MASP-2

Anjana Chandrasekhar¹, Ashok Reddy Dinasarapu¹, Nicole Thiedens², Shankar Subramaniam¹

MASP-2 (mannose/mannan binding lectin (MBL) associated serine protease-2) is a serum protein predominantly synthesized by the liver as a ~75kDa protein and is one of the key molecules of the innate immune system. It is mainly bound to multimeric protein complexes, such as MBL, the three ficolins (M-ficolin, L-ficolin and H-ficolin) and collectin kidney 1 (CL-K1, alias CL-11). These complexes serve as pathogen receptors, which are further bound to MASP-1, a serine protease. Binding of these complexes to their appropriate pathogenic ligands auto-activates MASP-1. Active MASP-1 in turn acts on its substrate, MASP-2, and thereby activates it. In a cascade of proteolytic cleavage events, MASP-2 activates complement proteins C4 and C2 to form C4b2a (classical C3 convertase), thereby converging the lectin pathway with the classical pathway of complement activation. Further, MASP-2 activity is regulated by several factors, including the serine protease inhibitor C1INH and by interaction with other proteins of the lectin complement pathway.

KEYWORDS
Mannan-binding lectin serine peptidase 1 pseudogene 1; Mannan-binding lectin serine peptidase 2; Mannan-binding lectin serine protease 1 pseudogene 1; Mannan-binding lectin serine protease 2; Mannose-binding protein-associated serine protease 2; MAP19; MASP-2; MASP1P1; MASP2; MBL-associated plasma protein of 19 kD; MBL-associated serine protease 2; Small MBL-associated protein; sMAP

PROTEIN FUNCTION
MBL-associated serine protease-2 (MASP-2) was initially discovered in 1997 by Thiel et al. MASP-2 has the following domains: two CUB (C1r/C1s/Uegf/bmp1), one epidermal growth factor (EGF)-like, two complement control proteins (CCPs) and a serine protease (which is a chymotrypsin-like protease domain) (Fujita et al. 2002).

Activation of complement pathway: MASP-2 in complex with collectins such as mannose/mannan-binding lectin (MBL) or collectin kidney 1 (CL-K1, alias CL-11) and ficolins (M-ficolin, L-ficolin and H-ficolin) activates the complement pathway (Ali et al. 2012, Ma et al. 2013). Upon binding of collectins or ficolins to its appropriate pathogenic ligands, MASP-2 cleaves C4, followed by binding of C2 to C4b and subsequent cleavage of C2 forming C4b2a (C3 convertase), which cleaves C3 into C3a and C3b (Wallis et al. 2007, Vorup-Jensen et al. 1998, Matsushita et al. 2000). MASP-2 was previously believed to be autoactivated (Vorup-Jensen et al. 2000). However, as per current literature another serine protease, MASP-1, bound to MBL or ficolins activates MASP-2 to generate C3 convertase (see 'Regulation of Activity') (Møller-Kristensen et al. 2007). MASP-2, in comparison to C1s, has higher efficiency of C4 (~23-fold) and C2 (~3 fold) cleavage, which is attributed to better binding of the substrate through its CCP domains (Rossi et al. 2001, Rossi et al. 2005). Use of a randomized substrate phage display library revealed MASP-2 to be around 50 times more catalytically active than C1s (Kerr et al. 2008). MASP-2 also has very weak C3 cleaving activity (Rossi et al. 2001).

Opsonophagocytosis: MASP-2 in complex with MBL and ficolins have been documented to aid in opsonophagocytosis of Staphylococcus aureus (Neth et al. 2002) and group B streptococci (Aoyagi et al. 2005). However, it is not clear if MASP is required or if MBL/ficolins alone are sufficient for this function (Shiratsuchi et al. 2008). In mice however, MASP-2 knockout results in increased susceptibility to pneumococcal infection, due to a defect in opsonization of Streptococcus pneumoniae (Ali et al. 2012).

Other roles: MASP-2 has been shown to activate coagulation (Krarup et al. 2007) and studies in mice have shown MASP-2 to be involved in ischemia-reperfusion injury (Schwaebel et al. 2011). sMAP (also known as Map19) is a splice variant of MASP2 (see 'Splice Variants' section) with no enzymatic activity. Hence unlike MASP-2, sMAP cannot cleave C4 and C2.

REGULATION OF ACTIVITY
MASP-2 is synthesised as single chain proenzyme and activation proceeds through the cleavage of a single Arg-Ile bond, generating two disulfide-linked chains, A (N-terminal) and B (C-terminal serine protease domain). Isolated rat and human recombinant MASP-2 undergo autoactivation, which is enhanced by binding to target-bound MBL or ficolins (Chen and Wallis 2004, Gal et al. 2005). MBL was also proposed to occlude the C4 binding site on MASP-2, till activation occurs (Chen and Wallis 2004). Recent studies, including in a MASP-1 deficient patient and MASP-1 knockout mice, structural details and use of inhibitors demonstrate that MASP-1 cleaves and thereby activates MASP-2 (Degn et al. 2012, Megyeri et al. 2013, Kocsis et al. 2010, Heja et al. 2012a, Heja et al. 2012b, Takahashi et al. 2008).

MASP-2 activity is inhibited by C1 inhibitor (C1INH), an inhibitor for C1r, C1s and MASP-1. C1INH forms equimolar complexes with both MASP-1 and MASP-2 (Matsushita et al. 2000, Rossi et al. 2001, Ambrus et al. 2003, Presanis et al. 2004) and can inhibit MASP-2 fifty-fold faster than C1s, implying MASP-2 to be a major physiological target of C1INH (Kerr et al. 2008). Also, anti-thrombin III could inhibit activity in the presence of heparin (Presanis et al. 2004, Paréj et al. 2013). MASP-3 (a splice variant of MASP1) and sMAP have been shown to down-regulate C4 deposition, most likely by...

¹Department of Bioengineering, University of California, San Diego, CA 92093, US. ²IBS/IPAS, 38027, FR. ³Department of Bioengineering, University of California at San Diego, CA 92093, US.
Correspondence should be addressed to Anjana Chandrasekhar: 4chandra@ucsd.edu
Published online: 13 Sep 2013 | doi:10.6072/H0.MP.A004275.01
competing with MASP-2 binding to MBL or ficolins (Dahl et al. 2001, Moller-Kristensen et al. 2007, Iwaki et al. 2006, Skjoedt et al. 2010a). However, results obtained in vitro with human proteins suggest that sMAP has no inhibitory activity on MASP-2 mediated activation of the lectin pathway (Degen et al. 2011). Also, Map44 (also known as MAP-1), another splice variant of MASP1, can disrupt heterodimer interaction of MASP-1 and MASP-2 and thereby inhibit MASP-2 activity (Degen et al. 2013, Degen et al. 2009, Skjoedt et al. 2010b, Pavlov et al. 2012).

INTERACTIONS
Collectins and ficolins: Both MASP-2 and sMAP form homodimers in human and rat (Chen and Wallis 2001, Thielens et al. 2001, Feinberg et al. 2003) in a Ca\(^{2+}\) dependent manner. The homodimers then go on to interact with MBL and L-ficolin through its CUB1 domain in a Ca\(^{2+}\) dependent manner (Thielens et al. 2001, Gregory et al. 2004). Comparison of K_D values between MASP-2 and sMAP suggest MASP-2 to bind more efficiently to MBL (0.8 nM vs 13 nM). MASP-2 and sMAP bind to Lys55 (residue number corresponds to the mature protein) of MBL in presence of Ca\(^{2+}\) (Thiel et al. 2000, Teillet et al. 2007). Further, MASP-2 and sMAP compete with calreticulin (CRT) for the same binding site on MBL (Pagh et al. 2008). The oligomerization state of MBL has no influence on the interaction with the MASPs (similar K_D values for trimer and tetramer) (Teillet et al. 2005). MASP-2 interaction with L-ficolin and H-ficolin also requires Ca\(^{2+}\) (Ma et al. 2004, Matsushita and Fujita 2001, Cseh et al. 2002, Zacho et al. 2012, Csuka et al. 2013). Lys57 and Lys47 of L-ficolin and H-ficolin respectively (residue numbers correspond to the mature proteins) are important in binding to MASP-2 (LaCroix et al. 2009). M-ficolin was shown to mediate activation of the lectin pathway, which strongly suggests that, similarly to L- and H-ficolins, M-ficolin interacts with MASP-2 (Liu et al. 2005). MASP-2 can also interact with a novel collectin, CL-11 (CL-K1) to activate the complement pathway (Ali et al. 2012, Ma et al. 2013).

MASPs and other proteins: MASP-3 was found together with MASP-2 on large MBL oligomers whereas MASP-1 and sMAP were found on lower MBL oligomers, but no direct evidence of heterodimerization was provided (Thiel et al. 2000, Dahl et al. 2001, Tateishi et al. 2011). A recent study documents heterodimer formation between MASP-1 and MASP-2, which can be disrupted by Map44 (Degen et al. 2013). The CCP domains of MASP-2 positively co-operate with the active site to ensure effective binding to C4 and C4b (Duncan et al. 2012, Kidmose et al. 2012). The exosite contributed by both CCP domains of MASP-2 recognizes the C345C domain of C4.

The experimental methods used to characterize these interactions are documented in CMAP, a complement map database (Yang et al. 2013).

PHENOTYPES
Most inherent differences in the protein levels arise from single nucleotide polymorphisms (SNPs), several of which (D120G, R99N, V377A, R439H) have been documented in the recent years.

p.D120G: The SNP resulting in D120G substitution, found in Caucasians and Inuits from West-Greenland (Thiel et al. 2007) shows very low serum levels (5% and 45% of wild-type in homozygous and heterozygous mutants respectively) (Stengaard-Pedersen et al. 2003). A cystic fibrosis patient with homozygous D120G mutation was found to have a severe lung disease (Olesen et al. 2006). Further studies showed that MASP-2 with D120G substitution could not bind to MBL and hence could not activate the complement pathway (Thiel et al. 2009). The same mutation, when introduced in MAp19, also abolished its interaction with MBL and L-ficolin (Gregory et al. 2004).


p.P126L: This SNP, similar to R99N, is isolated in CUB1 domain and generally found in African and Amerindian populations (Lozano et al. 2005, Thiel et al. 2007). Individuals with homozygous p.126L showed non-functional MASP-2 (Thiel et al. 2007), while the isolated protein could cleave C4 efficiently (Thiel et al. 2009). p.126L has also been linked to Crohn’s disease haplotype with reduced MASP-2 levels and associated with chagasic cardiomyopathy (Boldt et al. 2011).

p.V377A: Similar to p.126L, p.V377A also shows reduced MASP-2 levels, is linked to Crohn’s disease haplotype and associated with chagasic cardiomyopathy (Boldt et al. 2011). However, the V377A protein (similar to wild type and p.126L) has a normal enzymatic activity and can cleave C4 (Thiel et al. 2007, Thiel et al. 2009).

p.R439H: This variant, common in Sub-Saharan Africans with a gene frequency of 10%, binds normally to MBL but is deficient in enzymatic activity (Thiel et al. 2009).

p.156-159 dupCHNH: This four amino-acid tandem duplication polymorphism, which results in poor secretion of the protein is found only in Chinese population with a gene frequency of 0.26%. It does not bind to MBL and hence does not result in deposition of C4 (Thiel et al. 2007, Thiel et al. 2009).

Additonally, p.D371Y is associated with susceptibility to hepatitis C virus infection (Tulio et al. 2011). Polymorphisms flanking MAp19 exon 5 and MASP2 haplotypes generating low MASP-2 levels were associated with susceptibility to leprosy (Boldt et al. 2013). MASP-2 levels and thereby activity have been associated with several diseases, including schizophrenia and septic shock induced mortality (Mayilyan et al. 2006, Charchaflieh et al. 2012). MASP-2 deficiency lead to increased risk of fever and neutropenia in pediatric cancer patients (Schlapbach et al. 2007), while higher MASP-2 level was associated with better event free survival in pediatric patients with hematologic malignancies, especially lymphoma (Zehnder et al. 2009). A study showed neonates with very low MASP-2 levels (below 42 ng/ml) to have a shorter mean gestational age and a higher incidence of premature and low birthweight babies. In contrast, babies with infections had higher MASP-2 concentrations (St Swierzko et al. 2005). Pre-mature infants with higher MASP-2 cord blood levels compared with controls developed necrotizing enterocolitis at a later stage (Schlapbach et al. 2008). Colorectal cancer patients showed higher MBL-MASP activity as compared to controls (Ytting et al. 2004) and high MASP-2 levels are significantly correlated with recurrent cancer disease and poor survival (Ytting et al. 2005, Ytting et al. 2008). MASP-2 levels are also increased in patients with acute lymphoblastic leukaemia, non-Hodgkin lymphoma, central nervous system (CNS) tumors (Fisch et al. 2011),
hematological infections (0.53 µg/ml compared to patients without infections 0.37 µg/ml) (Ameye et al. 2012).

MAJOR SITES OF EXPRESSION
MASP-2 is mainly expressed in the liver (Endo et al. 2002), with smaller amounts (~100-500 fold less compared to liver) found in the small intestine and testis (Seyfarth et al. 2006). MASP-2-specific mRNA expression, which is generally absent in healthy ovary tissues, was detected in the ovary tissues of patients with malignant reproductive disease (Swierzko et al. 2007). Increased MASP-2 expression was observed in esophageal squamous cancer cells in premalignant condition, dysplasia in comparison with the normal tissues and is associated with late clinical stage and nodal metastasis (Verma et al. 2006). The promoter activity of the MASP-2 gene was increased in the presence of IL-1β. However, this increase is nullified in the presence of IL-6 (Endo et al. 2002). MASP-2 gene expression is positively regulated by binding of Stat3 to its promoter region (Unterberger et al. 2007).

SPlice VeRiants
MASP2 located on chromosome 1p36.2–3 has one splice variant, MAp19 or sMAP, which is 19 kDa in size (Stover et al. 1999, Takahashi et al. 1999). MASP2 encompasses 12 exons (Stover et al. 2004), among which 11 encode the six domains of MASP-2: two CUB, an epidermal growth factor (EGF)-like, two complement control proteins (CCPs) and a serine protease domain (Fujita et al. 2002). Alternative splicing at exon 5 results in MAp19, which shares 4 exons with MASP-2 (encoding the N-terminal CUB and EGF domains) whereas exon 5 encodes a unique C-terminal extension of 4 a.a. (Schwaebel et al. 2002). MAp19 is enzymatically inactive (as it lacks the serine protease domain) and is believed to down-regulate lectin pathway in mice (Iwaki et al. 2006). However contradictory results were obtained in vitro using human proteins (Degn et al. 2011).

Regulation of Concentration
MASP-2 concentrations differ among the diverse populations. Africans from Zambia show the lowest levels of 0.196 µg/ml, while Hong Kong Chinese, Amerindians and Danish Caucasians show 0.262 µg/ml, 0.29 µg/ml and 0.416 µg/ml respectively (Thiel et al. 2007). Another study showed the levels in a danish donor population to be 0.534 µg/ml (Møller-Kristensen et al. 2003). It is likely that higher MASP-2 concentrations in individuals from a UK population, compared to Armenians, leads to 2-fold higher MBL-MASP-2 activity (Mayilyan et al. 2006b). The concentration of MAP19 was detected to be 0.217 µg/ml, (11nM, compared to the 7nM of MASP-2) (Degn et al. 2011). Both MASP-2 and MAP19 are generally found in complex with other proteins such as MBL and ficolins in serum (Thiel et al. 2000, Møller-Kristensen et al. 2003).

Serum levels of MASP-2 also differ with age. Cord sera shows a value of 0.093 µg/ml (St Swierzko et al. 2009), while newborns show serum levels of 0.126 µg/ml. The levels increase with age and peak at adulthood (0.416 µg/ml) (Sallenbach et al. 2011). However, the levels are stable over time in healthy adults, which makes them potential biomarkers (Ytting et al. 2007). Patients with hereditary angiodema, which is the clinical manifestation of C1INH deficiency, showed decreased MASP-2 levels (Varga et al. 2008).

Antibodies
MASP-2 antibodies are available from: Santa Cruz Biotechnology, Abcam, Novus Biologicals, Sigma Aldrich, Hycult Biotech, Biorbyt, LifeSpan Biosciences, Atlas Antibodies, Aviva, Genentech Biotech, GenTex, My BioSource.com, Origene Technologies, Antibodies-online, Abnova, Creative Biomart, Bioss Inc, USCN Life Science and Fitzgerald industries. MASP-2 antibody has been used as a therapeutic intervention in mice to prevent injury by gastrointestinal post-ischemic reperfusion (Schwaebel et al. 2011).
### Table 1: Functional States

<table>
<thead>
<tr>
<th>STATE DESCRIPTION</th>
<th>LOCATION</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>MASP-2</td>
<td>extracellular region</td>
<td>Kerr FK et al. 2008; Matsushita M et al. 2000</td>
</tr>
<tr>
<td>MASP-2/C4</td>
<td>extracellular region</td>
<td>Wallis R et al. 2007</td>
</tr>
<tr>
<td>2(MASP-2)</td>
<td>extracellular region</td>
<td>Chen CB and Wallis R 2001; Gregory LA et al. 2004; Thielens NM et al. 2001</td>
</tr>
<tr>
<td>sMAP</td>
<td>extracellular region</td>
<td>Stover CM et al. 1999; Takahashi M et al. 1999</td>
</tr>
<tr>
<td>2(sMAP)</td>
<td>extracellular region</td>
<td>Chen CB and Wallis R 2001; Cseh S et al. 2002; Gregory LA et al. 2004; Thielens NM et al. 2001</td>
</tr>
<tr>
<td>3(3MBL)/2(MASP-1)/2(sMAP)</td>
<td>extracellular region</td>
<td>Dahl MR et al. 2001; Degn SE et al. 2011; Gregory LA et al. 2004; Tateishi K et al. 2011; Teillet F et al. 2005</td>
</tr>
<tr>
<td>L-FCN/2(MASP-1)/2(MASP-2)/2(sMAP)</td>
<td>extracellular region</td>
<td>Lacroix M et al. 2009; Cseh S et al. 2002; Matsushita M et al. 2000; Ma YG et al. 2004</td>
</tr>
<tr>
<td>H-FCN/2(sMAP)</td>
<td>extracellular region</td>
<td>Lacroix M et al. 2009; Zacho RM et al. 2012; Csuka D et al. 2013</td>
</tr>
<tr>
<td>4(3MBL)/2(MASP-1)/2(MASP-2)/2(MASP-3)</td>
<td>extracellular region</td>
<td>Dahl MR et al. 2001; Sekine H et al.; Teillet F et al. 2005; Thielens NM et al. 2001</td>
</tr>
<tr>
<td>5(3MBL)/2(MASP-1)/2(MASP-2)/2(MASP-3)</td>
<td>extracellular region</td>
<td>Dahl MR et al. 2001; Sekine H et al.; Teillet F et al. 2005; Thielens NM et al. 2001; Wallis R et al. 2007</td>
</tr>
<tr>
<td>6(3MBL)/2(MASP-1)/2(MASP-2)/2(MASP-3)</td>
<td>extracellular region</td>
<td>Sekine H et al.; Teillet F et al. 2005; Thielens NM et al. 2001; Dahl MR et al. 2001; Wallis R et al. 2007</td>
</tr>
<tr>
<td>L-FCN/2(MASP-1)/2(MASP-2)</td>
<td>extracellular region</td>
<td>Cseh S et al. 2002; Lacroix M et al. 2009</td>
</tr>
<tr>
<td>CL-K1/2(MASP-1)/2(MASP-2)</td>
<td>extracellular region</td>
<td>Ali YM et al.; Ma YJ et al.</td>
</tr>
<tr>
<td>MBL,ficolins/active2(MASP-1)/active2(MASP-2)</td>
<td>extracellular region</td>
<td>Héja D et al. 2012; Héja D et al. 2012; Megyeri M et al. 2013</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS
The UCSD Signaling Gateway Molecule Pages (SGMP) is funded by NIH/NIGMS Grant 1 R01 GM078005-01. The authors thank Dr. John D. Lambris, University of Pennsylvania, Philadelphia, UCSD-SGMP editorial board member, for extensive discussions.

SUPPLEMENTARY
Supplementary information is available online.

REFERENCES


protease of the lectin complement pathway and identification of the enzyme as a major physiological target of the serpin, C1-inhibitor. Mol Immunol, 45, 3.


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, 3-4.


Schwaeble W, Dahl MR, Thiel S, Stover C, Jensenius JC (2002). The mannose-binding lectin-associated serine proteases (MASPs) and...


Wallis R, Dodds AW, Mitchell DA, Sim RB, Reid KB, Schweabe WJ (2007). Molecular interactions between MASP-2, C4, and C2 and their activation fragments leading to complement activation via the


This molecule exists in 18 states, has 21 transitions between these states and has 2 enzyme functions. (Please zoom in the pdf file to view details.)