Multiple roles of complement MASP-1 at the interface of innate immune response and coagulation

József Dobó[a,1], Verena Schroeder[b,1], Lorenz Jenny[b,1], László Cervenak[c,1], Péter Závodszky[a,1] and Péter Gál[a,1,*]

[a] Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Magyar tudósok krt. 2, H-1113 Budapest, Hungary

[b] Department of Clinical Research, University of Bern, and University Clinic of Haematology, University Hospital, Bern, Switzerland

[c] 3rd Department of Internal Medicine, Semmelweis University, Kútvölgyi út 4, H-1125 Budapest, Hungary

Footnotes:

[*] Corresponding author.

Tel.: +36-1-3826768, Fax: +36-1-3826295, e-mail address: gal.peter@ttk.mta.hu (P. Gál)

[1] All authors contributed equally to this article

Abstract

MASP-1 is a versatile serine protease that cleaves a number of substrates in human blood. In recent years it became evident that besides playing a crucial role in complement activation MASP-1 also triggers other cascade systems and even cells to mount a more powerful innate immune response. In this review we summarize the latest discoveries about the diverse functions of this multi-faceted protease. Recent studies revealed that among MBL-associated serine proteases, MASP-1 is the one responsible for triggering the lectin pathway via its ability to rapidly autoactivate then cleave MASP-2, and possibly MASP-3. The crystal structure of MASP-1 explains its more relaxed substrate specificity compared to the related complement enzymes. Due to the relaxed specificity, MASP-1 interacts with the coagulation cascade and the kinin generating system, and it can also activate endothelial cells eliciting pro-inflammatory signaling.

Keywords

complement system; lectin pathway; serine protease; blood coagulation; bradykinin; endothelial cell

Abbreviations

CCP = complement control protein domain; CUB = C1r/C1s-Uegf-BMP domain; EGF = epidermal growth factor domain; FPA = fibrinopeptide A; FPB = fibrinopeptide B; FXIII = coagulation factor XIII; HUVEC = human umbilical vein endothelial cell; ICAM-1 = intercellular adhesion molecule 1; MAp = MBL-associated protein; MAPK = mitogen activated protein kinase; MASP = MBL-associated serine protease; MBL = mannan-binding lectin; MI = myocardial infarction; PAR = protease activated receptor; SP = serine protease domain; TAFI = thrombin-activatable fibrinolysis inhibitor; t-PA = tissue-type plasminogen activator; T2DM = type-2 diabetes mellitus; VCAM-1 = vascular cell adhesion molecule 1

1. Introduction

In the blood a complex system of cells and molecules mediates the protection of the integrity of our body and maintains the homeostasis. In case of infection and/or trauma a number of response reactions are triggered that lead to the elimination of pathogens and repair of damaged tissues. A remarkable system that plays a central role in the above mentioned processes, is a network of serine proteases having chymotrypsin fold and trypsin-like specificity. Traditionally these proteases are considered members of independent proteolytic cascade systems, such as the complement, coagulation, kinin and fibrinolytic systems. In the recent years increasing number of evidences indicated that these cascade systems have common evolutionary origin and they interact with each other in various ways at numerous points (Markiewski et al., 2007; Amara et al., 2010; Oikonomopoulou et al., 2012). In this review we will concentrate on the role of MASP-1, a key protease of the lectin pathway of complement, in various cascade systems. We will discuss how MASP-1 initiates lectin pathway activation, how it contributes to inflammatory reactions through directly activating cells and releasing bradykinin from high molecular weight kininogen, and how it influences the activation of the coagulation system.

In the innate immune system pattern recognition receptors and molecules monitor the inter- and extracellular space to detect the appearance of invading pathogens or dangerously altered self structures. Such receptors can be found on the cell surface (Toll-like receptors), inside the cells (Toll-like receptors, NOD-like, RIG-like receptors) or they can freely circulate in different body fluids. C1q, MBL and ficolins are pattern recognition molecules in the blood that consist of C-terminal globular domains and N-terminal collagen-like arms (Degn and Thiel, 2013). The globular domains bind to the dangerous structures which results in the activation of serine proteases associated with the collagen-like arms. The activated serine proteases initiate the complement cascade, a central effector arm of the immune system. The initiation complexes of the classical and the lectin pathways of complement activation contain a pattern recognition molecule and several serine protease zymogens, depending on the pathway. C1, the first component of the classical pathway of the complement system, consists of a C1q molecule and a tetramer of C1r and C1s proteases (C1s-C1r-C1r-C1s) (Gaboriaud et al., 2004). C1r is the enzyme that converts the recognition function of C1q into an enzymatic signal: it can autoactivate and cleave zymogen C1s. Activated C1s then generates the C3 convertase complex (C4b2a) by cleaving C4 and C2. Cleavage of C3 is the central event of complement activation since it initiates the terminal reactions (i.e. formation of the membrane

attack complex, C3-opsonized particles and anaphylatoxins) and it also initiates the alternative pathway, which amplifies the original signal by a feedback loop. The alternative pathway C3 convertase (C3bBb) can also be formed as a result of the spontaneous and slow hydrolysis of C3 near a foreign surface.

The same C3 convertase as in the classical pathway (C4b2a) is formed during lectin pathway activation, but the situation is much more complicated. There are several pattern recognition molecules (MBL, Ficolin-1/-2/-3, CL-K1, CL-L1) that can exist in different oligomeric forms (from dimer to hexamer or even higher), three serine proteases: MASP-1 (alias MASP1 isoform 1), MASP-2 (alias MASP2 isoform 1), MASP-3 (alias MASP1 isoform 2) and two non-catalytic variants of these proteases MAp19 (alias MASP2 isoform2 or MAP-2 or sMAP) and MAp44 (alias MASP1 isoform3 or MAP-1), which are the alternative splicing products of the protease genes. Although we do not have a detailed picture, it seems very likely that the composition and the stoichiometry of the initiation complexes of the lectin pathway can be quite diverse. While in the classical pathway the C1r and C1s proteases are present in equimolar amounts (about 600 nM each), the concentrations of MASP-1 (143 nM), MASP-2 (6 nM) and MASP-3 (63 nM) in human serum are quite different (Thiel et al., 2012). MASP-2 has the lowest concentration, but it is essential, since it is the only lectin pathway protease that can cleave C4 for the C3 convertase. C2 is cleaved by both MASP-1 and MASP-2. MASP-1 is the most abundant protease of the lectin pathway, its concentration far exceeds that of the other proteases. It can autoactivate but it cannot initiate the lectin pathway alone. The physiological role of MASP-1 has been a debated issue since its discovery. In the recent years it became evident that due to its high concentration and relaxed substrate specificity MASP-1 plays not only a central role in lectin pathway activation, but it also has substrates outside the complement system (**Fig. 1A**) through which it can influence other proteolytic cascades and physiological processes.

2. The central role of MASP-1 in the initiation of the lectin pathway

2.1. The mystery surrounding the role of MASP-1

Due to its relatively high concentration MASP-1 was the first MBL-associated protease isolated and studied. At first MASP-1 was regarded as the only protease component of the initiation complex of the lectin pathway that has C1s-like activity cleaving C4 and C2 (Matsushita and Fujita, 1992). Later on a second MBL-associated protease, denoted as MASP-2 was discovered, which was only a minor component of the original MASP samples,

but it was shown to be responsible for the C1s-like activity (Thiel et al., 1997). The autoactivating capacity of MASP-2 was also demonstrated, and complexes reconstituted from MBL molecules and MASP-2 dimers were able to initiate the lectin pathway (Vorup-Jensen et al., 2000). These experiments supported the general view that persisted for many years in textbooks stating that MASP-2 is the independent activator of the lectin pathway, and MASP-1 has only a supportive role, if any. Paradoxically, the minor component proved to be essential, while the major protease component seemed to be unnecessary in the activation process. It was also suggested that MASP-1 can cleave C3, generating C3b before the assembly of the C3 convertase complexes (Matsushita et al., 2000). Detailed enzyme kinetic studies, however, revealed that although MASP-1 really cleaves C3, the catalytic efficiency (k_{cat}/K_M) of this reaction is very low ($3X10^2$ $M^{-1}s^{-1}$) (Ambrus et al., 2003) compared to the efficiency of a real C3 convertase, which is three orders of magnitude higher. This low C3 cleaving activity of MASP-1 might be able to initiate the alternative pathway on cell surface, however, this hypothesis has yet to be proven. Interestingly, MASP-1 cleaves C3 containing a reacted thiolester bond (C3i) 20 times more efficiently than it cleaves native C3, however, this cleavage is much less efficient than the C3i cleavage by Factor I in the presence of Factor H, casting doubt on the physiological relevance of this reaction. The only complement component that is cleaved rather efficiently by MASP-1 is C2 ($3X10^4$ $M^{-1}s^{-1}$). This catalytic efficiency combined with the high concentration of MASP-1 in human serum could result in significant C2 cleavage during lectin pathway activation. Since MASP-1 cannot cleave C4, it was suggested that it can enhance the C3 generating ability of MASP-2 via C2 cleavage (Chen and Wallis, 2004).

2.2. MASP-2 is activated by MASP-1

A clue suggesting that MASP-1 may have a more significant role in lectin pathway activation came from the experiments using MASP-1/-3 deficient mouse serum. In lectin pathway activation experiments the MASP-1/-3 deficient mouse serum gave C3 and C4 deposition on mannan-coated surface but the effect was lower compared to the normal mouse serum. The activation of MASP-2 was significantly delayed in the deficient serum. The above anomalies could be abolished by adding recombinant MASP-1 to the MASP-1/-3 deficient mouse serum. These results suggested that MASP-1 is not essential, but it facilitates the activation process. It was proposed that "MASP-1 contributes to the activation of the lectin pathway, probably through the activation of MASP-2" (Takahashi et al., 2008).

In order to unambiguously clarify the role of MASP-1 and MASP-2 in lectin pathway activation in normal human serum, we developed selective inhibitors against the MASPs (Kocsis et al., 2010; Héja et al., 2012a; Héja et al., 2012b). The physiological inhibitors of the lectin pathway (C1-inhibitor and antithrombin) inhibit both MASP-1 and MASP-2, therefore they are not suitable for selective inhibition (Paréj et al., 2013). We used directed evolution technique (phage display) to select highly specific and efficient inhibitors. As starting scaffolds we used naturally occurring canonical trypsin inhibitors and subjected the inhibitory loops to *in vitro* selection in the presence of the proteases. We managed to select two efficient inhibitors: SGMI-1 exclusively inhibits MASP-1 (K_i=7 nM) while SGMI-2 exclusively inhibits MASP-2 (K_i=6 nM). As expected, inhibition of MASP-2 completely abolished lectin pathway activation: no C4, C3 and MAC deposition could be detected on activating (mannan-coated or acetylated BSA-coated) surfaces in the presence of SGMI-2. The big surprise came when we used the MASP-1 selective inhibitor in our experiments: lectin pathway activity was completely and permanently blocked just like in the case of MASP-2 inhibition. These experiments proved that both MASP-1 and MASP-2 are essential for lectin pathway activation in normal human serum. In order to figure out the role of MASP-1 in the activation process we performed two types of C4 cleavage experiments. In one experimental set up we pre-activated the MASPs in the serum on the activating surface before adding C4 and the inhibitors. In this case only SGMI-2 could prevent C4 deposition since only MASP-2 can cleave C4. If the MASPs are activated inhibition of MASP-1 has no effect on C4 cleavage. This picture changed dramatically when we added the inhibitors into fresh serum, which contains zymogen proteases, before incubating it on the activating surface. Inhibition of MASP-1 efficiently prevented the MASP-2 mediated C4 cleavage. This experiment unequivocally proved that the activity of MASP-1 is the prerequisite of MASP-2 activity, in other words if no MASP-1 activation occurs there will be no MASP-2 activation either. We can conclude that MASP-1 is the exclusive activator of MASP-2 in normal human serum (**Fig. 1B**). It was also quite surprising that the inhibition of MASP-1 permanently blocked MASP-2 activity, i.e. MASP-2 could not autoactivate even during long-time incubation on the activating surface. This result was reinforced using serum from a MASP-1/-3 deficient patient where the MASP-2 remained inactive due to the lack of MASP-1 activity (Degn et al., 2012). It seems very likely that the autoactivation capacity of MASP-2 plays no role in lectin pathway activation under physiological circumstances.

2.3. Autoactivation and C2 cleavage

Measuring the kinetic constants of the individual proteolytic reactions supported the central role of MASP-1 in lectin pathway activation (Megyeri et al., 2013). The zymogen autoactivation rate of MASP-2 ($1.4X10^{-1}$ $M^{-1}s^{-1}$) is negligible compared to that of MASP-1 ($4.5X10^{2}$ $M^{-1}s^{-1}$). Active MASP-1 cleaves zymogen MASP-1 ($8.3X10^{4}$ $M^{-1}s^{-1}$) and zymogen MASP-2 ($1.2X10^{4}$ $M^{-1}s^{-1}$) extremely efficiently. Actually, MASP-1 cleaves zymogen MASP-2 20-times more efficiently than active MASP-2 cleaves its own zymogen. The initial enzymatic reaction in lectin pathway activation is the autoactivation of MASP-1. SGMI-1, which prevents autoactivation of MASP-1, is an efficient reagent to prevent lectin pathway activation. Using our selective inhibitors we could also determine the individual contributions of MASP-1 and MASP-2 to the C2 cleavage. It turned out that MASP-1 cleaves the majority (60%) of C2 which is incorporated into the C3 convertase (Héja et al., 2012b). This result validated the former hypothesis about the cooperation between MASP-1 and MASP-2 in the C3 convertase generation (Møller-Kristensen et al., 2007) and also underpinned the dominant role of MASP-1.

2.4. Inhibition in whole blood

An interesting observation is that while the K_i values of the two SGMIs are almost identical, the IC_{50} values in different assay systems are systematically smaller when using SGMI-1 than using SGMI-2, and this difference is much more pronounced in undiluted serum and in whole blood ($IC_{50\ SGMI-1}$ = 1.1 μM vs. $IC_{50\ SGMI-2}$ = 55.6 μM in C3 deposition assay) (Héja et al., 2012b). This phenomenon can be explained if we suppose that beside MASP-2, MASP-1 also activates another protease (or proteases) which exerts its function in concentrated serum. In this respect it is worth mentioning that we showed that MASP-1 cleaves and activates MASP-3 quite efficiently (Megyeri et al., 2013). Although we do not know the physiological role of MASP-3, it was suggested that it might contribute to alternative pathway activation which is efficient only in concentrated serum (Iwaki et al., 2011).

3. The structure of MASP-1 and related proteins

3.1 Structural basis of dimerization

MASPs (MASP-1, MASP-2, and MASP-3) along with the classical pathway proteases, C1r and C1s form a family with identical domain structures (Gál et al., 2009). The

serine protease (SP) domain is preceded by five regulatory domains in an array of CUB1-EGF-CUB2-CCP1-CCP2. The dimerization fragment of MASPs is the CUB-EGF region (Feinberg et al., 2003; Teillet et al., 2008) through which MASPs form tight Ca^{2+}-dependent dimers (Paréj et al., 2014, Rosbjerg et al., 2014). It is notable that two other proteins exist in circulation derived from the MASP genes, but lacking protease activity. MAp19 is composed of the CUB1-EGF domains of MASP-2 plus 4 unique C-terminal residues, while MAp44 has a domain sequence of CUB1-EGF-CUB2-CCP1 (identical with the MASP-1/3 domains) plus it has 17 unique C-terminal residues. Both proteins have the capacity to dimerize (Gregory et al., 2004; Skjoedt et al., 2012) and interact with the pattern recognition molecules analogously with MASPs. Probably the best quality structure is the 2.3 Å resolution structure of MASP-1/3 CUB1-EGF-CUB2 fragment, which clearly shows Ca^{2+} ions bound to all three domains. The CUB1-EGF segments dimerize in a head-to-tail arrangement, with the EGF module of each subunit forming extensive contact with the CUB1 module of the other subunit. The CUB2 domain does not participate in the dimerization, however, it provides additional binding sites for the collagen segment of the recognition molecules (Gingras et al., 2011). Interestingly *in vitro* MASP-1 and MASP-2 can form heterodimers with one another as efficiently as they form homodimers with themselves (Paréj et al., 2014). The relevance of this heterodimer formation *in vivo* is not yet known. Based on the structure of MASP-1/3 N-terminal fragment (Teillet et al., 2008), that of MAp44 (Skjoedt et al., 2012), and the structure of MASP-1 C-terminal fragment (Dobó et al., 2009) a model of full length MASP-1 dimer is presented in **Fig. 2A**.

3.2. Proenzyme MASP structures

By now the structures of the CCP1-CCP2-SP fragments for all three MASP proenzymes have been determined (Gál et al., 2005; Megyeri et al., 2013; Yongqing et al., 2013). The first in the series described the structure of zymogen MASP-2 determined at 2.4 Å resolution. The MASP-2 proenzyme fragment has a remarkably high activity on C4 substrate. The basis of this high activity could lie in the fact that MASP-2 interacts with C4, in addition to the canonical binding site, through two exosites that stabilize the MASP-2:C4 interaction (Kidmose et al., 2012; Kjaer et al., 2013). Interaction with C4 presumably induces a conformational change in proenzyme MASP-2 that renders the serine protease domain to active conformation by an induced fit mechanism. Nevertheless MBL-bound proenzyme MASP-2 does not cleave C4 in the fluid phase (Chen and Wallis, 2004), because MBL probably occludes the accessory binding sites. Proenzyme MASP-2 is rather inactive on small

substrates, and its zymogen autoactivation rate is very low. In contrast, MASP-1 zymogen is fairly active on small substrates, and it autoactivates rapidly due to its relatively fast zymogen autoactivation step (Megyeri et al., 2013). Because the zymogenicity of MASP-1 shows very little substrate dependence, it is presumable that zymogen MASP-1 fluctuates between proenzymic and active-like forms in solution via the conformational selection mechanism spending about 1/200 fraction of its time in the active-like conformation (Megyeri et al., 2013). The 2.5 Å structure of zymogen MASP-1 shows the enzyme "frozen" in the proenzyme conformation, however the structure provides some clues why it can easily turn into an active form (**Fig. 2B**). The catalytic triad of zymogen MASP-1 is in an active-like conformation, while the S1 pocket is blocked and the oxyanion hole is distorted, which are typical features of zymogen serine proteases. Upon activation, the new amino-terminus forms a salt-bridge with Asp^{645} (Asp^{c194}, "c" stands for chymotrypsin numbering) rearranging the surface loops and allowing the oxyanion hole and the S1 pocket to form. In the structure of zymogen MASP-1 two positive side chains might be able to substitute for the amino-terminus aiding Asp^{c194} to rotate to the active-like position (**Fig. 2B**).

Recently the structure of the zymogen MASP-3 G666E (Gly^{c197} to Glu) mutant has been solved at 2.6 Å resolution (Yongqing et al., 2013). This mutation is one among the several genetic alterations causing the 3MC syndrome, a developmental disorder caused by mutations in the *MASP1* or the *COLEC11* genes (Sirmaci et al., 2011; Rooryck et al., 2011). This mutation is found in the very conserved Gly-Asp-Ser^{c195}-Gly-Gly sequence, two residues away from the catalytic Ser residue, hence it is no wonder that this mutant is inactive in the cleaved ("activated") form of this enzyme. The large negative side chain removes the catalytic Ser side chain from its active position rendering the enzyme to be inactive. The structure of zymogen MASP-3 also shows that the CCP1-CCP2 and the CCP2-SP interfaces have some flexibility. Interestingly, while mutant proenzyme and inhibited active MASP-3 (Gaboriaud et al., 2013) fragments have been crystallized successfully, the wild-type enzyme by itself has so far resisted crystallization.

3.3. The structure of MASP-1 reflects its broad specificity

MASP-1, compared to the related proteases (MASP-2, MASP-3, C1r, C1s), has a high number of substrates. It autoactivates rapidly, cleaves MASP-2, MASP-3, C2, and C3i among complement components, and it was shown to cleave fibrinogen, FXIII, high molecular weight kininogen, PAR4, and even prothrombin (see later). From an evolutionary point of view the SP domain of MASP-1 differs from those of the other four members (Endo et al.,

1998; Dahl et al., 2001). The most important difference, from a structural point of view, is that MASP-1 has four disulfide bridges, while MASP-2, MASP-3, C1r, and C1s have only three in the SP domain, lacking the so-called histidine loop, a disulfide bond usually present in trypsin-like serine proteases. In many respect the SP domain of MASP-1 is closer to thrombin and in some respect to trypsin, than to the SP domains of the other members of the family. This difference is also reflected in its overall structure. Comparison of the surface area around the substrate-binding pocket (Dobó et al., 2009) revealed that MASP-1 has a relatively large open cleft allowing protein substrates to access the substrate-binding site (**Fig. 2C**). Although MASP-1 is relatively unspecific, it has some characteristics that restrict its activity and specificity. An internal salt bridge between the S1 Asp^{c189} and an Arg side chain, and a large surface loop on the substrate-binding side of the enzyme, the so-called 60-loop, are likely to contribute to the specific characteristics of MASP-1.

4. The role of MASP-1 in cell activation

4.1. Endothelial cell activation by MASP-1

Serine proteases are quite diverse in regard to their substrate selectivity. Thrombin is a promiscuous serine protease with an open long substrate binding cleft (Bode et al., 1989). It can cleave proteins belonging to the coagulation pathway as well as transmembrane receptors on various cells initiating pro-inflammatory signaling. As we have seen, MASP-1 also has an open, relatively wide substrate binding cleft, similar to that of thrombin (Dobó et al., 2009). Therefore, it is not surprising that MASP-1 also has multiple substrates. It can cleave several members of protease activated receptors (PARs): PAR-1, PAR-2 and PAR-4 (**Fig. 3**). However, the relative efficiency towards the specific PARs is somewhat different from that of thrombin. MASP-1 prefers PAR-4 over PAR-1 and PAR-2, whereas thrombin cleaves PAR-1 better than PAR-4 and has no effect on PAR-2 (Megyeri et al., 2009). Taking into account that distinct PARs have completely different expression profiles, signaling, recycling, desensitization and heterocomplex formation capacity (Adams et al., 2011), it is not unexpected that there are differences in the effects of thrombin and MASP-1 (**Fig. 3**). Thrombin induces a typical pro-inflammatory and pro-coagulant phenotype in endothelial cells. Thrombin activated endothelial cells produce cytokines/chemokines to attract and activate several types of white blood cells including monocytes and neutrophil granulocytes (Marin et al., 2001; Kawanami et al., 2011). In contrast, MASP-1 alone induces IL-6 and IL-8

production of endothelial cells mainly driven by p38 MAPK, which leads to chemotaxis of neutrophils but not monocytes (**Fig. 3**) (Jani et al., 2014). Moreover, MASP-1 elicits a transient elevation in E-Selectin expression in HUVECs leaving the levels of ICAM-1 and VCAM-1 unchanged (unpublished data), which also favors neutrophil transmigration. Various infections and other diseases are associated with the infiltration of very different white blood cell populations (e.g. in viral and intracellular bacterial infections as well as in atherosclerosis mostly monocytes/macrophages and T cells are recruited, whereas in extracellular bacterial and fungal infections, and also in tissue necrosis a massive neutrophil infiltration can be detected, followed by monocytes later on). It means that the difference in endothelial cell activation profiles elicited by thrombin and MASP-1 might be a sensitive regulatory mechanism by which the most relevant parts of the immune system are recruited. Thrombin is activated when blood vessels are injured, which requires a complete remodeling of the affected tissues coordinated by macrophages but also requires an active anti-bacterial protection by neutrophil granulocytes. MASP-1 is activated mainly by bacterial/fungal infections, where neutrophil chemotaxis and activation is indispensable. However, MASP-1 elevates mRNA expression of MCP-1, TNF-α, ICAM-1 and VCAM-1 (Jani et al., 2014, and unpublished data), which may potentiate monocyte and T cell attraction if the infection becomes more serious. Here we compared the differential effects of MASP-1 and thrombin on the cellular immune response for didactical purposes, however, *in vivo* these two proteases as well as the two respective cascade systems may act synergistically, or complement each other's function (see below, part "The role of MASP-1 in the coagulation").

4.2. Only MASP-1 activates endothelial cells among lectin pathway components

We have to note that amongst lectin pathway proteases only MASP-1 has the properties described above (Megyeri et al., 2009; 2014). In contrast to MASP-1, MASP-2 has a partly occluded substrate binding cleft, and has only two major substrates - C2 and C4 (Ambrus et al., 2003). Therefore, it is not unexpected that MASP-2 does not cleave any PARs nor has any effects on endothelial cells (Megyeri et al., 2009). We know much less about MASP-3 substrates and enzyme activity, but we know that it cannot activate endothelial cells similarly to MASP-2 (Megyeri et al., 2014). Although MBL binds to endothelial cells, it cannot activate them (Oroszlán et al., 2007). Moreover, MBL-MASP-1 complex formation does not affect the activation of endothelial cells by MASP-1 (Megyeri et al., 2014), which might not be evident considering the large size of the complex. Since the cellular effects of

MASP-1 can be completely abolished by C1-inhibitor or by using catalytically inactive S646A mutant MASP-1, it is reasonable to consider PARs as the only receptors of MASP-1 on endothelial cells. The putative *in vivo* relevance of MASP-1 induced endothelial cell activation would be questionable if zymogen and active forms of the serine protease could equivalently exert their action. However, stable proenzyme MASP-1 mutant (R448Q) has no effects on endothelial cells (Megyeri et al., 2014), which means that MASP-1 induces endothelial cells only if it becomes activated by pathogen- or danger-associated molecular patterns (PAMPs or DAMPs, respectively).

4.3. Importance of MASP-1 induced endothelial cell activation during various physiological and pathological conditions

It is important to define, which molecular patterns may trigger MASP-1 induced endothelial cell activation and, as a consequence, a boosted anti-microbial immune response. Microbial sugar motives are the targets of MBL, whereas DNA, modified lipids and sugars are recognized by CL-K1 and ficolins (Matsushita et al., 2013; Henriksen et al., 2013). These data together suggest that beside microbial components, necrotic debris and aberrantly modified self macromolecules may also trigger MASP-1 induced cell activation. Indeed, in necrotic tissues evolved upon infarction, burning, edema, inflammation etc. neutrophil granulocyte infiltration is a common phenomenon. Mitochondrial formyl-peptides have long been known to directly attract neutrophils (Schiffmann et al., 1975) but this process can possibly be enhanced by nucleic acid triggered CL-K1/MASP-1 activation and as a consequence, IL-8 production by endothelial cells.

4.4 Cleavage of high molecular weight kininogen by MASP-1

Bradykinin, a vasoactive pro-inflammatory peptide, has an unquestionable role in hereditary angioedema (HAE), however, several types of triggering factors are needed to provoke edematous attacks (Zotter et al., 2014). Microbial infection, trauma and menstruation are three well known triggering factors of HAE attacks. Fibrin clot formed during trauma or menstruation may also activate MASP-1 via ficolin (Endo et al., 2010). Recently we showed that recombinant MASP-1 catalytic fragment and also MBL-MASP complexes can cleave high molecular weight kininogen, and we also demonstrated the release of bradykinin (Dobó et al., 2011). Although the cleavage efficiency was two orders of magnitude lower than for kallikrein, it is presumable that in the absence of other triggers bradykinin released by MASP-1 can contribute to a more pronounced immune response in healthy individuals and elevated

levels of bradykinin in HAE patients. Since the majority of HAE cases are caused by functional C1-inhibitor deficiency, and C1-inhibitor is a major natural inhibitor of MASP-1, MASP-1 can be a contributing factor in the pathogenesis of HAE.

Taken together, the cellular effects of MASP-1 may be a two-edged sword. In most microbial infections MASP-1 can enhance the effectiveness of immune response by boosting neutrophil granulocytes chemotaxis and activation via endothelial cells. On the other hand, if potent lectin pathway activators, such as modified lipids or DNA, are released and/or the natural inhibitors are depleted, MASP-1 can enhance tissue destruction or edema formation in several diseases. If this latter presumption is proved to be true, highly specific inhibitors of MASP-1 may be considered as supplementary therapy in these cases.

5. The role of MASP-1 in blood coagulation

5.1 Interactions between complement and coagulation in cardiovascular diseases

Cardiovascular diseases still represent a major cause for morbidity and mortality in industrialized countries. We know that these diseases have a strong inflammatory component and there is increasing evidence for a prominent role of the complement system. In particular, many interactions between complement and coagulation seem to aggravate pathophysiological processes, promote the development of atherosclerosis and atherothrombosis, and lead to more severe complications (Oksjoki et al., 2007; Speidl et al., 2011; Frauenknecht and Schroeder, 2012; Duehrkop and Rieben, 2014).

For example, localized complement activation detected at the site of acute coronary thrombus formation is thought to amplify the vascular occlusion process in acute myocardial infarction (MI) via neutrophil recruitment (Distelmaier et al., 2009). But complement components also directly influence characteristics of fibrin clots (Shats-Tseytlina et al., 1994). This is of pathophysiological relevance since fibrin clots with tightly packed, thin fibers are more resistant to fibrinolysis and are associated with a higher risk for thrombotic complications (Undas and Ariens, 2011). We have recently shown that complement C3 becomes incorporated into fibrin clots and prolongs fibrinolysis (Richardson et al., 2013; Howes et al., 2012). Since C3 plasma levels are increased in patients with cardiovascular risk factors, especially with type-2 diabetes mellitus (T2DM), and vascular diseases, the incorporation of C3 into forming fibrin may contribute to the development of thrombotic complications (Schroeder et al., 2010; Hess et al., 2012a).

Not only do complement components influence fibrin clot formation and structure, also activated coagulation factors are able to cleave complement components and induce and/or alter their effects. Recently, described as a new complement activation pathway, thrombin is capable of activating C5 introducing a new cleavage site that leads to a membrane attack complex with significantly more lytic activity than C5b-9 generated by C5 convertase (Huber-Lang et al., 2006; Krisinger et al., 2012). Fibrinogen/fibrin may serve as a matrix for binding of complement components, in particular ficolins and MBL and thereby augment the lectin pathway (Endo et al., 2010) and this may have implications in thrombotic diseases as described below.

5.2 MASP-1 interacts with coagulation factors and affects clot formation

MASP-1 is a serine protease that shares many characteristics with thrombin, the executive serine protease in the coagulation cascade, in terms of structure, substrate specificity, and inhibitors (Ambrus et al., 2003; Presanis et al., 2004; Dobó et al., 2009). Furthermore, MASP-1 cleaves proteins which are thrombin substrates in the coagulation cascade such as fibrinogen, factor XIII (FXIII), and thrombin-activatable fibrinolysis inhibitor (TAFI) (Krarup et al., 2008; Gulla et al., 2010; Hess et al., 2012b). Thrombin activates fibrinogen by releasing fibrinopeptides A and B (FPA, FPB) from the N-termini of fibrinogen α- and β-chains, respectively. MASP-1, however, has been shown to release FPB, but not FPA cleaving the α-chain at different sites (Krarup et al., 2008). Therefore it is doubted that is capable of inducing fibrin formation directly. MASP-1 is able to activate FXIII, the fibrin crosslinking factor, directly, albeit at a lower catalytic efficiency compared with thrombin (Krarup et al., 2008; Hess et al., 2012b). Similarly, MASP-1 can also activate TAFI (Hess et al., 2012b) which inhibits fibrin-dependent fibrinolysis by tissue plasminogen activator (t-PA). It was reported earlier that MASP-2 is able to activate prothrombin (Krarup et al., 2007). We have shown recently that MASP-1 also activates prothrombin and there is evidence that its effects on clot formation are mainly mediated by prothrombin activation (Hess et al., 2012b; Jenny et al., 2013; and own unpublished data). Taken together, MASP-1 promotes fibrin formation, exerts synergistic effects to thrombin in plasma and whole blood clot formation, and also influences fibrin structure (**Fig. 4**) (Gulla et al., 2010; Hess et al., 2012b; Jenny et al., 2013). These effects on coagulation factors and clot formation are enhanced by activation of endothelial cells (Megyeri et al., 2009). As a consequence, a prothrombotic environment is created that is prone to vessel occlusion. First *in vivo* evidence for an involvement of MASP-1 in coagulation and thrombus formation was obtained from MASP-1

and MBL knockout mice which showed prolonged bleeding time on tail tip excision (Takahashi et al., 2011) and a significant decrease in $FeCl_3$-induced thrombogenesis (La Bonte et al., 2012).

5.3 MASPs in cardiovascular diseases

MASP-1 seems to be a major link between the complement system and coagulation, yet the physiological relevance of results obtained in purified and non-human systems remains to be confirmed. In a first step, clinical studies have therefore looked at plasma levels of MASP-1, as well as MASP-2, MASP-3, and MAp44 in patients with cardio- and cerebrovascular diseases. In a pilot study, we found increased MASP-1 plasma levels in patients in the subacute phase of MI, but decreased levels of MASP-1 in patients with acute ischemic stroke (Frauenknecht et al., 2013). MASP-2 levels were lower in both MI and stroke patients compared with healthy controls, while MASP-3 and MAp44 did not differ between groups (Frauenknecht et al., 2013). In a cohort study of individuals with T2DM, MASP-2 levels were significantly decreased in patients with acute cardiovascular events (Mellbin et al., 2012). MASP-2 levels were also lower in another study in patients with acute MI (Zhang et al., 2013). These first data suggest that MASP-1 and MASP-2, both of which interact with coagulation, are altered in patients with acute cardio- and cerebrovascular diseases. Whether these alterations are indeed associated with activation of the coagulation system and contribute to the vascular event, remains to be elucidated in further studies.

6. Conclusions

After more than two decades of its discovery, MASP-1 is still one of the most intriguing proteases of the complement system whose physiological importance and relevance to health and disease is not completely understood. During the last few years, new and important discoveries were made on the biochemistry and structure of MASP-1, and its central role in lectin pathway activation has been unambiguously demonstrated. Structural data and enzymatic measurements proved that MASP-1 has a relaxed substrate specificity, compared to the other complement proteases. While the other members of the C1r/C1s/MASP protease family have only a few physiological substrates, MASP-1 was shown to cleave more than a dozen proteins in the human blood (Table 1). This relaxed substrate specificity enables MASP-1 to establish connections between the different proteolytic cascade systems and to directly activate cells. In the future more translational studies will be needed to investigate the *in vivo* relevance of the putative functions of this interesting serine protease.

Acknowledgements

This work was supported by the Hungarian Scientific Research Fund (OTKA) grants NK100834, NK100769, K100684 and NK108642 and János Bolyai Research Fellowships of the Hungarian Academy of Sciences.

References

Adams MN, Ramachandran R, Yau MK, Suen JY, Fairlie DP, Hollenberg MD, Hooper JD., 2011. Structure, function and pathophysiology of protease activated receptors. Pharmacol Ther. 130(3):248-82.

Amara, U., Flierl, M.A., Rittirsch, D., Klos, A., Chen, H., Acker, B., Brückner, U.B., Nilsson, B., Gebhard, F., Lambris, J.D., Huber-Lang, M., 2010. Molecular intercommunication between the complement and coagulation systems. J. Immunol. 185, 5628-5636.

Ambrus, G., Gál, P., Kojima, M., Szilágyi, K., Balczer, J., Antal, J., Gráf, L., Laich, A., Moffatt, B.E., Schwaeble, W., Sim, R.B., Závodszky, P., 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.

Chen, C.B., Wallis, R., 2004. Two mechanisms for mannose-binding protein modulation of the activity of its associated serine proteases. J. Biol. Chem. 279, 26058-26065.

Bode, W., Mayr, I., Baumann, U., Huber, R., Stone, S.R., Hofsteenge, J., 1989. The refined 1.9 A crystal structure of human alpha-thrombin: interaction with D-Phe-Pro-Arg chloromethylketone and significance of the Tyr-Pro-Pro-Trp insertion segment. EMBO J. 8, 3467-3475.

Dahl, M.R., Thiel, S., Matsushita, M., Fujita, T., Willis, A.C., Christensen, T., Vorup-Jensen, T., Jensenius, J.C., 2001. MASP-3 and its association with distinct complexes of the mannan-binding lectin complement activation pathway. Immunity 15, 127-135.

Degn, S.E., Jensen, L., Hansen, A.G., Duman, D., Tekin, M., Jensenius, J,C., Thiel, S., 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.

Degn, S.E, Thiel, S., 2013. Humoral pattern recognition and the complement system. Scand. J. Immunol. 78, 181-193.

Distelmaier, K., Adlbrecht, C., Jakowitsch, J., Winkler, S., Dunkler, D., Gerner, C., Wagner, O., Lang, I.M., Kubicek, M., 2009. Local complement activation triggers neutrophil recruitment to the site of thrombus formation in acute myocardial infarction. Thromb. Haemost. 102, 564–572.

Dobó, J., Harmat, V., Beinrohr, L., Sebestyén, E., Závodszky, P., Gál, P., 2009. MASP-1, a promiscuous complement protease: structure of its catalytic region reveals the basis of its broad specificity. J. Immunol. 183, 1207-1214.

Dobó, J., Major, B., Kékesi, K.A., Szabó, I., Megyeri, M., Hajela, K., Juhász, G., Závodszky, P., Gál, P., 2011. Cleavage of kininogen and subsequent bradykinin release by the

complement component: mannose-binding lectin-associated serine protease (MASP)-1. PLoS One 6, e20036.

Duehrkop, C., Rieben, R., 2014. Ischemia/reperfusion injury: Effect of simultaneous inhibition of plasma cascade systems versus specific complement inhibition. Biochem. Pharmacol. 88, 12-22.

Endo, Y., Takahashi, M., Nakao, M., Saiga, H., Sekine, H., Matsushita, M., Nonaka, M., Fujita, T., 1998. Two lineages of mannose-binding lectin-associated serine protease (MASP) in vertebrates. J Immunol. 161, 4924-4930.

Endo, Y., Nakazawa, N., Iwaki, D., Takahashi, M., Matsushita, M., Fujita, T., 2010. Interactions of ficolin and mannose-binding lectin with fibrinogen/fibrin augment the lectin complement pathway. J. Innate Immun. 2, 33-42.

Feinberg, H., Uitdehaag, J.C., Davies, J.M., Wallis, R., Drickamer, K., Weis, W.I., 2003. Crystal structure of the CUB1-EGF-CUB2 region of mannose-binding protein associated serine protease-2. EMBO J. 22, 2348-2359.

Frauenknecht, V., Schroeder, V., 2012. Complement - a phylogenetically old system as a new player in the development of atherosclerosis. Hämostaseologie 32, 276-285. [Article in German]

Frauenknecht, V., Thiel, S., Storm, L., Meier, N., Arnold, M., Schmid, J.P., Saner, H., Schroeder, V., 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.

Gaboriaud, C., Thielens, N.M., Gregory, L.A., Rossi, V., Fontecilla-Camps, J.C., Arlaud, G.J., 2004. Structure and activation of the C1 complex of complement: unraveling the puzzle. Trends Immunol. 25, 368-373.

Gaboriaud, C., Gupta, R.K., Martin, L., Lacroix, M., Serre, L., Teillet, F., Arlaud, G.J., Rossi, V., Thielens, N.M., 2013. The serine protease domain of MASP-3: enzymatic properties and crystal structure in complex with ecotin. PLoS One. 8, e67962.

Gál, P., Harmat, V., Kocsis, A., Bián, T., Barna, L., Ambrus, G., Végh, B., Balczer, J., Sim, R.B., Náray-Szabó, G., Závodszky, P., 2005. A true autoactivating enzyme. Structural insight into mannose-binding lectin-associated serine protease-2 activations. J. Biol. Chem. 280, 33435-33444.

Gál, P., Dobó, J., Závodszky, P., Sim, R.B., 2009. Early complement proteases: C1r, C1s and MASPs. A structural insight into activation and functions. Mol. Immunol. 46, 2745-2752.

Gingras, A.R., Girija, U.V., Keeble, A.H., Panchal, R., Mitchell, D.A., Moody, P.C., Wallis, R., 2011. Structural basis of mannan-binding lectin recognition by its associated serine protease MASP-1: implications for complement activation. Structure 19, 1635-1643.

Gregory, L.A., Thielens, N.M., Matsushita, M., Sorensen, R., Arlaud, G.J., Fontecilla-Camps, J.C., Gaboriaud, C., 2004. The X-ray structure of human mannan-binding lectin-associated protein 19 (MAp19) and its interaction site with mannan-binding lectin and L-ficolin. J Biol Chem. 279, 29391-29397

Gulla, K.C., Gupta, K., Krarup, A., Gál, P., Schwaeble, W.J., Sim, R.B., O'Connor, C.D., Hajela, K., 2010. Activation of mannan-binding lectin-associated serine proteases leads to generation of a fibrin clot. Immunology 129, 482-495.

Héja, D., Harmat, V., Fodor, K., Wilmanns, M., Dobó, J., Kékesi, K.A., Závodszky, P., Gál, P., and Pál, G. (2012a) Monospecific inhibitors show that both mannan-binding lectin-associated serine protease-1 (MASP-1) and -2 Are essential for lectin pathway activation and reveal structural plasticity of MASP-2. *J. Biol. Chem.* **287**, 20290-20300

Héja, D., Kocsis, A., Dobó, J., Szilágyi, K., Szász, R., Závodszky, P., Pál, G., and Gál, P. (2012b) 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. USA* **109,** 10498-10503

Henriksen ML, Brandt J, Iyer SS, Thielens NM, Hansen S., 2013. Characterization of the interaction between collectin 11 (CL-11, CL-K1) and nucleic acids. Mol Immunol. 56, 757-67.

Hess, K., Alzahrani, S.H., Mathai, M., Schroeder, V., Carter, A.M., Howell, G., Koko, T., Strachan, M.W., Price, J.F., Smith, K.A., Grant, P.J., Ajjan, R.A., 2012a. A novel mechanism for hypofibrinolysis in diabetes: the role of complement C3. Diabetologia 55, 1103-1113.

Hess, K., Ajjan, R., Phoenix, F., Dobó, J., Gál, P., Schroeder, V., 2012b. Effects of MASP-1 of the complement system on activation of coagulation factors and plasma clot formation. PLoS One 7, e35690.

Howes, J.M., Richardson, V.R., Smith, K.A., Schroeder, V., Somani, R., Shore, A., Hess, K., Ajjan, R., Pease, R.J., Keen, J.N., Standeven, K.F., Carter, A.M., 2012. Complement C3 is a novel plasma clot component with anti-fibrinolytic properties. Diab. Vasc. Dis. Res. 9, 216-225.

Huber-Lang, M., Sarma, J.V., Zetoune, F.S., Rittirsch, D., Neff, T.A., McGuire, S.R., Lambris, J.D., Warner, R.L., Flierl, M.A., Hoesel, L.M., Gebhard, F., Younger, J.G., Drouin, S.M., Wetsel, R.A., Ward, P.A., 2006. Generation of C5a in the absence of C3: a new complement activation pathway. Nat. Med. 12, 682-687.

Iwaki, D., Kanno, K., Takahashi, M., Endo, Y., Matsushita, M., Fujita, T., 2011. The role of mannose-binding lectin-associated serine protease-3 in activation of the alternative complement pathway. J. Immunol. 187, 3751-3758.

Jani PK, Kajdácsi E, Megyeri M, Dobó J, Doleschall Z, Futosi K, Tímár CI, Mócsai A, Makó V, Gál P, Cervenak L., 2014. MASP-1 induces a unique cytokine pattern in endothelial cells: a novel link between complement system and neutrophil granulocytes. PLoS One. 9, e87104.

Jenny, L., Dobó, J., Gál, P., Schroeder, V., 2013. Thrombelastographic studies reveal a new mechanism in MASP-1-induced fibrin clotting. Mol. Immunol. 56, 266 [abstract].

Kawanami, D., Matoba, K., Kanazawa, Y., Ishizawa, S., Yokota, T., Utsunomiya, K., 2011. Thrombin induces MCP-1 expression through Rho-kinase and subsequent p38MAPK/NF-κB signaling pathway activation in vascular endothelial cells. Biochem. Biophys. Res. Commun. 411, 798-803.

Kidmose, R.T., Laursen, N.S., Dobó, J., Kjaer, T.R., Sirotkina, S., Yatime, L., Sottrup-Jensen, L., Thiel, S., Gál, P., Andersen, G.R., 2012. Structural basis for activation of the complement system by component C4 cleavage. Proc. Natl. Acad. Sci. USA 109, 15425-15430.

Kjaer, T.R., Thiel, S., Andersen, G.R., 2013. Toward a structure-based comprehension of the lectin pathway of complement. Mol. Immunol. 56, 413-422.

Krarup, A., Wallis, R., Presanis, J.S., Gál, P., Sim, R.B., 2007. Simultaneous activation of complement and coagulation by MBL-associated serine protease 2. PLoS One 2, e623.

Krarup, A., Gulla, K.C., Gál, P., Hajela, K., Sim, R.B., 2008. The action of MBL-associated serine protease 1 (MASP1) on factor XIII and fibrinogen. Biochim. Biophys. Acta 1784, 1294-1300.

Krisinger, M.J., Goebeler, V., Lu, Z., Meixner, S.C., Myles, T., Pryzdial, E.L., Conway, E.M., 2012. Thrombin generates previously unidentified C5 products that support the terminal complement activation pathway. Blood 120, 1717-1725.

La Bonte, L.R., Pavlov, V.I., Tan, Y.S., Takahashi, K., Takahashi, M., Banda, N.K., Zou, C., Fujita, T., Stahl, G.L., 2012. Mannose-binding lectin-associated serine protease-1 is a significant contributor to coagulation in a murine model of occlusive thrombosis. J. Immunol. 188, 885-891.

Marin, V., Farnarier, C., Grès, S., Kaplanski, S., Su, M.S., Dinarello, C.A., Kaplanski, G., 2001. The p38 mitogen-activated protein kinase pathway plays a critical role in thrombin-induced endothelial chemokine production and leukocyte recruitment. Blood. 98, 667-673.

Markiewski, M.M., Nilsson, B., Nilsson Ekdahl, K., Mollnes, T.E., Lambris, J.D., 2007. Complement and coagulation: strangers or partners in crime? Trends Immunol. 28, 184-192.

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.

Matsushita, M., Thiel, S., Jensenius, J.C., Terai, I., Fujita, T., 2000. Proteolytic activities of two types of mannose-binding lectin-associated serine protease. J. Immunol. 165, 2637-2642.

Matsushita, M., Endo, Y., Fujita, T., 2013. Structural and functional overview of the lectin complement pathway: its molecular basis and physiological implication. Arch. Immunol. Ther. Exp. 61, 273-283.

Megyeri, M., Harmat, V., Major, B., Végh, Á., Balczer, J., Héja, D., Szilágyi, K., Datz, D., Pál, G., Závodszky, P., Gál, P., Dobó, J., 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.

Megyeri, M., Makó, V., Beinrohr, L., Doleschall, Z., Prohászka, Z., Cervenak, L., Závodszky, P., Gál, P., 2009. Complement protease MASP-1 activates human endothelial cells: PAR4 activation is a link between complement and endothelial function. J. Immunol. 183, 3409-3416.

Megyeri M, Jani PK, Kajdácsi E, Dobó J, Schwaner E, Major B, Rigó J Jr, Závodszky P, Thiel S, Cervenak L, Gál P., 2014. Serum MASP-1 in complex with MBL activates endothelial cells. Mol Immunol. 59, 39-45.

Mellbin, L.G., Bjerre, M., Thiel, S., Hansen, T.K., 2012. Complement activation and prognosis in patients with type 2 diabetes and myocardial infarction: a report from the DIGAMI 2 trial. Diabetes Care 35, 911-917.

Møller-Kristensen, M., Thiel, S., Sjöholm, A., Matsushita, M., and Jensenius J. C., 2007. Cooperation between MASP-1 and MASP-2 in the generation of C3 convertase through the MBL pathway. Int. Immunol. 19, 141-149.

Oikonomopoulou, K., Ricklin, D., Ward, P.A., Lambris, J.D., 2012. Interactions between coagulation and complement - their role in inflammation. Semin. Immunopathol. 34, 151-165.

Oksjoki, R., Kovanen, P.T., Meri, S., Pentikainen, M.O., 2007. Function and regulation of the complement system in cardiovascular diseases. Front. Biosci. 12, 4696–4708.

Oroszlán M, Daha MR, Cervenak L, Prohászka Z, Füst G, Roos A., 2007. MBL and C1q compete for interaction with human endothelial cells. Mol Immunol. 44, 1150-8.

Paréj, K., Dobó, J., Závodszky, P., Gál, P., 2013. The control of the complement lectin pathway activation revisited: both C1-inhibitor and antithrombin are likely physiological inhibitors, while α2-macroglobulin is not. Mol. Immunol. 54, 415-422.

Paréj, K., Hermann, Á., Donáth, N., Závodszky, P., Gál, P., Dobó, J., 2014. Dissociation and re-association studies on the interaction domains of mannan-binding lectin (MBL)-associated serine proteases, MASP-1 and MASP-2, provide evidence for heterodimer formation. Mol Immunol. 59, 1-9.

Presanis, J.S., Hajela, K., Ambrus, G., Gál, P., Sim, R.B., 2004. Differential substrate and inhibitor profiles for human MASP-1 and MASP-2. Mol. Immunol. 40, 921-929.

Richardson, V.R., Schroeder, V., Grant, P.J., Standeven, K.F., Carter, A.M., 2013. Complement C3 is a substrate for activated factor XIII that is cross-linked to fibrin during clot formation. Br. J. Haematol. 160, 116-119.

Rooryck, C., Diaz-Font, A., Osborn, D.P., Chabchoub, E., Hernandez-Hernandez, V., Shamseldin, H., Kenny, J., Waters, A., Jenkins, D., Kaissi, A.A., Leal, G.F., Dallapiccola, B., Carnevale, F., Bitner-Glindzicz, M., Lees, M., Hennekam, R., Stanier, P., Burns, A.J., Peeters, H., Alkuraya, F.S., Beales, P.L., 2011. Mutations in lectin complement pathway genes COLEC11 and MASP1 cause 3MC syndrome. Nat. Genet. 43, 197-203.

Rosbjerg, **A.**, Munthe-Fog, L., Garred, P., Skjoedt, M.O., 2014 Heterocomplex Formation between MBL/Ficolin/CL-11-Associated Serine Protease-1 and -3 and MBL/Ficolin/CL-11-Associated Protein-1. J. Immunol. 192, 4352-4360.

Schiffmann, E., E. A. Corcoran, and S. M. Wahl., 1975. N-formylmethionyl peptides as chemoattractants for leukocytes. Proc. Natl. Acad. Sci. USA 72, 1059.

Schroeder, V., Carter, A.M., Dunne, J., Mansfield, M.W., Grant, P.J., 2010. Proinflammatory and hypofibrinolytic phenotype in healthy first-degree relatives of patients with type 2 diabetes. J. Thromb. Haemost. 8, 2080-2082.

Shats-Tseytlina, E.A., Nair, C.H., Dhall, D.P., 1994. Complement activation: a new participant in the modulation of fibrin gel characteristics and the progression of atherosclerosis? Blood Coagul. Fibrinolysis 5, 529–535.

Sirmaci, A., Walsh, T., Akay, H., Spiliopoulos, M., Sakalar, Y.B., Hasanefendioglu-Bayrak, A., Duman, D., Farooq, A., King, M.-C., Tekin, M., 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.

Skjoedt, M.O., Roversi, P., Hummelshøj, T., Palarasah, Y., Rosbjerg, A., Johnson, S., Lea, S.M., Garred, P., 2012. Crystal structure and functional characterization of the complement regulator mannose-binding lectin (MBL)/ficolin-associated protein-1 (MAP-1). J Biol Chem. 287, 32913-32921.

Speidl, W.S., Kastl, S.P., Huber, K., Wojta, J., 2011. Complement in atherosclerosis: friend or foe? J. Thromb. Haemost. 9, 428–440.

Takahashi, K., Chang, W.C., Takahashi, M., Pavlov, V., Ishida, Y., La Bonte, L., Shi, L., Fujita, T., Stahl, G.L., Van Cott, E.M., 2011. Mannose-binding lectin and its associated proteases (MASPs) mediate coagulation and its deficiency is a risk factor in developing complications from infection, including disseminated intravascular coagulation. Immunobiology 216, 96-102.

Takahashi, M., Iwaki, D., Kanno, K., Ishida, Y., Xiong, J., Matsushita, M., Endo, Y., Miura, S., Ishii, N., Sugamura, K, Fujita, T. 2008. Mannose-binding lectin (MBL)-Associated serine protease (MASP)-1 contributes to activation of the lectin complement pathway. J. Immunol. 180, 6132-6138.

Teillet, F., Gaboriaud, C., Lacroix, M., Martin, L., Arlaud, G.J., Thielens N.M., 2008. Crystal structure of the CUB1-EGF-CUB2 domain of human MASP-1/3 and identification of its interaction sites with mannan-binding lectin and ficolins. J. Biol. Chem. 283, 25715-25724.

Thiel, S., Jensen, L., Degn, S.E., Nielsen, H.J., Gál, P., Dobó, J., Jensenius, J.C., 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.

Thiel, S., Vorup-Jensen, T., Stover, C.M., Schwaeble, W., Laursen, S.B., Poulsen, K., Willis, A.C., Eggleton, P., Hansen, S., Holmskov, U., Reid, K.B.M., Jensenius, J.C., 1997. A second serine protease associated with mannan-binding lectin that activates complement. Nature, 386, 506-510.

Undas, A., Ariens, R.A.S., 2011. Fibrin clot structure and function. A role in the pathophysiology of arterial and venous thromboembolic diseases. Arterioscler. Thromb. Vasc. Biol. 31, e88-99.

Vorup-Jensen, T., Petersen, S.V., Hansen, A.G., Poulsen, K., Schwaeble, W., Sim, R.B., Reid K.B.M., Davis, S.J., Thiel, S., Jensenius, J.C., 2000. Distinct pathways of mannan-binding lectin (MBL)- and C1-complex autoactivation revealed by reconstitution of MBL with recombinant MBL-associated serine protease-2. J. Immunol. 165, 2093-2100.

Yongqing, T., Wilmann, P.G., Reeve, S.B., Coetzer, T.H., Smith, A.I., Whisstock, J.C., Pike, R.N., Wijeyewickrema, L.C., 2013. The x-ray crystal structure of mannose-binding lectin-associated serine proteinase-3 reveals the structural basis for enzyme inactivity associated with the Carnevale, Mingarelli, Malpuech, and Michels (3MC) syndrome. J Biol Chem. 288, 22399-22407.

Zhang, M., Hou, Y.J., Cavusoglu, E., Lee, D.C., Steffensen, R., Yang, L., Bashari, D., Villamil, J., Moussa, M., Fernaine, G., Jensenius, J.C., Marmur, J.D., Ko, W., Shevde, K., 2013. MASP-2 activation is involved in ischemia-related necrotic myocardial injury in humans. Int. J. Cardiol. 166, 499-504.

Zotter Z, Csuka D, Szabó E, Czaller I, Nébenführer Z, Temesszentandrási G, Fust G, Varga L, Farkas H., 2014. The influence of trigger factors on hereditary angioedema due to C1-inhibitor deficiency. Orphanet J Rare Dis. 9, 44.

Figure legends

Fig. 1. The multiple roles of MASP-1 in the initiation of the innate immune response. (A) MASP-1 cleaves both complement and non-complement substrates. MASP-1 is a serine protease with relaxed substrate specificity. After autoactivation it initiates lectin pathway activation via cleavage of complement proteins, while at the same time it contributes to the development of a more effective innate immune response through cleavage of non-complement substrates. (B) The central role of MASP-1 in the activation of the lectin pathway. After the pattern recognition molecules bind to the activation surface MASP-1 autoactivates. Active MASP-1 then activates MASP-2 and MASP-3. MASP-2 cleaves C4 for the C3 convertase but C2 is cleaved by both MASP-1 and MASP-2. MASP-3 might contribute to the complement activation by cleaving factor B.

Fig. 2. Structure of MASP-1. (A) Structural model of a MASP-1 dimer. Structures of MASP-1 CUB1-EGF-CUB2 (red, PDB code: 3dem), MAp44 (pink, PDB code: 4aqb) and MASP-1 CCP1-CCP2-SP (blue and green, PDB code: 3gov) were structurally overlaid showing the overall shape of the MASP-1 dimer. Ca^{2+} ions are depicted as bright green spheres on the ribbon representation (above). The dimer is shown by surface representation in the same orientation below. (B) Comparison of proenzyme and active MASP-1. Zymogen (PDB code: 4igd) and active (PDB code: 3gov) MASP-1 SP domains were structurally aligned. The catalytic triad (Asp^{552}, His^{490}, Ser^{646}) is in the same position in both structures. Asp^{645} forms a salt bridge with the new N-terminus (Ile^{449}) in active MASP-1. Loops in zymogen MASP-1 that change conformation upon activation are shown in green. Two positive residues that can potentially aid zymogen MASP-1 to attain and active-like conformation are shown in magenta. (C) The substrate binding cleft of MASP-1 is open and relatively wide. The substrate binding cleft is potentially accessible for a wide range of protein substrates, however, the large 60-loop (orange) can contribute to the specific characteristics of MASP-1. The catalytic triad is colored red, and the S1 pocket is found below the active site in this picture.

Fig. 3. Distinct effects of thrombin and MASP-1 signaling via PARs. MASP-1 and thrombin cleave protease activated receptors localized on the cell surface. Signaling of PARs leads to cytokine production amongst several outcomes. Finally, cytokines attract and activate different leukocyte populations depending on the pattern of the cytokines produced.

Thickness of the arrows from thrombin/MASP-1 to PARs represents the relative activity of the enzyme towards the specific PAR, whereas the size of the PARs is proportional to their relative mRNA expression in HUVECs.

Fig. 4. Role of MASP-1 in coagulation and thrombus formation. In the pro-inflammatory and pro-coagulant environment of atherosclerosis, low level activation of complement and coagulation occurs, possibly leading to MASP-1 activation and fibrin formation. MASP-1 activates endothelial cells (EC), which can then attract platelets leading to platelet adhesion and activation. Fibrin can act as a matrix for binding of MBL and ficolins, generating more autoactivated MASP-1. This in turn activates more endothelial cells. Furthermore, MASP-1 activates the coagulation factors prothrombin (PT) and factor XIII (FXIII), and the antifibrinolytic factor thrombin-activatable fibrinolysis inhibitor (TAFI). Thrombin (Thr), generated by MASP-1-induced activation of PT, in turn activates fibrinogen (Fbg), FXIII, TAFI, and platelets. These events eventually lead to formation of an occlusive thrombus. MASP-1 may play an important role in feeding and potentiating this vicious circle of coagulation activation and thrombus formation. (Platelets are shown in beige, erythrocytes in red, fibrin fibers in grey, and MBL in blue.)

Fig. 1.

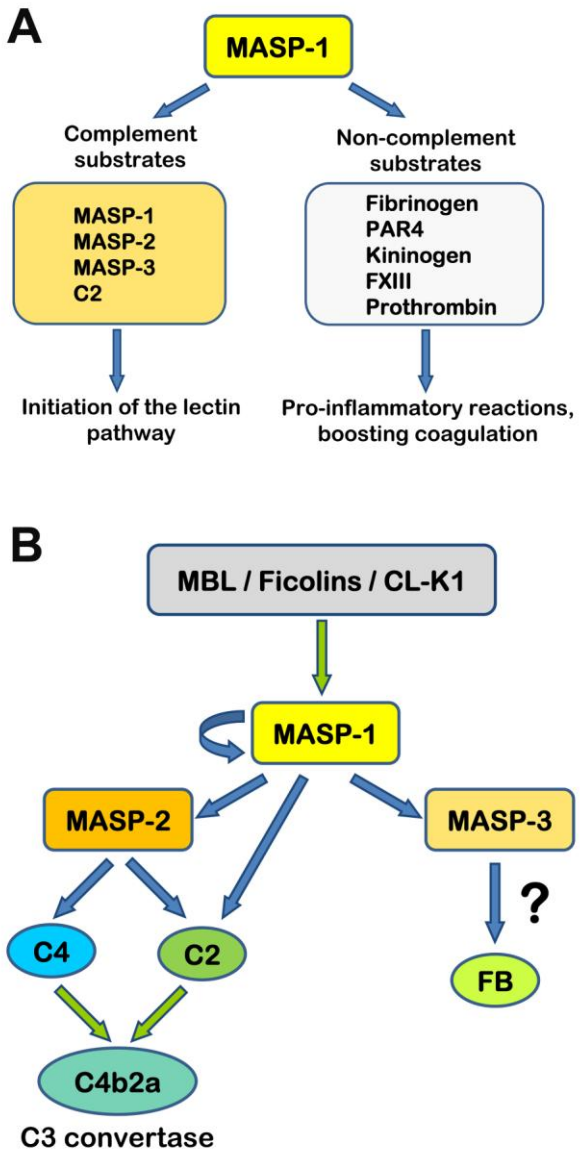

A
MASP-1
Complement substrates
Non-complement substrates
MASP-1
MASP-2
MASP-3
C2
Fibrinogen
PAR4
Kininogen
FXIII
Prothrombin
Initiation of the lectin pathway
Pro-inflammatory reactions, boosting coagulation
B
MBL / Ficolins / CL-K1
MASP-1
MASP-2
MASP-3
?
C4
C2
FB
C4b2a
C3 convertase

Fig. 2.

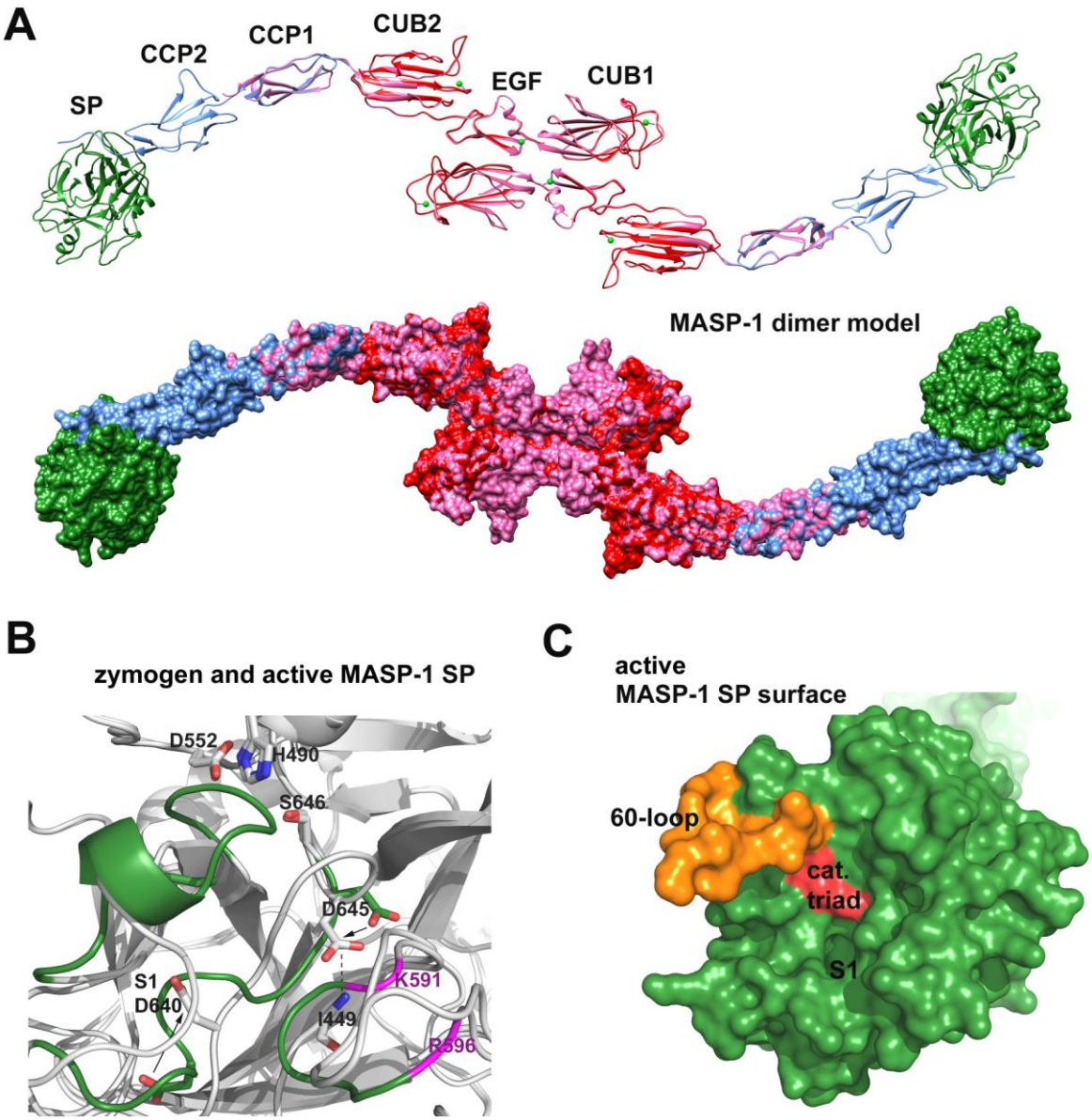

A
SP
CCP2
CCP1
CUB2
EGF
CUB1
MASP-1 dimer model
B
zymogen and active MASP-1 SP
D552
H490
S646
D645
S1
D640
K591
I449
R596
C
active
MASP-1 SP surface
60-loop
cat.
triad
S1

Fig. 3.

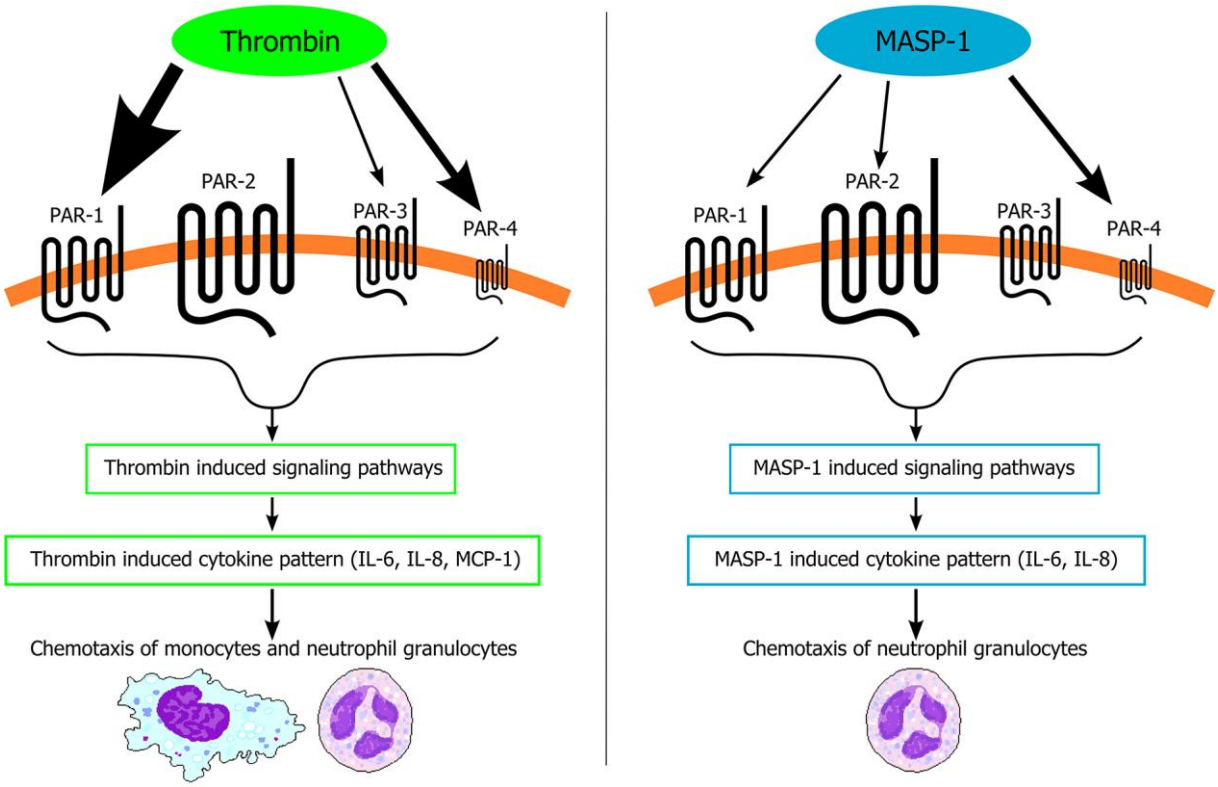
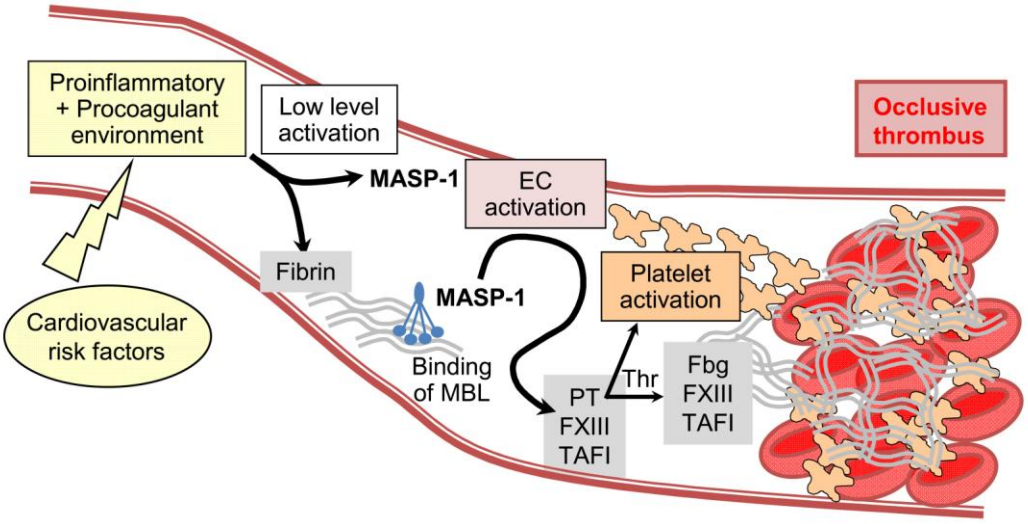

Thrombin
PAR-1
PAR-2
PAR-3
PAR-4
Thrombin induced signaling pathways
Thrombin induced cytokine pattern (IL-6, IL-8, MCP-1)
Chemotaxis of monocytes and neutrophil granulocytes
MASP-1
PAR-1
PAR-2
PAR-3
PAR-4
MASP-1 induced signaling pathways
MASP-1 induced cytokine pattern (IL-6, IL-8)
Chemotaxis of neutrophil granulocytes

Fig. 4.


Proinflammatory
+ Procoagulant
environment
Low level
activation
MASP-1
EC
activation
Occlusive
thrombus
Fibrin
MASP-1
Platelet
activation
Cardiovascular
risk factors
Binding
of MBL
Thr
PT
FXIII
TAFI
Fbg
FXIII
TAFI