenin-labeled lectins according to the instructions supplied by the manufacturer (DIG Glycan Differentiation Kit, Roche Diagnostics GmbH). A control gel was included as reference (Coomassie stain). DIG-conjugated lectins used in this analysis, and a description of their carbohydrate binding specificity, were: Datura stramonium agglutinin, recognizes unsubstituted galactose-beta(1,4)-N-acetylglucosamine; Maackia amurensis agglutinin, recognizes SA residues terminally linked alpha(2,3) to galactose; Sambucus nigra agglutinin, recognizes SA residues terminally linked alpha(2,6) to galactose or N-acetylgalactosamine. N-deglycosylated polypeptides were also tested in order to distinguish between sialic acids present on either N-linked glycans or O-linked glycans. Lectin binding was detected by anti-digoxigenin-alkaline phosphatase, followed by a staining reaction. Control glycoproteins were included to assess lectin binding efficiency and specificity.

Example 7

Virus preparations

The IAV preparations used are outlined in Table 1. IAV was grown in the chorioallantoic fluid of 10-day-old chicken eggs and purified on a discontinuous sucrose gradient as described previously (Hartshorn, K. L., et al. "Effects of influenza A virus on human neutrophil calcium metabolism." J.Immunol. 141.4 (1988): 1295-301.) Virus stocks were dialysed against PBS, aliquoted and stored at -70°C. A/Phillipines/82(H3N2) (Phil), A/Memphis71H-BelN(H3N1) (Mem) and their bovine serum beta-inhibitor-resistant variants, PhilBS and

MemBS, respectively, were provided by Dr. E. M. Anders (Department of Microbiology, University of Melbourne, Melbourne, Australia). The PR-8 strain was provided by Dr. J. Abramson(Department of Pediatrics, Bowman Gray School of Medicine, Wake Forest University,Winston Salem, NC). Both swine IAV strains, A/swine/Iowa/3421/90 'classical' H1N1 (Swine Cl) and A/swine/Iowa/8548-1/98 triple reassortant H3N2 (Swine Tr), were provided by Dr. R. G. Webster and Dr. S. Krauss (St. Jude's Hospital, Memphis, TN) and were inactivated with 0.025% (vol/vol) formalin for 3 days at 4°C (Katz, J. M. and R. G. Webster. "Efficacy of inactivated influenza A virus (H3N2) vaccines grown in mammalian cells or embryonated eggs." J.Infect.Dis. 160.2 (1989): 191-98.). A2/Aichi/2/68(Aichi) and A/equine2/Miami/1/63 (Equine) were purchased from the American Type Culture Collection (ATCC, Manassas, VA). HA titres of each strain were determined by titration of virus samples in PBS++ with thoroughly PBS-washed human type O, Rh(-) red blood cells as described (Hartshorn, K. L., K. et al. (1993) J. Clin. Invest. 91:1414-1420). Titration of swine IAV strains was carried out using PBS-washed chicken erythrocytes. The IAV strains were tested for the presence of N-linked high-mannose oligosaccharides by reducing SDS-PAGE analysis of 5 microgram IAV extract, followed by Western blotting and staining with DIG-conjugated Galanthus nivalis agglutinin, a lectin which specifically recognizes 'high-mannose' N-glycan chains. The procedure was carried out according to the instructions supplied by the manufacturer (DIG Glycan Differentiation kit, Roche Diagnostics GmbH).

Table 1. Influenza A viral strains used

Strain	Abbreviation	Subtype
A/Phillippines/82	Phil	H3N2
A/Phillippines/82/BS	PhilBS	H3N2
A/Puerto Rico/8/34	PR-8	H1N1
A/MEM71H-BelN	Mem	H3N1
A/MEM71H-BelN/BS	MemBS	H3N1
A2/Aichi/2/68	Aichi	H3N2
A/equine2/Miami/1/63	Equine	H3N8
A/swine/Iowa/3421/90	Swine Cl	H1N1
A/swine/Iowa/8548-1/98	Swine Tr	H3N2

Example 8

Hemagglutination inhibition assay

Hemagglutination (HAA) inhibition was measured by serially diluting polypeptide preparations in round-bottom 96-well plates (Serocluster U-Vinyl plates; Costar, Cambridge, MA) using PBS++ as diluent (25 microliter per well). The highest polypeptide concentration tested for HAA inhibitory activity was 100 ng/ml. After adding 25 microliter IAV solution, giving a final concentration of 40 HAA Units per ml or 4 HAA Units/well, the IAV/polypeptide mixture was pre-incubated for 15 min, followed by the addition of 50 microliter human erythrocyte suspension in PBS++; chicken erythrocytes were use for analysis of both swine IAVs. The entire procedure was performed at room temperature. The minimal concentration of a polypeptide, required to fully inhibit the HAA caused by the virus, was determined by reading the plates after 2h. HAA inhibition was detected as the formation of a pellet of red blood cells.

Results with the polypeptides obtained in example 2 are shown in the Table 2 below:

Table 2

	Virus				
Polypeptide	Phil	Phil/BS	PR-8	Aichi	Equine
PP-0	-	-	-	-	-
PP-1	++	+	+	+	+
PP-2	++	++	++	++	++
PP-D	+	0	0	0	+

HAA inhibitory activity is shown as very strong (++), strong (+), moderate (0), or weak (-). PP-0, PP-1 and PP-2 are the polypeptides obtained according to Example 2. PP-D is a polypeptide obtained from porcine SP-D cDNA (cDNA as described by van Eijk, M., et al. "Porcine lung surfactant protein D: complementary DNA cloning, chromosomal localization, and tissue distribution." J.Immunol. 164.3 (2000): 1442-50).

Comparable results can be obtained with other viruses described above and with other CRD's. Deglycosylation and desialylation reduces HAA inhibitory activity of PP-1, PP-2 and PP-D. Resialylation of PP-1 and PP-2 after desialylation, restored HAA inhibitory activity.

It is clear from the results that the polypeptides according to the invention have improved activity, for example with respect to binding to various micro-organisms, for example various types of IAV, not only with respect to the origin of the host (i.e. virus derived from for example human, pig, equine and the like), but also with respect to the subtype (H5N1, H3N1 etc.). The polypeptides according to the invention provide new and surprising means for the use of modified CRD's in the treatment of a wide variety of conditions.

C L A I M S

1. Polypeptide comprising a modified carbohydrate recognition domain, derived from a collectin carbohydrate recognition domain, wherein the modification comprises introduction of at least one glycosylation site in the said collectin carbohydrate recognition domain, the said glycosylation site not being present in the said collectin carbohydrate domain before the said introduction.

2. Polypeptide according to claim 1, further comprising a replacement of a glycosylation site present in the said collectin carbohydrate recognition domain by a non-glycosylation site.

3. Polypeptide according to any one of the previous claims, wherein said modified carbohydrate recognition domain comprises at least 2 glycosylation sites.

4. Polypeptide according to one of the previous claims, wherein said introduced glycosylation site has the amino acid sequence Asn-X-Ser or Asn-X-Thr, wherein X can be any amino acid except proline.

5. Polypeptide according to one of the previous claims, wherein said introduced glycosylation site is glycosylated with a carbohydrate.

6. Polypeptide according to claim 5, wherein said carbohydrate comprises at least one sialic acid residue, preferably at least one terminal sialic acid residue.

7. Polypeptide according to claim 6, wherein said sialic acid residue is linked to the carbohydrate by alpha(2,3)-linkage or alpha(2,6)-linkage, or a mixture thereof.

8. Polypeptide according to any one of the previous claims wherein said polypeptide is in the form of a trimer.

9. Polypeptide according to one of the previous claims wherein said collectin carbohydrate recognition domain is derived from

collectin, originally from any of the group consisting of rodents, human, porcine and bird.

10. Polypeptide according to one of the previous claims, wherein said collectin carbohydrate recognition domain is derived from any of the group consisting of mannan-binding lectin (MBL), surfactant protein A (SP-A), surfactant protein D (SP-D), collectin liver 1 (CL-L1), collectin placenta 1 (CL-P1), conglutinin, collectin-43 (CL-43), collectin-46 (CL-46) and collectin 11.

11. Polypeptide according to one of the previous claims, wherein said collectin carbohydrate recognition domain comprises any one of the amino acid sequences shown in figure 1.

12. Polypeptide according to one of the previous claims, wherein said introduced glycosylation site is introduced at a position between amino acid 240 and 260, preferably between 246 and 253, and/or between 267 and 298, preferably between 272 and 290, and/or between 304 and 331, preferably between 308 and 330, and/or between 331 and 344, preferably between 333 and 340 of an amino acid sequence of said collectin carbohydrate recognition domain.

13. Polypeptide according to one of the previous claims, wherein the polypeptide comprises a N-terminal region comprising cysteine residues and/or a collagen-like region characterised by repetitive Gly-Xaa-Yaa sequences and/or a neck-region.

14. Polypeptide according to one of the previous claims, wherein said polypeptide is a non-natural polypeptide.

15. Polypeptide according to one of the previous claims for use in the treatment of an animal body, preferably a human body.

16. Nucleic acid comprising a sequence of nucleotides encoding a polypeptide according to anyone of the previous claims.

17. Use of a polypeptide according to any one of the claims 1 - 15 for the manufacture of a medicament for the treatment of infection or inflammatory conditions.

18. Use of a polypeptide according to any one of the claims 1-15 for binding a hemagglutinin or a micro-organism comprising a hemagglutinin.

19. Method for obtaining a polypeptide according to any one of the claims 1 - 15, wherein said method comprises the step of introducing a glycosylation site at a non-glycosylation site in a collectin carbohydrate recognition domain.

20. Method according to claim 19, wherein the method further comprises the step of introducing a non-glycosylation site at a glycosylation site in a collectin carbohydrate recognition domain.

21. Method according to one of the claims 19-20, wherein introducing comprises substitution, deletion and/or addition of at least one amino acid residue in said collectin carbohydrate recognition domain.

22. Method for obtaining a polypeptide according to one of the claims 1 - 15 comprising the step of expressing a nucleic acid according to claim 16 in a host organism under conditions that the polypeptide is formed and isolating the said polypeptide.

23. Method according to claim 22, wherein said nucleic acid is expressed in a cell capable of N-glycosylation of proteins, preferably capable of both N-glycosylation and sialylation.

ALIGNMENT CARBOHYDRATE RECOGNITION DOMAINS COLLECTINS

```
Amino  2         2                   2                                                                                3
acid   3         4                   6                                                                                5
no.    2         0                   0                                                                                5
                 ----------------------
hSP-D  ELFPNGQS--VGE---KIFKTAGFVKPFTEAQLLCTQAGGQLASPRSAAENAALQQLVVAKNEA-AFLSMTDSKTEGKFTYPTGESLV-YSNWAPGEPNDDGGS----EDCVEIFTNGKWNDRACGEK-RLVVCEF
mSP-D  ALFPDGRS--VGD---KIFRTADSEKPFEDAQEMCKQAGGQLASPRSATENAAIQQLITAHNKA-AFLSMTDVGTEGKFTYPTGEPLV-YSNWAPGEPNNNGGA----ENCVEIFTNGQWNDKACGEQ-RLVICEF
pSP-D  ELFPNGRG--VGE---KIFKTGGFEKTFQDAQQVCTQAGGQMASPRSETENEALSQLVTAQNKA-AFLSMTDIKTEGNFTYPTGEPLV-YANWAPGEPNNNGGS-SGAENCVEIFPNGKWNDKACGEL-RLVICEF
bSP-D  MLFPNGRS--VGE---KIFKTVGSEKTFQDAQQICTQAGGQLPSPRSGAENEALTQLATAQNKA-AFLSMSDTRKEGTFIYPTGEPLV-YSNWAPQEPNNDGGS----ENCVEIFPNGKWNDKVCGEQ-RLVICEF
rSP-D  ALFPDGQS--VGD---KIFRAANSEEPFEDAKEMCRQAGGQLASPRSATENAAVQQLVTAHSKA-AFLSMTDVGTEGKFTYPTGEALV-YSNWAPGEPNNNGGA----ENCVEIFTNGQWNDKACGEQ-RLVICEF
CL-43  VLFPDGQA--VGE---KIFKTAGAVKSYSDAEQLCREAKGQLASPRSSAENEAVTQLVRAKNKH-AYLSMNDISKEGKFTYPTGGSLD-YSNWAPGEPNNRAKDEGP-ENCLEIYSDGNWNDIECREE-RLVICEF
CL-46  VLFPDGQA--VGK---KIFKTAGAVKSYSDAQQLCREAKGQLASPRSAAENEAVAQLVRAKNND-AFLSMNDISTEGKFTYPTGESLV-YSNWASGEPNNNNAGQP--ENCVQIYREGKWNDVPCSEP-LLVICEF
Congl. VLFPDGQA--VGE---KIFKTAGAVKSYSDAVQLCREAKGQLASPRSSAENEAVTQMVRAQEKN-AYLSMNDISTEGRFTYPTGEILV-YSNWADGEPNNSDEGQP--ENCVEIFPDGKWNDVPCSKQ-LLVICEF
hSP-A  -----GSIMTVGE---KVFSSNGQSITFDAIQEACARAGGRIAVPRNPEENEAIASFVKKYNTY-AYVGLTEGPSPGDFRYSDGTPVN-YTNWYRGEPA----GR-GKEQCVEMYTDGQWNDRNCLYS-RLTICEF
h-MBL  LTFSLGKQ--VGN---KFFLTNGEIMTFEKVKALCVKFQASVATPRNAAENGAIQNLIKEE----AFLGITDEKTEGQFVDLTGNRLT-YTNWNEGEPN----NAGSDEDCVLLLKNGQWNDVPCSTS-HLAVCEFPI
hCL-L1 --IA-GIRETEE----KFYYIVQEEKNYRESLTHCRIRGGMLAMPKDEAANTLIADYVAKSGFFRVFIGVNDLEREGQYMFTDNTPLQNYSNWNEGEPS----DPYGHEDCVEMLSSGRWNDTECHLT-MYFVCEFIKKKK
hC 11  --VA-GVRETES----KIYLLVKEEKRYADAQLSCQGRGGTLSMPKDEAANGLMAAYLAQAGLARVFIGINDLEKEGAFVYSDHSPMRTFNKWRSGEPN----NAYDEEDCVEMVASGGWNDVACHTT-MYFMCEFDKENM
hCL-P1 -----GCPPHWKNFTDKCYYFSVEKEIFEDAKLFCEDKSSHLVFINTREEQQWIKKQMVGRESH--WIGLTDSERENEWKWLDGTSPD-YKNWKAGQPDNWGHGHGPGEDCAGLIYAGQWNDFQCEDV-NNFICEKDRETVLSSAL
                                 Ω       Φ  θ   C     θ  θ O  E  Ωθ            ΦΘ θ          Φ    G     Ω  W   ZP          EOCθ Ω    G WND  C      Ω C
                                                                                  1  1                      2 2    1   21          22
```

Conserved (grey shaded): letter below sequence, Φ = aromatic, θ = aliphatic, Ω = aromatic or aliphatic, O = contains oxygen, Z = either E or Q, ligands calcium 1 and 2 denoted with 1 or 2,

Figure based upon Hogenkamp et al, Mol. Immun. 43: 1604-1616 (2006)

Fig. 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2006/000159

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/47 C12N15/12 A61K38/17 A61P31/16

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)
C07K C12N C12P

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 practical, search terms used)
EPO-Internal, BIOSIS, Sequence Search, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VAN EIJK M ET AL: "Collectins and the effect of different N-linked glycan profiles on their antiviral activity" FEBS JOURNAL, vol. 272, no. Suppl. 1, July 2005 (2005-07), page 287, XP002389969 & 30TH CONGRESS OF THE FEDERATION-OF-EUROPEAN-BIOCHEMICAL-SOCIETIES (FEBS)/9TH IUBMB CONFERENCE; BUDAPEST, HUNGARY; JULY 02 -07, 2005 ISSN: 1742-464X(print) 1742-4658(ele the whole document	1-23

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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 July 2006	31/07/2006

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, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Wiame, I

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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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