Serine Proteases in the Lectin Pathway of the Complement System

18

Fabiana A. Andrade, Kárita C.F. Lidani, Sandra J. Catarino, and Iara J. Messias-Reason

Abstract

The complement system plays a crucial role in host defense against pathogen infections and in the recognition and removal of damaged or altered *self*-components. Complement system activation can be initiated by three different pathways—classical, alternative, and lectin pathways—resulting in a proteolytic cascade, which culminates in multiple biological processes including opsonization and phagocytosis of intruders, inflammation, cell lysis, and removal of immune complexes and apoptotic cells. Furthermore, it also functions as a link between the innate and adaptive immune responses. The lectin pathway (LP) activation is mediated by serine proteases, termed mannan-binding lectin (MBL)-associated serine proteases (MASPs), which are associated with the pattern recognition molecules (PRMs) that recognize carbohydrates or acetylated compounds on surfaces of pathogens or apoptotic cells. These result in the proteolysis of complement C2 and C4 generating C3 convertase (C4b2a), which carries forward the activation cascade of complements, culminating in the elimination of foreign molecules. This chapter presents an overview of the complement system focusing on the characterization of MASPs and its genes, as well as its functions in the immune response.

Keywords

Serine proteases • Complement system • Lectin pathway

F.A. Andrade • K.C.F. Lidani • S.J. Catarino • I.J. Messias-Reason (✉)
Department of Clinical Pathology, Hospital de Clínicas, Universidade Federal do Paraná (UFPR), General Carneiro, 181 Curitiba, PR, Brazil
e-mail: iarareason@hc.ufpr.br

S. Chakraborti, N.S. Dhalla (eds.), *Proteases in Physiology and Pathology*,
DOI 10.1007/978-981-10-2513-6_18

18.1 The Complement System

The human immune system is an extraordinary complex of biochemical mechanisms that provides effective defense against a large number of pathogens while also protecting against improper responses to *self*-components. The immune system exhibits innate and adaptive responses that cooperate together to facilitate appropriate host defense. Innate immunity provides the first line of defense by recognizing specific patterns present on the surface of microbes (PAMPs, pathogen-associated molecular patterns) or damaged cells (DAMPs, damage-associated molecular patterns) through innate pattern recognition molecules and receptors (PRMs and PRRs, respectively). The effectors of innate immunity include epithelial barriers, phagocytes and natural killer cells, cytokines, and a whole complex of proteins known as the complement system [1, 2].

The complement system is comprised of more than 35 plasma proteins and cell surface receptors/regulators, which enables the recognition, tagging, and elimination of various microbial intruders and foreign cells. Most of the soluble proteins circulate in functionally inactive forms called proenzymes or zymogens, which share identical domain organization and overall structure, but differ in enzymatic properties and physiological significance in health and disease [1]. Upon proteolytic cleavage, inactive proteins become activated, resulting in a proteolytic cascade that culminates in multiple biological processes such as opsonization and phagocytosis of intruders, inflammation, cell lysis, and removal of immune complexes in addition to being a link between the innate and adaptive immune responses [3]. Furthermore, the complement system plays an important role in the removal of apoptotic cells by recognizing damaged or altered *self*-components, thereby contributing to tissue homeostasis and preventing autoimmunity [4, 5]. However, excessive complement activation may be deleterious and is associated with tissue damage in certain diseases. Conversely, insufficient activity has also been associated with susceptibility to infection and autoimmune diseases [6]. Complement system is also involved in noninflammatory functions in the brain, such as basal and ischemia-induced neurogenesis [7] and synapse remodeling and pruning [8]. Further, the complement system also interacts with the coagulation system, although the precise molecular mechanism underlying the interaction has not been elucidated [9].

Complement activation involves a remarkably powerful degree of amplification and thus requires an appropriate and efficient checking system of regulatory molecules to maintain homeostatic balance to ensure efficient destruction of pathogens and recognition of *self*-components. The regulation predominantly occurs at the level of the convertases and during assembly of the membrane attack complex (MAC) [4]. The regulatory proteins, both, soluble proteins (such as Factor H and Factor I) and proteins on host cell membranes (such as CR1, CD46, CD55, and CD59) are necessary to ensure that complement activation is not exacerbated or deficient to prevent tissue damage or physiological disorders, respectively [6].

18.2 Pathways of Complement Activation

Complement activation can be initiated by three different pathways: classical, alternative, and lectin pathways [9]. Each pathway is activated by different components that converge in the formation of active enzyme complexes (C3 and C5 convertases), followed by the assembly of the terminal pathway and MAC (C5b-9), which is inserted into to the target cell membrane to lyse the cell. Complement activation also results in the release of chemoattractants (C4a, C3a, and C5a), which are potent inflammatory molecules, and opsonins (C3b and C4b), which mediate phagocytosis (Fig. 18.1). Serine proteases play an important role in human physiology and pathology, activating each other to promote initiation and amplification of the complement cascade [10]. They present a common domain containing the catalytic triad of histidine, aspartic acid and serine residues [11]. The serine proteases of the complement system include Clr (85 KDa) and Cls (85 KDa) of the classical pathway (CP),

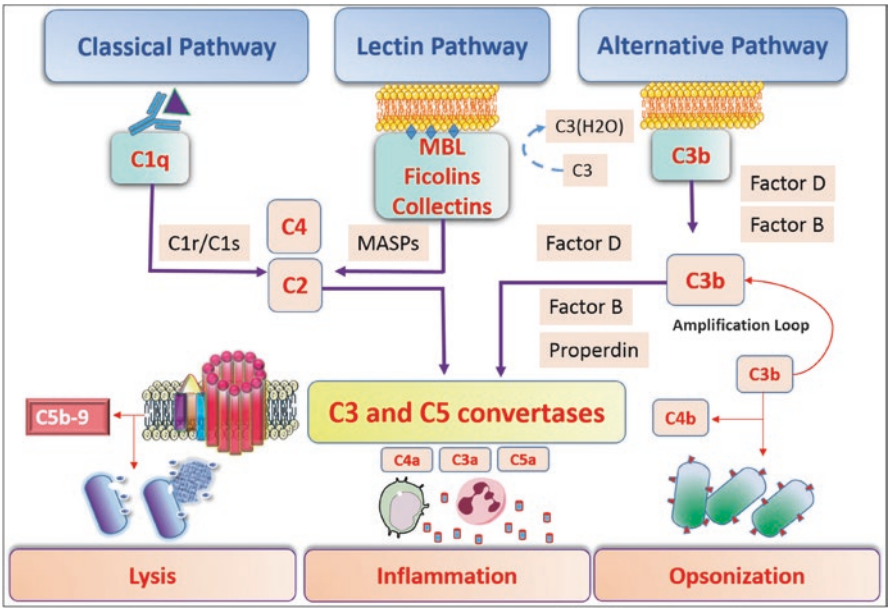

Fig. 18.1 Complement activation by the classical pathway (CP), lectin pathway (LP), and alternative pathway (AP). CP typically requires an antigen-antibody complex on pathogen surface and binding to C1 complex (C1q, C1r, and C1s) for its activation. LP recognizes mannose-terminating glycan or acetylated residues on pathogens leading to MBL/ficolins/collectins-MASP complex activation. Both pathways induce the formation of C3 convertase, C4b2a. AP is permanently activated at a low level by spontaneous hydrolysis of C3 into C3(H2O). Lack of complement inhibitors on pathogens induces AP activation by the C3bBb assembly. Complement activation leads to opsonization and phagocytosis of pathogens owing to C3b and C4b deposition, bacterial lysis by C5b-9 complex formation, and inflammation by C4a, C3a, and C5a, leading to recruitment of immune cells, endothelial and epithelial cell activation, and platelet activation

Table 18.1 Serine proteases of the complement system [9, 32]

Protease	Complement pathway	Active form	Function
C1r	Classical	C1 complex (C1q, C1r, C1s)	Clr autoactivation and Cls cleavage
C1s	Classical	C1 complex (C1q, C1r, C1s)	C2 and C4 cleavage
Factor I	Alternative	Factor I complex with C3b or C4b	C3b and C4b cleavage
Factor B	Alternative	C3bBb	C3 and C5 cleavage
Factor D	Alternative	C3bBD complex	Cleaves factor B bound to C3b
MASP-1	Lectin	MBL/MASPs complex	C2 (but not C4), C3 and MASP-2 cleavage and MASP-1 autoactivation
MASP-2	Lectin	MBL/MASPs complex	C2 and C4 cleavage
MASP-3	Lectin	MBL/MASPs complex	Remains unclear
C2	Classical/ lectin	C4b2a	C3 and C5 cleavage

MASPs 1–3 (mannan-binding lectin (MBL)-associated serine proteases; 80–90 KDa) of the lectin pathway (LP), C2 (110 KDa) of the classical/lectin pathway, and Factor B (93 KDa), Factor D (25 KDa), and Factor I (88 KDa) of the alternative pathway (AP) (Table 18.1) [12].

The CP activation is typically antibody dependent and requires the presence of C1 complex (C1q, C1r, and C1s subunits) by the binding of subcomponent C1q to the Fc portion (CH2 domain) of immunoglobulins M or G [13]. In the absence of antibody, C1q can also directly recognize other molecules of the bacterial cell wall, viral envelope membrane, C-reactive protein, etc. [1, 14]. Autocatalytic activation of the serine protease C1r leads to subsequent activation of C1s, that in turn cleaves C4 and C2 into larger (C4b, C2a) and smaller (C4a, C2b) fragments to form the enzyme complex C4bC2a (C3 convertase) [4]. The formation of C3 convertase leads to C3 activation and formation of C3a (anaphylatoxin) and C3b (opsonin), with C3 as the convergence point of the cascade [15]. C3b exposes an internal thioester bond that allows stable covalent binding to hydroxyl groups of any carbohydrates and proteins on the target surface. C3 convertase activity is very efficient, leading to the formation of approximately 1000 molecules of C3b that are able to bind to targets in the vicinity [16]. This process allows pathogens to be recognized as foreign bodies, resulting in phagocytosis and complement activation. Subsequently, additional C3b molecules bind to C3 convertase forming the C5 convertase (C4bC2aC3b) that cleaves C5 in to C5a and C5b, initiating the terminal pathway and assembly of MAC (Fig. 18.1) [4, 17].

The AP occurs on microbial surfaces in the absence of specific antibody. The AP activation occurs on the surface of foreign bodies at a low level by the spontaneous hydrolysis of the internal thioester bond in C3, leading to the formation of C3b analog, C3(H2O). Factor B binds the C3(H2O) and is then cleaved by Factor D, generating a distinct C3 convertase (C3bBb) that further cleaves C3 molecules. In the presence of an activating surface (e.g., a bacterial cell wall), C3b is protected from inactivation by regulatory proteins such as Factors I and H. However, in the

AP, a more active C3 convertase (C3bBb) is formed instead, which is further stabilized by properdin. In contrast to other pathways, AP functions as an amplification loop providing a strong positive feedback activation of C3, thereby increasing the production of pro-inflammatory mediators [18]. In fact, 80–90% of pathological complement activation in disease is driven by the AP [19]. Furthermore, the alternative convertase assembly may also be initiated by non-covalent attachment of properdin to some target surfaces (Fig. 18.1) [20, 21].

18.3 The Lectin Pathway

The existence of the LP was first discovered in the 1970s when the plant lectin mannose-binding protein (concanavalin A) was found to activate the complement system [22]. This pathway was further characterized by using proteins isolated from rabbit liver and serum; however, its function remained unclear initially [23, 24]. In 1992, Matsushita and Fujita reported that MBL and MASPs activated the LP, which was a landmark study on the mechanism of LP activation [25]. Thus far, 6 different PRMs that initiate the activation of the LP have been identified: 3 ficolins (M-ficolin, L-ficolin, and H-ficolin, also known as ficolin-1, ficolin-2, and ficolin-3, respectively), and 3 collectins (MBL, collectin 11 or collectin kidney-1 or CL-K1, and collectin 10 or colletin-L1or CL-L1). Similar to AP, the LP may be activated in the absence of immune complexes by the binding of PRMs to carbohydrates or acetylated compounds on the surfaces of pathogens (PAMPs) or apoptotic cells (DAMPs) (Fig. 18.2). The PRMs form complexes with the serine proteases, MASPs (MASPs

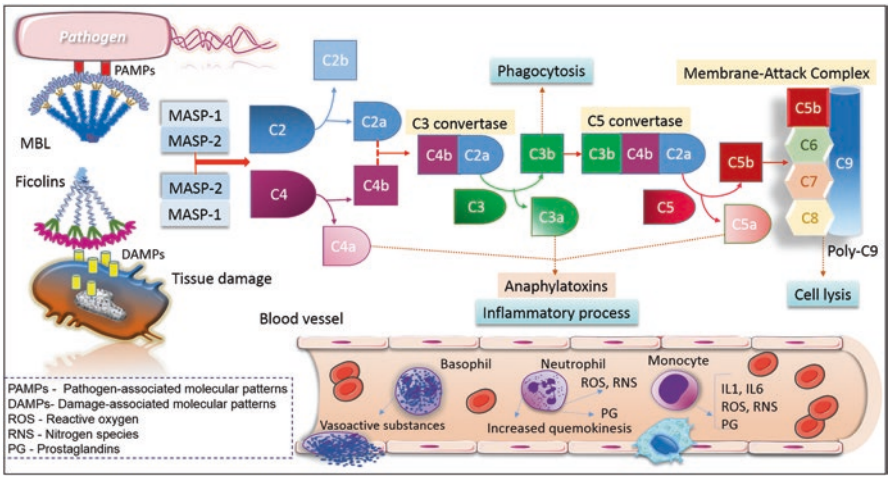

Fig. 18.2 The lectin pathway of complement activation. MBL and ficolins undergo conformational changes upon interaction with PAMPs and DAMPs by binding MBL and ficolin, respectively. This activates MASP-1, followed by MASP-2, which initiates a cleavage cascade of complement factors, with anaphylatoxins C4a, C3a, and C5a playing important roles in the inflammatory process

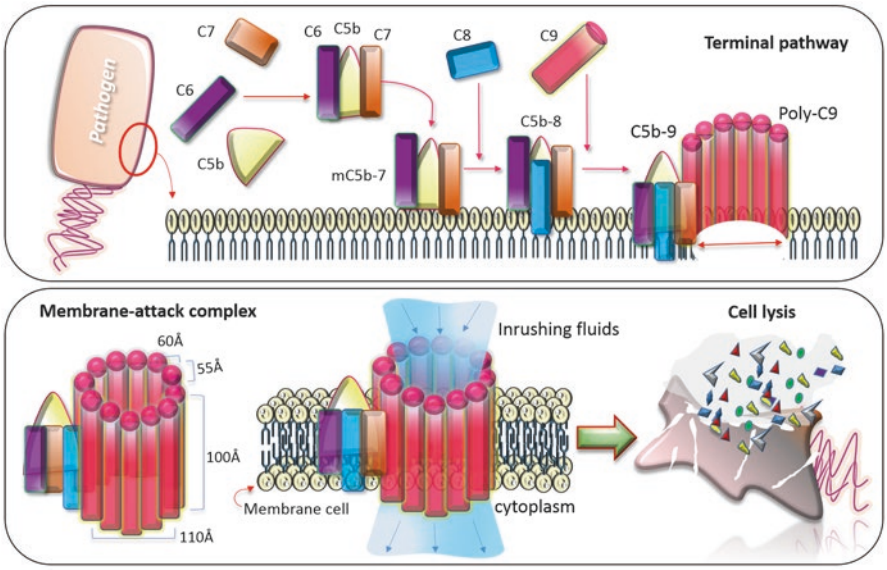

Fig. 18.3 Membrane attack complex (MAC) formation and the resultant consequences in target cell. Newly formed C5b reacts with C6 to form the stable C5b6 complex. Binding of C7 results in a hydrophobic complex that targets the membrane (mC5b-7). Membrane insertion is initiated upon binding of C8 (C5b-8) after which 12–18 copies of C9 polymerize to form the pore-forming ring structure to induce lysis of microbial membranes

1, 2, and 3), and two nonenzymatic splice products MBL-associated proteins (MAps19 and 44) [26–28]. Upon binding of PRM/MASP complexes to appropriate targets, MASPs get activated from pro-enzymes (zymogens) to active forms catalyzing the cleavage of C4 and C2, to generate C3 convertase (C4bC2a), which carries the complement activation cascade forward, culminating in the elimination of microbial intruders by phagocytosis or cells lysis [29].

The terminal pathway occurs in a similar manner in all three activation pathways and results in the assembly of the MAC, initiated by the interaction of C5b with C6 and C7 molecules, yielding the C5bC6C7 (C5b-7) complex. The membrane insertion event is initiated upon binding of C8 to C5b-7 complex. Subsequently, 12–18 copies of C9 molecules bind to the C5b-7, forming the lytic pore (C5b-9) inducing cell death by causing imbalance in cell osmolarity (Fig. 18.2) [14, 30]. Multiple MACs are required for complement-mediated lysis of nucleated cells; however, in erythrocytes it has been demonstrated that a single pore could cause cell lysis [31] (Fig. 18.3).

18.4 MBL-Associated Serine Proteases (MASPs) of the Lectin Pathway

There are three serine proteases associated with the LP PRMs, MASP-1, MASP-2, and MASP-3, in addition to two nonenzymatic proteins MAp19 and MAp44 [1]. The three MASP enzymes have an identical domain organization (Fig. 18.4), which is also similar to that of the two classical pathway serine proteases, C1r and C1s. The regulatory domain (A-chain) is composed of C1r/C1s, Uegf, and bone morphogenetic protein 1 (CUB1), followed by the epidermal growth factor (EGF), a second CUB domain (CUB2), and two contiguous complement control proteins (CCPs) 1 and 2 [33, 34]. The regulatory domain is responsible for dimerization of MASP polypeptides and binding to PRMs [35–37]. The regulatory domain is followed by the module with the catalytic activity (B-chain), the serine protease (SP) domain [33, 34]. The CCP2 and SP domains are connected through a linker peptide (also termed the activation peptide), where an Arg-Ile bond is cleaved through autolysis when MASP/PRM complexes bind to pathogens, linking the A- and B-chain connected via a disulfide bond [33].

All MASPs are generated from two genes. MASP-1, MASP-3, and MAp44 are encoded by the *MASP1* gene through an alternative splicing process [38, 39], while MASP-2 and MAp19 are alternatively spliced products of *MASP2* gene [40].

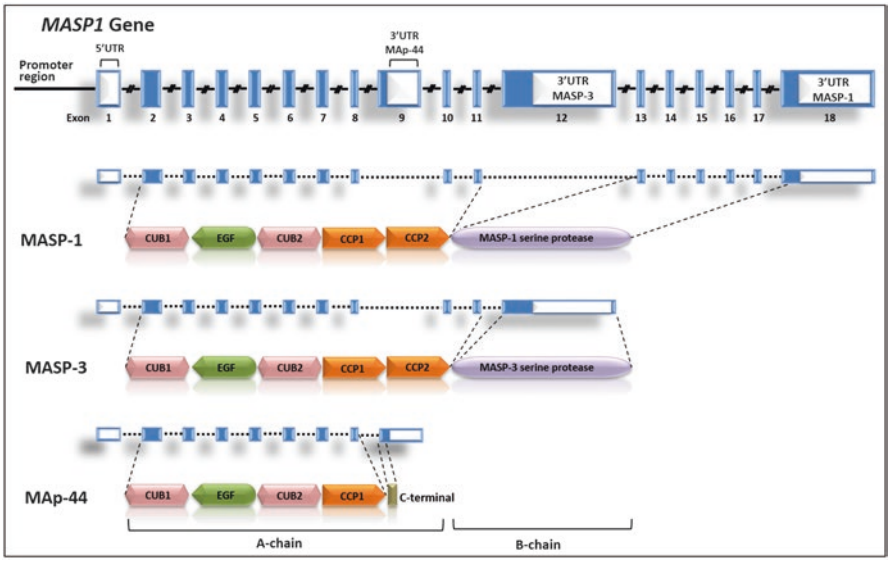

Fig. 18.4 *MASP1* gene and transcripts. The primary transcript can be spliced into three different mRNAs encoding the MASP-1, MASP-3, and MAp44 proteins. *Blue boxes* indicate the translated part of the exons. MASP: mannose-binding lectin associated serine protease. MAp44: mannose-binding lectin-associated protein of 44 kDa. CUB, C1r/C1s, Uegf, and bone morphogenetic protein; EGF, epidermal growth factor; CCP, complement control protein. Exons are drawn to scale and introns are truncated

18.4.1 *MASP1* Gene

The *MASP1* gene contains 18 exons and is located on chromosome 3q27–q28 spanning 76 kb (Fig. 18.4) [41, 42]. The gene encodes a primary pre-mRNA transcript, which is spliced differentially to yield three distinct mRNAs encoding the MASP-1, MASP-3, and MAp44 (also termed MAp 1) proteins [38, 39]. MASP-1 and MASP-3 regulatory domains (CUB1-EGF-CUB2-CCP1-CCP2) are encoded by exons 2–8 and exons 10 and 11, while the SP domain is encoded by exons 13–18 and exon 12 in MASP-1 and MASP-3, respectively. MAp44 lacks the SP domain but shares the first four domains (CUB1-EGF-CUB2-CCP) with MASP-1 and MASP-3 that are encoded by exons 2–8. Exon 9 is unique to MAp44 [39, 43]. The mRNA encoding MASP-1 is largely observed in the liver, while mRNA for MASP-3 is primarily observed in the liver and cervix, followed by bladder, brain, colon prostate, and placenta [39]. The highest expression of MAp44 is observed in the heart; it was weakly expressed in cervix, colon, and liver [39].

Some *MASP1* gene polymorphisms are associated with the serum levels of MASP-1, MASP-3, and MAp44 (Table 18.2); most associations were observed in healthy individuals. In Danish blood donors, heterozygotes of rs190590338 (G > A) lead to increase in MASP-1 median concentration, while the minor allele of rs7625133 (A > C) decreased MAp44 concentration. The minor alleles of SNPs rs3774275 (A > G), rs698090 (T > C), and rs67143992 (G > A) result in an increase in MASP-1 and MAp44 and a decrease in MASP-3 serum concentrations; SNPs rs72549154 (G > T) and rs35089177 (T > A) showed the opposite effect—the minor alleles result in an increase of MASP-3 and a decrease of MASP-1 and MAp44 [44]. The additive effect of some *MASP1* SNPs in haplotypes on MASP-1, MASP-3, and MAp44 serum concentrations has also been described. The *MASP1 TGAG* haplotype (rs35089177 (T > A), rs62292785 (G > A), rs7625133 (A > C), and rs72549254 (G > A)), for example, leads to an increase in MASP-1 and MAp44 and decrease in MASP-3 concentration in healthy blood donors [44].

In patients with cystic fibrosis homozygous (A/A) and heterozygous (G/A) alleles, SNP rs850312 (G > A) was associated with the earlier onset of *Pseudomonas aeruginosa* colonization [45]. These same genotypes were associated with higher on-admission MASP-3 levels in critically ill children, exhibiting a protective effect, as higher MASP-3 levels are related to a better outcome [46]. The T/T genotype of rs710469 (C > T) was also considered a protective genotype in critically ill children by increasing on-admission MASP-3 levels, although the genotype was equally distributed among controls and patients [46]. A non-synonymous polymorphism (rs38343199) in exon 10 (G > A) located in the MASP-1 and MASP-3 CCP2 domain was evaluated in systemic lupus erythematosus (SLE), systemic inflammatory response syndrome (SIRS), and/or sepsis patients. However, no association was found between this amino acid substitution and the diseases [47]. Some mutations in *MASP1* gene are also related to the autosomal-recessive 3MC syndrome (Carnevale, Mingarelli, Malpuech, and Michels) [48–50].

Table 18.2 *MASP1* gene polymorphisms associated with MASP-1, MASP-3, and MAp44 concentration and diseases

dbSNP	Allele	MAF	Gene region	Gene position	Amino acid position	Protein region	Serum levels[a]	Disease association
rs190590338	G > A	<1%	Promoter	−2464	n.a.	n.a.	G/A: Increase MASP-1 levels	–
rs7625133	A > C	3%	Promoter	−961	n.a.	n.a.	A/C, C/C: Decrease MAp44 levels	–
rs35089177	T > A	28%	Promoter	−1418	n.a.	n.a.	T/A, AA: Decrease MASP-1 and MAp44 levels	–
rs75284004	A > G	1%	Promoter	−1479	n.a.	n.a.	A/G decrease MASP-3 levels	–
rs62292785	G > A	10%	Promoter	−1251	n.a.	n.a.	G/A: Decrease MASP-1 levels	–
rs72549254	G > A	17%	Intron 1	9	n.a.	n.a.	A/G: Increase MASP-3 levels AG, AA: Decrease MAp44 levels	–
rs710469	C > T	49%	Intron 2	24,903	n.a.	n.a.	T/T: Higher on-admission MASP-3 levels in critically ill children	Protective effect on critically ill children [46]
rs3774275	A > G	24%	Intron 8	44,153	n.a.	n.a.	A/G, G/G: Increase MASP-1 and MAp44 and decrease MASP-3 levels	–

(continued)

Table 18.2 (continued)

dbSNP	Allele	MAF	Gene region	Gene position	Amino acid position	Protein region	Serum levels[a]	Disease association
rs113938200	C > T	<1%	Exon 9	44,259	p.Asn368Asp	C-terminal MAp44	C/T: Decrease MAp44 levels	–
rs698090	C > T	46%	Exon 9	45,121	n.a.	3'UTR MAp44	C/T: Increase MAp44 levels	–
rs850312	G > A	21%	Exon 12	55,613	p.Leu617Leu	CCP2 MASP-3	A/A, A/G: Higher on-admission MASP-3 levels in critically ill children	Earlier *Pseudomonas aeruginosa* colonization [45], protective effect on critically ill children [46]
rs72549154	G > T	7%	Exon 12	55,489	p.Arg576Met	SP MASP-3	G/T: Decrease MASP-1 levels	–
rs67143992	G > A	9%	Exon12	56,100	n.a.	3′ UTR MASP-3	G/A: Increase MASP-1, MAp44 and decrease of MASP-3 levels	–
							A/A: Increase MAp44 and decrease MASP-3 levels	

dbSNP, Single Nucleotide Polymorphism Database; n.a., not applicable; MAF, minor allele frequency of 1000 genomes project (all populations); CCP, complement control protein; SP, serine protease; UTR, untranslated

[a]Compared to the homozygote state of the major allele in [44, 46]

18.4.2 MASP-1

MASP-1 was characterized by Matsushita and Fujita (1992) as the first serine protease C1s-like and was designated as mannose-binding protein (MBP)-associated serine protease (MASP). This serine protease plays a central role in the initiation of the LP, by carrying out the activation of MASP-2. It is considered a promiscuous protease since its substrate binding groove is wide and resembles that of trypsin rather than early complement proteases [51].

Recent findings supported MASP-1 as an essential component of the LP, whose concentration is 20-fold higher than MASP-2 in the plasma. MASP-1 undergoes autoactivation to subsequently activate MASP-2 efficiently—acting in a manner analogous to that of C1r and C1s in the CP, being responsible for 60% of the C2 cleaved and C3 convertase formation [52, 53]. MASP-1 autoactivation seems to control the initiation of the LP [54], but does not cleave C4, being not capable of generating C3 convertase by itself, although direct activation of C3 by MASP-1 can occur at a relatively low efficiency [55, 56].

MASP-1 is primarily expressed in the liver, with mean plasma levels of 11 μg/ml (range 4–30 μg/ml) [57], and significantly contributes to the development of the inflammatory reaction by proteolytic activity. MASP-1 induces Ca^{2+} signaling, NF-κB and p38 MAPK pathways in endothelial cells through protease-activated receptor 4 (PAR4) [58]. This activity leads to the release of IL-6 and IL-8, activating the chemotaxis of neutrophil granulocytes [59]. MASP-1 is also able to modulate the immune response by the release of pro-inflammatory bradykinin from high-molecular-weight kininogen [60].

MASP-1 is immediately activated after microbial infection by the binding of PRM complexes to targets leading to opsonization, cell lysis, release of anaphylatoxins, chemotaxis of neutrophils, and inflammation. In fact, MASP-1 plasma levels have been associated with some inflammatory disorders, and the activity of MBL/MASP-1 complex has been associated to disease severity in post-streptococcal acute glomerulonephritis and hepatitis C virus (HCV) infection, leading to glomerular fibrinogen deposits and sustained hematuria [61], and liver fibrosis [62], respectively. In addition, MASP-1 plasma levels were also higher in patients who suffered myocardial infarction and lower in patients with acute ischemic stroke [63]. High levels of MASP-1 were also observed in patients with type 1 diabetes mellitus [64].

In autoimmune diseases, high plasma levels of MASP-1 were associated with SLE [65]. In contrast, MASP-1 levels were reduced in patients with hereditary angioedema in response to the degree of complement C4 consumption, which was expected to contribute to the pathophysiology and severity of the disease [66].

Furthermore, MASP-1 was shown to play a role in coagulation, cleaving factor XIII and fibrinogen and mediating the formation of cross-linked fibrin, although with lower catalytic efficiency compared to thrombin [67]. In fact, antithrombin in the presence of heparin is a more potent inhibitor of MASP-1 then C1 inhibitor. The ancient origin of MASP-1 and its thrombin-like activity suggests its involvement in a coagulation-based defense mechanism in the early evolution of innate immunity [68]. Interestingly, components of the coagulation cascade amplify complement

activation in such a manner that both complement and coagulation cascade are interconnected through an important crosstalk [9]. In addition, MASP-1 was associated with thrombus formation in a mouse model of arterial injury [69], and in patients with diabetes, contributing to an enhanced thrombotic environment and consequent vascular complications [64].

18.4.3 MASP-3

MASP-3 is an alternative spliced product of *MASP1* gene, which contains an identical A-chain, but an entirely different B chain and is highly conserved [70]. MASP-3 is mainly expressed in the pancreas, skeletal muscle, spleen, thymus, prostate, and ovary [56]. The mean serum concentration is 5.2 μg/ml (range 1.8–10.6 μg/ml) [71], mainly occurring in association with ficolin-3 and in lower amounts with ficolin-2 and MBL [38].

MASP-3 does not cleave any complement components and it is not inhibited by C1-inhibitor [56, 72]. MASP-3 may reduce the LP activity as it has to compete for MASP binding sites on the LP recognition molecules [39]. Similar to C1s, MASP-3 cleaves insulin-like growth factor-binding protein-5 (IGFBP-5), an important regulator of physiological processes in the bone, kidney, and mammary glands [73]. MASP-3 has also been implicated in the activation of the AP in mice [74]; however, in humans MASP-3 is not required for activation of AP [52].

Along with CL-K1, MASP-1, and MAp44, MASP-3 seems to have an important role in early embryonic development, as shown by the effect of five rare MASP-3 exon 12 mutations in four independent families with autosomal recessive 3MC syndrome, characterized by several development disorders. All the implicated mutations are predicted to damage the SP domain, eliminating the enzymatic activity [49, 75]. According to Venkatraman et al., this disorder is probably a result of structural defects caused by disruption of $Ca^{(2+)}$ binding during biosynthesis of CL-K1, causing structural changes in the protein and in the consequent CL-K1/MASP-3 complexes [76]. In this context, MASP-3 also cleaves IGFBP-5 [73], regulating physiological processes in kidney, bone, among others, and interestingly, is expressed in the craniofacial region during mouse embryonic development [49].

In addition, MASP-3 levels were associated to infections in children admitted to the intensive care unit (ICU). Low MASP-3 levels on-admission were associated with an increased risk of acquiring new infection in critically ill children [46].

18.4.4 MAp44

MAp44 is an alternative splice product of the *MASP1* gene, which lacks the SP domain and consequently, its functional activity. The polypeptide was named MAp44 due to its molecular mass of 44 kDa. MAp44 is mainly expressed in the heart and skeletal muscle, with a mean serum concentration of 1.7 μg/ml (range 0.8–3.2 μg/ml) [39, 43].

Although MAp44 does not contain the SP domain, the other domains interact with MBL or ficolins, thereby competing with MASP-1, MASP-2, and MASP-3 and resulting in the inhibition of C4 deposition and consequently the inhibition of downstream complement activation [39, 43, 77]. In addition to inhibiting the incorporation of MASPs into MBL/ficolin complexes, MAp44 was shown to prevent MBL deposition on MBL ligands and restricting complement activation and C3 deposition [78].

MAp44 has been associated with cardioprotective effects, preserving cardiac function, decreasing infarct area, and preventing thrombogenesis in murine models of ischemia/reperfusion injury by inhibiting MBL and C3 deposition [69, 78]. Due to its protective effects on cardiovascular system, MAp44 has been suggested to be used in a therapeutic approach for the treatment of myocardial ischemia/reperfusion injury and thrombogenesis [78]. In contrast, Frauenknecht et al. demonstrated that MAp44 levels were not directly related to the pathophysiology of cardio- and cerebrovascular diseases, but instead was associated with cardiovascular risk factors such as dyslipidemia, obesity, and hypertension [63].

18.4.5 *MASP2* Gene

The *MASP2* gene comprises 12 exons and is located on chromosome 1p36.23–31 spanning about 20 kb [79, 80]. The primary gene transcript gives rise to two different mRNAs generated by alternative splicing/polyadenylation, encoding the MASP-2 serine protease and a truncated MASP-2-related plasma protein, termed MAp19 or sMAP (Fig. 18.5) [79]. For MASP-2, the regulatory domains, CUB1-EGF-CUB2-CCP1-CCP2, are encoded by exons 2–4 and exons 6–11, while the serine protease domains are encoded by exon 12. MAp19 is encoded by 4 exons, of which 3 (exons 2–4) are shared with MASP-2 and encode the CUB1-EGF regulatory domains, whereas exon 5 encodes four specific C-terminal amino acids. MAp19 does not have a serine protease domain [40, 81].

Some *MASP2* polymorphisms are associated with modulation of MASP-2 and MAp19 serum levels (Table 18.3). The rs72550870 (T > C) responsible for the Asp > Gly substitution in residue 120 (p.D120G) occurs in the CUB1 domain [82] and affects both MASP-2 and MAp19 leading to a reduced serum concentration by eliminating the binding to MBL and ficolins and affecting complement activation [83]. The MASP-2 levels in heterozygous p.D120G healthy subjects is about half of those in subjects with the wild-type allele [82]. The rs12085877 (G > A) leads to an amino acid substitution (p.R439H) in the MASP-2 serine protease domain leading to a reduction in MASP-2 concentration in heterozygotes. MASP-2 with the p.R439H polymorphism is able to bind to MBL, however, showing reduced enzymatic activity in the MBL-MASP2 complexes [84, 85]. Several other *MASP2* polymorphisms, including rs7548659 (G > T) in the promoter region, rs61735600 (C > T) and rs56392418 (G > A) in exon 3, rs2273344(C > T) in intron 4, rs9430347 (T > C) in intron 5, rs17409276 (G > A) in intron 9, rs12711521(C > A) and rs2273346 (A > G) in exon 10, and rs12085877 (C > T) and rs1782455 (G > A) in

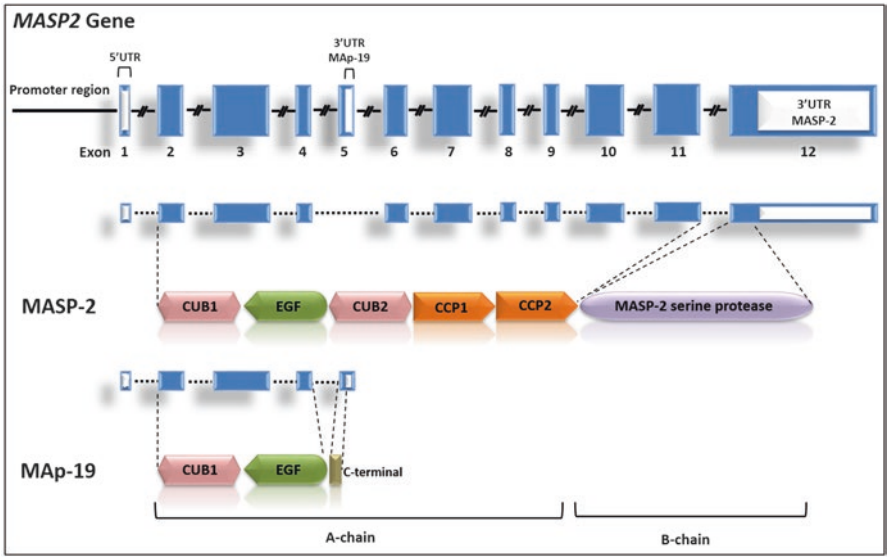

Fig. 18.5 *MASP2* gene and transcripts. Alternative splicing of primary transcript gives rise to two different mRNAs encoding MASP-2 and MAp19 proteins. *Blue boxes* indicate translated part of the exons. MASP: mannose-binding lectin-associated serine protease. MAp19: mannose-binding lectin-associated protein of 19 kDa. CUB, C1r/C1s, Uegf, and bone morphogenetic protein; EGF, epidermal growth factor; CCP, complement control protein. Exons are numbered and drawn to scale; introns are truncated

exon 12, were found to be associated with the modulation of serum levels [84–87]. Some of them were associated with the susceptibility to leprosy [87], hepatitis C [88], malaria [89], bacterial infections after orthotopic liver transplantation [90], Chagas disease [91], rheumatoid arthritis [92], tuberculosis [93], rheumatic fever [94], and endemic pemphigus foliaceus [95].

18.4.6 MASP-2

The second MASP was identified in 1997 by Thiel et al., which showed notable homology with the first reported MASP (MASP-1) and the serine proteases, C1s and C1r, of the CP. Nevertheless, subsequent analysis demonstrated that despite the homology, MASP-2 was entirely different in assembly and function to C1s [56, 68, 96] with a 1000-fold higher catalytic activity and could be inhibited by C1-inhibitor 50-fold more rapidly [97]. The almost identical substrate specificity of MASP-2 and C1s is mediated through different group of enzyme-substrate interactions, and it is very probable that the major functional difference between them is reflected in the different loop structures of the two enzymes [96].

MASP-2 is synthesized as single-chain proenzyme, and its activation proceeds through the cleavage of a single Arg-Ile bond, generating the two disulfide-linked

Table 18.3 *MASP2* gene polymorphisms associated with MASP-2 and MAp19 concentration and diseases

dbSNP	Allele	MAF	Gene region	Gene position	Amino acid position	Protein region	Serum levels[a]	Disease associations
rs7548659	G > T	43%	Promoter	−175	n.a.	n.a.	High MASP-2 and low MAp19 concentration	Susceptibility to leprosy [87]
rs72550870	T > C	1%	Exon 3	599	p.D120G	CUB1	Low MASP-2 and MAp19 concentration	Endemic pemphigus foliaceus [95], rheumatic fever [94], persistent inflammatory disease, and severe pneumococcal pneumonia [82]
rs61735600	C > T	2%	Exon 3	537	p.R99Q	CUB1	High MASP-2 concentration	Susceptibility to leprosy [87]
rs56392418	C > T	4%	Exon 3	620	p.P126L	CUB1	Low MASP-2 concentration	–
rs2273343	T > C	1%	Exon 4	1689	p.H155R	EGF	Low MASP-2 concentration	–
rs2273344	C > T	16%	Intron 4	2143	n.a.	n.a.	High MASP-2 and low MAp19 concentration	–
rs9430347	G > A	15%	Intron 5	2420	n.a.	n.a.	High MASP-2 and low MAp19 concentration	–
rs17409276	G > A	16%	Intron 9	16,060	n.a.	n.a.	High MASP-2 and low MAp19 concentration	–
rs2273346	A > G	12%	Exon 10	16,368	p.V377A	CCP2	Low MASP-2 concentration	Susceptibility to tuberculosis [93], rheumatic fever [94]

(continued)

Table 18.3 (continued)

dbSNP	Allele	MAF	Gene region	Gene position	Amino acid position	Protein region	Serum levels[a]	Disease associations
rs12711521	C > A	42%	Exon 10	16,349	p.D371Y	CCP2	High MASP-2 and low MAp19 concentration	Susceptibility to leprosy [87], HCV [88], complications after orthotopic liver transplantation [90]
rs12085877	G > A	3%	Exon 12	19,578	p.R439H	SP	Low MASP-2 concentration	Susceptibility to leprosy [87] and rheumatic fever [94], protective to malaria [89]
rs1782455	G > A	31%	Exon 12	19,741	p.S493=	SP	High MASP-2 and low MAp19 concentration	Susceptibility to leprosy [87]

dbSNP Single Nucleotide Polymorphism Database, *n.a.* not applicable, *MAF* minor allele frequency of 1000 genomes project (all populations), *CCP* complement control protein, *SP* serine protease

[a]Effect of the homozygote of the minor allele in [83–85, 87, 91]

chains, A and B [3]. The MASP-2 protease is comprised of 3 N-terminal non-catalytic domains (CUB1-EGF-CUB2) and 3 catalytic domains (CCP1-CCP2-SP). The non-catalytic domain is responsible for the binding of the protease to the recognition molecules, such as MBL. The catalytic domains are responsible for protein conformation and help to ensure the narrow selectivity for protein substrates by restricting access to the substrate binding [36, 56, 96]. The binding interface of the protease is located on all the fragments of CCP1-CCP2-SP, binding C4 with similar affinity [98].

In contrast to MASP-1, MASP-2 is a very specific protease, which very efficiently cleaves C4 and proconvertase C2, thus having the ability to generate the C3 convertase on its own [29, 34, 99]. MASP-2 can autoactivate, but under physiological conditions, MASP-1 is the essential MASP-2 activator [34]. MASP-1 is 20-fold more abundant than MASP-2 [57], having a much higher propensity for autoactivation, thus causing a dramatic increase in the rate of activation of MASP-2 [52].

MASP-2 is mainly expressed in the liver [80, 100] and is stable over time in healthy individuals, with concentration around 400–500 ng/mL in serum/plasma (range 70–1200 ng/mL) [101, 102].

The first clinical effect of MASP-2 deficiency was reported in 2003 when a patient with an inherited deficiency of MASP-2 showed several and recurrent infectious and autoimmune disease manifestations. Sequence analysis of DNA revealed a point mutation in exon 3, causing substitution of glycine for aspartic acid at position 105 (D105G) [82]. In 2005 another report with the same mutation and similar clinical condition confirmed the importance of MASP-2 deficiency in human health [83].

Further investigations showed that MASP-2 levels may be associated with several other diseases, with levels lower than 100 ng/mL being considered deficient [3]. Low MASP-2 levels were reported in acute stroke when compared with normal coronary vessel individuals [63]. This finding is in line with the observation that myocardial infarction induces complement activation with MASP-2 consumption [63, 103]. In contrast, MASP-2 deficiency appears to protect mice from gastrointestinal post-ischemic reperfusion injury [104].

Furthermore, low MASP-2 levels were associated with malignancy among critically ill children [46] and with rheumatic fever [94]. The authors suggested that low MASP-2 levels may reflect protein consumption due to complement activation, which may be involved in the establishment of rheumatic heart disease [94].

On the other hand, high MASP-2 levels appear to protect against rheumatoid arthritis and articular symptoms suggesting that MASP-2 levels might be used as a biomarker in the follow-up of individuals with familial predisposition to the disease [92]. High MASP-2 levels were also associated with the development of severe infections in adult patients with hematological cancer undergoing chemotherapy [105], type 1 diabetes mellitus [64], and juvenile idiopathic arthritis [106]. Similar to the complement system as a whole, MASP-2 represents a dual role in diseases. In general, low MASP-2 can lead to a compromised immune response against pathogens, thereby facilitating infection and disease progression, but on the other hand, high MASP-2 level can lead to exacerbated inflammatory response and tissue injury.

Additionally, MASP-2 levels have been related to a number of other diseases, including schizophrenia [107], septic shock [108], acute lymphoblastic leukemia, non-Hodgkin lymphoma, central nervous system tumors [109], and colorectal cancer [110, 111].

Finally, MASP-2 is also known to trigger the coagulation cascade by cleaving prothrombin to thrombin in a similar manner as factor Xa, generating cross-linked fibrin covalently bound on bacterial surfaces. This MASP-2 function may be protective by limiting the dissemination of infection [67, 112].

18.4.7 MAp19

MAp19 is a truncated 19 kDa product of alternative splicing and polyadenylation of the primary RNA transcript of the *MASP2* gene [56]. It contains the same CUB1 and EGF domain as MASP-2, but has an additional four unique amino acids at the C-terminal end of the protein, with no serine protease activity [29, 56, 113]. MAp19 forms homodimers via the CUB1 and EGF domains, like MASP-2, and associates with MBL and ficolins in a calcium-dependent manner [37]. It is secreted by the liver in to the plasma and expressed by Kupffer cells with a similar median level as MASP-2 (217 ng/ml, 26–675 ng/ml) [114].

The function of MAp19 is not entirely understood, but because of its ability to bind to MBL and ficolins, it was speculated that MAp19 competes with MASPs, thus acting as a downregulator to the LP. In fact, MAp19 was shown to reduce the activation of C4, by being an attenuator of the activation of LP [115]. Nevertheless, only a minor fraction of MAp19 is associated with MBL and ficolins, and binding to MBL/ficolins occurs with about ten times lower affinity compared with MASP-2 [116].

In a recent study, MAp19 was not related to inflammatory markers in patients with systemic and oligoarticular juvenile idiopathic arthritis differently as observed for the others MASPs [106].

Finally, MAp19 is excreted in human urine and may play a role in the inhibition of calcium oxalate renal stone formation [114, 117]. The nucleocapsid N protein of severe acute respiratory syndrome coronavirus interacts with MAp19 in vitro, but the functional significance of this remains unknown [118].

18.5 Conclusions

This chapter discussed several aspects and research findings that point out the importance of serine proteases of the LP and its gene polymorphisms in the human physiology and pathology. The activation of complement by complex PRMs/MASP-1/2 has been associated not only with immune response but also with other biological processes, such as coagulation and embryonic development. However, future studies are required in order to clarify the role of MASP-3, MAp19, and MAp44 proteins in the activation of the LP.

In conclusion, serine proteases of the LP have an essential role in maintaining physiological homeostasis. The activation of complement requires an effective regulatory system that is able to perform a complex checking mechanism in order to prevent pathological disorders. The impact of plasma MASP levels and its genetic polymorphisms in health and diseases processes should be encouraged in order to improve the knowledge about its real role in the maintenance of homeostasis and development of diseases. This may disclose new therapeutic and/or preventive strategies.

References

1. Degn SE, Thiel S (2013) Humoral pattern recognition and the complement system. Scand J Immunol 78:181–193
2. Hoffmann JA, Kafatos FC, Janeway CA, Ezekowitz RA (1999) Phylogenetic perspectives in innate immunity. Science 284:1313–1318
3. Thiel S (2007) Complement activating soluble pattern recognition molecules with collagen-like regions, mannan-binding lectin, ficolins and associated proteins. Mol Immunol 44:3875–3888
4. Dunkelberger JR, Song W-C (2010) Complement and its role in innate and adaptive immune responses. Cell Res 20:34–50
5. Trouw LA, Blom AM, Gasque P (2008) Role of complement and complement regulators in the removal of apoptotic cells. Mol Immunol 45:1199–1207
6. Carroll MV, Sim RB (2011) Complement in health and disease. Adv Drug Deliv Rev 63:965–975
7. Rahpeymai Y, Hietala MA, Wilhelmsson U et al (2006) Complement: a novel factor in basal and ischemia-induced neurogenesis. EMBO J 25:1364–1374
8. Stevens B, Allen N, Vazquez L et al (2007) The classical complement cascade mediates CNS synapse elimination. Cell 131:1164–1178
9. Ricklin D, Hajishengallis G, Yang K, Lambris JD (2010) Complement: a key system for immune surveillance and homeostasis. Nat Immunol 11:785–797
10. Gál P, Dobó J, Beinrohr L et al (2013) Inhibition of the serine proteases of the complement system. Adv Exp Med Biol 735:23–40
11. Wetsel R (2000) C5. In: Morley B, Walport M (eds) Complement Factsb. London, pp 104–109
12. Sim RB, Laich A (2000) Serine proteases of the complement system. Biochem Soc Trans 28:545–550
13. Duncan AR, Winter G (1988) The binding site for C1q on IgG. Nature 332:738–740
14. Tegla CA, Cudrici C, Patel S et al (2011) Membrane attack by complement: the assembly and biology of terminal complement complexes. Immunol Res 51:45–60
15. Janeway CA, Travers P, Walport M, Shlomchk M (2005) Immunobiology: the immune system in health and disease, 6th edn. Garland Science Taylor & Francis Group, New York
16. Khan FH (2009) The elements of immunology. Pearson Education, Delhi
17. Walport MJ (2001) Complement. First of two parts. N Engl J Med 344:1058–1066
18. Emlen W, Li W, Kirschfink M (2010) Therapeutic complement inhibition: new developments. Semin Thromb Hemost 36:660–668
19. Harboe M, Mollnes TE (2008) The alternative complement pathway revisited. J Cell Mol Med 12:1074–1084
20. Spitzer D, Mitchell LM, Atkinson JP, Hourcade DE (2007) Properdin can initiate complement activation by binding specific target surfaces and providing a platform for de novo convertase assembly. J Immunol 179:2600–2608

21. Xu W, Berger SP, Trouw LA et al (2008) Properdin binds to late apoptotic and necrotic cells independently of C3b and regulates alternative pathway complement activation. J Immunol 180:7613–7621
22. Milthorp P, Forsdyke DR (1970) Inhibition of lymphocyte activation at high ratios of concanavalin A to serum depends on complement. Nature 227:1351–1352
23. Kawasaki T, Etoh RYI (1978) Isolation and characterization of a mannan-binding protein from rabbit liver. Biochem Biophys Res Commun 81:1018–1024
24. Kozutsumi Y, Kawasaki T, Yamashina I (1980) Isolation and characterization of a mannan-binding protein from rabbit serum. Biochem Biophys Res Commun 95:658–664
25. Matsushita M, Fujita T (1992) Activation of the classical complement pathway by mannose-binding protein in association with a novel C1s-like serine protease. J Exp Med 176:1497–1502
26. Matsushita M, Endo Y, Taira S et al (1996) A novel human serum lectin with collagen- and fibrinogen-like domains that functions as an opsonin. J Biol Chem 271:2448–2454
27. Honoré C, Hummelshoj T, Hansen BE et al (2007) The innate immune component ficolin 3 (Hakata antigen) mediates the clearance of late apoptotic cells. Arthritis Rheum 56:1598–1607
28. Drickamer K (1988) Two distinct classes of carbohydrate-recognition domains in animal lectins. J Biol Chem 263:9557–9560
29. Thiel S, Vorup-Jensen T, Stover CM et al (1997) A second serine protease associated with mannan-binding lectin that activates complement. Nature 386:506–510
30. Hadders MA, Bubeck D, Roversi P et al (2012) Assembly and regulation of the membrane attack complex based on structures of C5b6 and sC5b9. Cell Rep 1:200–207
31. Koski CL, Ramm LE, Hammer CH et al (1983) Cytolysis of nucleated cells by complement: cell death displays multi-hit characteristics. Proc Natl Acad Sci U S A 80:3816–3820
32. Sim RB, Tsiftsoglou SA (2004) Proteases of the complement system. Biochem Soc Trans 32:21–27
33. Sato T, Endo Y, Matsushita M, Fujita T (1994) Molecular characterization of a novel serine protease involved in activation of the complement system by mannose-binding protein. Int Immunol 6:665–669
34. Kjaer TR, Thiel S, Andersen GR (2013) Toward a structure-based comprehension of the lectin pathway of complement. Mol Immunol 56:413–422
35. Thielens NM, Enrie K, Lacroix M et al (1999) The N-terminal CUB-epidermal growth factor module pair of human complement protease C1r binds Ca^{2+} with high affinity and mediates Ca^{2+}−dependent interaction with C1s. J Biol Chem 274:9149–9159
36. Wallis R, Dodd RB (2000) Interaction of mannose-binding protein with associated serine proteases: effects of naturally occurring mutations. J Biol Chem 275:30962–30969
37. Gregory LA, Thielens NM, Matsushita M et al (2004) The X-ray structure of human mannan-binding lectin-associated protein 19 (MAp 19) and its interaction site with mannan-binding lectin and L-ficolin. J Biol Chem 279:29391–29397
38. Skjoedt M-O, Palarasah Y, Munthe-Fog L, et al (2010) MBL-associated serine protease-3 circulates in high serum concentrations predominantly in complex with Ficolin-3 and regulates Ficolin-3 mediated complement activation. Immunobiology 215:921–931
39. Degn SE, Hansen AG, Steffensen R et al (2009) MAp 44, a human protein associated with pattern recognition molecules of the complement system and regulating the lectin pathway of complement activation. J Immunol 183:7371–7378
40. Stover CM, Thiel S, Thelen M et al (1999) Two constituents of the initiation complex of the mannan-binding lectin activation pathway of complement are encoded by a single structural gene. J Immunol 162:3481–3490
41. Takada F, Seki N, Matsuda Y et al (1995) Localization of the genes for the 100-kDa complement-activating components of Ra-reactive factor (CRARF and Crarf) to human 3q27-q28 and mouse 16B2-B3. Genomics 25:757–759
42. Skjoedt M, Hummelshoj T, Palarasah Y et al (2011) Serum concentration and interaction properties of MBL/ficolin associated protein-1. Immunobiology 216:625–632

43. Skjoedt M-O, Hummelshoj T, Palarasah Y et al (2010) A novel mannose-binding lectin/ficolin-associated protein is highly expressed in heart and skeletal muscle tissues and inhibits complement activation. J Biol Chem 285:8234–8243
44. Ammitzbøll CG, Steffensen R, Jørgen Nielsen H et al (2013) Polymorphisms in the MASP1 gene are associated with serum levels of MASP-1, MASP-3, and MAp 44. PLoS One 8:e73317
45. Haerynck F, Van Steen K, Cattaert T et al (2012) Polymorphisms in the lectin pathway genes as a possible cause of early chronic Pseudomonas Aeruginosa colonization in cystic fibrosis patients. Hum Immunol 73:1175–1183
46. Ingels C, Vanhorebeek I, Steffensen R et al (2014) Lectin pathway of complement activation and relation with clinical complications in critically ill children. Pediatr Res 75:99–108
47. Weiss G, Madsen HO, Garred P (2007) A novel mannose-binding lectin-associated serine protease 1/3 gene variant. Scand J Immunol 65:430–434
48. Degn SE, Jensenius JC, Thiel S (2011) Disease-causing mutations in genes of the complement system. Am J Hum Genet 88:689–705
49. Sirmaci A, Walsh T, Akay H et al (2010) MASP1 mutations in patients with facial, umbilical, coccygeal, and auditory findings of Carnevale, Malpuech, OSA, and Michels syndromes. Am J Hum Genet 87:679–686
50. Atik T, Koparir A, Bademci G et al (2015) Novel MASP1 mutations are associated with an expanded phenotype in 3MC1 syndrome. Orphanet J Rare Dis 10:128
51. Dobó J, Harmat V, Beinrohr L et al (2009) MASP-1, a promiscuous complement protease: structure of its catalytic region reveals the basis of its broad specificity. J Immunol 183:1207–1214
52. Degn SE, Jensen L, Hansen AG et al (2012) Mannan-binding lectin-associated serine protease (MASP)-1 is crucial for lectin pathway activation in human serum, whereas neither MASP-1 nor MASP-3 is required for alternative pathway function. J Immunol 189:3957–3969
53. Héja D, Kocsis A, Dobó J et al (2012) Revised mechanism of complement lectin-pathway activation revealing the role of serine protease MASP-1 as the exclusive activator of MASP-2. Proc Natl Acad Sci U S A 109:10498–10503
54. Megyeri M, Harmat V, Major B et al (2013) Quantitative characterization of the activation steps of mannan-binding lectin (MBL)-associated serine proteases (MASPs) points to the central role of MASP-1 in the initiation of the complement lectin pathway. J Biol Chem 288:8922–8934
55. Ambrus G, Gál P, Kojima M et al (2003) Natural substrates and inhibitors of mannan-binding lectin-associated serine protease-1 and -2: a study on recombinant catalytic fragments. J Immunol 170:1374–1382
56. Yongqing T, Drentin N, Duncan RC et al (2012) Mannose-binding lectin serine proteases and associated proteins of the lectin pathway of complement: two genes, five proteins and many functions? Biochim Biophys Acta 1824:253–262
57. Thiel S, Jensen L, Degn SE et al (2012) Mannan-binding lectin (MBL)-associated serine protease-1 (MASP-1), a serine protease associated with humoral pattern-recognition molecules: normal and acute-phase levels in serum and stoichiometry of lectin pathway components. Clin Exp Immunol 169:38–48
58. Megyeri M, Makó V, Beinrohr L et al (2009) Complement protease MASP-1 activates human endothelial cells: PAR4 activation is a link between complement and endothelial function. J Immunol 183:3409–3416
59. Jani PK, Kajdacsi E, Megyeri M et al (2014) MASP-1 induces a unique cytokine pattern in endothelial cells: a novel link between complement system and neutrophil granulocytes. PLoS One 9:10–13
60. Dobó J, Major B, Kékesi KA et al (2011) Cleavage of Kininogen and subsequent Bradykinin release by the complement component: mannose-binding lectin-associated serine protease (MASP)-1. PLoS One 6:1–8
61. Hisano S, Matsushita M, Fujita T et al (2007) Activation of the lectin complement pathway in post-streptococcal acute glomerulonephritis. Pathol Int 57:351–357

62. Brown KS, Ryder SD, Irving WL et al (2007) Mannan binding lectin and viral hepatitis. Immunol Lett 108:34–44
63. Frauenknecht V, Thiel S, Storm L et al (2013) Plasma levels of mannan-binding lectin (MBL)-associated serine proteases (MASPs) and MBL-associated protein in cardio- and cerebrovascular diseases. Clin Exp Immunol 173:112–120
64. Jenny L, Ajjan R, King R et al (2015) Plasma levels of mannan-binding lectin-associated serine proteases MASP-1 and MASP-2 are elevated in type 1 diabetes and correlate with glycaemic control. Clin Exp Immunol 180:227–232
65. Troldborg A, Thiel S, Laska MJ et al (2015) Levels in plasma of the serine proteases and associated proteins of the lectin pathway are altered in patients with systemic lupus erythematosus. J Rheumatol 42:948–951
66. Hansen CB, Csuka D, Munthe-Fog L et al (2015) The levels of the lectin pathway serine protease MASP-1 and its complex formation with C1 inhibitor are linked to the severity of hereditary angioedema. J Immunol 195:3596–3604
67. Krarup A, Wallis R, Presanis JS et al (2007) Simultaneous activation of complement and coagulation by MBL-associated serine protease 2. PLoS One 2:e623
68. Presanis JS, Hajela K, Ambrus G et al (2004) Differential substrate and inhibitor profiles for human MASP-1 and MASP-2. Mol Immunol 40:921–929
69. La Bonte LR, Pavlov VI, Tan YS et al (2012) MBL-associated serine protease -1 (MASP-1) is a significant contributor to coagulation in a murine model of Occlusive thrombosis. J Immunol 188:885–891
70. Stover C, Lynch NJ, Dahl M et al (2003) Murine serine proteases MASP-1 and MASP-3, components of the lectin pathway activation complex of complement, are encoded by a single structural gene. Genes Immun 4:374–384
71. Degn SE, Jensenius JC, Bjerre M (2011) The lectin pathway and its implications in coagulation, infections and auto-immunity. Curr Opin Organ Transpl 16:21–27
72. Zundel S, Cseh S, Lacroix M et al (2004) Characterization of recombinant Mannan-binding lectin-associated serine protease (MASP)-3 suggests an activation mechanism different from that of MASP-1 and MASP-2. J Immunol 172:4342–4350
73. Cortesio CL, Jiang W (2006) Mannan-binding lectin-associated serine protease 3 cleaves synthetic peptides and insulin-like growth factor-binding protein 5. Arch Biochem Biophys 449:164–170
74. Iwaki D, Kanno K, Takahashi M et al (2011) The role of mannose-binding lectin-associated serine protease-3 in activation of the alternative complement pathway. J Immunol 187:3751–3758
75. Rooryck C, Diaz-Font A, Osborn DPS et al (2011) Mutations in the lectin complement pathway genes COLEC11 and MASP1 cause 3MC syndrome. Nat Genet 43:197–203
76. Venkatraman Girija U, Furze CM, Gingras AR et al (2015) Molecular basis of sugar recognition by collectin-K1 and the effects of mutations associated with 3MC syndrome. BMC Biol 13:27
77. Degn SE, Jensen L, Olszowski T et al (2013) Co-complexes of MASP-1 and MASP-2 associated with the soluble pattern-recognition molecules drive lectin pathway activation in a manner inhibitable by MAp 44. J Immunol 191:1334–1345
78. Pavlov VI, Skjoedt M-O, Siow Tan Y et al (2012) Endogenous and natural complement inhibitor attenuates myocardial injury and arterial thrombogenesis. Circulation 126:2227–2235
79. Stover CM, Schwaeble WJ, Lynch NJ et al (1999) Assignment of the gene encoding mannan-binding lectin-associated serine protease 2 (MASP2) to human chromosome 1p36.3-->p36.2 by in situ hybridization and somatic cell hybrid analysis. Cytogenet Cell Genet 84:148–149
80. Stover C, Endo Y, Takahashi M et al (2001) The human gene for mannan-binding lectin-associated serine protease-2 (MASP-2), the effector component of the lectin route of complement activation, is part of a tightly linked gene cluster on chromosome 1p36.2-3. Genes Immun 2:119–127

81. Schwaeble W, Dahl MR, Thiel S et al (2002) The Mannan-binding lectin-associated serine proteases (MASPs) and MAp 19: four components of the lectin pathway activation complex encoded by two genes. Immunobiology 205:455–466
82. Stengaard-Pedersen K, Thiel S, Gadjeva M et al (2003) Inherited deficiency of mannan-binding lectin-associated serine protease 2. N Engl J Med 349:554–560
83. Sørensen R, Thiel S, Jensenius JC (2005) Mannan-binding-lectin-associated serine proteases, characteristics and disease associations. Springer Semin Immunopathol 27:299–319
84. Thiel S, Steffensen R, Christensen IJ et al (2007) Deficiency of mannan-binding lectin associated serine protease-2 due to missense polymorphisms. Genes Immun 8:154–163
85. Thiel S, Kolev M, Degn S et al (2009) Polymorphisms in mannan-binding lectin (MBL)-associated serine protease 2 affect stability, binding to MBL, and enzymatic activity. J Immunol 182:2939–2947
86. Boldt AB, Grisbach C, Steffensen R et al (2011) Multiplex sequence-specific polymerase chain reaction reveals new MASP2 haplotypes associated with MASP-2 and MAp 19 serum levels. Hum Immunol 72:753–760
87. Boldt ABW, Goeldner I, Stahlke ERS et al (2013) Leprosy association with low MASP-2 levels generated by MASP2 haplotypes and polymorphisms flanking MAp 19 exon 5. PLoS One 8:e69054
88. Tulio S, Faucz FR, Werneck RI et al (2011) MASP2 gene polymorphism is associated with susceptibility to hepatitis C virus infection. Hum Immunol 72:912–915
89. Holmberg V, Onkamo P, Lahtela E et al (2012) Mutations of complement lectin pathway genes MBL2 and MASP2 associated with placental malaria. Malar J 11:61
90. de Rooij B-JF, van Hoek B, ten Hove WR et al (2010) Lectin complement pathway gene profile of donor and recipient determine the risk of bacterial infections after orthotopic liver transplantation. Hepatology 52:1100–1110
91. Boldt A, Luz PR, Messias-Reason IJ (2011) MASP2 haplotypes are associated with high risk of cardiomyopathy in chronic Chagas disease. Clin Immunol 140:63–70
92. Goeldner I, Skare T, Boldt ABW et al (2014) Association of MASP-2 levels and MASP2 Gene polymorphisms with rheumatoid arthritis in patients and their relatives. PLoS One 9:e90979
93. Chen M, Liang Y, Li W et al (2015) Impact of MBL and MASP-2 gene polymorphism and its interaction on susceptibility to tuberculosis. BMC Infect Dis 15:151
94. Catarino SJ, Boldt ABW, Beltrame MH, et al (2014) Association of MASP2 polymorphisms and protein levels with rheumatic fever and rheumatic heart disease. Hum Immunol.
95. Messias-Reason I, Bosco DG, Nisihara RM et al (2008) Circulating levels of mannan-binding lectin (MBL) and MBL-associated serine protease 2 in endemic pemphigus foliaceus. Clin Exp Dermatol 33:495–497
96. Gál P, Dobó J, Závodszky P, Sim RBM (2009) Early complement proteases: C1r, C1s and MASPs. A structural insight into activation and functions. Mol Immunol 46:2745–2752
97. Kerr FK, Thomas AR, Wijeyewickrema LC et al (2008) Elucidation of the substrate specificity of the MASP-2 protease of the lectin complement pathway and identification of the enzyme as a major physiological target of the serpin, C1-inhibitor. Mol Immunol 45:670–677
98. Duncan RC, Bergström F, Coetzer TH et al (2012) Multiple domains of MASP-2, an initiating complement protease, are required for interaction with its substrate C4. Mol Immunol 49:593–600
99. Rossi V, Cseh S, Bally I et al (2001) Substrate specificities of recombinant mannan-binding lectin-associated serine proteases-1 and -2. J Biol Chem 276:40880–40887
100. Stover CM, Lynch NJ, Hanson SJ et al (2004) Organization of the MASP2 locus and its expression profile in mouse and rat. Mamm Genome 15:887–900
101. Møller-Kristensen M, Jensenius JC, Jensen L et al (2003) Levels of mannan-binding lectin-associated serine protease-2 in healthy individuals. J Immunol Methods 282:159–167
102. Ytting H, Christensen IJ, Thiel S et al (2007) Biological variation in circulating levels of mannan-binding lectin (MBL) and MBL-associated serine protease-2 and the influence of age, gender and physical exercise. Scand J Immunol 66:458–464

103. Zhang M, Hou YJ, Cavusoglu E et al (2011) MASP-2 activation is involved in ischemia-related necrotic myocardial injury in humans. Int J Cardiol 166:499–504
104. Schwaeble WJ, Lynch NJ, Clark JE et al (2011) Targeting of mannan-binding lectin-associated serine protease-2 confers protection from myocardial and gastrointestinal ischemia/reperfusion injury. Proc Natl Acad Sci U S A 108:7523–7528
105. Ameye L, Paesmans M, Thiel S et al (2012) M-ficolin levels are associated with the occurrence of severe infections in patients with haematological cancer undergoing chemotherapy. Clin Exp Immunol 167:303–308
106. Petri C, Thiel S, Jensenius JC, Herlin T (2015) Investigation of complement-activating pattern recognition molecules and associated enzymes as possible inflammatory markers in oligoarticular and systemic juvenile idiopathic arthritis. J Rheumatol 42:1252–1258
107. Mayilyan KR, Arnold JN, Presanis JS et al (2006) Increased complement classical and mannan-binding lectin pathway activities in schizophrenia. Neurosci Lett 404:336–341
108. Charchaflieh J, Wei J, Labaze G et al (2012) The role of complement system in septic shock. Clin Dev Immunol 2012:407324
109. Fisch UP, Zehnder A, Hirt A et al (2011) Mannan-binding lectin (MBL) and MBL-associated serine protease-2 in children with cancer. Swiss Med Wkly 141:w13191
110. Ytting H, Christensen IJ, Thiel S et al (2005) Serum mannan-binding lectin-associated serine protease 2 levels in colorectal cancer: relation to recurrence and mortality. Clin Cancer Res 11:1441–1446
111. Ytting H, Christensen IJ, Thiel S et al (2008) Pre- and postoperative levels in serum of mannan-binding lectin associated serine protease-2 -a prognostic marker in colorectal cancer. Hum Immunol 69:414–420
112. Gulla KC, Gupta K, Krarup A et al (2010) Activation of mannan-binding lectin-associated serine proteases leads to generation of a fibrin clot. Immunology 129:482–495
113. Takahashi M, Endo Y, Fujita T, Matsushita M (1999) A truncated form of mannose-binding lectin-associated serine protease (MASP)-2 expressed by alternative polyadenylation is a component of the lectin complement pathway. Int Immunol 11:859–863
114. Degn S, Thiel S, Nielsen O (2011) MAp 19, the alternative splice product of the MASP2 gene. J Immunol Methods:89–101
115. Iwaki D, Kanno K, Takahashi M et al (2006) Small mannose-binding lectin-associated protein plays a regulatory role in the lectin complement pathway. J Immunol 177:8626–8632
116. Degn SE, Thiel S, Nielsen O et al (2011) MAp 19, the alternative splice product of the MASP2 gene. J Immunol Methods 373:89–101
117. Kang I, Kim J, Chang S et al (1999) Mannan-binding lectin (MBL)-associated plasma protein present in human urine inhibits calcium oxalate crystal growth. FEBS Lett 462:89–93
118. Liu J, Ali MAM, Shi Y et al (2009) Specifically binding of L-ficolin to N-glycans of HCV envelope glycoproteins E1 and E2 leads to complement activation. Cell Mol Immunol 6:235–244