Ficolins: Complement-Activating Lectins Involved in Innate Immunity

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Key Words
Complement · Carbohydrates · Ficolins · Homeostasis · Innate immunity · Lectin pathway · MBL-associated serine protease · Pattern recognition molecules

Introduction
In 1993, two new transforming growth factor-β1-binding proteins from pig uterus membranes were cloned and characterized [1]. They had a unique chimeric structure consisting of collagen-like and fibrinogen-like domains and were termed ficolin α and ficolin β. The collagen-like domain sequence contains glycine residues at every third position. The fibrinogen-like domain consists of approximately 230 amino acids similar to the COOH-terminal halves of fibrinogen β and γ chains. Since the first report on pig ficolins, many proteins having this chimeric structure have been discovered in both vertebrates and invertebrates (table 1).

Accumulating evidence shows that ficolins constitute a family of lectins, the majority of which bind to N-acetylated saccharides such as N-acetylglucosamine (GlcNAc). Ficolins have tissue-dependent distributions, suggesting that their functions are also tissue-specific. To date, ficolins in serum have been intensively characterized and found to act as pattern recognition molecules that recognize carbohydrates on the surface of microbial pathogens. Following pathogen recognition, serum ficolins activate the complement system – an effector system in host defense – thereby playing a crucial role in innate immunity [2]. This review discusses ficolins with a focus on their ability to activate complement and their role in innate immunity.

Abstract
Ficolins are a group of oligomeric lectins with subunits consisting of both collagen-like and fibrinogen-like domains. The majority of ficolins identified in vertebrates and invertebrates to date recognize N-acetylglucosamine (GlcNAc). X-ray crystallographic analysis of human ficolins has shown that the fibrinogen-like domain binds to the N-acetylated moiety. Ficolins in serum are associated with MBL-associated serine protease (MASP). The ficolin-MASP complex binds directly to carbohydrates present on the surface of a variety of Gram-positive and Gram-negative bacteria through ficolin. Binding of the complex initiates complement activation via the lectin pathway, leading to generation of opsonic fragments of complement components, such as C3b, and to lysis of the bacteria by the membrane attack complex. Thus, serum ficolins play an important role in innate immunity.
Table 1. Characteristics of ficolins

<table>
<thead>
<tr>
<th>Species</th>
<th>Ficolins</th>
<th>mRNA expression</th>
<th>Protein identified</th>
<th>Binding substance</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>L-ficolin</td>
<td>liver</td>
<td>serum/plasma</td>
<td>GlcNAc (acetyl group); β-(1→3)-D-glucan; N-acetylmuramic acid; lipoteichoic acid; C-reactive protein; fibrinogen, fibrin; DNA; elastin; corticosteroid</td>
<td>complement activation; opsonin</td>
</tr>
<tr>
<td></td>
<td>H-ficolin</td>
<td>liver, lung, glioma cell</td>
<td>serum/plasma, bile duct, bronchus, alveolus</td>
<td>GlcNAc, GalNAc, fucose; lipopolysaccharide; polysaccharide preparations</td>
<td>complement activation; opsonin</td>
</tr>
<tr>
<td></td>
<td>M-ficolin</td>
<td>lung, monocyte, spleen</td>
<td>plasma, monocyte, neutrophil, alveolar epithelial cell</td>
<td>GlcNAc, GalNAc; sialic acid</td>
<td>complement activation; phagocytic receptor</td>
</tr>
<tr>
<td>Mouse</td>
<td>Ficolin A</td>
<td>liver, spleen</td>
<td>serum/plasma</td>
<td>GlcNAc, GalNAc, elastin</td>
<td>complement activation</td>
</tr>
<tr>
<td></td>
<td>Ficolin B</td>
<td>bone marrow, spleen</td>
<td>macrophage</td>
<td>GlcNAc, GalNAc; sialic acid</td>
<td>opsonin</td>
</tr>
<tr>
<td>Pig</td>
<td>Ficolin α</td>
<td>liver, lung, bone marrow</td>
<td>serum/plasma, uterus membranes</td>
<td>GlcNAc; lipopolysaccharide, lipoteichoic acid, elastin, TGF-β1</td>
<td>antiviral activity</td>
</tr>
<tr>
<td></td>
<td>Ficolin β</td>
<td>neutrophil, bone marrow</td>
<td>neutrophil, uterus membranes</td>
<td>TGF-β1</td>
<td>ND</td>
</tr>
<tr>
<td>Hedgehog</td>
<td>Erinacin</td>
<td>ND</td>
<td>muscle</td>
<td>metalloprotease</td>
<td>antihemorrhagic activity</td>
</tr>
<tr>
<td>Xenopus</td>
<td>XeFCN1</td>
<td>liver, spleen, heart</td>
<td>serum</td>
<td>GlcNAc, GalNAc</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>XeFCN2</td>
<td>lung, spleen, leukocyte</td>
<td>NI</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>XeFCN3</td>
<td>ND</td>
<td>NI</td>
<td>ND</td>
<td>ND</td>
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<tr>
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<td>XeFCN4</td>
<td>lung, spleen</td>
<td>NI</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ascidian</td>
<td>p40</td>
<td>hepatopancreas</td>
<td>hemolymph plasma</td>
<td>GlcNAc, GalNAc</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>p50</td>
<td>hepatopancreas</td>
<td>hemolymph plasma</td>
<td>GlcNAc</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = Not determined; NI = not identified.

Human Ficolins

Three types of ficolins, L, H and M, are present in humans.

L-Ficolin

L-ficolin (synonymous with L-ficolin/P35, ficolin 2, ficolin L, EBP-37, or hucoolin) is an oligomeric protein consisting of 35-kDa subunits [3]. Each subunit is composed of an NH2-terminal region with 2 cysteine residues (Cys7 and Cys27), which is followed by a collagen-like sequence and the COOH-terminus of a fibrinogen-like domain (fig. 1). The fibrinogen-like domain is globular, and the overall structure of L-ficolin resembles a ‘bouquet’. The proposed structure is a tetramer consisting of 4 triple helices formed by 12 subunits [4]. The oligomeric structure of L-ficolin is formed by the crosslinking of subunits via disulfide bridges involving Cys7 and Cys27 residues.

The L-ficolin gene (FCN2) is located on chromosome 9q34 and contains 8 exons [5]. The mRNA of L-ficolin is mainly expressed in the liver and its protein product is secreted into serum.

L-ficolin exhibits lectin activity toward GlcNAc [3]. L-ficolin has been found to recognize an acetyl group [6–8]. It also binds to β-(1→3)-D-glucan [9]. Binding sites for carbohydrates are located in the fibrinogen-like domain. X-ray crystallographic analysis of the fibrinogen-like do-
main in complex with various ligands has revealed that L-ficolin has 4 different sites involved in binding for N-acetylated carbohydrates and neutral carbohydrates such as \( \beta -(1\rightarrow 3) \) -D-glucan [10]. Two single nucleotide polymorphisms, which result in the substitution of threonine with methionine at codon 236 and alanine for serine at codon 258, have been found in exon 8 of FCN2 [11, 12]. These polymorphisms are associated with decreased and increased GlcNAc binding, respectively, in comparison to wild type L-ficolin.

L-ficolin binds to \textit{Salmonella typhimurium} TV119, which is an Ra chemotype strain containing a lipopolysaccharide with a GlcNAc residue at the non-reducing terminus. It also binds to \textit{Escherichia coli}, several capsulated \textit{Staphylococcus aureus} serotypes, and capsulated \textit{Staphylococcus pneumoniae} serotypes; however, L-ficolin does not bind to noncapsulated strains of bacteria. In addition to its lectin activity, L-ficolin has been reported to bind to elastin [13] and corticosteroids [14].

\textbf{H-Ficolin}

H-ficolin (synonymous with Hakata antigen, ficolin H, ficolin 3, or \( \beta 2 \) thermolabile macroglycoprotein) was first identified as a serum antigen recognized by an auto-antibody present in patients with systemic lupus erythematosus [15]. H-ficolin is an oligomer of 34-kDa subunits and exhibits more than 10 bands on SDS-PAGE under nonreducing conditions, thereby suggesting a mixture of oligomers of different sizes [16].

The H-ficolin gene (\textit{FCN3}) is located on chromosome 1p35.3 and consists of 8 exons with similar organization as the L-ficolin gene. H-ficolin mRNA is expressed in the liver and lung. In the liver, H-ficolin is produced by bile duct epithelial cells and hepatocytes and is secreted into bile and serum [17]. In the lungs, H-ficolin is produced by both ciliated bronchial epithelial cells and type II alveolar epithelial cells and is secreted into the bronchus and alveolus. H-ficolin is also produced in glioma cell lines [18].

H-ficolin has been shown to bind to GlcNAc, \( N \)-acetylgalactosamine (GalNAc), and fucose, but not to mannose and lactose [19]. Apparently, however, the binding affinity of H-ficolin for GlcNAc may be very weak compared to that of L-ficolin. In accordance with this supposition, structural studies demonstrated that the fibrinogen-like domain of H-ficolin could not be co-crystallized with any of the acetylated compounds under investigation, including GlcNAc [10].

H-ficolin agglutinates human erythrocytes coated with lipopolysaccharide derived from \textit{S. typhimurium}, \textit{Salmonella Minnesota} and \textit{Escherichia coli} (O111) [19]. Studies to date have shown that the only bacterium to which H-ficolin binds is \textit{Aerococcus viridans}; H-ficolin binds to polysaccharide preparations (PSA) derived from this bacterium, which are composed of glucose, mannose, GlcNAc, and xylose [20]. However, the chemical moiety in PSA that is recognized by H-ficolin remains unknown because none of the monosaccharides inhibit H-ficolin binding to PSA.

\textbf{M-Ficolin}

M-ficolin (synonymous with L-ficolin/P35 related protein, ficolin M or ficolin 1) mRNA is expressed in monocytes, the lungs and the spleen [5, 21–23]. The M-ficolin gene (\textit{FCN1}) is located on chromosome 9q34, as is L-ficolin, and its exon organization resembles that of L-ficolin [5]. M-ficolin has an extra exon encoding an additional segment of 4 Gly-Xaa-Yaa repeats. M-ficolin is reported to be expressed on the surface of peripheral blood monocytes and promonocytic U937 cells [23]. In contrast, M-ficolin has been found to be located in secretory granules in the cytoplasm of peripheral neutrophils and monocytes and in type II alveolar epithelial cells in the lungs, suggesting that M-ficolin is a secretory protein [24]. In accordance with this supposition, M-ficolin has

\begin{figure}
\centering
\includegraphics[width=\linewidth]{ficolin_structure.png}
\caption{Structure of ficolin. \textbf{a} Domain structure. \textbf{b} The tetrameric structure consisting of 12 subunits proposed for L-ficolin.}
\end{figure}
Ficolins as Complement-Activating Lectins

Recently been found to be present in human plasma at very low levels [25]. Recombinant M-ficolin exhibits binding activity toward acetylated compounds, including GlcNAc, GalNAc and sialic acid [24, 26], and it binds to bacteria including *S. aureus* and *S. typhimurium* LT2. Crystallization analysis supports the binding specificity for acetylated compounds [10].

**Ficolins of Nonhuman Species**

Besides human ficolins, proteins belonging to the ficolin family have been isolated from several vertebrates, including mice, pigs and *Xenopus*, and from the invertebrate ascidians (table 1).

Mice have 2 types of ficolins (ficolin A and ficolin B). Ficolin A mRNA is highly expressed in liver and spleen. The ficolin A protein is present in serum as a tetramer with 12 subunits [27], and it binds to GlcNAc [27] and elastin [28]. Ficolin B mRNA is expressed in bone marrow and spleen. The ficolin B protein is reported to be localized in lysosomes of activated macrophages [29]. Recombinant ficolin A and ficolin B exhibit lectin activities toward GlcNAc and GalNAc, whereas ficolin B also recognizes sialic acid like human M-ficolin [30]. Both ficolin A and B genes are located on chromosome 2A3. The ficolin B gene is close to the region homologous to the human L-ficolin and M-ficolin gene locus. The ficolin A gene consists of 10 exons, while ficolin B consists of 9 exons and its organization resembles that of the ficolin A gene, except it lacks the fifth exon. The exon organization of the ficolin B gene is very similar to that of the M-ficolin gene. It is speculated that ficolin A and ficolin B are homologous to human L-ficolin and M-ficolin, respectively. The mouse homologue of the H-ficolin gene exists as a pseudogene on chromosome 4 [31].

**Three Activation Pathways of the Complement System**

The complement system consists of many proteins involved in a chain reaction of proteolysis and protein complex assembly that culminates in the elimination of the invading pathogens. It is activated via 3 pathways (the classical, alternative and lectin pathways; fig. 2). In the classical pathway, a collagensous subcomponent of the first component (C1), C1q, binds to immunoglobulins within immune complexes, and this activates the associated serine proteases; C1r is activated first, followed by C1s. Activated C1s then cleaves C4 and C2 to generate C3 convertase C4bC2a, which activates C3. The resulting C3b fragment not only acts as an opsonin but also initiates the formation of the membrane attack complex in the lytic pathway (C5 to C9). The alternative pathway consists of C3, properdin, factor B, factor D and regulatory proteins, and is activated on the surface of pathogens without involvement of immunoglobulins. The classical and alternative pathways are involved in adaptive immunity and innate immunity, respectively.

The lectin pathway was first discovered as a third activation pathway of complement mediated by the mannos-binding lectin (MBL) [32]. MBL is an oligomeric lectin with identical subunits; each subunit consists of a collagen-like domain and a carbohydrate-recognition domain. MBL oligomers are formed by disulfide bonds between subunits. MBL recognizes mannose, GlcNAc, glucose and fucose. Human MBL is complexed with 3 types of serine protease, named MBL-associated serine protease (MASP-1, MASP-2, MASP-3) and sMAP (also called MAP19) [33]. MASP-1, 2 and 3 share the domain structures CUB1, EGF, CUB2, CCP1, CCP2 and the serine protease domain. Thus, MASP, C1r and C1s constitute a subfamily of serine protease (MASP/C1r/C1s family). sMAP is a truncated protein of MASP-2 and consists of the first 2 modules (CUB1-EGF) of MASP-2 as well as an additional 4 amino acids at the C-terminus.

Upon binding of the MBL-MASP complex to carbohydrates on the surface of microorganisms, MASP-2 undergoes autoactivation, which is concomitant with the conversion from an unactivated pro-enzyme form (single polypeptide chain) to an active form (2 polypeptide chains linked by a disulfide bond), thereby acquiring proteolytic activity against C4 and C2 [34]. MASP-1 is reported to collaborate with MASP-2 in the generation of C3 convertase C4bC2a by activating C2 [35]. Another report has shown that MASP-1 functions to activate MASP-2 in the MBL-MASP complex [36]. MASP-3 and sMAP have been found to have a regulatory role in the lectin pathway [37, 38]. However, these functions of MASP-1, MASP-3 and sMAP in the complex have yet to be established.
Serum Ficolin-Mediated Activation of the Lectin Pathway

Like MBL, L- and H-ficolin present in human serum are complexed with the 3 MASPs and sMAP [39, 40]. The binding sites on MASP-1 and MASP-3 for ficolins and MBL are located at the CUB1-EGF module [41]. Sequence alignments and site-directed mutagenesis studies have revealed that L-ficolin, H-ficolin and MBL share homologous binding sites for MASPs and calreticulin in the collagen-like regions [42]. Recent research has shown that recombinant M-ficolin also forms complexes with MASP-1 and MASP-2 [24].

Comparison of MBL and 3 ficolins (L, H and M) in terms of their ability to activate the lectin pathway revealed that H-ficolin exhibited the greatest capacity. L-ficolin and MBL had similar capacities. M-ficolin had minor activating potential [43]. The L-ficolin-MASP complex binds to acetylated LDL and activates C4 [7]. It also activates C4 upon binding to S. typhimurium TV119.
The L-ficolin-MASP complex also binds to N-acetyl-neuraminic acid in the capsular polysaccharides of group B streptococci (S. agalactiae) of many serotypes, including serotype III, a common cause of neonatal sepsis and meningitis, and initiates neutrophil-mediated opsonophagocytosis in the presence of human serum [45]. As described above, L-ficolin recognizes β-(1→3)-D-glucan as well as acetyl groups. Upon binding to β-(1→3)-D-glucan, the L-ficolin-MASP complex activates the lectin pathway [9].

C-reactive protein is a plasma protein that binds to specific carbohydrates on the surface of microorganisms. C1 binds to C-reactive protein through Clq, initiating the classical pathway without involvement of immunoglobulins. Recent studies have shown that L-ficolin interacts with C-reactive protein under conditions of local infection/inflammation, and this amplifies complement activation [46]. This finding suggests a collaboration of ficolins and C-reactive protein in innate immunity.

The H-ficolin-MASP complex binds to PSA derived from A. viridans coated on microplates and activates C4 [40]. It also exhibits in vitro bactericidal activity against A. viridans in the presence of human serum [47].

The recombinant M-ficolin-MASP complex binds to GlcNAc or acetylated compounds, resulting in C4 activation [24]. Since M-ficolin is found in plasma, it may function as a complement-activating ficolin in blood like L- and H-ficolin, although whether or not M-ficolin in plasma is complexed with MASP remains to be elucidated. As described above, however, M-ficolin is reported to be expressed on U937 cells. Antibodies against the protein prevent these cells from phagocytosing E. coli, suggesting that M-ficolin plays a role in innate immunity by acting as a lectin-like phagocytic receptor for pathogens [23]. Further research is required to clarify the physiologic roles of M-ficolin.

Recombinant ficolin A, but not ficolin B, binds to mouse MASP-2 and sMAP, resulting in complement activation [30]. These findings suggest that, similar to human L-ficolin and H-ficolin, ficolin A in serum functions as a recognition molecule in the lectin pathway, while ficolin B lacks such a function.

Human MBL-MASP has recently been reported to activate C3 not only via the lectin pathway, in which C3 convertase C4bC2a is formed, but without involvement of C2 [48]. The latter activation is dependent on the alternative pathway. Although the precise mechanisms underlying MBL-MASP-mediated activation of the alternative pathway are not known, MASPs such as MASP-1 may be involved in that activation. Whether or not ficolin-MASP has a similar function to MBL-MASP remains unknown.

C1 inhibitor (C1-INH) is a protease inhibitor belonging to the serpin family. C1-INH inhibits Clr and Cls in the C1 complex by binding covalently to their active forms, thereby regulating the activation of the classical pathway. C1-INH also binds to MASP-1 and MASP-2 but not MASP-3 in the fluid phase. When the lectin pathway is activated via MBL-MASP, an MBL-MASP-C1-INH complex is formed [49]. Preliminary studies by the current authors have revealed that L-ficolin-MASP-C1-INH complex is formed upon activation of the lectin pathway via L-ficolin-MASP in human serum, which suggests that complement activation by ficolin-MASP and MBL-MASP is regulated by C1-INH in a similar manner (unpubl. data).

Endo et al. have recently found that recombinant mouse ficolins and MBL enhance activation of the lectin pathway by interacting with fibrinogen and fibrin [50]. This implies that the lectin pathway collaborates with the coagulation system in immunity. This collaboration of these 2 systems is also seen in the proteolytic activity of MASP-1 with respect to coagulation components such as factor XIII and fibrinogen [51].

The components and functions of the lectin pathway and classical pathway are very similar. Collagenous lectins (ficolin and MBL) are associated with MASP of the MASP/Clr/Cls family in the former pathway, whereas Clq is associated with Clr and Cls in the latter pathway. Binding sites of the corresponding proteases of the MASP/Clr/Cls family are located in the collagen-like domains of the lectins and Clq. C4 and C2 are activated by proteases of the MASP/Clr/Cls family in both pathways. However, the striking difference between the 2 pathways is that ficolin and MBL act as pattern recognition molecules in innate immunity and bind to carbohydrates on pathogens, whereas Clq binds to immunoglobulins in adaptive immunity. These findings suggest that the lectin pathway evolved into the classical pathway.

Other Functions of Serum Ficolins

The ability of L-ficolin to enhance the phagocytosis of S. typhimurium by neutrophils suggests that serum ficolins not only activate the lectin complement pathway but also act as opsonins [3]. In addition to their important role in innate immunity, ficolins are involved in apoptosis. Binding of serum ficolins to apoptotic cells activates...
the complement system and, consequently, generates opsonic C3 [52]. DNA is a potential L-ficolin ligand because it inhibits L-ficolin binding to late apoptotic and necrotic cells [53]. Similar to their role in innate immunity, ficolins have opsonin-like activity during apoptosis [54]. The opsonic activity of human ficolins might be mediated by receptors such as CR1 and C1q receptor/calreticulin on phagocytes.

Pig plasma ficolin α binds to and neutralizes porcine reproductive and respiratory viruses [55]. This finding indicates that ficolins in plasma/serum play a role in innate immunity through complement activation as well as antiviral and opsonic activity.

**Conclusions**

Binding specificity for carbohydrates seems to vary among ficolins. Apparently, however, the N-acetyl group is recognized by a majority of ficolins. The carbohydrate GlcNAc contains an N-acetyl group and is widely distributed on the surface of microbes. Therefore, ficolins bind a variety of microbial pathogens bearing GlcNAc.

Accumulating evidence has shown that upon binding to the carbohydrates present on microbes, ficolins present in the serum, such as human L-ficolin, H-ficolin and mouse ficolin A, activate the lectin complement pathway in association with MASP, thereby playing an important role as a pattern recognition molecule in innate immunity. The presence of ficolins in ascidian hemolymph plasma raises the possibility that these proteins play a role in defense against pathogens in invertebrates as well as vertebrates. Mouse ficolin B cannot form complexes with MASP-2, implying that ficolin B lacks the ability to activate complement. On the other hand, mouse ficolin B is reported to aggregate S. aureus, which enhanced phagocytosis of the bacteria by macrophages [2]. Human H-ficolin in the lung is likely to act as an opsonin, similar to 2 types of pulmonary collectins (SP-A and SP-D), whereas as serum H-ficolin, which is associated with MASP, may have dual functions as both an opsonin and as an initiator of complement activation. Therefore, a major function of ficolins, regardless of whether or not they are complexed with MASP, may be to contribute to innate immunity by activating complement and/or enhancing phagocytosis and eliminating pathogens. The importance of ficolins in host defense is exemplified by the association of L-ficolin deficiency with premature, low birthweight neonates, and perinatal infections [56]. Taken together with the finding that ficolins are involved in apoptosis in vitro, ficolins are conceivably responsible for both host defense and homeostasis.

**References**


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