Protein Science (1994), *3*:1143–1158. Cambridge University Press. Printed in the USA.

REVIEW

Collectins—soluble proteins containing collagenous regions and lectin domains—and their roles in innate immunity

HANS-JÜRGEN HOPPE AND KENNETH B.M. REID

MRC Immunochemistry Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, United Kingdom

(RECEIVED March 17, 1994; ACCEPTED May 5, 1994)

Abstract

The collectins are a group of mammalian lectins containing collagen-like regions. They include mannan binding protein, bovine conglutinin, lung surfactant protein A, lung surfactant protein D, and a newly discovered bovine protein named collectin-43. These proteins share a very similar modular domain composition and overall 3-dimensional structure. They also appear to play similar biological roles in the preimmune defense against microorganisms in both serum and lung surfactant. The close evolutionary relationship between the collectins is further emphasized by a common pattern of exons in their genomic structures and the presence of a gene cluster on chromosome 10 in humans that contains the genes known for the human collectins. Studies on the structure/function relationships within the collectins could provide insight into the properties of a growing number of proteins also containing collagenous regions such as C1q, the hibernation protein, the α- and β-ficolins, as well as the membrane acetylcholinesterase and the macrophage scavenger receptor.

Keywords: collagen-like; collectins; innate immunity; lectin; mannan binding protein; lung surfactant protein

A growing number of mammalian plasma and cell-surface proteins have been found to contain collagen-like regions attached to noncollagenous domains; however, they are not included in the definition of "collagens," which exclusively refers to proteins of the extracellular matrix. The proteins with collagenous domains found in other locations include not only the "collectins," but also 6 other proteins, namely serum complement protein C1q (Brodsky-Doyle et al., 1976; Sellar et al., 1991), α- and β-ficolin (Ichijo et al., 1993), the "hibernation protein" from squirrel (Takamatsu et al., 1993), the macrophage scavenger receptor (Kodama et al., 1990), and the membrane acetylcholinesterase (MacPhee-Quiley et al., 1986; Krejci et al., 1991). Among these collagenous molecules are the collectins, which form a family of oligomeric proteins, which contain a C-type lectin domain at the C-terminal end of each of their polypeptide chains. The collectins include serum mannan binding protein (MBP), bovine conglutinin and collectin-43 (CL-43), and the lung surfactant proteins SP-A and SP-D (Holmskov et al., 1994). The structure/function relationships within the collectins are at present under intensive study and it is becoming clear that the C-type lectin domains, by binding to carbohydrate ligands on the cell-surface of pathogens, fulfill a recognition function that can elicit effector functions via the collagen-like region, such as complement activation (in the case of MBP) or binding to cell-surface receptors to trigger phagocytosis or oxidative killing. These relationships resemble closely the well-established correlation between the structure of C1q and its functions, such as binding to immune complexes, activating complement and interacting with cell-surface receptors, with the collagen-like regions and the globular domains playing very distinct roles (Reid, 1983; Tenner, 1993). Because some of the collectins display similar functions to C1q, but do so without the involvement of antibody, it is probable that they play an important role in innate immunity.

The overall domain organization seen in the collectins is illustrated in Figure 1A. Four distinct regions can be identified within these proteins, three of which are structurally well defined. An independently folding C-type lectin domain is located at the C-terminal end of each polypeptide chain (Drickamer, 1988). The lectin domain is preceded by a stretch of about 40 amino acids, which have the potential to form a 3-stranded

Reprint requests to: Kenneth B.M. Reid, MRC Immunochemistry Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK.

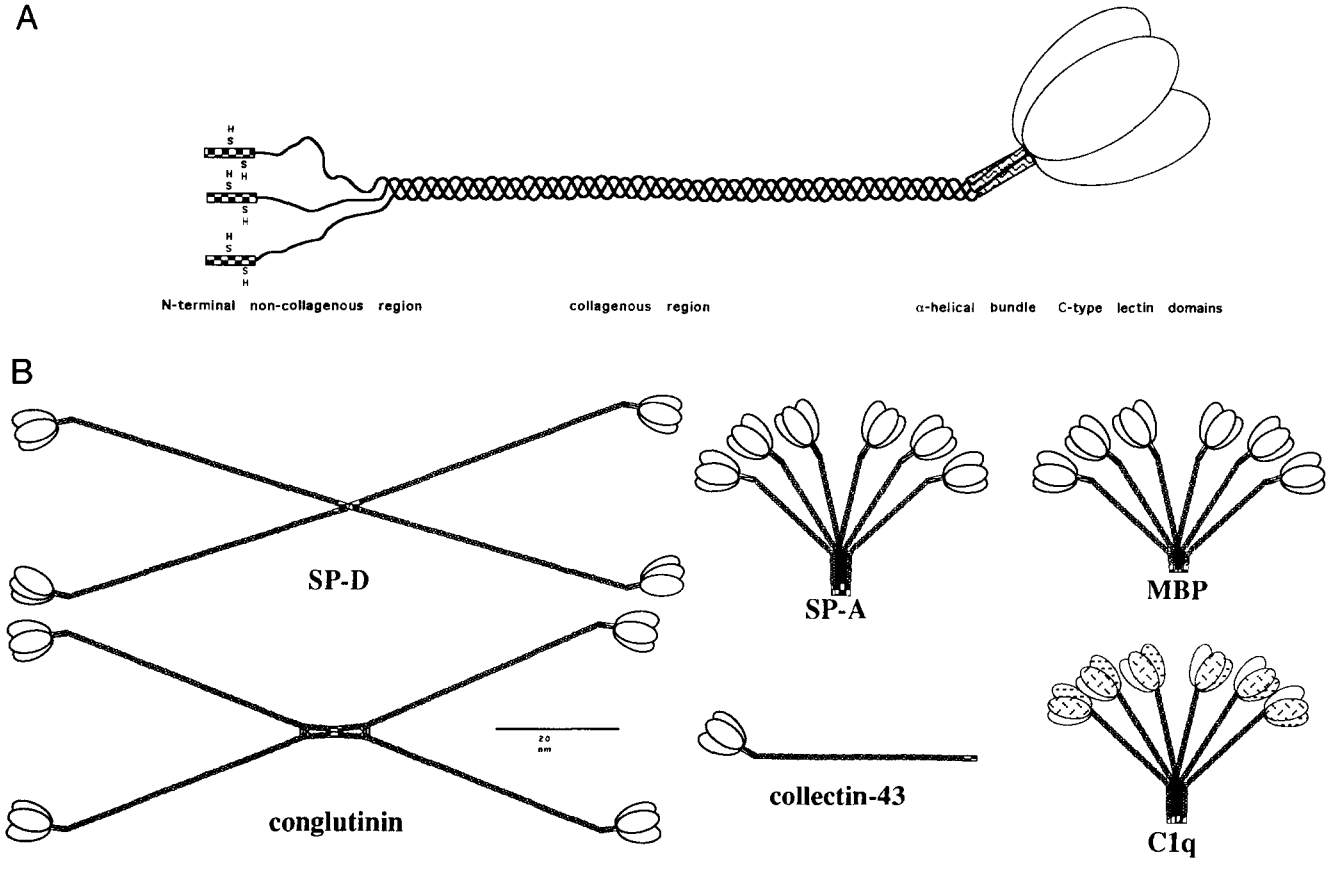


Fig. 1. Domain organization and assembly of the collectin molecules and C1q. **A:** Three polypeptide chains, each having a C-terminal C-type lectin domain, are considered to associate through strong hydrophobic interactions via the α-helical bundle forming the "neck" region. The collagen triple-helix forms in a zipper-like fashion from the C-terminal end to the N-terminal end, where a short noncollagenous region is believed to mediate oligomerization, involving disulfide bridges, to the higher oligomers shown in B. **B:** Schematic drawings of C1q and the molecules belonging to the collectin family of proteins (drawn to scale). The overall dimensions were determined by electron microscopy. However, very few side views are available of SP-A to allow the estimation of the length of the collagenous parts, which appear aligned in parallel at the foot of the molecule and thus hold the 6 triple-helical rods together to give a hexameric structure. No oligomers have as yet been found in the case of collectin-43. The globular domains of the 3 chains of C1q are not identical.

α-helical coiled-coil when associated with the equivalent regions in 2 other chains (Hoppe et al., 1994). The third domain is the collagenous region, which is always found directly N-terminal to the α-helical bundle. The repeating Gly-Xaa-Yaa triplet regions from 3 chains of any collectin form a collagen triple-helix of varying length. In SP-A and MBP, the sequences include an interruption, at triplets 13 and 8, respectively, and therefore the collagen helices are considered to bend at these points (Voss et al., 1988; Fig. 1B). The N-terminal portion in each molecule is short, containing at least 1 cysteine residue per chain, and these cysteines are thought to play an important role in the assembly of the trimeric, collagen-containing rods into higher oligomers (Fig. 1B). However, little is known about the secondary structure of this region.

The collectins are considered to act as a "bridge" between very large particles, which have on their surface certain exposed carbohydrates (Lu et al., 1990) (such as those found on invading microorganisms or, indeed, allergens derived from house dust mites or pollen grains), and the host's immune system. Their functions can be divided into 2 categories, namely those that rely on the carbohydrate recognition specificities of the lectin domains involved and those of the subsequent interactions with the host's effector systems or cells (Malhotra et al., 1990).

The collectins have been characterized at the cDNA and the genomic levels and the sequences, and intron/exon positions, are consistent with the domain organization outlined in Figure 1. Bacterial expression of distinct regions of the collectins has been crucial to the structure determination of both the C-type lectin domain (of MBP) and the 3-stranded α-helical bundle (of human SP-D). Analysis of the gene sequence of MBP suggests that the opsonic defects seen in infants suffering from recurrent infections correlate with point mutations in the MBP genes, a finding that may have important implications for the analysis of inflammatory disorders of the lung, possibly involving the collectins SP-D and SP-A and their genes.

Structure of the collectins

The lectin domain

Lectins are defined as proteins other than antibodies or enzymes that bind carbohydrates (Barondes, 1988). The specificity of a

given lectin is usually determined by the method of binding-site saturation in which different sugars are tested for their ability to inhibit lectin binding to immobilized carbohydrates and is defined in terms of the most potent carbohydrate inhibitor. Animal lectins are not a homogeneous class of proteins, but can be divided into 2 major groups: the C-type and the S-type lectins (Drickamer, 1988). C-type lectins are extracellular proteins that bind to a variety of carbohydrates in a strictly Ca^{2+}-dependent manner, whereas S-type lectins have no requirement for divalent cations; they occur both intra- and extracellularly and bind predominantly β-galactosides (Barondes, 1984; Liao et al., 1994). The main exceptions, in terms of carbohydrate binding proteins, which do not fit into either of these categories of lectins, are the extracellular proteins laminin and fibronectin (Yamada, 1983); the mannose-6-phosphate receptor (Kornfeld, 1986), responsible for targeting proteins to the lysosomal compartments; and the viral hemagglutinins (Wiley & Skehel, 1987). The pentraxin C-reactive protein (Kolb-Bachofen, 1991; Kottgen et al., 1992) and serum amyloid component P (SAP) (Kubak et al., 1988) also show lectin activities. The crystal structure of SAP has recently been determined and a high degree of similarity was seen in comparison to the 3-dimensional structure of legume lectins, such as concanavalin A (Emsley et al., 1994).

Collectins contain lectin domains of the calcium-dependent type that are between 114 and 118 amino acids long with 14 invariant and 18 highly conserved residues, including 4 cysteine residues giving rise to the disulfide pattern 1-4 and 2-3 (Drickamer, 1988). C-type lectin domains occur in a wide variety of modular proteins (Table 1). The 3-dimensional structure of the recombinant C-type lectin domain of rat MBP has been determined by X-ray crystallography (Weis et al., 1991b) and was found to have the following features: (1) the N- and C-terminal ends of the lectin domain are located very close to one another, thus making it an ideal domain to link to the N- or C-terminal end of a polypeptide chain; and (2) the structure appears divided by 2 transverse β-strands that separate a compact scaffold of 2 helices and 2 β-sheets, with the N- and C-termini, from 1 very extensive loop, carrying the binding sites for 2 calcium ions as well as for the carbohydrate ligand. Thus, half of the domain, consisting of nonregular secondary structure, is responsible for carbohydrate binding and is attached to a very tight core structure. One of the calcium ions bound by the lectin domain was shown in the crystal structure to be complexed by both conserved residues within the lectin domain and the 3 and 4 hydroxyl groups of the terminal α-D-mannose residue bound by the domain (Weis et al., 1992). Modeling different carbohydrate structures onto the binding site of the MBP lectin domain showed the importance of other residues in the domain, which are in close proximity to the binding site and sterically restrict the access of differently shaped carbohydrates. However, the binding site is not buried within the protein structure but appears to be located at the surface of the $2.5 \times 2.5 \times 4.0$-nm lectin domain, allowing for binding to accessible sugars contained within complex oligosaccharide chains as well as recognition of terminal nonreducing sugars. However, in contrast to the interactions seen in S-type and legume lectin-type protein–carbohydrate binding, the crystal structure of the C-type lectin domain of MBP provided little evidence for van der Waals or hydrophobic interactions with the complexed carbohydrate.

The molecular architecture of C-type lectin domains permits site-specific alterations to be made in the carbohydrate-binding region, within the flexible loop, which do not appear to affect the integrity of the structural scaffold holding the loop in place (Quessenberry & Drickamer, 1991). Such alterations can transfer a galactose-binding activity into the mannose-binding protein C-type lectin domain (Drickamer, 1992). Two of the 5 residues dues responsible for complexing the calcium ion involved in carbohydrate binding, namely E185 and N187 within the sequence EPN-E-WND (where E185, N187, E193, N205, and D206 are considered to be of importance in binding Ca^{2+}), were shown to be predominantly involved in determining the specificity for hydroxyl groups in positions equivalent to the 3- and 4-OH groups of D-mannose ($^4C_1$ form) in monosaccharide recognition (Drickamer, 1992). Change of these 2 residues to Q185 and D187 resulted in a large increase in the affinity for D-galactose as opposed to D-mannose seen for the E/N wild type, reflecting the steric requirements of bi-equatorial vs. equatorial-axial positioned hydroxyl groups at positions 3 and 4 of the ligand. The correlation between specificity and residue pair was found in sequence comparisons to be in excellent agreement with other C-type lectin domains of known sequence and monosaccharide specificity (Table 1). Point mutations as well as peptide/mAb inhibition studies (Geng et al., 1991, 1992) were also used to localize the binding site(s) within the E- and P-selectin molecules (Erbe et al., 1992, 1993). However, the possibility of more than 1 binding site present within these adhesion molecules, or additional protein–protein interactions involved in selectin–glycoprotein recognition, cannot be ruled out, and should also be considered in the definition of ligands for the collectins.

Using the criteria discussed above in sequence alignments, the collectins MBP, conglutinin, CL-43, and SP-D were all found to belong to the mannose-type carbohydrate binding family, whereas SP-A assumes a hybrid position between the rest of the collectins and the domains of other lectins specific for galactose-type sugars, e.g., the cartilage proteoglycans, which is in good agreement with the observation that both types of sugars, galactose and mannose, can inhibit SP-A binding equally well (Haagsman et al., 1987). The macrophage galactose-receptor (Sato et al., 1992) and the Kupffer cell fucose receptor (Hoyle & Hill, 1988) also exhibit galactose-type selectivity, however they contain within their binding regions a 4-amino acid-long insertion between the 2 residues found to direct mannose-type or galactose-type hydroxyl-group specificity and residue E193. This may influence the conformation of the lectin domain at the binding site and thus define the steric requirements determining the binding activity for the natural polysaccharide ligands of these proteins. A similar observation can be made in case of the selectins (Lasky et al., 1989), which have a highly charged and conserved 5-residue-long insertion between E193 and N205/D206. Therefore, although the requirement for hydroxyl-groups of the mannose- or galactose-type within the binding-site on the carbohydrate molecules is determined by the E-N/Q-D pair of residues, other differences seen in the sequences of the lectin domains, and of the extensive loop carrying the binding sites in particular, seem to be also involved in ligand selection in the in vivo interaction of C-type lectins with complex carbohydrate structures. Domains identified by sequence comparison and predicted to have an overall folding similar to that seen in the crystal structure of the C-type lectin domain of MBP (Weis et al., 1991b), but lacking some of the characteristic residues involved in calcium/carbohydrate binding, are seen in a number of type II membrane proteins (Table 1). These exceptions include the mu-

Table 1. *Proteins containing C-type lectin domains or homologous regions*[a]

	Specificity known	Reference	Gal/Man type
1. Proteoglycans			
Rat cartilage proteoglycan core protein	Gal-Fuc	Doege et al., 1987	QPD-E-WND gal
Human cartilage proteoglycan core protein	Gal-Fuc	Doege et al., 1991	QPD-E-WND gal
Chicken cartilage proteoglycan core protein	Gal-Fuc	Tanaka et al., 1988	QPD-E-WND gal
Fibroblast proteoglycan protein	Gal-Fuc	Zimmerman & Ruoslahti, 1989	QPD-E-WND gal
2. Type II membrane proteins			
Chicken hepatic lectin	GlcNAc	Drickamer, 1981	EPN-E-WND man
Human asialoglycoprotein receptors 1 and 2	Gal-GalNAc	Spiess & Lodish, 1985	QPD-E-WND gal
Rat asialoglycoprotein receptor	Gal-GalNAc	Drickamer et al., 1984	QPD-E-WND gal
Rat peritoneal macrophage lectin	Gal-GalNAc	Ii et al., 1990	QPD-E-WND gal
Rat Kupffer cell lectin	Gal-Fuc	Hoyle & Hill, 1991	QPD-E-WND gal
Murine macrophage lectin	Gal-GalNAc	Sato et al., 1992	QPD-E-WND gal
Human placenta-derived gp120 binding lectin	Man	Curtis et al., 1992	EPN-E-WND man
Human CD23	*	Suter et al., 1987	EPN-E-WND
Murine T lymphocyte antigen (A1)	*	Yokoyama et al., 1989	----G-RLD
Murine T lymphocyte antigen (YE1/48)	*	Chan & Takei, 1989	---------
Rat NKR-P1	*	Giorda et al., 1990	----D-VLS
Human Lyb-2	*	Van Hoegen et al., 1990	KPF-D-WIM
Human NKG2 (A–D)	*	Houchins et al., 1991	----L-LKS
Murine NK cell protein Ly49	*	Wong et al., 1991	----G-LDN
Human T cell hybridoma lectin homologue	*	Yoshimatsu et al., 1992	QPW-G-WRR
3. The collectins			
Bovine conglutinin	GlcNAc	Lee et al., 1991	EPN-E-WND man
Human mannan binding protein	Man-Fuc	Taylor et al., 1989	EPN-E-WND man
Rat mannan binding proteins A and C	Man-Fuc	Drickamer et al., 1986	EPN-E-WND man
Human lung surfactant protein A	Man-Fuc	White et al., 1985	EPA-E-WND
Dog lung surfactant protein A	*	Benson et al., 1985	EPR-E-WND
Human lung surfactant protein D	Malt-Man	Rust et al., 1991	EPN-E-WND man
Bovine collectin-43	Man-ManNAc	Holmskov et al., 1993b	EPN-E-WND man
Bovine mannan binding protein	Man-Fuc	Holmskov et al., 1993a	EPN-E-WND man
4. The selectins			
L-selectin	NeuNAc-Fuc	Lasky et al., 1989	EPN-E-WND man
E-selectin	NeuNAc-Fuc	Bevilacqua et al., 1991	EPN-E-WND man
P-selectin	NeuNAc-Fuc	Johnson et al., 1989	EPN-E-WND man
5. Other C-type lectins			
Human HIP	Lac	Christa et al., 1994	EPN-G-WKD
Sea raven antifreeze protein	Ice	Ng & Hew, 1992	KPD-A-WDD
Smelt antifreeze protein	Ice	Ewart et al., 1992	QPD-E-WND gal
Rat pancreatic thread protein	$CaCO_3$	Rouquier et al., 1991	YPN-G-WRD
Macrophage mannose receptor	Man	Taylor et al., 1990	
CRD-1			SPS-K-WEN
CRD-2			EPS-E-WAD
CRD-3			MPG---WDV
CRD-4			EPN-W-WND man
CRD-5			EPN-W-WND man
CRD-6			YPG-A-WMD
CRD-7			EPK---WKT
CRD-8			DPS---WSN

[a] Proteins containing C-type lectin domains or regions with homology to C-type lectin domains are listed and are divided into 5 groups (Drickamer, 1992). Not all of the carbohydrate specificities have been determined (*), and certain proteins, although they contain the conserved residues consistent with a C-type lectin domain being present, do not appear to bind carbohydrate (e.g., the sea raven antifreeze protein). The mannose or galactose types indicate the presence of residues (Drickamer, 1992) shown to correlate with the binding specificity of the particular lectin domain for either galactose or mannose.

rine T lymphocyte antigens A1 (Yokoyama et al., 1989) and YE1/48 (Chan & Takei, 1989), the murine natural killer cell protein Ly49 (Wong et al., 1991), and its rat homologue NKR-P1 (Giorda et al., 1990), as well as the human and murine Lyb-2 proteins (Van Hoegen et al., 1990). Also lacking one of the conserved residues considered to be involved in MBP-carbohydrate interaction is the human IgE receptor CD 23, which binds in a calcium-dependent manner to IgE, but also to nonglycosylated

IgE (Vercelli & Geha, 1989). The macrophage mannose receptor, which is the only protein known to contain within its primary sequence more than a single C-type lectin domain, also falls into this category. Only 2 of the 8 domains show all of the calcium-binding residues required for interacting with a carbohydrate ligand seen in the MBP crystal structure. Interestingly, significant sequence homology to C-type lectin domains is also seen in a group of antifreeze proteins from herring, sea raven, and smelt (Ewart et al., 1992; Ng & Hew, 1992). These polypeptides inhibit the growth of ice crystals in a calcium-dependent manner; however, there is no evidence yet whether the interaction with ice crystals is similar to that of C-type lectins to hydroxyl groups of carbohydrates.

The α-helical bundle

Recently the structural characteristics of the domain located immediately N-terminal to the lectin domain, and which is common to all of the collectins, were established (Hoppe et al., 1994). The short stretch of 34–41 amino acids between the N-terminal end of the lectin domain and the beginning of the collagen-like triple helix contains hydrophobic residues that show a heptad repeat (Fig. 2). A recombinant peptide equivalent to this region in human SP-D was analyzed, by size exclusion chromatography and crosslinking experiments, and found to form a trimer in solution. CD measurements indicated mostly α-helical structure and multidimensional NMR revealed the parallel nonstaggered orientation of the α-helices in a 3-stranded coiled-coil. The structure is held together by very strong, hydrophobic forces and proved to be very stable against denaturation by heat ($T_m > 55$ °C) or pH (pH 3.0–8.5). Coiled-coils are found in a variety of modular proteins, and can contain 2, 3, or 4 α-helices in parallel and nonstaggered, or antiparallel and staggered, orientation. The strong and noncovalent forces that hold these helices together result from hydrophobic side chains (Harbury et al., 1993), in a and d positions of the heptad repeat, forming the interface between the helices (Fig. 2). However, other side chains also play an important role in the interactions between the helices and may be crucial to the interchain recognition process in the folding pathway of these molecules. SP-D, conglutinin, and CL-43 show a good agreement with the heptad repeat pattern, whereas irregularities occur in the sequences of MBP and SP-A (Fig. 2). However, hydrophilic or charged residues in positions a and d can also be found in the chains of other α-helices of known coiled-coil structure, such as laminin (Timpl et al., 1979) and the macrophage scavenger receptors (Kodama et al., 1990).

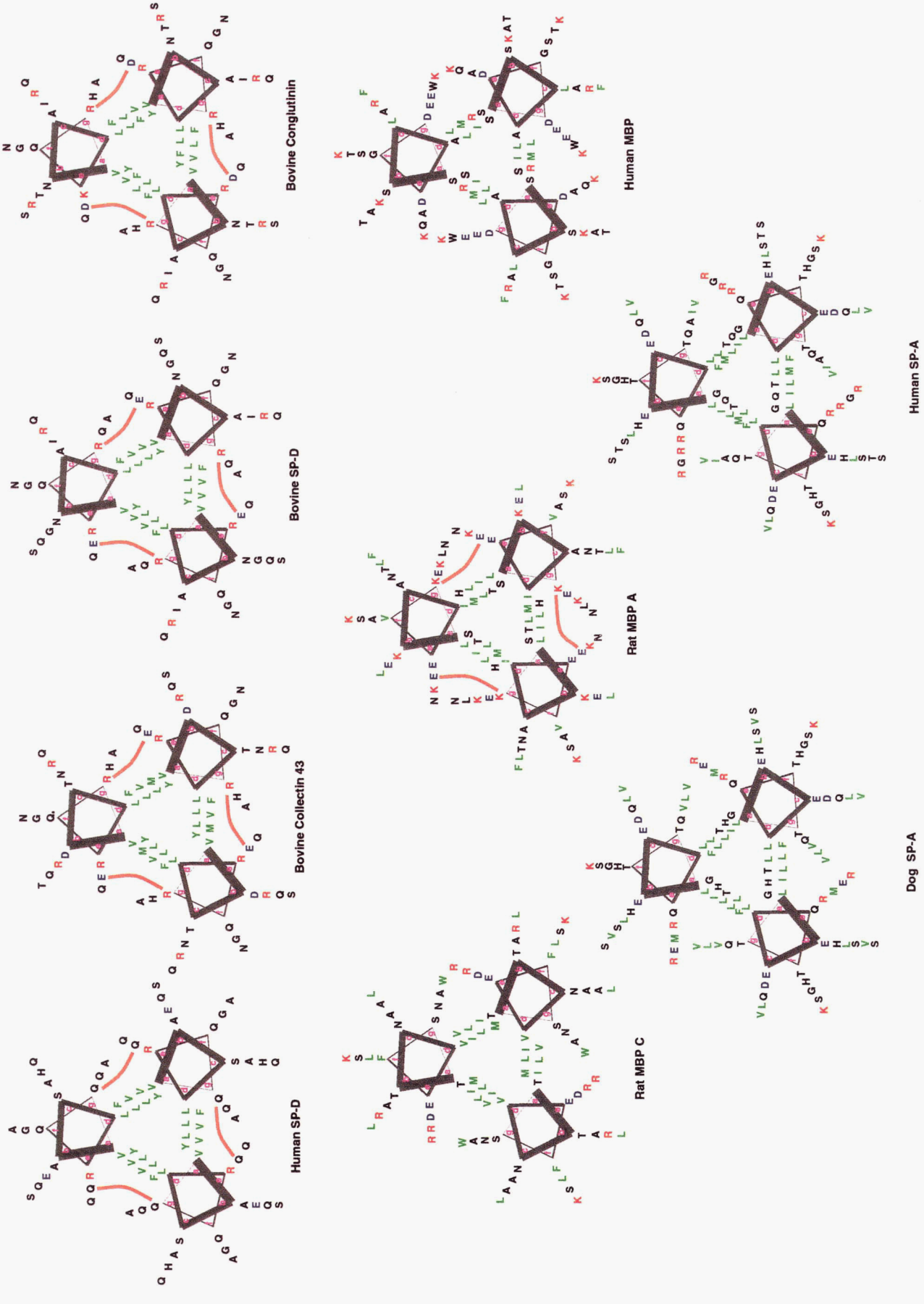
The collagenous domain

The collectins are a family of soluble proteins containing collagenous sequence. Most collagens are insoluble constituents of the extracellular matrix, such as the fibril-associated collagens with interrupted triple-helix (FACITs) (collagen types IX, XII, XIV, and XVI) and the collagens of striated fibrils (types I, II, III, V, and XI) (Van der Rest & Garrone, 1991). C1q is well known as a serum protein containing collagen-like triple helical structures (Reid, 1983), and its C-terminal globular domains show significant similarity to the C-terminal noncollagenous regions of the chains of the ground squirrel hibernation protein (Takamatsu et al., 1993) as well as to the C-terminal regions of the type VIII and X collagens (Sellar et al., 1991), and to the precerebellin molecule (Urade et al., 1991) (Table 2). However, none of these molecules shows a C-type lectin activity or sequence similarity to the lectin domain or appears to contain an α-helical bundle. This also holds for the ficolins (Ichijo et al., 1993), a newly discovered class of molecules bearing fibrinogen-like domains at their C-terminal ends that, like the C1q B chain, conglutinin, and SP-D, contain noninterrupted collagenous regions within the N-terminal halves of their polypeptide chains. The features of the collagenous regions of the collectins and other soluble collagen-containing proteins are compared in Table 2A.

The biosynthesis of collagen-containing molecules requires the correct alignment of 3 polypeptides of repeating Gly-Xaa-Yaa triplets to form the triple-helix (Traub & Piez, 1971). As the formation of the collagen helix proceeds in a zipperlike fashion from a single nucleation point at the C-terminal end of the 3 chains (Engel & Prockop, 1991) and as no direct association between the C-type lectin domains can be observed under physiological conditions (Weis et al., 1991a) it is possible that, in the collectin family of proteins, the tight association of the 3 chains in the α-helical bundle serves as the nucleation point for the formation of the triple-helix (Hoppe et al., 1994). Characteristic side-chain modifications known from collagen molecules are also present in the collagenous portion of the collectins, as for example in the case of MBP, which was shown to carry glycosyl-galactosyl modifications on a lysine residue (Colley & Baenzinger, 1987) similar to those seen in other collagens and C1q. Many of the proline and lysine residues in the Yaa-position are hydroxylated to give 4-hydroxyproline and hydroxylysine, respectively. Hydroxyproline residues are known to stabilize the collagen triple-helix (Sakakibara et al., 1973) and presumably carry the same function within the collectins. However, the hydroxylysine residues do not appear to participate in the covalent crosslinking reactions mediated by lysyl oxidases as is seen in the collagens of the extracellular matrix (Eyre et al., 1984). In the collectin family of proteins the association of the trimeric collagen-containing rods to form higher order oligomers appears to be mediated predominantly by the N-terminal noncollagenous sequences and often involves disulfide bridges between the subunits (Lu et al., 1993c). The association between the heterotrimeric subunits in the C1q molecule, as well as in the SP-A molecule, involves almost a third of the collagen triple-helix and is characterized by not only disulfide bonds but predominantly by hydrophobic residues thought to face those of the neighboring triple-helices in the collagenous region up to the bend in the bunch-of-tulips-like structure (Kilchherr et al., 1988). However, in the C1q molecule, a region of charged amino acids, in the Xaa- and Yaa-positions, exhibits a significant degree of homology to the regions in the triple-helical domain following the N-terminal domains of the collectin molecules. This region has been postulated to be involved in the interaction between both C1q and the collectins with the C1q receptor (Malhotra et al., 1993b).

The N-terminal region of the collectins

The N-terminal, noncollagenous, sequences of the collectins show only limited similarity to each other and no homologous regions in other proteins were retrieved from the databank in searches with these sequences. However, the conservation of the cysteine residues between conglutinin, CL-43, MBP, and SP-D


Bovine Conglutinin
Bovine SP-D
Bovine Collectin 43
Human SP-D
Human MBP
Rat MBP A
Rat MBP C
Human SP-A
Dog SP-A

is indicative of a similar role for these residues, in the association to higher oligomeric forms (as illustrated in Fig. 1B).

Bovine conglutinin was found to contain an additional cysteine residue within its collagenous domain. This cysteine residue in the interruption of the triple-helix at triplet 8 has been implicated in the increased stability of the disulfide-linked oligomers seen in the electron micrographs and in SDS-PAGE analysis of conglutinin when compared to bovine SP-D (Lu et al., 1993c). The purification of intact oligomers proved to be more difficult in the case of SP-D than of conglutinin and a large excess of monomers is seen in electron microscopy pictures, depending on the preparation; no higher oligomers have as yet been detected in preparations of the recently discovered CL-43 molecule from bovine serum (Holmskov et al., 1993b).

Function

Carbohydrate recognition by the collectins

An important feature of C-type lectin domains is the very low affinity of a single domain for its ligand. Thus, it is not surprising that C-type lectin domains have mainly been found in molecules containing regions mediating oligomerization or membrane attachment. Native MBP, for example, contains 18 lectin domains and exhibits a K_D for mannan of 2×10^{-9} M (Kawasaki et al., 1983), whereas the binding of single recombinant lectin domains of human SP-D to immobilized carbohydrate ligands on plates or resins can be completely inhibited by 0.1 mM glucose (Hoppe et al., unpubl. obs.). Lectin domains in trimeric forms, such as those found in dissociated single subunits of collectins (Fig. 1A) or in recombinant molecules containing the α-helical bundle of human SP-D, can be bound to immobilized monosccharides and can only be completely eluted when a concentration of free monosaccharide of 20 mM is used (Hoppe et al., unpubl. obs.). However, the degree of multivalency is only 1 structural factor determining ligand specificity of the collectin molecules.

Structural factors influencing carbohydrate binding appear to operate at 3 distinct levels: at the level of the residues directly involved at the binding sites of both the lectin domains and the carbohydrate; at the level of steric hindrance by either protein or carbohydrate structure restricting access to the respective binding sites; and at the level of epitope spacing on the ligand, which has to match the position of the lectin domains in a given collectin as determined by the overall 3-dimensional assembly. Therefore, the conformation of the neighboring hydroxyl groups at the exposed sites of the carbohydrate has to match the subtype of the C-type lectin (this explains the failure of galactose and the success of L-fucose in inhibiting MBP binding to mannan), and the conformation of the carbohydrate around the hydroxyl groups directly involved in binding has to be sterically favored by other side chains within the lectin domain (e.g., the increasing size of the groups substituted at the 2-hydroxyl group position in glucose reduces the ability of the sugar to inhibit binding of SP-D to maltosyl-BSA [Lu et al., 1993a], with 50% inhibition being shown by 10 mM glucose or by 19 mM glucosamine or by 29 mM *N*-acetyl glucosamine). Also, a suitable binding site must be present within the carbohydrate moiety and must not be masked by other parts of the structure of the glycoproteins or complex carbohydrates (the recognition of the carbohydrate moiety in iC3b but not in the native C3 by conglutinin in the fluid phase is a good example of the biological significance of this level of regulation of specificity [Lachmann & Müller-Eberhard, 1968]).

However, the unusual overall structures of the collectin molecules may very well have an additional specificity-determining influence in the in vivo situation; for example, conglutinin spans almost 100 nm with its collagenous regions compared to a maximal distance of 28 nm between the lectin domains in the MBP molecule (Strang et al., 1986) (Fig. 1B). It seems unlikely, therefore, that both of these serum collectins exhibit identical binding to the carbohydrate moieties on target particles of different size and shape, such as influenza virus (100 nm), poliomyelitis virus (30 nm), or *Escherichia coli* (1,000 nm), even if those particles contain accessible carbohydrate molecules with the required OH configuration in a comparable density on their surface. The requirement for multiple binding sites on the same particle becomes especially relevant in the case of lectin–ligand interaction where only a single lectin domain, or a lectin "head" composed of a trimeric group of lectin domains, is involved in binding, such as would be required for the crosslinking by collectin molecules of small nonaggregated ligands, e.g., soluble asialoglycoproteins. These interactions should be readily inhibitable by normal serum concentrations of glucose, and this thus represents another factor determining ligand specificity in the in vivo situation. Truncated conglutinin is the major form of conglutinin in heat-inactivated bovine serum and lacks the N-terminal 40 residues, resembling a trimeric molecule containing a collagenous triple-helix and a single lectin "head," and cannot form higher oligomers. This truncated conglutinin molecule was shown to exhibit neither conglutination activity nor binding activity to the sensitized erythrocyte–solid phase iC3b complex (EAiC3b), but it retained the binding activity to mannan in solution and the original binding specificity for *N*-acetylglucosamine (Kawasaki et al., 1983; Lu et al., 1993c). The different shapes of the collectins as well as the length of the collagenous region and their oligomeric assembly all seem capable of influencing the binding specificity for carbohydrate ligands on biologically relevant particles, yet comparative studies identifying distinct targets for the binding of different collectins on an appropriate target

Fig. 2. Wheel projection of the amino acids forming the α-helical bundle. The structure of the α-helical bundle present in human SP-D has been determined by multidimensional NMR, and the equivalent regions of the other collectins are shown for comparison. The sequences begin at the N-terminal end of the α-helical region, with hydrophobic residues shown in green, positively charged residues shown in red, and negatively charged residues shown in blue. The brown line indicates a possible interaction between residues in e and g positions. The wheel projections for bovine SP-D, conglutinin, and collectin-43 are in excellent agreement with the observed structure of human SP-D and illustrate the strong hydrophobic interactions between the helices. The wheels representing MBP and SP-A show a number of hydrophobic residues in positions b, c, e, f, and g, pointing "outward," and also contain hydrophilic residues in the buried positions a and d. The model drawing of these 2 collectins therefore indicates that the forces holding the α-helical bundle together might not be as strong as those seen in the human SP-D molecule.

Table 2. *Comparison of soluble proteins containing collagenous regions*

A. Soluble proteins containing collagenous regions[a]

Protein, origin	Leader length (a.a.)	N-terminal region		Collagen region				Neck region length (a.a.)	Globular domain	
		Length (a.a.)	Cysteines	Length (a.a.)	Cysteines	Interrupted triplet (no.)	Length (EM or calc.)		Length (a.a.)	Domain type
SP-D, human	20	25	2	177	–	–		38	115	C-type lectin
SP-D, rat	19	25	2	177	–	–		38	115	C-type lectin
SP-D, bovine	20	25	2	171	–	–	46 nm	38	115	C-type lectin
Conglutinin, bovine	20	25	2	171	1	GRECPH (4)	39 nm	38	117	C-type lectin
Collectin-43, bovine	20	28	2	114	–	–	37.4 nm	41	118	C-type lectin
SP-A, human	17	10	1	71	–	GEMPCPP (13)	10 + 10 nm	34	114	C-type lectin
MPB, human	21	21	3	59	–	GGTGQ (8)	Ca. 13.2 nm	34	113	C-type lectin
Ficolin α	29	19	1	54	1	–	16 nm (calc.)	NK	220	Fibrinogen
Ficolin β	32	19	1	57	1	–		NK	217	Fibrinogen
HP-20	23	1	0	39	–	–	11 nm (calc.)	NK	133	C1q-like
HP-25	28	11	1	42	–	–		NK	135	C1q-like
HP-27	30	12	1	39	–	–		NK	134	C1q-like
C1q A	22	8	1	79	–	GIRT (10)	11 + 12 nm	NK	136	C1q
C1q B	27	11	1	78	–	–		NK	136	C1q
C1q C	28	5	1	81	–	GIPAIK (11)		NK	131	C1q

B. Cysteines in the N-terminal region[b]

Protein	Sequence	Origin
SP-D	AEMTTFSQKILANACTLVMCSPPEDGLPG	Bovine
SP-D	AEMKTYSHRTTPSACTLVMCSSVESGLPG	Human
SP-D	AEMKTLSQRSITNTCTLVLCSPTENGLPG	Rat
Conglutinin	AEMTTFSQKILANACTLVMCSPLESGLPG	Bovine
Collectin-43	EEMDVYWEKTLTDPCTLVVCAPPADSLRG	Bovine
MBP	ADTETETNEENIRKTCPVIACGPGING	Bovine
MBP	ETVTCEDAQKTCPAVIACSSPGING	Human
MBPc	AVYAETLTEGAQSSCPVIACSSPAWNG	Rat
MBPc	ETLTEGAQSSCPVIACSSPGLNG	Mouse
MBPa	VSSSGSQTCEETLKTCSVIACGRDGRDGPK	Rat
MBPa	SGSQTCEDTLKTCSVIAC	Mouse
SP-A	EVKDVCVGSPGIPGTP	Human
SP-A	VSGIENNTKDVCVGNPGIPGTP	Dog
C1q A	MVTEDLCRAPDGKKGEAGRPGRR	Human
C1q B	GLIDISQAQLSCTGPPAIPGIPGIPGTP	Human
C1q C	ALRGQANTGCYGIPGMPGLPGAPGKD	Human

[a] Domain organization of the collectins and comparison of their sequences with those of the ficolin molecules, the 3 chains of C1q, and the hibernation protein. a.a., Amino acids; EM, determined by electron microscopy; NK, not known.

[b] Conserved cysteine residues present in the N-terminal regions of the collectins and comparison of these regions with the N-terminal sequences of the 3 chains of C1q.

microorganism, such as *Salmonella* spp., have not yet been published.

As the activation of the C1 molecule requires only 2 immobilized IgG molecules to interact with 2 (heterotrimeric) globular "heads" of the C1q molecule (Kilchherr et al., 1986), it seems reasonable to assume a similar situation for the interaction of the serum MBP-MASP (MASP = MBP-associated serine protease) complex (see below) with activating carbohydrate-bearing particles (Matsushita & Fujita, 1992). Complement-related, functionally relevant binding levels of MBP to surface carbohydrate structures might thus be considerably lower than those required for the opsonizing and phagocytosis-enhancing functions common to MBP and other collectins via their interaction with the C1q receptor, present on a variety of cells. A single molecule of MBP can activate the complement system and thereby cause the deposition of iC3b molecules on the activating surface, whereas much higher numbers of MBP molecules have to be bound to a surface of a particle to trigger the phagocytosis-enhancing functions involving the C1q receptor.

Binding of the collectins to bacteria and viruses

MBP is part of the complement-dependent RaRF bactericidal factor that is present in the sera of a wide range of vertebrates (Kawakami et al., 1984; Ji et al., 1993). This factor is composed of the MBP plus the enzyme MASP. MASP, like C1s, can activate components C4 and C2 of the classical pathway of com-

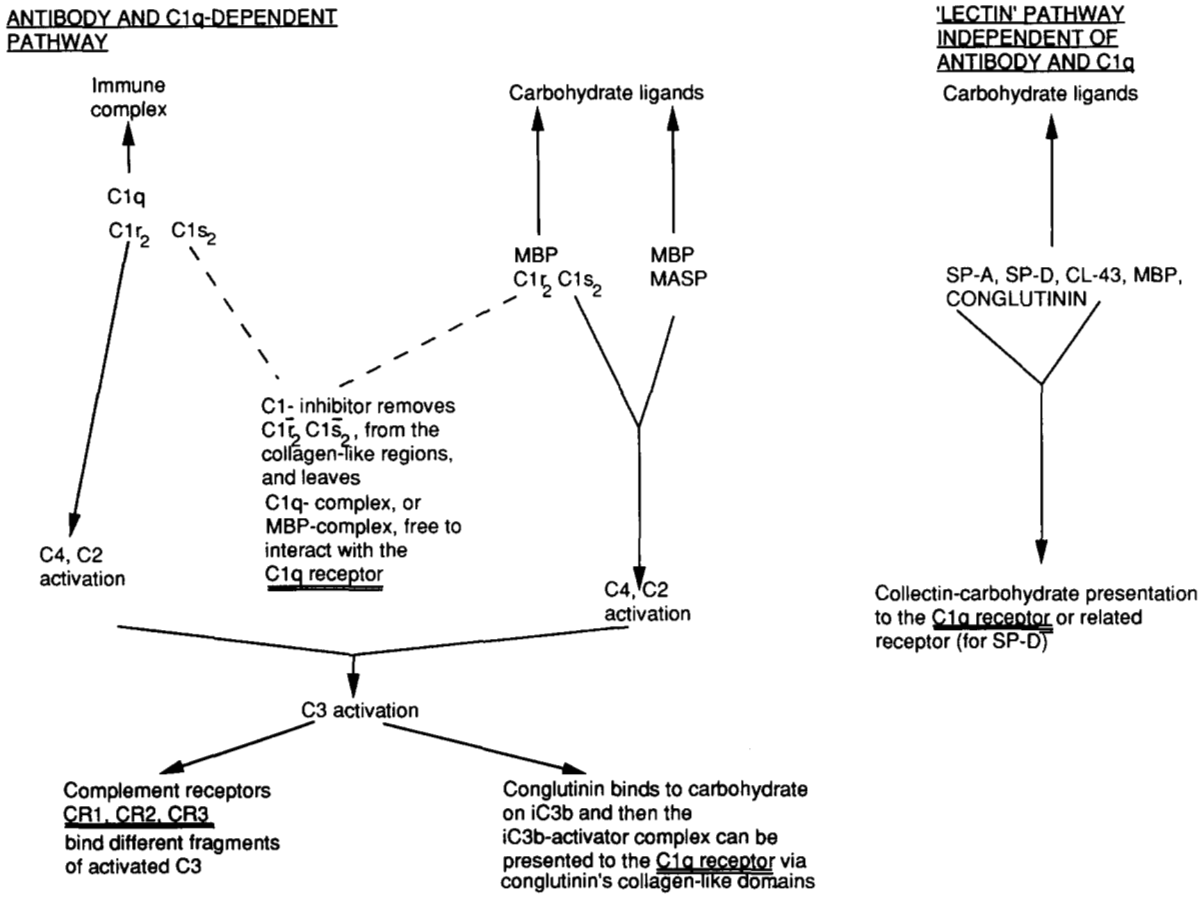


Fig. 3. Interaction of C1q and the collectins with the complement system. Activation of the classical pathway of complement via C1q binding to immune complexes or via the collectins binding to carbohydrate ligands. MASP is considered to be the protease associated with MBP in physiological conditions; however, it is not yet known if MASP associates with the collagen-like region of MBP. It is not known if MASP is bound and inactivated by the serum C1-inhibitor.

plement (Ji et al., 1993) (Fig. 3). The RaRF factor was first isolated by virtue of its calcium-dependent lectin binding to the rough Ra-chemotype strains of *Salmonella* and the rough R2 strains of *E. coli*. Enhanced phagocytosis of MBP-coated *Salmonella montevideo* is shown by human monocytes (Kulhman et al., 1989), but this appears to be due to a direct interaction of MBP with monocyte receptors rather than by means of complement activation and complement receptors. MBP interacts with envelope glycoproteins of HIV-1 and HIV-2 and, in serum, this results in activation of the classical pathway (Haurum et al., 1993), which could lead to lysis and/or opsonizing of the virus. On the other hand, inhibition of infection of a T-cell line by HIV-1 was achieved by prior incubation of HIV-1 with MBP, in the absence of complement (Ezekowitz et al., 1989). Bovine serum conglutinin has been shown, in vitro, to have antibacterial activity against *Salmonella typhimurium* and *E. coli*, with the activity requiring the presence of an intact complement system and macrophages (Friis-Christiansen et al., 1990), thus suggesting that the conglutinin was playing a crosslinking role between the iC3b-coated microorganisms and complement/collectin receptors. Bovine conglutinin binds, in a calcium-dependent and lectin fashion, to the recombinant form of the gp160 envelope glycoprotein of HIV-1 and inhibits the binding of the gp160 to the CD4 receptor on CEM 13 cells (Andersen et al., 1990). Recently, Ushijima et al. (1992) have purified a human conglutinin-like protein that bound to gp120 of HIV-1 and prevented infection of CD4 positive a CEM lymphoblastoid cell line by HIV-1. Conglutinin and MBP also appear likely to be the β-inhibitors present in bovine serum that have been reported to inhibit the infectivity and hemagglutinating activity of different influenza viruses (Andersen et al., 1990). The ability of bovine serum CL-43 to bind to microorganisms has not yet been reported.

SP-A is a major protein in the pulmonary surfactant, which is a complex of lipids and proteins that form an interface between the aqueous film on the alveolar epithelium surface and the air in the alveolar space (Weaver & Whitsett, 1991). SP-A appears to enhance the activities of the hydrophobic surfactant proteins SP-B and SP-C and it may also be involved in the recycling of surfactant and inhibition of surfactant secretion. However, in addition to these properties, SP-A also has been shown to enhance the phagocytosis of bacteria and viruses by alveolar macrophages (Van Iwarden et al., 1991) and to induce the production of oxygen radicals by these cells. The binding of SP-A, via its lectin domain, has been shown to take place with the gp120 glycoprotein of *Pneumocystis carinii* (Zimmerman et al., 1992) and with *Staphylococcus aureus* (McNeely & Coon-

rod, 1993), the latter binding enhancing the adherence of the SP-A coated bacteria to alveolar macrophages. SP-D is present at an approximately 10-fold lower level in the lung than SP-A, as judged by analysis of bronchoalveolar lavage. SP-D, unlike SP-A, does not show a strong affinity for phospholipids and therefore it is found in the lipid-free portion of bronchoalveolar lavage fluid. SP-A is perhaps a stronger candidate for playing a role in antibody-independent defense against lung infections or in the removal or presentation of allergens. For example, SP-D can bind to, and agglutinate, *E. coli*, probably via carbohydrate structures within the surface lipopolysaccharides (Kuan et al., 1992), which is strongly suggestive that SP-D plays a role in the lung's defence against Gram-negative bacteria.

Interaction of the collectins with the complement system and the C1q/collectin receptor

C1q, along with the calcium-dependent proenzyme, $C1r_2C1s_2$ complex, forms C1, the first component of the classical pathway of complement. The C1 component is activated upon its interaction with immune complexes by the binding of the globular heads of C1q to specific sites on the C_H2 domains of antibody IgG, or the C_H3 domains of antibody IgM. This binding of C1q, to the Fc regions of antibodies, results in activation of both C1r and C1s and the initiation of the complement cascade via activation of both C4 and C2 by C1s. The $C1r_2C1s_2$ complex is known to be bound to the collagen-like regions of C1q and, after activation, to be rapidly removed and inactivated by the control protein C1-inhibitor (Reid, 1983). This leaves the collagen-like regions of C1q free to interact with the C1q receptor (which is known to also interact with many of the collectins). In view of the striking structural similarity between C1q and the collectins (especially SP-A and MBP), it is perhaps not too surprising that the collectins show certain functional similarities to C1q (Holmskov et al., 1994). For example, it is now clear that MBP can mimic the action of C1q in bringing about the activation of the classical pathway of complement but it appears that it is the only collectin that has this property. On the other hand, all the collectins, with the probable exception of SP-D, are bound by the C1q receptor.

MBP, after binding to carbohydrate ligands, has been shown to activate the proenzyme $C1r_2C1s_2$ complex (Lu et al., 1990; Ohta et al., 1990) and thus bring about activation of components C4 and C2 of the classical pathway of complement. However, it seems likely that in plasma MBP is normally complexed with the proenzyme MASP and that, upon binding of the MBP–MASP complex to suitable ligands, the MASP is activated and it is this enzyme that normally brings about complement activation mediated via MBP (Matsushita & Fujita, 1992; Ji et al., 1993; Takada et al., 1993). Although it has been shown that the 80-kDa MASP enzyme is about 36% identical in amino acid sequence to both C1r and C1s, and has the same overall domain organization, it is still not known with certainty what the stoichiometry is with respect to MBP and MASP in the MBP–MASP complex, although the most recent studies suggest a ratio of 1:1 (Ji et al., 1993). Also it is not clear if the MASP is bound to the collagen-like regions of MBP or if the activated MASP is, like activated C1r and C1s, controlled by the C1 inhibitor. As discussed below, the MBP–MASP complex thus provides a route for activation of the classical pathway of complement that is triggered by carbohydrate ligands and is independent of both antibody and the C1 complex.

Conglutinin also exhibits leucocyte- and complement-dependent bactericidal activity but in contrast to MBP this does not appear to involve a direct activation of complement by the carbohydrate ligand–conglutinin complex. The opsonic effects seen with conglutinin are dependent upon prior activation of complement taking place up to at least the C3b stage so that the bacterial target becomes coated with large numbers of C3b molecules. The C3b is converted to iC3b by the enzyme factor I, in the presence of the cofactor H, and this exposes a carbohydrate structure on the α' chain of iC3b, which is recognized and bound by conglutinin. It seems likely that some of the opsonic effects and triggering of other effector functions are mediated by the interaction of the collagen-like regions of conglutinin with the C1q/collectin receptor as outlined below.

MBP, conglutinin, CL-43, and SP-A all appear to bind to the cell-surface C1q receptor, which is consequently also referred to as the "collectin receptor" (Malhotra et al., 1992). The C1q receptor is found on a wide variety of cell types, including most leukocytes, endothelial cells, eosinophils, platelets, fibroblasts, and neutrophils. A number of C1q-mediated cellular responses have been described, including the enhancement of monocyte phagocytic activity, stimulation of fibroblast adhesion, stimulation of an oxidative burst in neutrophils, enhancement of phagocytosis by pulmonary endothelial cells, inhibition of collagen-induced platelet aggregation, and serotonin release (Tenner, 1993). If these, and other, responses attributed to triggering of the C1q receptor by C1q are also set in motion by binding of complexes, composed of collectins bound to carbohydrate ligands on the surfaces of microorganisms, then a wide variety of cellular responses in the blood and lung fluids might be expected as a result of collectins interacting with the C1q receptor. The C1q receptor is an acidic protein, which behaves as an elongated molecule of 115 kDa after purification using detergents (Malhotra et al., 1993c). The detergent-solubilized molecule is composed of 2 identical chains, each of approximately 60 kDa, which show a remarkable degree of sequence identity to the intracellular, calcium-binding, protein calreticulin. However, it is considered, in view of certain differences in the structural and antigenic properties between the 2 proteins, that although they are very similar they may not be identical (Malhotra et al., 1993c). The binding of C1q to its receptor, and the collectins known to bind the C1q receptor, is considered to take place via their collagen-like regions in a Ca^{2+}-independent manner. The collagen-like regions of C1q are masked by the $C1r_2C1s_2$ proenzyme complex and upon activation the activated C1r and C1s are rapidly removed by the C1-inhibitor thus exposing the collagen-like regions and allowing the antibody–antigen–C1q complex to be presented to the C1q receptor. A similar situation may apply to MBP due to its association in plasma with the proenzyme form of the MASP protease. Thus, it is likely that upon activation the MASP enzyme is removed, allowing the carbohydrate ligand–MBP complex to be presented to the C1q receptor via the collagen-like regions in MBP. Studies on the binding of intact and truncated conglutinin to the C1q receptor indicate that the receptor binding site in conglutinin lies within the first 54 N-terminal amino acid residues in the molecule (Malhotra et al., 1993b). Comparison of the N-terminal regions of the chains of C1q, MBP, conglutinin, CL-43, and SP-A has led to the identification of a possible receptor bind-

ing site, composed of 5 Gly-Xaa-Yaa triplets with many charged residues in the X and Y positions, within the N-terminal portions of the collagenous regions of all these molecules (Malhotra et al., 1993b). However, this site is also present in SP-D, which does not appear to bind to the C1q receptor but to bind to a different, but probably related, receptor on alveolar macrophages (Miyamura et al., 1994).

SP-A has been found to bind to pollen grains, and also to specific proteins in an aqueous extract of pollen grains, in a calcium-dependent and mannose-type fashion (Malhotra et al., 1993a). The interaction between SP-A and the pollen grains/extract allows the presentation and adhesion of the pollen grains/extract to A549 alveolar type II cells to take place, probably via the collagen-like regions in SP-A binding to the C1q/collectin receptor, thus suggesting that this receptor may play a role in removal of potential allergens.

Biosynthesis, tissue location, and genetics

cDNA and genomic cloning of the collectins

The liver appears to be the major site of synthesis of both the tissue and serum forms of MBP, since this collectin was first characterized from rabbit, rat, and human liver (Kawasaki et al., 1978; Wild et al., 1983) and subsequently, in higher oligomeric forms, from the sera of these and other species. MBP has also been shown to be synthesized by hepatocytes and liver cell lines. As judged by cDNA, and genomic cloning studies, there are 2 forms of rat and mouse MBP (the A and C forms), which show 55% identity in amino acid sequence within species (Drickamer et al., 1986; Sastry et al., 1991; Wada et al., 1992). Only 1 sequence for human MBP has been described so far, by cDNA and genomic cloning, and it shows approximately 62% identity in amino acid sequence to both the A and C forms of rat MBP (Ezekowitz et al., 1988; Sastry et al., 1989; Taylor et al., 1989). The human MBP gene (Fig. 4) is 7 kb long and contains 4 exons: exon 1 encodes the leader peptide, N-terminal region and part of the collagen-like region; exon 2 encodes the remainder of the collagen-like region; and exon 3 encodes the sequences that form the short α-helical bundle located just prior to the C-terminal C-type lectin domain, which is encoded by exon 4.

SP-A is synthesized in the lung by the alveolar type II cells and, as judged by cDNA and genomic cloning, there appear to be at least 2 expressed human SP-A genes (SP-A I and SP-A II; White et al., 1985; Floros et al., 1986; Katyal et al., 1992). The human SP-A structural gene (gene symbol SFTP 1) is 4.5 kb long and contains 5 exons, with exon 2 encoding the signal peptide, the amino-terminal region, and the first 10 Gly-Xaa-Yaa triplets, and exon 3 encoding the remaining 13 Gly-Xaa-Yaa triplets. Thus, as in MBP, C1q, and type IV collagen, an intron is found within the codon for the glycine located between 2 and 9 residues before an interruption to the repeating nature of the Gly-Xaa-Yaa sequence (since in SP-A the repeats are interrupted after the 13th triplet by the sequence Pro-Cys-Pro-Pro). Exon 4 and part of exon 5 encode a putative amphipathic α-helical region, whereas the remainder of exon 5 encodes the C-type lectin domain. The 2 SP-A genes that are expressed encode for polypeptides differing at only 6 amino acid positions (White et al., 1985; Katyal et al., 1992). SP-A has been cloned at the cDNA level in a variety of other species, such as dog, rabbit, rat, and mouse (Korfhagen et al., 1991). So far, only single SP-A genes have been reported to be present in the rat, rabbit, and mouse.

SP-D is synthesized by alveolar type II cells and has also been reported to be present in alveolar macrophages (in endocytic and lysosomal structures) and in the secretory granules of bronchiolar "Clara" cells. Human SP-D has been cloned at the cDNA (Rust et al., 1991; Lu et al., 1992) and genomic (Crouch et al., 1993) levels. The human SP-D gene (gene symbol SFTP 4) is approximately 11 kb long and contains 8 exons (Fig. 4): exon 2 encodes the signal peptide, the N-terminal region, and the first 7 Gly-Xaa-Yaa triplets; exons 3–6 are all 117 bp in length and encode the remainder of the collagen-like region; and exon 7 encodes the sequences that form the α-helical bundle that links the collagenous regions to the C-type lectin carbohydrate recognition domain (which is encoded by exon 8). SP-D has been cloned at the cDNA level in rat and bovine systems, but no data are yet available at the gene level in these species.

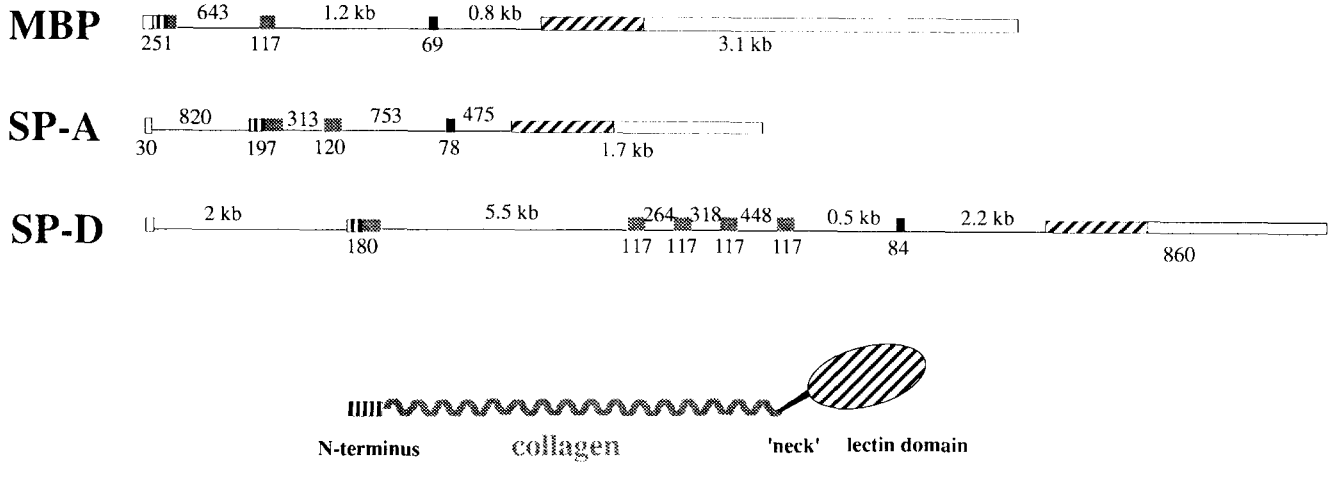


Fig. 4. Organization of the genes of MBP, SP-A, and SP-D on the genomic level. The genes for SP-A, SP-D, and MBP have been mapped to chromosome 10 in humans. Four exons were found to encode MBP, whereas the genes encoding the surfactant proteins A and D span 5 and 8 exons, respectively.

Cluster of genes encoding collectins on human chromosome 10

The genes for MBP, SP-A, and SP-D have all been mapped to the long arm of human chromosome 10. The human MBP gene (gene symbol -MBL) has been localized to 10q11.2–q21 (Sastry et al., 1989), and the human SP-A gene(s) (gene symbol SFTP 1) have been localized to 10q21–q24 (Fisher et al., 1987). There appear to be at least 2 SP-A genes (SP-AI and SP-AII; White et al., 1985; Floros et al., 1986; Katyal et al., 1992) and 1 SP-A pseudogene (Korfhagen et al., 1991) forming a closely linked cluster. STS mapping has also allowed localization of the human SP-D gene (gene symbol SFTP 4) to 10q23–q23, close to the SP-A genes and a 430-kb genomic Mlu1 fragment has been identified that contains both the SP-D gene and the SP-A gene cluster (Kölble et al., 1993). The presence of the gene for the α 1 chain of human collagen XIII (gene symbol COL 13A1) at 10q 22 is of some interest because this non-fibril-forming collagen has 4 noncollagenous regions of unknown function and therefore the presence of this gene along with the genes for the collectins suggests that an ancestor for a superfamily of genes containing collagen-like regions and carbohydrate recognition domains may have originated on the long arm of human chromosome 10.

Susceptibility to disease associated with genetic deficiency of MBP

It has proved difficult to obtain reliable measurements of SP-A and SP-D levels in bronchoalveolar lavage samples from healthy individuals, or patients, and measurements of conglutinin and CL-43 serum levels have, to date, only been made in cows. However, the serum levels of human MBP in Caucasians are well studied and have been found to vary quite considerably (0–5 μg/mL with an average value of 1.23 μg/mL) with quite a high percentage of individuals showing close to zero and therefore suggesting at least a partial deficiency of MBP.

It has been established that, in humans, a low serum concentration of MBP correlates with a common opsonic defect (Super et al., 1989) and that deficiency of MBP is associated with recurrent infections in the very young (Turner, 1991). It is considered that low serum levels or lack of MBP results in an inability to activate the classical pathway of complement (via MASP or $C1r_2C1s_2$), by an antibody-independent route, and this prevents efficient coating of pathogenic organisms with the activated C4 and C3 fragments that are required for efficient opsonization by cells carrying C4b and/or C3b receptors.

Four allelic forms of MBP have been described: the normal, A, allele; the B allele, which shows a single nucleotide substitution within codon 54 in exon 1, which results in the replacement of a glycine by aspartic acid within the collagen-like region (Sumiya et al., 1991); the C allele, which also shows a single nucleotide substitution, but within codon 57 in exon 1, which results in the replacement of a glycine by glutamic acid (Lipscombe et al., 1992) within the collagen-like region; and the D allele, another point mutation, but within codon 52 of exon 1, resulting in the replacement of an arginine by a cysteine, again within the collagen-like region (Madsen et al., 1994). The substitution of glycine in alleles B and C by charged residues results in disruption of the Gly-Xaa-Yaa repeating structure, and this is considered to prevent the correct formation of a triple-helical structure containing 1 or more of the abnormal alleles. Although the amino acid substitution in the D allele is also within the collagen-like region, it does not disrupt the continuity of the Gly-Xaa-Yaa repeating structure. However, the introduction of a cysteine residue could also cause structural problems due to possible aberrant disulfide bond formation. It is clear that individuals who are homozygous with respect to any of the B, C, or D alleles have undetectable, or trace, amounts of MBP antigenic activity in their sera as judged by sensitive immunochemical assays. The allele frequencies seen in Caucasians for A:B:C:D are 0.81:0.13:0.02:0.05. People who are heterozygous for the normal allele and one of the variant alleles (e.g., A/B heterozygous individuals) do have MBP in their sera that has the same biochemical and functional characteristics as MBP isolated from A/A homozygous individuals. Thus, it is generally agreed that the presence of any of the variant alleles, along with the A allele, decreases the serum concentration of MBP to about 15% of that expected in A/A homozygous individuals. This is consistent with the view that in heterozygous individuals only 1 in any 8 of the MBP "structural units" (composed of 2 triple-helices of disulfide-bonded AAA chains) would be of the normal type while the remaining 7/8 would be abnormal (containing at least one of the variant chains) and probably susceptible to rapid degradation (Sumiya et al., 1991). The fact that the point mutations giving rise to the different allelic forms are clustered within codons 52 and 57 found in exon 1 of the MBP gene is quite striking and indicates that the correct folding of the N-terminal section of the collagen-like region is critical for the stability of MBP.

The identification of the B, C, and D alleles of MBP does not, however, explain the very wide variation seen in serum MBP levels within apparently healthy A/A genotype individuals (0.13–5.0 μg/mL). It is possible that other variants have yet to be identified. Such variants need not necessarily influence the structure of the MBP, but may be affected in the area of the gene responsible for regulation of the level of biosynthesis of MBP.

Conglutinin has, to date, only been fully characterized in the bovine system. Hepatocytes seem likely to be the major site of synthesis, as judged by both immunohistochemical staining (Holmskov et al., 1992) and Northern blotting (Lu et al., 1993b). Bovine conglutinin has been cloned at the cDNA (Lu et al., 1993b) and genomic (Liou et al., 1994) levels. The intron/exon organization of the bovine gene (Liou et al., 1994) is very similar to that of the human SP-D gene, reflecting the high degree of amino acid sequence similarity (86% identity) seen between bovine SP-D and bovine conglutinin (Lim et al., 1993). Although a human lectin with similar structure and functional properties to conglutinin has been described (Thiel et al., 1987; Ushijima et al., 1992), it is still not clear if conglutinin is found in species other than the Bovidae.

Collectin-43, like conglutinin, has only been characterized in the bovine system, where, as judged by Northern blot analysis, it is synthesized in the liver (Lim et al., 1994). It has been cloned at the cDNA (Lim et al., 1994), but not the genomic, level.

Perspectives

The exact compositions of naturally occurring oligosaccharide ligands, for any of the collectins, have not yet been fully identified. The overlapping monosaccharide specificities (Table 3)

Table 3. *Comparative functional activities of the collectins*[a]

Collectin	Principal carbohydrate specificities	C1q receptor binding	Complement activation	iC3b binding	Organisms bound
Human					
MBP	GlcNAc > Man	Yes	Yes	No	*S. montevideo*, *S. typhimurium*, *E. coli*, HIV
SP-A	ManNAc > Fuc	Yes	No	No	*P. carinii*, pollen extract, *S. aureus*, herpes simplex virus
SP-D	Mal > Gluc	No (SP-D receptor?)	No	No	*E. coli*
Bovine					
MBP	Fuc > GlcNAc	Yes	Yes	No	Influenza virus
Conglutinin	GlcNAc > Gluc	Yes	No	Yes	Influenza virus
Collectin-43	Man > ManNAc	Yes	No	No	Not determined

[a] The carbohydrate-binding properties of all collectins are of the mannose type and the monosaccharide specificities indicated; although informative in their own right, they do not allow for speculation about the natural ligands for the collectins (Lu et al., 1992; Haurum et al., 1993; Holmskov et al., 1993a, 1993b). The organisms listed do not reflect comparative studies with more than 1 collectin (Ezekowitz et al., 1989; Andersen et al., 1990; Friis-Christiansen et al., 1990; Van Iwarden et al., 1991; Kuan et al., 1992; Zimmerman et al., 1992; Haurum et al., 1993; McNeely & Coonrod, 1993).

for all of the collectins suggest that the various native oligosaccharide ligands for these molecules may be found on the surface of quite a wide range of microorganisms and target particles. In addition to this, oligosaccharides of known composition can assume in solution a range of conformations, of which even energetically unfavorable structures were shown to act as ligands for lectin binding (Imberty et al., 1993), which is reflected in the lack of success in carbohydrate structure determination using X-ray diffraction. The flexible nature of oligosaccharides clearly makes structural analysis by NMR a more suitable approach to determine lectin–carbohydrate interactions. The occurrence of unordered side chains within close proximity to the binding site of MBP seen in the crystal structure also allows for speculation on there being some degree of flexibility of the protein structure, especially within the extended loop of nonregular secondary structure. Obviously, the determination of the monosaccharide specificities is only a step in the right direction towards fully understanding the interaction between collectins and their biologically relevant targets. The well-defined groups of oligosaccharide ligands, like the sialyl Lewis x family of carbohydrate structures (Tyrrell et al., 1991), were available in the study of selectin mediated cell adhesion, but no such well-defined groups of oligosaccharide ligands have yet been identified to allow study of collectin function. It is important to characterize the natural oligosaccharide ligands for the collectins to allow assessment of collectin-mediated reactions, such as complement activation or opsonization. The determination of ligand specificity of the collectins, beyond monosaccharide recognition, should therefore be a promising future direction of investigation.

All the collectins are soluble molecules and are readily secreted in mammalian expression systems, thus avoiding the use of fusion proteins, which is necessary for producing soluble selectins (Foxall et al., 1992). Collectins also have a constant and defined degree of oligomerization and well characterized overall structures. Study of normal and mutated recombinant molecules should allow the establishment of binding sites for carbohydrates, proteins, or even lipids. The interaction with the complement system and host cell receptors via the collagenous/N-terminal regions of the collectins might allow for the classification of microorganisms according to their ability to be recognized by different collectins. The collectin–microorganism interaction could direct the innate immune response toward either complement activation, opsonization, phagocytosis via the C1q receptor, or utilization of a recently postulated distinct receptor for SP-D (Miyamura et al., 1994). A combination of these reactions may take place, depending on the collectin in question, and the state of inflammation already in progress, especially, since MBP, for example, behaves as an actue phase reactant. The very specific recognition of the high mannose structure in iC3b, but not C3, by bovine conglutinin is a further interaction of this collectin with the complement system. Recent evidence also suggests that allergic reactions to carbohydrate-bearing particles released from pollen grains, or house dust mites, may be affected by the binding of SP-A (Malhotra et al., 1993a) or SP-D to the carbohydrate on the allergen.

As more data become available on proteins showing homology with the C-type lectin domains, it should be borne in mind that not all of the domains identified by sequence alignment as "C-type lectin" domains necessarily bind carbohydrate structures. Some of these proteins have been shown to bind to structures other than carbohydrates and lack some (if not all) of the residues shown in the C-type lectin domains to be responsible for interaction with the calcium ions as well as the carbohydrate molecules, while retaining most of the hydrophobic residues of the core structure, including the disulfide pattern. The pancreatic thread protein binds to $CaCO_3$ crystals, and binding to ice crystals is seen in the case of antifreeze proteins of smelt (calcium-dependent) and sea raven (no calcium requirement) protein. It might therefore be necessary to consider also studying noncarbohydrate ligands on target organisms and particles, especially in the case of SP-A, which lacks one of the residues thought to be involved in ligand binding.

In view of the possible medical importance of collectins in the lung and plasma, the definition of the natural oligosaccharide structures recognized by the collectins, the further characterization of receptors for collectins, and the precise identification of carbohydrate/glycoprotein structures on the surface of microorganisms that are important in collectin-mediated immune re-

actions seem likely to be the dominant lines of investigation in this area in the near future.

References

Andersen O, Sørensen AM, Svehag SE, Fenouillet E. 1990. Conglutinin binds the envelope glycoprotein gp160 and inhibits its interaction with cell membrane CD4. *Scand J Immunol 32*:81–88.

Barondes SH. 1984. Soluble lectins: A new class of extracellular proteins. *Science 223*:1259–1264.

Barondes SH. 1988. Bifunctional properties of lectins: Lectins redefined. *Trends Biochem Sci 13*:480–482.

Benson B, Hawgood S, Schilling J, Clements J, Damm D, Cordell B, Seed B. 1985. Structure of canine pulmonary surfactant apoprotein: cDNA and complete amino acid sequence. *Proc Natl Acad Sci USA 82*:6379–6383.

Bevilacqua M, Butcher E, Furie B, Gallatin M, Gimbrone M, Harlan J, Kishimoto K, Lasky L, McEver R, Paulson J, Rosen S, Seed B, Spiegelman M, Springer T, Stoolman L, Tedder T, Varki A, Wagner D, Weissman I, Zimmerman G. 1991. Selectins: A family of adhesion receptors. *Cell 67*:233.

Brodsky-Doyle B, Leonard KR, Reid KBM. 1976. Circular-dichroism and electron-microscopy studies of human subcomponent C1q before and after limited proteolysis with pepsin. *Biochem J 159*:279–286.

Chan PY, Takei F. 1989. Molecular cloning and characterisation of a novel murine T cell surface antigen, YE1/48. *J Immunol 142*:1727–1736.

Christa L, Felin M, Morali O, Simon M, Lasserre C, Brechot C, Seve A. 1994. The human HIP gene, overexpressed in primary liver cancer encodes for a C-type carbohydrate binding protein with lactose binding activity. *FEBS Lett 337*:114–118.

Colley KJ, Baenzinger JU. 1987. Biosynthesis and secretion of the rat core-specific lectin. *J Biol Chem 262*:3415–3421.

Crouch E, Rust K, Veile R, Donis-Keller H, Grosso L. 1993. Genomic organisation of human surfactant D(SP-D). SP-D is encoded on chromosome 10q22.2–23.1. *J Biol Chem 268*:2976–2983.

Curtis B, Scharnowske S, Watson AJ. 1992. Sequence and expression of a membrane-associated C-type lectin that exhibits CD4-independent binding of human immunodeficiency virus envelope glycoprotein gp120. *Proc Natl Acad Sci USA 89*:8356–8360.

Doege K, Sasaki M, Horigan E, Hassell JR, Yamada Y. 1987. Complete primary structure of the rat cartilage proteoglycan core protein deduced from cDNA clones. *J Biol Chem 262*:17757–17767.

Doege KJ, Sasaki M, Kimura T, Yamada Y. 1991. Complete coding sequence and deduced primary structure of the human cartilage large aggregating proteoglycan, aggrecan. *J Biol Chem 266*:894–902.

Drickamer K. 1981. Complete amino acid sequence of a membrane receptor for glycoproteins. Sequence of the chicken hepatic lectin. *J Biol Chem 256*:5827–5839.

Drickamer K. 1988. Two distinct classes of carbohydrate recognition domains in animal lectins. *J Biol Chem 263*:955–956.

Drickamer K. 1992. Engineering galactose-binding activity into a C-type mannose-binding protein. *Nature 360*:183–186.

Drickamer K, Dordal MS, Reynolds L. 1986. Mannose-binding proteins isolated from rat liver contain carbohydrate-recognition domains linked to collagenous tails. *J Biol Chem 261*:6878–6887.

Drickamer K, Mamon JF, Binns G, Leung JO. 1984. Primary structure of the liver asialoglycoprotein receptor: Structural evidence for multiple polypeptide species. *J Biol Chem 259*:770–778.

Emsley J, White HE, O'Hara BP, Oliva G, Srinivasan N, Tickle IJ, Blundell TL, Pepys MB, Wood SP. 1994. Structure of pentameric human serum amyloid P component. *Nature 367*:338–345.

Engel J, Prockop DJ. 1991. The zipper-like folding of collagen triple helices and the effects of mutations that disrupt the zipper. *Annu Rev Biochem Biophys Chem 20*:137–152.

Erbe DV, Watson SR, Presta LG, Wolitzky BA, Foxall C, Brandley BK, Lasky LA. 1993. P- and E-selectin use common sites for carbohydrate ligand recognition and cell adhesion. *J Cell Biol 120*:1227–1235.

Erbe DV, Wolitzky BA, Presta LG, Norton CR, Ramos RJ, Burns DK, Rumberger JM, Rao BNN, Foxall C, Brandley BK, Lasky LA. 1992. Identification of an E-selectin region critical for carbohydrate recognition and cell adhesion. *J Cell Biol 119*:215–227.

Ewart KV, Rubinsky B, Fletcher GL. 1992. Structural and functional similarity between fish antifreeze proteins and calcium-dependent lectins. *Biochem Biophys Res Commun 185*:335–340.

Eyre DR, Paz MA, Gallop PM. 1984. Cross-linking in collagen and elastin. *Annu Rev Biochem 53*:717–748.

Ezekowitz RAB, Day LE, Herman GA. 1988. A human mannose-binding protein is an acute-phase reactant that shares sequence homology with other vertebrate lectins. *J Exp Med 167*:1034–1046.

Ezekowitz RAB, Kulhman M, Groopman JE, Byrn RA. 1989. A human mannose-binding protein inhibits in vitro infection by the human immunodeficiency virus. *J Exp Med 169*:185–196.

Fisher JH, Kao FT, Jones C, White RT, Benson BJ, Mason RJ. 1987. The coding sequence for the 32 000-dalton pulmonary surfactant protein A is located on chromosome 10 and identifies two separate restriction-fragment-length polymorphisms. *Am J Hum Genet 40*:503–511.

Floros J, Steinbrink R, Jacobs K, Phelps DS, Kriz R, Recny R, Sultzman L, Jones S, Taeusch HW, Frank HA, Fritsch EF. 1986. Isolation and characterisation of cDNA clones for the 35 kDa pulmonary surfactant-associated protein. *J Biol Chem 261*:9020–9033.

Foxall C, Watson SR, Dowbenko D, Fennie C, Lasky LA, Kiso M, Hasegawa A, Asa D, Brandley BK. 1992. The three members of the selectin receptor family recognize a common carbohydrate epito pe, the sialyl Lewis x oligosaccharide. *J Cell Biol 117*:895–902.

Friis-Christiansen P, Thiel S, Svehag SE, Dessau R, Svendsen P, Andersen O, Laursen SB, Jensenius JC. 1990. In vivo and in vitro antibacterial activity of conglutinin, a mammalian plasma lectin. *Scand J Immunol 31*:453–460.

Geng JG, Heavner GA, McEver RP. 1992. Lectin-domain peptides from selectins interact with both cell surface ligands and Ca^{2+} ions. *J Biol Chem 267*:19846–19856.

Geng JG, Moore KL, Johnson AE, McEver RP. 1991. Neutrophil recognition requires a Ca^{2+} induced conformational change in the lectin domain of GMP-140. *J Biol Chem 266*:22313–22318.

Giorda, R, Rudert, WA, Vavassori, C, Chambers, WH, Hiserodt, JC, Trucco, M. 1990. NKR-P1, a signal transduction molecule on natural killer cells. *Science 249*:1298-1300.

Haagsman HP, Hawgood S, Sargeant T, Buckley D, White RT, Drickamer K, Benson BJ. 1987. The major lung surfactant protein, SP 28–36, is a calcium-dependent, carbohydrate-binding protein. *J Biol Chem 262*: 13877–13880.

Harbury PB, Zhang T, Kim PS, Alber T. 1993. A switch between two-, three, and four-stranded coiled-coils in GCN4 leucine zipper mutants. *Science 262*:1401–1407.

Haurum JS, Thiel S, Jones IM, Fischer PB, Laursen SB, Jensenius JC. 1993. Complement activation through binding of mannan-binding protein to human retrovirus glycoproteins. *AIDS 7*:1307–1313.

Holmskov U, Holt P, Reid KBM, Willis AC, Teisner B, Jensenius JC. 1993a. Purification and characterization of bovine mannan-binding protein. *Glycobiology 3*:147–153.

Holmskov U, Malhotra R, Sim RB, Jensenius JC. 1994. Collectins: Collagenous C-type lectins of the innate immune defense system. *Immunol Today 15*:67–73.

Holmskov U, Teisner B, Laursen SB, Rasmussen HB, Jensenius JC. 1992. Tissue localization of conglutinin, a bovine C-type lectin. *Immunology 76*:169–173.

Holmskov U, Teisner B, Willis AC, Reid KBM, Jensenius JC. 1993b. Purification and characterization of a bovine serum lectin (CL-43) with structural homology to conglutinin and SP-D, and carbohydrate specificity similar to mannan-binding protein. *J Biol Chem 268*:10120–10125.

Hoppe HJ, Barlow PN, Reid KBM. 1994. A three stranded α-helical bundle at the nucleation site of collagen triple-helix formation. *FEBS Lett 344*:191–195.

Houchins J, Yabe T, McSherry C, Bach FH. 1991. DNA sequence of NKG2, a family of related cDNA clones encoding type II integral membrane proteins on human natural killer cells. *J Exp Med 173*:1017–1020.

Hoyle GW, Hill RL. 1988. Molecular cloning and sequencing of a cDNA for a carbohydrate binding receptor unique to rat Kupffer cells. *J Biol Chem 266*:1850–1857.

Hoyle GW, Hill RL. 1991. Structure of the gene for a carbohydrate-binding receptor unique to rat Kupffer cells. *J Biol Chem 266*:1850–1857.

Ichijo H, Hellman U, Wernstedt C, Gonez LJ, Claesson-Welsh L, Heldin C, Miyazono K. 1993. Molecular cloning and characterization of ficolin, a multimeric protein with fibrinogen- and collagen-like domains. *J Biol Chem 268*:14505–14513.

Ii M, Kurata H, Itoh N, Yamashina I, Kawasaki T. 1990. Molecular cloning and sequence analysis of a cDNA encoding the macrophage lectin specific for galactose and *N*-galactosamine. *J Biol Chem 265*:11295–11298.

Imberty A, Bourne Y, Cambillau C, Rouge P, Perez S. 1993. Oligosaccharide conformation in protein/carbohydrate complexes. *Adv Biophys Chem 3*:71–118.

Ji YH, Fujita T, Hatsuse H, Takahasi A, Matsushita M, Kawakami M. 1993. Activation of the C4 and C2 components of components of complement

by a proteinase in serum bactericidal factor, Ra reactive factor. *J Immunol 150*:571–578.

Johnson GI, Cook RG, Le Beau MM, McEver RP. 1989. Cloning of GMP-140, a granule membrane protein of platelets and endothelium: Sequence similarity to proteins involved in cell adhesion and inflammation. *Cell 56*:1033–1044.

Katyal SL, Singh G, Locker J. 1992. Characterization of a second human pulmonary surfactant-associated protein SP-A gene. *Am J Respir Cell Mol Biol 6*:446–452.

Kawakami M, Ihara I, Ihara S, Suzuki A, Fukui K. 1984. A group of bactericidal factors conserved by vertebrates for more than 300 million years. *J Immunol 132*:2578–2581.

Kawasaki N, Kawasaki T, Yamashina I. 1983. Isolation and characterization of a mannan-binding protein from human serum. *J Biochem 94*:937–947.

Kawasaki T, Etoh R, Yamashina I. 1978. Isolation and characterization of a mannan-binding protein from rabbit liver. *Biochem Biophys Res Commun 81*:1018–1024.

Kilchherr E, Hofmann H, Steigemann W, Engel J. 1988. A structural model of the collagen-like regions of C1q comprising the kink region and the fibre-like packing of the six triple helices. *J Mol Biol 186*:403–415.

Kilchherr E, Schumaker VN, Phillips ML, Curtiss LK. 1986. Activation of the first component of human complement, C1, by monoclonal antibodies directed against different domains of subcomponent C1q. *J Immunol 137*:255–262.

Kodama T, Freeman M, Rohrer L, Zabrcky J. 1990. Type I macrophage scavenger receptor contains α-helical and collagen-like coiled-coils. *Nature 343*:531–535.

Kolb-Bachofen V. 1991. A review on the biological properties of C-reactive protein. *Immunobiology 183*:133–145.

Kölble K, Lu J, Mole SE, Kaluz S, Reid KBM. 1993. Assignment of the human pulmonary surfactant protein D gene (SFTP4) to 10q22–q23 close to the surfactant protein A gene cluster. *Genomics 17*:294–298.

Korfhagen TR, Glasser SW, Bruno MD, McMahon MJ, Whitsett JA. 1991. A portion of the human surfactant protein A (SP-A) gene locus consists of a pseudogene. *Am J Respir Cell Biol 4*:463–469.

Kornfeld S. 1986. Trafficking of lysosomal enzymes in normal and disease states. *J Clin Invest 77*:1–6.

Kottgen E, Hell B, Kage A, Tauber R. 1992. Lectin specificity and binding characteristics of human C-reactive protein. *J Immunol 149*:445–453.

Krejci E, Coussen F, Duval N, Chatel J, Legay C, Puype M, Vandekerckhove J, Cartaud J, Bon S, Massoulie J. 1991. Primary structure of a collagenic tail peptide of *Torpedo* acetylcholinesterase: Co-expression with catalytic subunit induces the production of collagen-tailed forms in transfected cells. *EMBO J 10*:1285–1293.

Kuan SF, Rust K, Crouch E. 1992. Interaction of surfactant protein D with bacterial lipopolysaccharides; SP-D is an *E. coli*-binding protein in bronchoalveolar lavage. *J Clin Invest 90*:97–106.

Kubak BM, Potempa LA, Anderson B, Mahkloud S, Venegas M, Gewurz H, Gewurz AT. 1988. Evidence that serum amyloid P component binds to mannose-terminated sequences of polysaccharides and glycoproteins. *Mol Immunol 25*:851–858.

Kulhman M, Joiner K, Ezekowitz RAB. 1989. The human mannose-binding protein. *J Exp Med 169*:1733–1745.

Lachmann PJ, Müller-Eberhard HJ. 1968. The demonstration in human serum of conglutinogen-activating factor and its effect on the third component of complement. *J Immunol 100*:691–698.

Lasky LA, Singer MS, Yednock TA, Dowbenko D, Fennie C, Rodriguez H, Nguyen T, Stachel S, Rosen SD. 1989. Cloning of a lymphocyte homing receptor reveals a lectin domain. *Cell 56*:1045–1055.

Lee YM, Leiby KR, Allar J, Paris K, Lerch B, Okarma TB. 1991. Primary structure of bovine conglutinin, a member of the C-type animal lectin family. *J Biol Chem 266*:2715–2723.

Liao DI, Kapadia G, Ahmed H, Vasta R, Herzberg, O. 1994. Structure of S-lectin, a developmentally regulated vertebrate β-galactoside-binding protein. *Proc Natl Acad Sci USA 91*:1428–1432.

Lim BL, Lu J, Reid KBM. 1993. Structural similarity between bovine conglutinin and bovine lung surfactant protein D and demonstration of liver as a site of synthesis of conglutinin. *Immunology 78*:159–165.

Lim BL, Willis AC, Reid KBM, Lu J, Laursen SB, Jensenius JC, Holmskov U. 1994. Primary structure of bovine collectin-43 (CL-43). Comparison with conglutinin and lung surfactant protein-D. *J Biol Chem 269*. Forthcoming.

Liou LS, Sastry R, Hartsholm KL, Lee YM, Okarma TB, Tauber AI, Sastry KN. 1994. Bovine conglutinin gene structure reveals its evolutionary relationship to surfactant protein D. *J Immunol*. Forthcoming.

Lipscombe RJ, Sumiya M, Hill AVS, Lau YL, Levinsky RJ, Summerfield JA, Turner MW. 1992. High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene. *Hum Mol Genet 1*:709–715.

Lu J, Day AJ, Sim RB. 1993a. Mannan-binding protein and the C-type carbohydrate recognition domain. *Behring Inst Mitt 93*:25–30.

Lu J, Thiel S, Laursen SB, Jensenius JC, Reid KBM. 1993b. Molecular cloning of a cDNA coding for bovine conglutinin and evidence for the presence of a structurally similar molecule in sheep. *Biochem J 292*:157–162.

Lu J, Thiel S, Weidemann H, Timpl R, Reid KBM. 1990. Binding of the pentamer/hexamer forms of mannan binding protein to zymosan activates the proenzyme $C1r_2C1s_2$ complex of the classical pathway of complement without the involvement of C1q. *J Immunol 144*:2287–2294.

Lu J, Weidemann H, Holmskov U, Thiel S, Timpl R, Reid KBM. 1993c. Structural similarity between lung surfactant protein D and conglutinin. *Eur J Biochem 215*:793–799.

Lu J, Weidemann H, Timpl R, Reid KBM. 1993d. Similarity in structure between C1q and the collectins as judged by electron microscopy. *Behring Inst Mitt 93*:6–16.

Lu J, Willis AC, Reid KBM. 1992. Purification, characterization, and cDNA cloning of human lung surfactant protein D. *Biochem J 284*:795–802.

MacPhee-Quiley K, Taylor P, Taylor S. 1986. Primary structures of the catalytic subunits from two molecular forms of acetylcholinesterase: A comparison of NH_2-terminal and active center sequences. *J Biol Chem 260*:12185–12189.

Madsen HO, Garred P, Kurtzhals JAL, Lamm LU, Ryder LP, Thiel S, Svejgaard A. 1994. A new frequent allele is the missing link in the structural polymorphism of the human mannan-binding protein. *Immunogenetics 40*:37–44.

Malhotra R, Haurum J, Thiel S, Jensenius JC, Sim RB. 1993a. Pollen grains bind to lung alveolar type II cells (A549) via lung surfactant protein A (SP-A). *Biosci Rep 13*:79–90.

Malhotra R, Haurum J, Thiel S, Sim RB. 1992. Interaction of C1q receptor with lung surfactant protein A. *Eur J Immunol 22*:1437–1445.

Malhotra R, Laursen SB, Willis AC, Sim RB. 1993b. Localization of the receptor-binding site in the collectin family of proteins. *Biochem J 293*: 15–19.

Malhotra R, Thiel S, Reid KBM, Sim RB. 1990. Human leukocyte C1q receptor binds other soluble proteins with collagen domains. *J Exp Med 172*:955–959.

Malhotra R, Willis AC, Jensenius JC, Jackson J, Sim RB. 1993c. Structure and homology of human C1q receptor (collectin receptor). *Immunology 78*:341–348.

Matsushita M, Fujita T. 1992. Activation of the classical complement pathway by mannose binding protein with a novel C1s-like serine protease. *J Exp Med 176*:1497–1502.

McNeely TB, Coonrod JD. 1993. Comparison of the opsonic activity of human surfactant protein A for *Staphylococceus aureus* and *Streptomyces pneumoniae* with rabbit and human macrophages. *J Infect Dis 6*:91–97.

Miyamura K, Leigh LEA, Lu J, Hopkin J, Lopez-Bernal A, Reid KBM. 1994. Surfactant protein D (SP-D) binding to alveolar macrophages. *Biochem J 300*:237–242.

Ng NFL, Hew CL. 1992. Structure of an antifreeze polypeptide from sea raven. *J Biol Chem 267*:16069–16075.

Ohta M, Okada M, Yamashina I, Kawasaki T. 1990. The mechanism of carbohydrate-mediated complement activation by the serum mannan-binding protein. *J Biol Chem 265*:1980–1984.

Quesenberry MS, Drickamer K. 1992. Role of conserved and nonconserved residues in the Ca^{2+}-dependent carbohydrate-recognition domain of a rat mannose-binding protein. Analysis by random cassette mutagenesis. *J Biol Chem 267*:10831–10841.

Reid KBM. 1983. Proteins involved in the activation and control of the two pathways of human complement. *Biochem Soc Trans 11*:1–12.

Rouquier S, Verdier JM, Iovanna J, Dagorn JC, Giorgi D. 1991. Rat pancreatic stone protein messenger RNA. Abundant expression in endocrine cells, regulation by food content, and sequence identity with the endocrine reg transcript. *J Biol Chem 266*:786–791.

Rust K, Grosso L, Zhang V, Chang D, Persson A, Langmore W, Cai GZ, Crouch E. 1991. Human surfactant protein D: SP-D contains a C-type lectin carbohydrate recognition domain. *Arch Biochem Biophys 290*: 116–126.

Sakakibara S, Inouye K, Shudo K, Kishida Y, Kobayashi Y, Prockop DJ. 1973. Synthesis of (Pro-Hyp-Gly)n of defined molecular weights. Evidence for the stabilization of collagen triple helix by hydroxyproline. *Biochim Biophys Acta 303*:2852–2858.

Sastry K, Herman GA, Day L, Deignan E, Burns G, Morton CC, Ezekowitz RAB. 1989. The human mannose-binding gene. Exon structure reveals its evolutionary relationship to a human pulmonary surfactant gene and localisation to chromosome 10. *J Exp Med 170*:1175–1189.

Sastry K, Zahedi K, Lelias JM, Whitehead AS, Ezekowitz RAB. 1991. Molecular characterization of the mouse mannose-binding proteins: The mannose-binding protein A but not C is an acute phase reactant. *J Immunol 147*:692–697.

Sato M, Kawakami K, Osawa T, Toyoshima S. 1992. Molecular cloning and expression of a cDNA encoding a galactose/*N*-acetylgalactosamine-specific lectin on mouse tumoricidal macrophages. *J Biochem 111*: 331–336.

Sellar GC, Blake DJ, Reid KBM. 1991. Characterization and organisation of the genes encoding the A-, B- and C-chains of human complement subcomponent C1q. The complete derived sequence of human C1q. *Biochem J 274*:481–490.

Spiess M, Lodish HF. 1985. Sequence of a second human asialoglycoprotein receptor: Conservation of two receptor genes during evolution. *Proc Natl Acad Sci USA 82*:6465–6469.

Strang CJ, Slayter HS, Lachmann PJ, Davis A.E III. 1986. Ultrastructure and composition of bovine conglutinin. *Biochem J 234*:381–389.

Sumiya M, Super M, Tabona P, Levinsky RJ, Arai T, Turner MW, Summerfield JA. 1991. Molecular basis of opsonic defect in immunodeficient children. *Lancet 337*:1569–1570.

Super M, Thiel S, Lu J, Levinsky RJ, Turner MW. 1989. Association of low levels of mannan-binding protein with a common defect in opsonization. *Lancet 2*:1236–1239.

Suter U, Bastos R, Hofstetter H. 1987. Molecular structure of the gene and the 5′ flanking region of the human lymphocyte immunoglobulin E receptor. *Nucleic Acids Res 15*:7295–7308.

Takada F, Takayama Y, Hatsuse H, Kawakami M. 1993. A new member of the C1s family of complement proteins found in a bactericidal factor Ra-reactive factor, in human serum. *Biochem Biophys Res Commun 196*:1003–1009.

Takamatsu N, Ohba KI, Kondo J, Shiba T. 1993. Hibernation-associated gene regulation of plasma proteins with a collagen-like domain in mammalian hibernators. *Mol Cell Biol 13*:1516–1521.

Tanaka T, Har-El T, Tanzer ML. 1987. Partial structure of the gene for chicken cartilage proteoglycan core protein. *J Biol Chem 263*:15831–15835.

Taylor ME, Brickell PM. Craig RK, Summerfield JA. 1989. Structure and evolutionary origin of the gene encoding a human serum mannose-binding protein. *Biochem J 262*:763–771.

Taylor ME, Conary JT, Lennartz MR, Stahl PD, Drickamer K. 1990. Primary structure of the mannose receptor contains multiple motifs resembling carbohydrate-recognition domains. *J Biol Chem 265*:12156–12162.

Tenner AJ. 1993. Functional aspects of the C1q receptors. *Behring Inst Mitt 93*:241–253.

Thiel S, Baatrup G, Friis-Christiansen P, Svehag SE, Jensenius JC. 1987. Characterization of a lectin in human plasma analogous to bovine conglutinin. *Scand J Immunol 26*:461–468.

Timpl R, Rhode H, Robey PG. 1979. Laminin—A glycoprotein from basement membranes. *J Biol Chem 254*:9933–9937.

Traub W, Piez KA. 1971. The chemistry and structure of collagen. *Adv Protein Chem 25*:243–352.

Turner MW. 1991. Deficiency of mannan binding protein—A new complement deficiency syndrome. *Clin Exp Immunol 86*(Suppl 1):53–56.

Tyrrell D, James P, Ra N, Foxall, C, Abbas S, Dasgupta F, Nashed M, Kasegawa A, Kiso M, Asa D, Kidd J, Brandley BK. 1991. Structural requirements for the carbohydrate ligand of E-selectin. *Proc Natl Acad Sci USA 88*:10372–10376.

Urade Y, Oberdick J, Molinar-Rode R, Morgan JI. 1991. Precerebellin is a cerebellum specific protein with similarity to the globular heads of complement C1q B chain. *Proc Natl Acad Sci USA 88*:1069–1073.

Ushijima H, Schröder HC, Poznanovic S, Gasié MJ, Matthes E, Muller WEG. 1992. Inhibition of human immunodeficiency virus-1 infection by human conglutinin-like protein: In vitro studies. *Jpn J Cancer Res 83*:458–464.

Van der Rest M, Garrone R. 1991 Collagen family of proteins. *FASEB J 5*:2814–2823.

Van Hoegen I, Nakayama E, Parnes JR. 1990. Identification of a human protein homologous to the mouse Lyb-2 B cell differentiation antigen and sequence of the corresponding cDNA. *J Immunol 144*:4870–4877.

Van Iwarden JF, Strish JAG, Ebskamp MJM, Wehners AC, Verhoef I, van Golde LMG. 1991. Surfactant protein A is opsonin in phagocytosis of herpes simplex virus type I by rat alveolar macrophages. *Am J Physiol 261*(Lung Cell Mol Physiol 5):L204–L209.

Vercelli D, Geha RS. 1989. The IgE system. *Ann Allergy 63*:4–11.

Voss T, Eistetter H, Schäfer KP, Engel J. 1988. Macromolecular organization of natural and recombinant lung surfactant protein SP-A derived from two human coding sequences: Implications for the chain composition of natural human SP-A. *Am J Respir Cell Mol Biol 4*:88–94.

Wada M, Itoh N, Ohta M, Kawasaki T. 1992. Characterization of rat liver mannan-binding protein gene. *J Biochem 111*:66–74.

Weaver TE, Whitsett JA. 1991. Function and regulation of pulmonary surfactant-associated proteins. *Biochem J 273*:249–264.

Weis WI, Crichlow GV, Murthy HMK, Hendrickson WA, Drickamer K. 1991a. Physicochemical characterization and crystallization of the carbohydrate-recognition domain of a mannose-binding protein from rat. *J Biol Chem 266*:20678–20686.

Weis WI, Drickamer K, Hendrickson WA. 1992. Structure of C-type mannose-binding protein complexed with an oligosaccharide. *Nature 360*:127–134.

Weis WI, Kahn R, Fourme R, Drickamer K, Hendrickson WA. 1991b. Structure of the calcium-dependent lectin domain from a rat mannose-binding protein determined by MAD phasing. *Science 254*:1608–1615.

White RT, Damm D, Miller J, Spratt K, Schilling J, Hawgood S, Benson B, Cordell B. 1985. Isolation and characterization of the human surfactant apoprotein gene. *Nature 317*:361–363.

Wild J, Robinson D, Winchester B. 1983. Isolation of mannose-binding proteins from human and rat liver. *Biochem J 210*:167–173.

Wiley DC, Skehel JJ. 1987. The structure and function of the hemagglutinin membrane glycoprotein of influenza virus. *Annu Rev Biochem 56*: 365–394.

Wong S, Freeman JD, Kellerher C, Mager D, Takei F. 1991. Ly-49 multigene family. A new member of a superfamily of type II membrane proteins with lectin like domains. *J Immunol 147*:1417–1423.

Yamada KM. 1983. Cell surface interactions with extracellular materials. *Annu Rev Biochem 52*:761–799.

Yokoyama WM, Jacobs LB, Kanagawa O, Shevach EM, Cohen DI. 1989. A murine T lymphocyte antigen belongs to a supergene family of type II integral membrane proteins. *J Immunol 149*:1379–1386.

Yoshimatsu K, Ohya Y, Shikata Y, Seto T, Hasegawa Y, Tanaka I, Kawamura T, Kitoh K, Toyoshima S, Osawa T. 1992. Purification and cDNA cloning of a novel factor produced by a human T-cell hybridoma: Sequence homology with animal lectins. *Mol Immunol 29*:537–546.

Zimmerman DR, Ruoslahti E. 1989. Multiple domains of the large fibroblast proteoglycan, versican. *EMBO J 8*:2975–2981.

Zimmerman PE, Voelker DR, McCormack FX, Praulsrud JR, Martin WJ. 1992. 120-kD surface glycoprotein of *Pneumocystis carinii* is a ligand for surfactant protein A. *J Clin Invest 89*:143–149.