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REVIEW

Structural and Functional Overview of the Lectin Complement Pathway: Its Molecular Basis and Physiological Implication

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Abstract The complement system is an effector mechanism in immunity. It is activated in three ways, the classical, alternative and lectin pathways. The lectin pathway is initiated by the binding of mannose-binding lectin (MBL) or ficolins to carbohydrates on the surfaces of pathogens. In humans, MBL and three types of ficolins (L-ficolin, H-ficolin, and M-ficolin) are present in plasma. Of these lectins, at least, MBL, L-ficolin, and H-ficolin are complexed with three types of MBL-associated serine proteases (MASPs), MASP-1, MASP-2, and MASP-3 and their truncated proteins (MAp44 and sMAP). In the lectin pathway, the lectin–MASP complex (i.e., a complex of lectin, MASPs and their truncated proteins) binds to pathogens, resulting in the activation of C4 and C2 to generate a C3 convertase capable of activating C3. MASP-2 is involved in the activation of C4 and C2. MASP-1 activates C2 and MASP-2. The functions of MASP-3, sMAP, and MAp44 in the lectin pathway remain unknown. MASP-1 and MASP-3 also have a role in the alternative pathway. MBL and ficolins are able to bind to a variety of pathogens depending on their carbohydrate binding specificity, resulting in the activation of the lectin pathway. Deficiencies of the components of the lectin pathway are associated to susceptibility to infection, indicating an important role of the lectin pathway in innate immunity. The lectin-MASP complex is also involved in innate immunity by activating the coagulation system. Recent findings suggest a crucial role of MASP-3 in development.

Keywords Complement · Lectin pathway · Mannose-binding lectin (MBL)-associated serine protease (MASP) · Ficolin

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Introduction

The complement system is activated through three different pathways: the classical, alternative, and lectin pathways. The lectin pathway plays a role in innate immunity. It is initiated by binding of mannose-binding lectin (MBL) or ficolins to carbohydrates on the surfaces of pathogens. These lectins are complexed with serine proteases named MBL-associated serine proteases (MASPs) (Fujita 2002; Matsushita et al. 2000a, 2002). Three types of MASPs, MASP-1 (Matsushita and Fujita 1992; Sato et al. 1994), MASP-2 (Thiel et al. 1997) and MASP-3 (Dahl et al. 2001) have been identified in many vertebrates. In addition to MASPs, MBL, and ficolins are also complexed with two truncated forms of MASP (MAp44 and sMAP) (Table 1). Recently, CL-K1, a newly identified collectin (Keshi et al. 2006), was found to be complexed with MASP-1 or MASP-3 in human plasma (Hansen et al. 2010). Thus, at least in mammals, the lectin pathway includes three types of lectins, MBL, ficolin, and CL-K1, which act as recognition molecules in the lectin pathway. The lectin pathway is structurally similar to the classical pathway. The recognition molecule C1q of the classical pathway is similar to MBL, ficolin and CL-K1 in having a collagen-like

Table 1 Characterization of MASPs and their truncated proteins

Protein	Gene and locus[a]	mRNA	Serum level (ug/ml)	Function	Inhibitor
MASP-1	*MASP1* 3p27	Liver	6.27 ± 1.85	Df activation (initiation of alternative pathway), C2 and C3 activation, MASP-2 activation MASP-3 activation, thrombin-like activity Activation of protease-activated receptor 4 Cleavage of IGFBP-5	C1-INH
MASP-2	*MASP2* 1q36	Liver	0.305 ± 0.015	C2 and C4 activation, Competitive binding to MBL with MASP-3 and sMAP prothrombin activation	C1-INH
MASP-3	*MASP1* 3p27	Liver cervix brain, colon	ca. 5.0 (range 1.8–10.6)	Df activation Bf activation Cleavage of IGFBP-5?	ND
sMAP (MAp19)	*MASP2* 1q36	Liver	ca. 0.217	Competitive binding to MBL with MASP-2 (down-regulation of lectin pathway)	–
MAp44 (MAP-1)	*MASP1* 3p27	Heart Liver Skeletal muscle	ca. 1.7 (range 0.8–3.2)	Competitive binding to MBL with MASP-2 (inhibition of C4 deposition)	–

ND not determined

[a] Locus in human chromosome

domain, which serves to generate the multivalent recognition molecules, though the recognition domains of C1q are unrelated to the lectins and have specificity mainly for the Fc region of immunoglobulins. Two serine protease components of the classical pathway, C1r, and C1s, are the members of the same serine protease family as MASPs. Thus, the component molecules of the lectin pathway are closely related in phylogeny to and therefore structurally similar to the components of the classical pathway. In addition, the phylogeny of the components revealed that the classical pathway have evolved from the ancestral lectin pathway (Fujita 2002; Matsushita et al. 2004).

In this review, we focus the molecular basis and physiological role of the lectin pathway, especially the structure and function of MASPs, the key enzymes of the lectin pathway, and the function of ficolin, one of the recognition molecules of this system.

Structure of MASPs and Their Truncated Proteins

The domain structures of MASP-1, MASP-2, and MASP-3 resemble C1r and C1s of the classical pathway, thus constituting a subfamily of the serine protease (MASP/C1r/C1s family). These five members of the MASP/C1r/C1s family each consist of six domains, C1r/C1s/Uegf/bone morphogenetic protein 1 (CUB) 1, epidermal growth factor (EGF)-like, CUB2, complement control protein (CCP) 1, CCP2 and serine protease domain. MASP-1 and MASP-3 are generated from the same gene (*MASP1*) by alternative splicing mechanisms (Fig. 1). The *MASP1* gene has ten exons that encode the CUB1–EGF–CUB2–CCP1–CCP2 domains common to both MASP-1 and MASP-3. These domains constitute the heavy chain of the active forms of MASP-1 and MASP-3. The exons coding the serine protease domain, which constitutes the light chain of the active forms, are different between MASP-1 and MASP-3. MAp44 (also called MAP-1) is also generated from the *MASP1* gene by alternative splicing (Degn et al. 2009; Skjoedt et al. 2010). MAp44 consists of the first four domains, CUB1–EGF–CUB2–CCP1, followed by an extra 17 amino acids sequence encoded by an exon that is not used for MASP-1 and MASP-3. MASP-2 and sMAP (also called MAp19) (Stover et al. 1999; Takahashi et al. 1999) are generated from the same gene (*MASP2*). sMAP is produced by alternative splicing and consists of CUB1 domain, EGF-like domain and an extra 4 amino acids at the C-terminal end encoded by a sMAP-specific exon.

The recombinant proteins of MASPs form a head-to-tail homodimer (Chen and Wallis 2001; Teillet et al. 2008; Thielens et al. 2001). The homodimers of MASP-1 and MASP-3 are made through the interaction between the

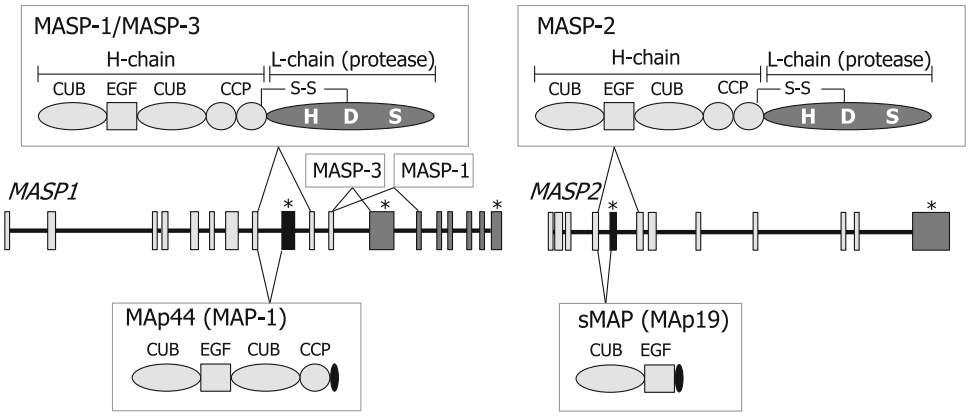

Fig. 1 Domain structures of MASPs and their truncated proteins and exon organization of the *MASP1* and *MASP2* genes. The human *MASP1* gene includes a total of 18 exons. Three different alternative splicings of the *MASP1* gene produce MASP-1, MASP-3, and Map44. The 9th and 12th exons, which include in frame termination codons, are specific for Map44 and MASP-3, respectively. The serine protease domain of MASP-1 is encoded by 6 exons but for MASP-3 these are fused into a single exon. The human *MASP2* gene includes 12 exons, and alternative splicings produce MASP-2 and sMAP. The fifth exon, which includes a termination codon, is specific for sMAP. Letters H, D and S in the L-chains of MASP-1 and MASP-2 represent three amino acid residues essential for the serine protease catalytic sites, histidine, aspartic acid and serine, respectively. Each exon is depicted by a *box* and the termination codon by an *asterisk* above the box

CUB1 and EGF domains of each monomer in the presence of calcium ions (Thielens et al. 2001). The MASPs homodimers bind to the collagen domain of MBL and ficolins. The collagen binding sites on MASPs are located in the first three domains, CUB1–EGF–CUB2 (Cseh et al. 2002; Wallis 2007).

Lectin Pathway Activation by MBL/Ficolin-MASP

Upon binding of MBL and ficolins to carbohydrates on the surface of pathogens, the proenzyme form of MASP is cleaved between the CCP2 and the serine protease domains, resulting in the generation of the active form comprising of two polypeptides, heavy (H)- and light (L)-chains, also called A and B chains (Fujita 2002) (Fig. 1). In the classical pathway, the binding of C1q to antibodies in immune complex leads to the autoactivation of C1r, followed by C1s activation by activated C1r. The activated C1s in turn cleaves C4 and C2, generating the C3 convertase C4bC2a. In contrast to the classical pathway, the roles of MASPs and their truncated proteins and the activation mechanisms of the lectin pathway have not been fully understood. The functional features of MASPs and their truncated proteins are summarized in Table 1. MASP-2 autoactivates and, like C1s, its active form is able to cleave C4 and C2 to generate the C3 convertase (Chen and Wallis 2004; Matsushita et al. 2000a; Vorup-Jensen et al. 2000) (Fig. 2). MASP-1 appears to facilitate lectin pathway activation by either direct cleavage of complex-bound MASP-2 or cleavage of C2 bound to C4b (Moller-Kristensen et al. 2007; Schwaeble et al. 2011; Takahashi et al. 2008). Very recently, it was reported that in normal human serum, autoactivation of MASP-2 does not occur and MASP-1 is responsible for the activation of MASP-2 (Héja et al. 2012a, b). Degn et al. (2012) reported that lectin pathway activation does not occur in a patient serum lacking MASP-1 and MASP-3 and that after reconstitution with MASP-1 the serum is able to activate the lectin pathway. MASP-1 also has the ability to cleave C3 (H_2O), the C3 molecule whose thioester bond is spontaneously hydrolyzed (Matsushita and Fujita 1995). The physiological significance of this remains unsolved. The role of MASP-3 in the MBL/ficolin complex remains unknown, although it was reported that MASP-3 competes for binding to MBL with MASP-2 resulting in the inhibition of C4 activation by MASP-2. There exists a difference in the interaction with C1 inhibitor (C1-INH) with MASPs. C1-INH binds covalently to activated MASP-1 and MASP-2, resulting in the inactivation of their proteolytic activities (Matsushita et al. 2000b). On the other hand, C1-INH does not bind to activated MASP-3 (Zundel et al. 2004).

Using sMAP-deficient mice generated by targeted disruption of the sMAP-specific exon, Iwaki and others demonstrated that sMAP and MASP-2 compete to bind MBL/ficolins and sMAP has the ability to down-regulate the lectin pathway (Iwaki et al. 2006). Similarly, MAp44 competes for binding to MBL with MASP-2, resulting in inhibition of C4 deposition (Degn et al. 2009; Skjoedt et al. 2010).

Human MBL is composed of a mixture of oligomers including mainly trimers, tetramers, pentamers, and hexamers. Analyses by synchrotron X-ray scattering, analytical ultracentrifugation, and constrained modeling have revealed that the carbohydrate recognition domains in the MBL dimers, trimers, and tetramers form near-planar fan-

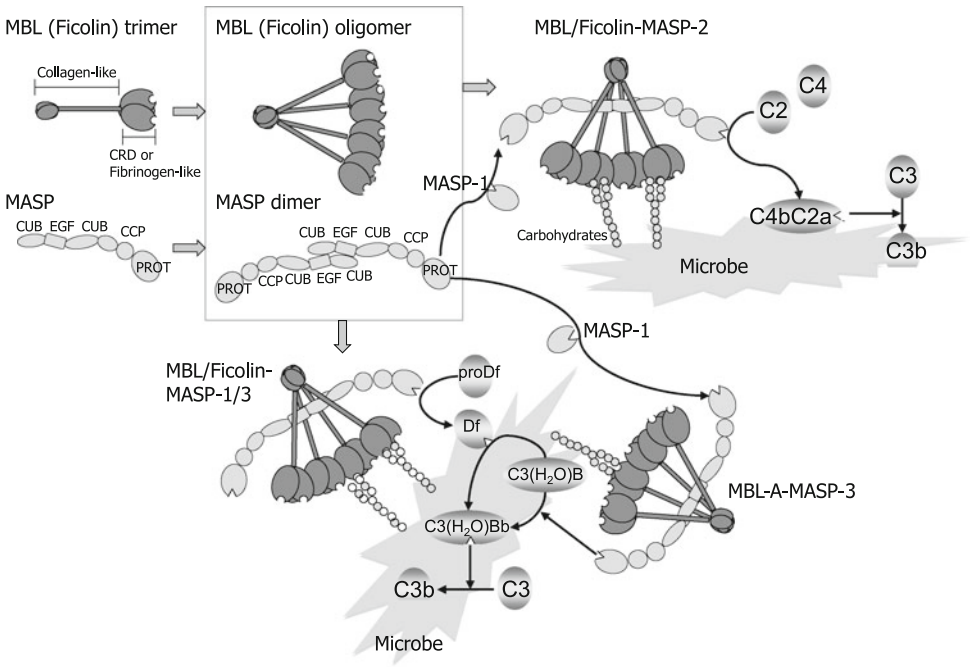

Fig. 2 Schematic representation of the lectin pathway driven by MBL (ficolin)-MASPs. MBL (or ficolin)–MASPs complexes, which are composed of MBL (or ficolin), MASPs, sMAP, and Map44, are depicted simply without sMAP and MAp44 in this figure, because the precise stoichiometry of the complexes is still unknown

like solution structures (Miller et al. 2012). Each oligomeric form of MBL appears to be complexed with different types of MASP and truncated proteins. For instance, it was shown that the MBL trimer is complexed with MASP-1 and sMAP (Tateishi et al. 2011). The MBL trimer complex activates C2 but not C4. MASP-2 and MASP-3 are found in oligomers larger than the MBL trimer. These facts suggest that MBL may have distinct functions depending upon the size of oligomer and hence the type of MASP bound.

Involvement of MASPs in the Alternative Pathway

The alternative complement pathway consists of C3, factor B, factor D, properdin, and regulatory proteins such as factor H and factor I. The activation mechanism of the alternative pathway is as follows; the thioester bond in the C3 molecule constantly undergoes hydrolysis to some extent in the plasma to generate C3 (H_2O). Factor B binds to C3 (H_2O) and is then cleaved by factor D to generate Ba and Bb. The bimolecular complex C3 (H_2O) Bb acts as the initial C3 convertase that can split C3 into C3a and C3b. C3b thus generated binds covalently to the surfaces of pathogens. Factor B binds to C3b on the pathogens and is cleaved by factor D, generating the C3 convertase C3bBb capable of activating C3.

A recent report has shown that alternative pathway activation does not occur in the serum of MASP-1 and MASP-3 knockout mice (MASP-1/3 KO mice) (Takahashi et al. 2010a, b). Factor D is produced mainly in adipocytes as the proenzyme with the activation peptide at the N-termini and is secreted in blood. Factor D in plasma is in the active from, suggesting that it is cleaved after secretion. Factor D in MASP-1/3 KO mice was found to be in the proenzyme form with the activation peptide. It seemed, therefore, likely that MASP-1 and/or MASP-3 is involved in the cleavage of the activation peptide from proenzyme factor D. Recombinant mouse MASP-1 was able to directly cleave the activation peptide in recombinant proenzyme factor D. Thus, MASP-1 is the enzyme for complement activation that not only enhances the activation of MASP-2 as mentioned above, but also activates proenzyme factor D in the alternative pathway (Takahashi et al. 2010b) (Fig. 2).

In addition to MASP-1, MASP-3 is also involved in the alternative pathway activation. Incubation of mouse rMASP-3 with heat-killed *S. aureus* and mouse rMBL-A led to the activation of rMASP-3, suggesting that rMASP-3 associated with rMBL-A autoactivates upon binding of rMBL-A to *S. aureus* (Iwaki et al. 2011). When these components, factor B and C3 (H_2O) were incubated, factor B was activated to generate Ba and Bb. These results indicate that MASP-3 in its active form has an ability to activate factor B bound to C3 (H_2O). Like rMASP-1, mouse rMASP-3 in its active form cleaved the activation peptide from proenzyme factor D to generate the active factor D. In contrast to MASP-1, however, MASP-3 in its unactivated form was also able to activate factor D. This might be a possible explanation of why factor D circulates in plasma in its active form (Rosen et al. 1989). Mouse rMASP-1 was unable to activate the endogenous proenzyme factor D in MASP-1/3 KO serum, suggesting that

rMASP-1 activity was blocked by its physiological inhibitors in the serum (i.e., C1 inhibitor and alpha 2-macroglobulin) (Takahashi et al. 2010a). In contrast, mouse rMASP-3 converted the endogenous proenzyme factor D in all MASPs KO serum to the active form. MASP-3, therefore, may be more effective than MASP-1 in activating factor D in plasma. As described above, MASP-3 in complex with MBL could autoactivate when MBL binds to *S. aureus*. MASP-3 also can be activated by MASP-1 (Iwaki et al. 2011). In contrast to these reports, Degn et al. (2012) suggested that neither MASP-1 nor MASP-3 is required for alternative pathway function.

The MBL-MASP complex activates the alternative pathway upon binding to mannan (Tateishi and Matsushita 2011). This activation is ascribed to the C2-bypass pathway in which C4b generated by the action of MASP-2 in the MBL-MASP complex triggers alternative pathway activation.

Physiological Roles of MASPs

It was reported that a patient with MASP-2 deficiency caused by a point mutation in the CUB1 domain of the *MASP-2* gene had a history of infections and chronic inflammatory disease (Stengaard-Pedersen et al. 2003).

MASP-1/3 KO mice are susceptible to influenza virus infection (Takahashi et al. 2007). MASP-1/3 deficiency protects mice from collagen antibody-induced inflammatory arthritis, an autoimmune-associated inflammatory tissue injury, which is caused by activation of the alternative pathway (Banda et al. 2010). MASP-2 KO mice are susceptible to infections with *S. pneumoniae* (Ali et al. 2012) and West Nile virus (Fucks et al. 2011), but not to *Pseudomonas aeruginosa* infection (Kenawy et al. 2012). MASP-2 deficiency protects mice from myocardial and gastrointestinal ischemia/reperfusion injury (Schwaeble et al. 2011), which is induced through lectin pathway-mediated C3 activation. Thus, MASPs not only protect against infections with some pathogens, but also induce tissue injuries through complement activation, suggesting their roles in both physiology and pathophysiology.

MASPs play a role in innate immunity by activating the coagulation system as well as the complement system (Hajela et al. 2002; Banda et al. 2010; La Bonte et al. 2012; Takahashi et al. 2010a, b). MASP-1 resembles thrombin in that it cleaves factor XIII and fibrinogen and thrombin-activatable fibrinolysis inhibitor (TAFI), while MASP-2 cleaves prothrombin (Hess et al. 2012; Krarup et al. 2007, 2008a). Gulla et al. (2010) demonstrated that the MBL-MASP and L-ficolin-MASP complexes activate the coagulation system upon binding to their respective ligands, leading to the generation of plasma fibrin clots. The clots could surround microbes and inhibit their spreading.

As with thrombin, MASP-1 is able to activate protease-activated receptor 4 that is a mediator of platelet activation and inflammation (Megyeri et al. 2009). MASP-3 cleaves insulin-like growth factor-binding protein 5 (IGFBP-5), which binds to insulin-like growth factors (IGFs) and modulates IGF actions on cell proliferation, differentiation, survival, and motility (Cortesio and Jiang 2006). IGFBP-5 also regulates these cellular events through IGF-independent mechanisms. A recent report has shown that human cases with MASP-3 deficiency due to mutation in the *MASP-1* gene are associated with the 3MC syndrome, a group of developmental disorders, one of whose major symptoms is facial clefting (Rooryck et al. 2011). This suggests that MASP-3 has a crucial role in development.

Structure of Ficolins and Orthology Between Human and Mouse Homologues

Ficolins are multimers of trimeric subunits, in which a single chain consists of a collagenous domain at the N-terminal and a fibrinogen-like domain at the C-terminal. Thus, ficolins are similar to MBL, in which carbohydrate recognition domain (CRD) replaces the fibrinogen-like domain of ficolins (Garred et al. 2010) (Fig. 2). Most of the ficolins identified to date bind to acetylated compounds such as *N*-acetylglucosamine (GlcNAc) and *N*-acetylgalactosamine (GalNAc), suggesting that the acetyl group is the binding moiety. Like MBL, ficolins in plasma are complexed with MASPs and their truncated proteins. Binding of the ficolin–MASP complex to acetylated residues present on the surface of microbes initiates complement activation via the lectin pathway (Endo et al. 2011; Matsushita 2010) (Fig. 2).

Three types of ficolins (L-ficolin, H-ficolin, and M-ficolin) are present in humans (Table 2). L-ficolin (also called P35, ficolin-2, EBP-37 or hucolin) is produced in the liver and circulates in the blood (Matsushita et al. 1996). Its proposed structure is a tetramer consisting of four triple helices formed by twelve subunits. H-ficolin (also called ficolin-3, Hakata antigen) mRNA is expressed in the liver and lung (Sugimoto et al. 1998). In the liver, H-ficolin is produced by bile duct epithelial cells and hepatocytes and is secreted into bile and plasma (Akaiwa et al. 1999; Inaba and Okochi 1978). Plasma H-ficolin is a mixture of different sizes of oligomers (Yae et al. 1991). H-ficolin produced in the lung is secreted into the bronchus and alveolus. M-ficolin (also called ficolin-1, P35-related protein) mRNA is expressed in monocytes, lung, and spleen (Endo et al. 1996; Lu et al. 1996a, b; Teh et al. 2000). The M-ficolin protein is detected on membranes of monocytes and granulocytes (Honoré et al. 2010). It is also present in plasma (Honoré et al. 2008; Wittenborn et al. 2010) at low concentration.

Table 2 Characterization of human and mouse ficolins

Species	Protein	Gene and locus	mRNA	Protein location	Binding substance	Complement activation	Opsonization
Human	Ficolin-2 (FCN-2) L-ficolin (L-FCN) P35	*FCN2* 9q34	Liver	Serum/plasma	GlcNAc *Salmonella typhimurium* TV119 elastin *Escherichia coli*, *Staphylococcus aureus* corticosteroid, capsulated *Streptococcus pneumoniae* β-D-glucan, lipoteichoic acid, DNA acetylated LDL	+	+
	Ficolin-1 (FCN-1) M-ficolin (M-FCN) P35-related	*FCN1* 9q34	Monocyte Lung Spleen	Neutrophil Monocyte Alveolar epithelial cell	GlcNAc, *S. aureus* GalNAc, *S. typhimurium LT2* Sialic acid	+	ND
	Ficolin-3 (FCN-3) H-ficolin (H-FCN) Hakata antigen	*FCN3* lp35.3	Liver Lung	Serum/plasma Bile duct Bronchus, alveolus	GlcNAc *Aerococcus viridans* GalNAc LPS from *Hafnia alvei* Fucose *S. minnesota*, *E. coli* (O111)	+	ND
Mouse	Ficolin A (FcnA)	*Fcna* 2A3	Liver Spleen	Serum/plasma	GlcNAc, *S. aureus* GalNAc, elastin, *S. pneumoniae*	+	ND
	Ficolin B (FcnB)	*Fcnb* 2A3	Bone marrow Spleen	Macrophage	GlcNAc Sialic acid	+	ND
	–	*Fcn3* (*pseudogene*) 4D2					

ND not determined

Mice have two types of ficolins, ficolin A and ficolin B (Table 2). Ficolin A mRNA is expressed in the liver and spleen and its protein is present in plasma (Fujimori et al. 1998; Ohashi and Erickson 1998). Ficolin B mRNA is expressed in cells of myeloid cell lineage. The mouse ficolin A gene (*Fcna*) is located far from the ficolin B gene (*Fcnb*) at the interval distance of more than 2 Mb on chromosome 2, whereas the human L-ficolin gene (*FCN2*) and M-ficolin gene (*FCN1*) gene are close in the region homologous to *Fcnb* locus (Endo et al. 2004) (Fig. 3). The exon organization of *Fcnb* is very similar to *FCN1*, while that of *Fcna* is different from the others as well as from *FCN2*. Together with the phylogenetic tree constructed using the amino acid sequences, these results suggest that human M-ficolin is the orthologue of mouse ficolin B and that the genes encoding plasma-type ficolins, ficolin A and L-ficolin, have evolved independently from the ficolin B/M-ficolin lineage in mice and primates, respectively. Human and mouse ficolins are roughly classified into two groups: a plasma type including L-ficolin, H-ficolin, and ficolin A that is produced mainly in the liver and is secreted into plasma and a non-plasma type including M-ficolin and ficolin B that is undetectable or detected at the low level in plasma. Recently, a similar function shared between plasma ficolins, L-ficolin and ficolin A, was suggested by Hummelshoj et al. (2012), based on their bindings to bacteria and fungus. Mice do not have an orthologue of H-ficolin, though a non-expressed pseudogene is present (Endo et al. 2004).

Recognition Specificity and Physiological Roles of Ficolins

Human L-ficolin recognizes acetyl-group containing substances including GlcNAc (Faro et al. 2008; Krarup et al. 2004, 2008b) and also recognizes β-(1-3)-D-glucan (Garlatti et al. 2007; Ma et al. 2004). In addition to sugars, it also binds to substances such as elastin and DNA. L-ficolin binds to many bacteria including *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus* (*S. aureus*) and *S. pneumoniae* by recognizing their lipopolysaccharides, lipoteichoic acid and capsule polysaccharides. The L-ficolin-MASP complex binds to acetylated low density lipoprotein, GlcNAc-containing neoglycolipid, *Salmonella typhimurium* TV119, lipoteichoic acid from *Streptococcus pyogenes*, *S. agalactiae*, and *S. aureus* and β-(1 → 3)-D-glucan, resulting in C4 activation (Faro et al. 2008; Inoshita

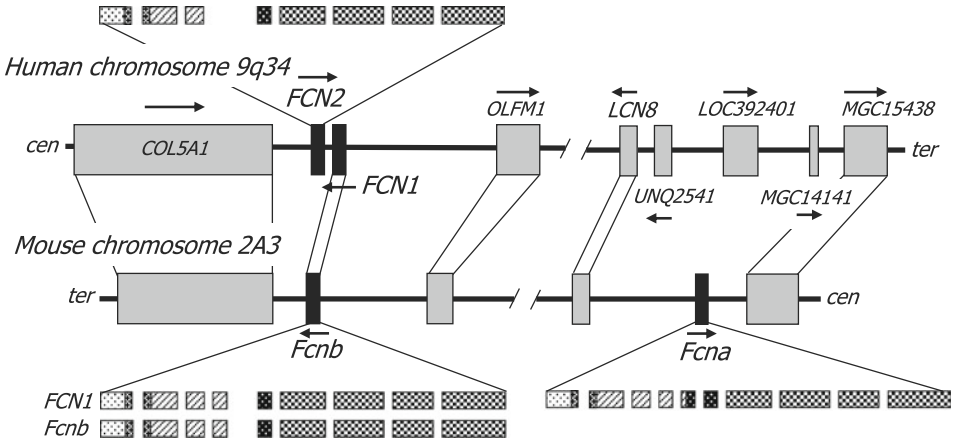

Fig. 3 Vicinities around the human ficolin genes (*FCN1* and *FCN2*) and mouse ficolin genes (*Fcna* and *Fcnb*) on chromosomes 9q34 and 2A3, respectively, and their exon organization. The locus of mouse *Fcnb* is syntenic to that of human *FCN1* and their exon–intron structures are very similar. In contrast, the locus of *Fcna* is located far from the homologous region of *FCN2*, and their exon–intron structures are different to each other

et al. 2009; Ma et al. 2004). The L-ficolin-MASP complex also binds to *N*-acetylneuraminic acid in the capsular polysaccharides of group B streptococci (*Streptococcus agalactiae*) of many serotypes, including serotype III, and initiates neutrophil-mediated opsonophagocytosis in a complement-dependent manner (Aoyagi et al. 2008). L-ficolin also acts as an opsonin itself (Matsushita et al. 1996).

Human H-ficolin recognizes the acetyl group (Hein et al. 2010; Munthe-Fog et al. 2009; Zacho et al. 2012). The H-ficolin–MASP complex binds to a polysaccharide called PSA derived from *Aerococcus viridans*, LPS from *Hafnia alvei* and acetylated compounds, leading to C4 activation (Hein et al. 2010; Matsushita et al. 2002; Swierzko et al. 2012; Tsujimura et al. 2001; Zacho et al. 2012). The H-ficolin–MASP complex exhibits in vitro bactericidal activity against *Aerococcus viridans* in the presence of human serum (Tsujimura et al. 2002). The role of H-ficolin present in the lung remains to be elucidated. However, it likely acts as an opsonin, similar to two types of pulmonary collectins (SP-A and SP-D). L-ficolin recognizes the acetyl group and binds to *Salmonella typhimurium* TV119 whose LPS has a GlcNAc residue at the non-reducing terminus. H-ficolin also recognizes the acetyl group as does L-ficolin, but it does not bind to *Salmonella typhimurium* TV119. Compared with L-ficolin, H-ficolin shows a narrow binding specificity for bacteria (Krarup et al. 2005).

M-ficolin recognizes acetylated compounds including GlcNAc, GalNAc, and sialic acid and binds to bacteria such as *S. aureus* and *S. typhimurium* LT2. It remains unknown whether plasma M-ficolin is complexed with MASP, although recombinant M-ficolin was shown to form a complex with MASP, resulting in complement activation upon binding to GlcNAc or the acetyl group (Frederiksen et al. 2005; Liu et al. 2005). M-ficolin is also present on the surface of monocytes and granulocytes where it is bound to sialic acid through its fibrinogen domain and is speculated to have immune-modulating functions (Honoré et al. 2010).

Mouse ficolin A binds to GlcNAc and GalNAc. Using recombinant proteins, it was shown that rficolin A is able to associate with MASP-2, resulting in C4 activation (Endo et al. 2005). This result strongly suggests that ficolin A is present in plasma in a form complexed with MASPs as is human L-ficolin. However, there have been no reports presenting the direct evidence for the association of ficolin A and MASPs in mouse plasma. Recently, we isolated mouse ficolin A and found that it is complexed with MASP-1, MASP-2, and sMAP (manuscript in preparation). Ficolin A-MASP activated C4 upon binding of the complex to GlcNAc.

Recombinant mouse ficolin B binds to GlcNAc and GalNAc. It also recognizes sialic acid similar to human M-ficolin (Endo et al. 2005). It was shown that a ficolin B preparation purified from bone marrow using GlcNAc-agarose contained trace amounts of MASP-2 and sMAP and exhibited C4-deposition activity on GlcNAc-coated microplates (Endo et al. 2012a). Furthermore, mouse recombinant ficolin B produced by CHO cells was able to form complexes with rMASP-1, rMASP-2 and rsMAP. In addition, the rficolin B-rMASP-2 complex activated C4 on GlcNAc-coated microplates. Similar to mouse ficolin B, rat recombinant ficolin B associates with MASPs and activates the lectin pathway (Girija et al. 2011).

Two Associations of H-Ficolin deficiency with diseases have been reported to date. Munthe-Fog et al. (2009) first reported a patient with repeated infections who was homozygous for a mutation (1637*delC*) of the H-ficolin gene (*FCN3*). This mutation leads to H-ficolin deficiency in plasma. The same mutation of the *FCN3* gene associated with H-ficolin deficiency was also found in a premature neonate with necrotizing enterocolitis (Schlapbach et al. 2011). Unlike H-ficolin, absolute deficiency of L-ficolin has not been reported so far. Low serum L-ficolin levels (L-ficolin insufficiency), however, have been reported to be associated with perinatal infection, preterm/premature birth and low birth weight (Kilpatrick and Chalmers 2012). L-ficolin insufficiency is also associated with bronchiectasis (Kilpatrick et al. 2009).

Ficolins are involved in not only innate immunity but also in apoptosis. L-ficolin binds to late apoptotic and necrotic cells (Jensen et al. 2007). Binding of plasma ficolins to apoptotic cells activates complement, resulting in the generation of opsonic C3 fragments which increase uptake of the cells by macrophages (Honoré et al. 2007; Kuraya et al. 2005). Ficolins also might have opsonin-like activity during apoptosis independent of complement activation.

Recently, to investigate the role of ficolin in vivo, the model mice genetically lacking ficolins were generated. The FcnA-deficient ($Fcna^{-/-}$) and FcnA/FcnB-double deficient ($Fcna^{-/-}b^{-/-}$) mice lacked FcnA-mediated complement activation in the sera, due to the absence of complexes comprising FcnA and MASPs (Endo et al. 2012b). When the host defense was evaluated by infection with a *Streptococcus pneumoniae* strain, which was recognized by ficolins but not by MBLs, the survival rate was significantly reduced in all three ficolin-deficient ($Fcna^{-/-}$, $Fcnb^{-/-}$ and $Fcna^{-/-}b^{-/-}$) mice compared with wild-type mice. Reconstitution of the FcnA-mediated lectin pathway in vivo improved survival rate in $Fcna^{-/-}$ mice. Ali et al. (2012) confirmed our observation that $Fcna^{-/-}$ mice were susceptible to *S. pneumonia* infection, and further demonstrated that MASP-2-deficient mice failed to opsonize this pathogen, suggesting that both ficolinA and MASP-2 are essential for defense against *S. pneumonia*.

Collaboration of Ficolins with Other Components in Complement Activation

C-reactive protein (CRP) and pentraxin 3 (PTX3) are plasma proteins belonging to the pentraxin family. The C1 complex binds to these pentraxins through C1q, initiating the classical pathway without involvement of immunoglobulins. Recent findings suggest a collaboration between ficolins and these pentraxins in innate immunity. L-ficolin interacts with CRP under conditions of local infection/inflammation, and this amplifies complement activation (Zhang et al. 2009). L-ficolin and M-ficolin bind to PTX3 (Gout et al. 2011; Ma et al. 2009). Both L-ficolin and PTX3 bind to *Aspergillus fumigatus* (Ma et al. 2009). The binding of L-ficolin to *Aspergillus fumigatus* is enhanced by PTX3 and vice versa. L-ficolin-dependent complement activation on *Aspergillus fumigatus* is enhanced by PTX3. These results suggest that L-ficolin and PTX3 synergically activate complement.

Endo et al. (2010) have shown that mouse rficolin A and rMBL bind to Aα- and Bβ-chains of fibrinogen and the α- and β-chains of fibrin. Binding of rficolin A and rMBL to *S. aureus* was enhanced by fibrinogen and fibrin. When C3 deposition on *S. aureus* was assessed in the ficolin A/MBL-depleted serum supplemented with purified ficolin A-MASP complex, it was enhanced by addition of fibrinogen. This pathway collaborates with the coagulation system in innate immunity under certain conditions such as those arising from injury and inflammation.

Concluding Remarks

Plasma ficolins in association with MASPs and their truncated proteins activate the lectin pathway of complement. Ficolins function as recognition molecules with lectin activity, while MASPs have proteolytic activity towards complement components. The activation mechanism of the lectin pathway is not fully understood. The roles of MASPs and their truncated proteins in the complex remain to be elucidated. Experimental or inherited deficiency of ficolins and MASPs are associated with susceptibility to infection, indicating their important role in innate immunity. The MBL-MASP and ficolin-MASP complexes also have a role in innate immunity by activating and cooperating with the coagulation system. They are responsible not only for innate immunity but also for apoptosis. The fact that MASP-3 is essential in development suggests a variety of physiological roles of the lectin pathway proteins.

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