Molecular Immunology 114 (2019) 1–9

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

MASP-1 of the complement system alters fibrinolytic behaviour of blood clots

Lorenz Jenny[a,1], Danilo Noser[a,1], Julie Brogaard Larsen[b], József Dobó[c], Péter Gál[c], Gábor Pál[d], Verena Schroeder[a,*]

[a] Experimental Haemostasis Group, Department for BioMedical Research, University of Bern, Bern, Switzerland
[b] Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark
[c] Institute of Enzymology, Biological Research Centre, Hungarian Academy of Sciences, Budapest, Hungary
[d] Department of Biochemistry, Eötvös Loránd University, Budapest, Hungary

ARTICLE INFO

Keywords:
Complement
Coagulation
Fibrinolysis
MASP-1
Clot formation
Cardiovascular disease

ABSTRACT

Background: The lectin pathway serine protease mannan-binding lectin-associated serine protease 1 (MASP-1) has been demonstrated to be a major link between complement and coagulation, yet little is known about its interactions with the fibrinolytic system. The aim of this work was to assess the effects of MASP-1 on fibrin clot lysis in different experimental settings.

Methods: Rotational thrombelastometry was used to evaluate the effect of MASP-1 on the lysis of clots formed in whole blood under static conditions. Whole blood clots were also formed in the presence and absence of MASP-1 under flow conditions in the Chandler loop and their lysis was analysed separately by fluorescence release of incorporated labelled fibrin. Real-time observation by laser scanning confocal microscopy was used to investigate the lysis of plasma clots where MASP-1 was present either during clot formation or lysis. Cleavage of tPA or plasminogen by MASP-1 was analysed by gel electrophoresis. We performed a turbidimetric clot lysis assay in the presence and absence of the MASP-1 inhibitor SGMI-1 (Schistocerca gregaria protease inhibitor (SGPI)-based MASP inhibitor-1) to evaluate the effect of endogenous MASP-1 in normal plasma and plasma samples from sepsis patients.

Results: In the thrombelastometric experiments, where MASP-1 was present during the entire clotting and lysis process, MASP-1 had a significant profibrinolytic effect and accelerated clot lysis. When clots were formed in the presence of MASP-1 under flow in the Chandler loop, the effects on fibrinolysis were heterogenous with impaired fibrinolysis in some individuals (n = 5) and no (n = 3) or even the opposite effect (n = 2) in others. In plasma clot lysis observed by confocal microscopy, lysis was prolonged when MASP-1 was added to the lysis solution, yet there was no difference in lysis time when MASP-1 was present during clot formation. When MASP-1 was incubated with tPA or plasminogen, respectively, cleavage of single-chain tPA into two-chain tPA and a slight reduction of plasminogen were observed. SGMI-1 significantly prolonged clot lysis in the turbidimetric clot lysis assay suggesting that MASP-1 accelerated lysis in plasma samples.

Conclusion: MASP-1 is able to alter the susceptibility of blood clots to the fibrinolytic system. MASP-1 has complex, mostly promoting effects on fibrinolysis with high inter-individual variation. Interactions of MASP-1 with the fibrinolytic system may be relevant in the development and therapy of cardiovascular and thrombotic diseases.

Abbreviations: MASP-1, mannan-binding lectin-associated serine protease 1; PAI-1, plasminogen activator inhibitor 1; FXIII, coagulation factor XIII; TAFI, thrombin activatable fibrinolysis inhibitor; CVD, cardio- and cerebrovascular disease; DIC, disseminated intravascular coagulation; rMASP-1cf, recombinant MASP-1 catalytic fragment; tPA, tissue plasminogen activator; CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness; MCFt, time to maximum clot firmness; LI30/45/60, lysis index after 30/45/60 min; LOT, lysis onset time; LT, lysis time; CLR, clot lysis rate; TLT, total lysis time; PAR4, protease-activated receptor 4; SGMI-1, Schistocerca gregaria protease inhibitor (SGPI)-based MASP inhibitor-1

* Corresponding author at: Department for BioMedical Research, Experimental Haemostasis Group, University of Bern, Murtenstrasse 40, 3008, Bern, Switzerland.
E-mail address: verena.schroeder@dbmr.unibe.ch (V. Schroeder).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.molimm.2019.07.005
Received 1 February 2019; Received in revised form 11 June 2019; Accepted 8 July 2019
Available online 17 July 2019

1. Introduction

The complement system is part of the innate immune system and serves to remove pathogens and foreign or altered-self particles from the human body. It is one example for the strongly conserved and highly regulated cascades among plasmatic proteolytic networks, such as the contact activation system (kinin-kallikrein system), the coagulation and fibrinolytic systems. These systems share partially overlapping stimuli, exhibit a high degree of interaction, and are derived from a common evolutionary ancestor (Conway, 2015; Ekdahl et al., 2016).

Interactions between complement, coagulation and fibrinolysis, which aggravate the underlying proinflammatory and prothrombotic condition, are thought to play an important role in the pathophysiology of cardio- and cerebrovascular diseases (CVDs), diabetes mellitus/metabolic syndrome, and sepsis-associated disseminated intravascular coagulation (DIC) (Carter, 2012; Hertle et al., 2014; Hess et al., 2012b; Kurosawa and Stearns-Kurosawa, 2014). Numerous direct interactions between proteins of the complement and coagulation cascades have been reported, platelets and endothelial cells serve as an important interface to mediate the cross-talk of both cascades, and complement components have been shown to be associated with and affect fibrin clots (Conway, 2015; Ekdahl et al., 2016). With the fibrinolytic system, responsible to regulate fibrin clot growth, remove clots from the vascular system and to enable tissue repair (Chapin and Hajjar, 2015), another proteolytic cascade has been shown to be tightly interwoven with the complement system, yet less is known about these interactions. Plasmin, the central enzyme of the fibrinolytic system, has been suggested to exhibit a complex role in regulating the complement system (Foley, 2017). On the one hand it can activate complement C3 and C5 generating the bioactive anaphylatoxins C3a and C5a (Amara et al., 2008). On the other hand, plasminogen activated by streptokinase can also inhibit complement activation by cleaving complement factors (Pillemer et al., 1953). Plasminogen has been shown to bind to and enhance inactivation of C3, C3b and C3d (Barthel et al., 2012). C5a has been shown to upregulate plasminogen activator inhibitor-1 (PAI-1) on macrophages and mast cells inducing their switch from a profibrinolytic to a prothrombotic phenotype (Kastl et al., 2006; Wojta et al., 2003). Some components of the complement cascade are also able to modulate the structural and functional characteristics of a fibrin clot. The incorporation of C3 into fibrin prolongs lysis time in a dose-dependent manner (Howes et al., 2012), and this effect is even greater in patients with diabetes (Hess et al., 2012b). Complement activation via the alternative pathway has also been shown to lead to the formation of a denser fibrin clot with prolonged clot lysis time (Shats-Tseytlina et al., 1994).

More recently, mannan-binding lectin-associated serine protease 1 (MASP-1) has been identified as an important link between complement and coagulation (Dobó et al., 2014). MASP-1 resembles thrombin in terms of its structure and its substrate specificity (Dobó et al., 2009) and it is therefore not surprising that it interacts with coagulation factors prothrombin, factor XIII (FXIII), fibrinogen, and thrombin-activatable fibrinolysis inhibitor (TAFI) (Hess et al., 2012a; Jenny et al., 2015a, 2015b; Kozarcanin et al., 2016; Krarup et al., 2008). We have also shown procoagulant effects of MASP-1 in a close-to-physiological microvascular whole blood flow model (Jenny et al., 2018). We have also suggested effects of MASP-1 on clot structure and lysis. Although plasma clots formed in presence of MASP-1 had a less dense structure, larger pores and thicker fibres, they surprisingly seemed to be more resistant to lysis in a turbidimetric lysis assay, possibly due to TAFI activation (Hess et al., 2012a). In MASP-1 knock-out mice, prolonged tail-bleeding (Takahashi et al., 2011) and protection from occlusive thrombus formation (La Bonte et al., 2012) were observed, supporting a procoagulant effect of MASP-1, but fibrinolysis was not studied. Thus, the possible effects of MASP-1 on fibrinolysis are not yet understood and need further investigation.

Here we study for the first time the effects of MASP-1 on fibrinolysis in whole blood and plasma using rotation thrombelastometry (ROTEM), the Chandler loop to generate blood clots under flow, and a laser scanning microscopic system to observe fibrinolysis in real-time. We also evaluate the inhibition of endogenous MASP-1 by a specific MASP-1 inhibitor in whole blood using ROTEM and in plasma samples from sepsis patients using a turbidimetric clot lysis assay. These different experimental settings will also allow us to distinguish between indirect effects on fibrinolysis via clot structure and direct effects on the activity of the fibrinolytic system.

2. Materials and methods

2.1. Blood collection and sample preparation

Whole blood (WB) was freshly drawn from healthy volunteers into Sarstedt Monovette® tubes containing 0.106 mol/l sodium citrate (Sarstedt AG, Nümbrecht, Germany) and used for experiments within 90 min after blood sampling. All volunteers gave written informed consent to the study protocol approved by the local ethics committee.

Septic shock patients were recruited at the intensive care unit, Aarhus University Hospital, Aarhus, Denmark (Larsen et al., 2019). Briefly, inclusion criteria were adult patients with a diagnosis of sepsis with a need for vasopressor treatment to maintain mean arterial pressure > 65 mmHg. Exclusion criteria were active cancer or chemotherapy within 3 months, pregnancy, major trauma or surgery within 24 h, and plasma transfusion or anticoagulant treatment within the last 72 h. Blood was taken within 24 h of admission to the intensive care unit into 3.6 ml citrated tubes (3.2% sodium citrate, Terumo®, Leuven, Belgium), centrifuged at 3000 *g* for 25 min to obtain platelet-poor plasma, aliquoted and stored at −80 °C until analysis. The project was approved by the Danish Data Protection Agency (file no 1–16–02–505–16) and conducted in accordance with the Helsinki Declaration and the Danish Health Care Act. Clinical characteristics and coagulation parameters of the septic shock cohort have been described earlier (Larsen et al., 2019). Plasma for the turbidimetric clot lysis assay performed as described below at the Department for BioMedical Research, University of Bern, was available from 33 patients.

2.2. Recombinant MASP-1

For the experimental settings used in this study, large amounts of active MASP-1 were necessary. Since isolation or expression of pure and stable full-length MASP-1 in sufficient quantities has not been achieved so far, a recombinant MASP-1 catalytic fragment (rMASP-1cf) was used. This truncated form of MASP-1 consists of the CCP1 − CCP2-SP domains while it is deprived of the N-terminal CUB1-EGF-CUB2 domains (Dobó et al., 2009). We have demonstrated earlier that rMASP-1cf has the same effects on clot formation as the isolated full-length enzyme (Jenny et al., 2015a). We used a final concentration of 10 µg/ml (corresponding to 220 nmol/l at a molecular weight of approx. 45 kDa) rMASP-1cf in the fibrinolysis experiments. This is within the physiological range of 4–30 µg/ml (corresponding to 53–400 nmol/l at a molecular weight of approx. 75 kDa), with a median concentration of 11 µg/ml (147 nmol/l), as determined in healthy adult donors (Thiel et al., 2012).

2.3. Rotation thrombelastometry (ROTEM)

The experiments were performed on a rotation thrombelastometry system (ROTEM®, Tem International, Munich, Germany). In a thrombelastometry cup, 248 µl of freshly drawn WB were recalcified with Star-TEM reagent ($CaCl_2$, 200 mmol/l, Tem International) to a final concentration of 12.4 mmol/l and activated with 8 µl Ex-Tem reagent (recombinant tissue factor, Tem International) to ensure a standardised coagulation activation. Tissue plasminogen activator (tPA;

Technoclone, Vienna, Austria) dissolved in Hepes buffer (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid, 25 mmol/l, 137 mmol/l NaCl, 3.5 mmol/l KCl, 1% bovine serum albumin, pH 7.4) was added to the sample (final tPA concentration 2.06 nmol/l) in presence or absence of r-MASP-1cf (final concentration 220 nmol/l) in TBS (Tris-buffered saline, 50 mmol/l Tris, 100 mmol/l NaCl, pH 7.4) and adjusted to 290 µl with 0.9% NaCl solution. The sample was mixed, applied to the ROTEM machine and the following kinetic and viscoelastic parameters were recorded over 90 min: Clotting time (CT; representing the lag time until the onset of clotting), clot formation time (CFT; representing the duration from onset of clotting until 20 mm amplitude are reached), maximum clot firmness (MCF; maximum amplitude of the formed clot), time to MCF (MCFt; time until the MCF is achieved), lysis index at 30/45/60 min (LI30/LI45/LI60; indicates the amplitude of the remaining clot at 30, 45, 60 min after CT, expressed in percentage of MCF), lysis onset time (LOT, measures the time span from CT to the start of significant lysis (i.e. reduction of the amplitude of 15% as compared to MCF)), lysis time (LT, time measured from CT until the clot is reduced to 10% of MCF, i.e. 90% of the clot is lysed). Additionally, we calculated the clot lysis rate (CLR, maximum lysis rate, calculated as (LT-LOT) / (MCF/maximal lysis)) and the total lysis time (TLT; LT – MCFt, i.e. the time from MCF to 90% lysis). Each sample (from 11 different individuals) was measured four times in the presence and absence of rMASP-1cf. Two measurements were excluded because the sample clotted immediately.

In another experimental series, we performed parallel ROTEM measurements as described above with recalcified whole blood only (control), recalcified whole blood with rMASP-1cf (final concentration 200 nmol/l), and recalcified whole blood with the MASP-1 inhibitor SGMI-1 (Schistocerca gregaria protease inhibitor (SGPI)-based MASP inhibitor-1) (obtained from G. Pál) at a final concentration of 5 µmol/l (Héja et al., 2012a, b).

2.4. Chandler loop

Thrombi were formed under flow according to an adapted Chandler loop protocol (Mutch et al., 2010). Nine hundred microlitres of freshly drawn WB were supplemented with prestained human fibrinogen (final concentration 126.5 nmol/l, Alexa Fluor® 488 conjugate, excitation/emission 495/519 nm; Molecular Probes, Eugene, USA) and $CaCl_2$ (final concentration 10.8 mmol/l) either in presence or absence of rMASP-1cf (final concentration 220 nmol/l) and adjusted to a total volume of 1150 µl with 0.9% NaCl. The sample was mixed by pipetting, drawn into syringes with blunt needles (Gauge 20, 1/2″, AMP Technica, Heerbrugg, Switzerland) and injected into PVC-tubing (internal diameter of 3 mm; VWR International GmbH, Dietikon, Switzerland). The tubing was connected to a loop (circumference 34.5 cm, diameter 10.5 cm), covered in tinfoil, and mounted on a rotating device which at 30 rpm exhibited a shear rate of $428 s^{-1}$ (Fig. 1A). After 90 min the clots were removed from the tubing, washed for 30 s in 0.9% NaCl solution and dripped off on filter paper (Fig. 1B). Subsequently the clots were incubated for 4 h at 37 °C in bathing buffer (10 mmol/l Tris, 0.01% Tween20, pH 7.4) containing tPA (final concentration 250 ng/ml corresponding to 3.67 nmol/l). At 30 min intervals, 5 µl from each sample was transferred to 245 µl of bathing buffer in a flat bottom 96-well plate (Nunc, F96 black MicroWell, ThermoFisher Scientific, Waltham, USA) and fluorescence released from the clot during lysis was measured at excitation/emission wavelengths of 485 nm and 535 nm and a manual gain of 90 on a Tecan Spark 10 M microplate reader (Tecan Group Ltd., Männedorf, Switzerland). Freshly drawn whole blood from ten individuals was used to form two clots each in presence (total n = 20) or absence (total n = 19, because one sample did not clot) of rMASP-1cf.

2.5. Laser scanning confocal microscopy (LSM) real-time fibrinolysis

Plasma clots were formed as described by Hess et al., 2012b, from 7.5 µl of pooled normal plasma (Precision BioLogic, Dartmouth, Canada) incubated for 30 min at room temperature with prestained human fibrinogen (final concentration 36.8 nmol/l, Alexa Fluor® 488 conjugate, Molecular Probes) in 21.75 µl permeation buffer (50 mmol/l Tris, 100 mmol/l NaCl, pH 7.4). Clot formation was induced with 5 µl of permeation buffer containing $CaCl_2$ (final concentration 35 mmol/l) and human thrombin (final concentration 0.35 U/ml; Merck, Darmstadt, Germany) either in presence or absence of rMASP-1cf (final concentration 220 nmol/l). Immediately, 30 µl of the activated plasma sample were transferred to an Ibidi-chamber slide (µ-Slide VI, hydrophobic, uncoated, Ibidi GmBH, Martinsried, Germany) shown in Fig. 2A and incubated for 1 h in a moist chamber at room temperature in the dark. Clot lysis was initiated with 10 µl of freshly prepared lysis solution consisting of permeation buffer containing tPA (final concentration 147.1 nmol/l; Technoclone), Glu-plasminogen (final concentration 1.14 µmol/l; Haematologic Technologies Inc., Essex Junction, USA) which was incubated for 10 min in presence or absence of rMASP-1cf (final concentration 220 nmol/l). The lysis solution was pipetted to the border of the clot formed within the Ibidi-chamber slide and time course images of an 212.3 μm^2 section of the clot were taken at 40x magnification (oil) at cycles of 9 s (LSM 710 confocal microscope with Zen software Version 2.1.; Carl Zeiss AG, Oberkochen, Germany). The time was recorded from the first appearance of the lysis front until the clot in the area was completely dissolved. This lysis velocity was compared between three experimental settings (with 15 clots for each setting): no MASP-1, MASP-1 present only during clot formation, MASP-1 present only during clot lysis.

2.6. Cleavage of tPA and plasminogen by MASP-1

rMASP-1cf (final concentration 1.1 µmol/l) was incubated with either tPA (final concentration 441 nmol/l), Technoclone, Vienna,

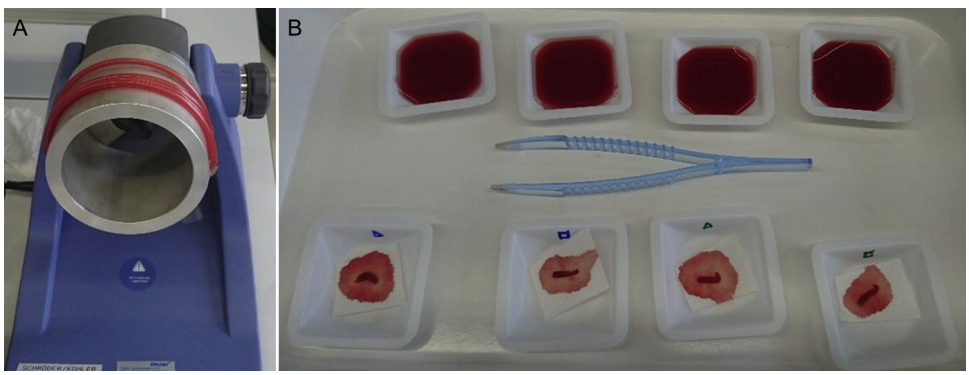

Fig. 1. Chandler loop. A) Image of a Chandler loop experimental setting with loops of PVC tubing filled with recalcified whole blood, mounted on a rotator to form clots under flow conditions. B) Whole blood clots formed in the Chandler loop.

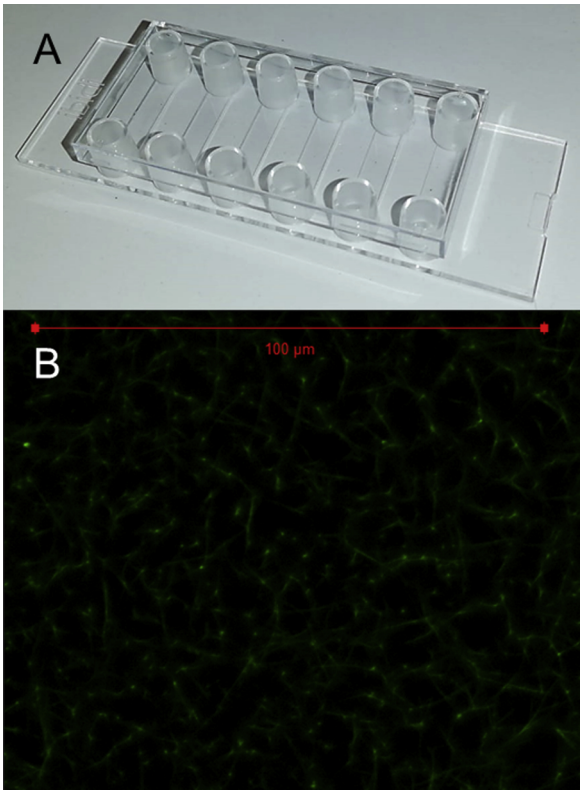

Fig. 2. Laser scanning microscopy. A) Image of an empty Ibidi-chamber slide. B) Exemplary image of fibrin formed from plasma in an Ibidi-chamber slide before induction of fibrinolysis. The picture was taken at 40x magnification with an LSM 710 confocal microscope and Zen software version 2.1 (Carl Zeiss AG, Oberkochen, Germany).

Austria) or Glu-plasminogen (final concentration 227 nmol/l, Haematologic Technologies Inc., Essex Junction, USA) for up to 60 min at 37 °C in a final volume of 42 µl Tris-buffered saline (TBS, 50 mmol/l Tris, 100 mmol/l NaCl, pH 7.4). Subsequently, the samples were mixed with 4x Laemmli buffer (Bio-Rad, Hercules, USA), vortexed briefly, boiled for 10 min at 70 °C and loaded onto Bolt™ 8% Bis-Tris precast gels (Thermo Scientific, Waltham, USA). The protein gel electrophoresis was carried out in Bolt™ MES running buffer (2-(N-Morpholino)-ethanesulfonic acid, Thermo Scientific) for 22 min at 220 V, using Precision Plus Protein™ Dual Color Standards (10–250 kDa, Bio-Rad) as protein marker. After separation, the gels were washed 3x for 5 min in MilliQ H_2O and the proteins bands were visualised by Coomassie staining for 1 h (Imperial™ Protein Stain, Thermo Scientific), followed by overnight destaining at 4 °C in MilliQ H_2O.

2.7. Turbidimetric clot lysis assay with/without SGMI-1 in plasma from sepsis patients

The turbidimetric clot formation and lysis assay was performed similar to a published protocol by Lisman et al., 2001. Tissue factor and tPA concentrations were titrated to obtain a median 50% lysis time around 700 s in pooled normal plasma (Precision BioLogic, Dartmouth, Canada), in accordance with a clot formation and lysis assay used at the Thrombosis and Haemostasis Research Unit, Department of Clinical Biochemistry, Aarhus University Hospital, Denmark (Neergaard-Petersen et al., 2018).

Citrated plasma samples from septic shock patients were thawed in a water bath at 37 °C for 5 min, vortexed and centrifuged at 13,500 rpm for 3 min. Pooled normal plasma was used as control. Then, 70 µl plasma and 49 µl assay buffer (25 mmol/l Hepes, 137 mmol/l NaCl, 3.5 mmol/l KCl, 0.1% bovine serum albumin, all from Sigma-Aldrich®, pH 7.4) per well were added to a low-binding 96-well microplate (Greiner Bio-One GmbH, Kremsmünster, Austria), (plate 1), and kept on ice until use. Two different activation solutions containing assay buffer, EXTEM reagent as tissue factor source, phospholipids (Rossix, Mölndal, Sweden), $CaCl_2$, tPA (Technoclone) and either SGMI-1 or no SGMI-1 were mixed on ice. The activation solutions were added to a separate plate (plate 2) which was kept on ice until reading. Immediately before reading, 85 µl plasma-buffer mix from plate 1 was pipetted onto the corresponding rows of plate 2 using a multi-channel pipette. Final concentrations of the reagents were: EXTEM reagent 1/3615 dilution, tPA 260 ng/ml, phospholipids 4 µmol/l, $CaCl_2$ 26 mmol/l, SGMI-1 5 µmol/l. Plate 2 was shaken for 10 s, and absorbance at 405 nm was read every 45 s for 3 h on a SparkTM 10 M reader (Tecan, Männedorf, Switzerland). Samples and controls were run in duplicate both with and without SGMI-1. Intra- and inter-assay coefficient of variation (CV) were < 13% for all parameters except for integral with no SGMI-1 which had an inter-assay CV of 18%. A CV ≤ 15% for the patient samples were deemed acceptable, otherwise the sample was repeated. Peak absorbance, the area under the clot formation and lysis curve over time (AUC), and time from peak to 50% lysis were calculated using the Shiny App Tool (Longstaff and Subcommittee on Fibrinolysis, 2017).

2.8. Statistical analysis

Statistical analysis was performed with SPSS (version 24.0) software (IBM Corporation, Chicago, USA). Continuous variables were tested for normal distribution with the Kolmogorov-Smirnov and Shapiro-Wilk tests. Normally distributed variables were compared between groups using the *t*-test or paired *t*-test, not normally distributed variables were compared using the Wilcoxon test.

3. Results

In order to better understand possible effects of MASP-1 on fibrinolysis and elucidate the underlying mechanisms, we performed clot lysis experiments in whole blood and plasma where active rMASP-1cf was present during clot formation, during clot lysis or both.

3.1. Effects of rMASP-1cf on fibrinolysis in whole blood studied by thrombelastometry

Thrombelastometry allows to study kinetics of clot formation and lysis and viscoelastic properties of the clot. The advantage of this system is that measurements can be performed in whole blood and MASP-1 is present during the entire clot formation and lysis process and can interact with all plasma components and blood cells. With this experiment we investigated if MASP-1 has an overall effect on clot formation, structure and lysis.

The results from the thrombelastometry measurements are shown in Table 1. As expected, MASP-1 had no significant effect on the kinetics of clot formation (clotting time CT, clot formation time CFT, time to maximum clot firmness MCFt) or the maximum clot firmness MCF, since we used tissue factor in this setting to standardise the clot formation process. Meanwhile, all lysis parameters showed significant differences between absence and presence of MASP-1, suggesting that lysis occurs faster in the presence of MASP-1.

In another experimental series, we investigated the effect of the MASP-1 inhibitor SGMI-1 on endogenous MASP-1, i.e. without adding exogenous rMASP-1cf. As shown in Table 2, addition of rMASP-1cf again accelerated clot lysis compared to recalcified whole blood only (control). Addition of SGMI-1, however, had no effect on any clot formation or lysis parameter compared with the controls.

3.2. Lysis of clots formed in the presence of rMASP-1cf in the Chandler loop

Flow vs. static conditions have an impact on clot structure which in

Table 1
Results from the thrombelastometry experiments. Mean and standard deviation (SD) from a total of 42 measurements in whole blood with MASP-1 or without MASP-1 are shown.

	rMASP-1cf		No MASP-1		p-value
	Mean (n = 42)	SD	Mean (n = 42)	SD	
CT (sec)	64	8.5	66	11.4	n.s.[a]
CFT (sec)	93	21.5	92	22.6	n.s.[b]
MCF (mm)	56	4.7	56	4.4	n.s.[a]
MCFt (sec)	1121	122.2	1155	165.9	n.s.[b]
LI30 (%)	92	7.0	95	4.4	< 0.001[b]
LI45 (%)	56	25.3	69	23.7	< 0.001[b]
LI60 (%)	14	18.9	34	27.5	< 0.001[b]
LOT (sec)	2196	284.0	2417	363.1	< 0.001[a]
LT (sec)	3399	557.5	4024	856.1	< 0.001[a]
CLR (mm/sec)	0.038	0.011	0.029	0.011	< 0.001[a]
TLT (sec)	2276	524.4	2867	791.3	< 0.001[a]

p-value for pairwise comparisons.
[a] Paired *t*-test.
[b] Wilcoxon test; n.s. = not significant.

turn affects fibrinolysis (Campbell et al., 2010). The Chandler loop allows to form a whole blood clot under flow conditions at similar shear rates as in the vasculature *in vivo*. During clot formation, fluorescent fibrinogen is incorporated into the clot, this later allows to quantify fibrinolysis represented by the amount of released fluorescence over time. In this experiment, where clot formation and lysis occur separately, we investigated whether MASP-1 present during clot formation under flow would alter the properties of a whole blood clot which would then translate into a different behaviour towards fibrinolysis.

Overall, fibrinolysis was similar of the clots formed in presence of MASP-1 compared with control clots without MASP-1 (Fig. 3A). Over time, there was a tendency to less fluorescence release and thus slightly impaired fibrinolysis of clots formed in presence of MASP-1, but the difference was not statistically significant. However, when clots from the same donor were compared pairwise (Fig. 3B), in five out of ten cases the clots with MASP-1 showed reduced fibrinolysis than the control clots without MASP-1. In three cases, there was almost no difference, and in two cases the clots with MASP-1 showed an increased lysis compared with their control clots. These results suggest that there are considerable interindividual differences in the effects of MASP-1 on clot properties which might affect fibrinolysis.

3.3. Real-time observation of fibrinolysis by laser scanning confocal microscopy

In this experiment, a fibrin clot is formed from plasma on a special microscopic slide. Incorporation of fluorescent fibrinogen makes the fibrin visible under the microscope as shown in Fig. 2B. When fibrinolysis is induced by adding tPA and plasminogen, lysis can be observed in real-time and the movement of the lysis front can be measured (Fig. 4). In this setting, we investigated the effects on fibrinolysis when active MASP-1 was already present during fibrin formation or only present during fibrinolysis. In the light of the high interindividual differences observed in the ROTEM and Chandler loop experiments, here we used a standard pool plasma in order to exclude any interindividual differences and possible interactions with blood cells to evaluate the pure effect of MASP-1 on fibrinolysis.

Fibrin clots (control clots) formed from standard plasma took an average of 140 s (SD 34.7 s, n = 15) to get fully lysed. When MASP-1 was added to the same standard plasma during fibrin formation, fibrinolysis occurred slightly but not significantly slower (mean 149 s, SD 46.9 s, n = 15, p = 0.555 compared with the controls clots without MASP-1). When MASP-1 was only added to the lysis solution, fibrinolysis was significantly slower (mean 210 s, SD 69.4 s, n = 15) compared with the control clots without MASP-1 (p = 0.002) and also compared with the clots where MASP-1 was added during fibrin formation (p = 0.008).

3.4. Cleavage of tPA and plasminogen by MASP-1

Because of the above observation, that MASP-1 prolonged fibrinolysis significantly when added to the lysis solution containing tPA and plasminogen, we next sought to investigate whether MASP-1 may cleave tPA and/or plasminogen directly. As shown in Fig. 5A, the single-chain form of tPA (band a) was reduced and the two-chain form (bands b and c) was increasing when tPA was incubated with MASP-1,

Table 2
Thrombelastometry experiments with/without SGMI-1. Mean and standard deviation (SD) from a total of 6 measurements in control whole blood (recalcified only, no MASP-1), with rMASP-1cf, or with the MASP-1 inhibitor SGMI-1 are shown.

	Control Mean (SD) n = 6	rMASP-1cf Mean (SD) n = 6	SGMI-1 Mean (SD) n = 6	p-values
CT (sec)	66 (10.3)	62 (7.0)	61 (4.7)	n.s.[a]
CFT (sec)	83 (16.8)	83 (15.4)	85 (20.5)	n.s.[a]
MCF (mm)	60 (1.3)	59 (2.1)	60 (2.3)	n.s.[b]
MCFt (sec)	1368 (272.7)	1227 (186.3)	1428 (273.0)	rMASP-1cf vs. Control: 0.028 [b] SGMI-1 vs. rMASP-1cf: 0.028 [b] SGMI-1 vs. Control: n.s.[b]
LI30 (%)	99 (2.2)	98 (2.3)	99 (1.3)	rMASP-1cf vs. Control: 0.038[b] SGMI-1 vs. rMASP-1cf: 0.024[b] SGMI-1 vs. Control: n.s.[b]
LI45 (%)	88 (8.5)	76 (15.3)	93 (4.1)	rMASP-1cf vs. Control: n.s.[a] SGMI-1 vs. rMASP-1cf: 0.027[a] SGMI-1 vs. Control: n.s.[a]
LI60 (%)	58 (26.0)	39 (30.4)	72 (17.6)	rMASP-1cf vs. Control: n.s.[a] SGMI-1 vs. rMASP-1cf: 0.003[a] SGMI-1 vs. Control: n.s.[a]
LOT (sec)	2874 (357.0)	2363 (257.9)	3048 (267.0)	rMASP-1cf vs. Control: n.s.[a] SGMI-1 vs. rMASP-1cf: < 0.001[a] SGMI-1 vs. Control: n.s.[a]
LT (sec)	4645 (716.9)	3798 (331.3)	5036 (951.6)	rMASP-1cf vs. Control: n.s.[a] SGMI-1 vs. rMASP-1cf: 0.049[a] SGMI-1 vs. Control: n.s.[a]
TLT (sec)	3270 (476.4)	2585 (254.7)	3566 (869.2)	n.s.[a]

p-value for pairwise comparisons.
[a] Paired *t*-test.
[b] Wilcoxon test; n.s. = not significant.

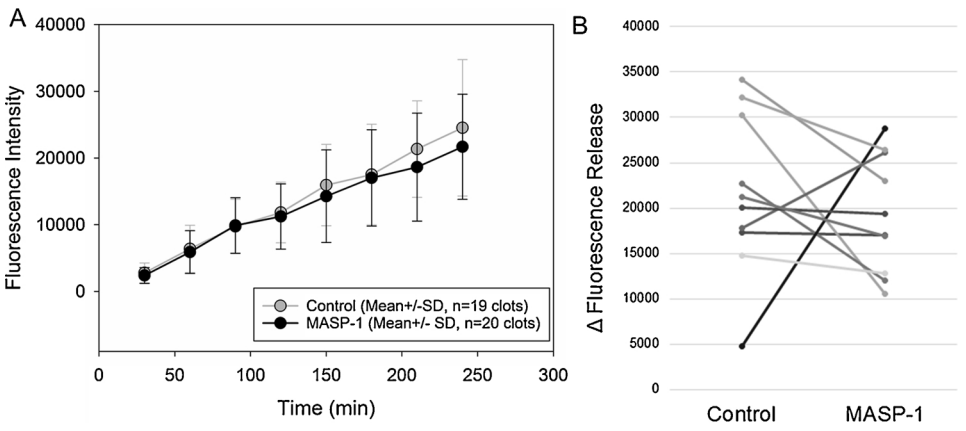

Fig. 3. Lysis of clots formed in the Chandler loop. A) Mean fluorescence release over time from whole blood clots formed under flow conditions in the absence (control) or presence of rMASP-1cf. B) Pairwise comparison of the fluorescence release after 4 h from whole blood clots from the same donor formed without (control) or with rMASP-1cf.

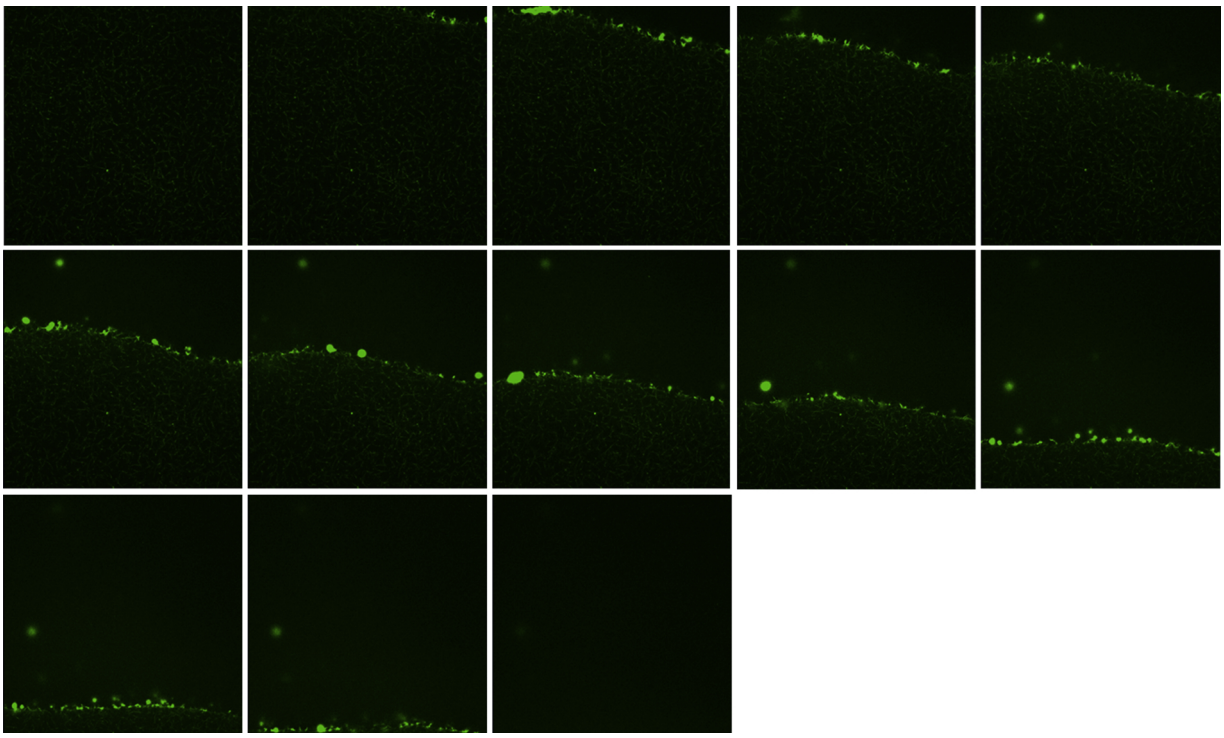

Fig. 4. Real-time fibrinolysis recorded by laser scanning microscopy. Exemplary time course images at cycles of 9 s showing a 212.3 μm^2 sized section of a plasma fibrin clot formed in an Ibidi-chamber slide. The lysis front moves downwards from the upper right corner. Pictures were taken at 40x magnification with an LSM 710 confocal microscope and Zen software version 2.1 (Carl Zeiss AG, Oberkochen, Germany).

suggesting that MASP-1 indeed cleaves tPA. As shown in Fig. 5B, there was also a reduction of plasminogen (band a) when incubated with MASP-1, however, no cleavage products could be clearly detected. This suggests that MASP-1 may cleave plasminogen to a small extent.

3.5. Turbidimetric clot lysis assay with/without SGMI-1 in plasma from sepsis patients

Finally, in order to get some information on the possible biological relevance, we have performed a clot lysis assay in plasma samples from sepsis patients. In these patients, the immune response and plasma cascades such as coagulation/fibrinolysis and complement are all activated (Larsen et al., 2019). We were reasoning that endogenous MASP-1 should also be activated (as shown by its increased consumption) and its inhibition by a specific MASP-1 inhibitor may have effects on clot lysis.

Of the 33 patients, 15 showed flat curves (no clot formation), two were completely resistant to lysis despite high tPA concentrations and three hours' reading time, and four had a CV > 15% despite repeated runs. Therefore, 12 patients were included in the data analysis. In these 12 patients, we found significantly increased peak absorbance, increased area under curve, and prolonged 50% lysis time in the presence of SGMI-1 compared with the same samples without SGMI-1 (Table 3). In normal plasma, the area under the curve and 50% lysis time were increased in the presence of SGMI-1. These results suggest, that endogenous MASP-1 may promote fibrinolysis.

4. Discussion

MASP-1 interacts with coagulation factors and promotes clot formation, but it is not yet fully understood if and how MASP-1 also affects clot lysis. In vivo, clot lysis itself is influenced by many factors, including clot structure, concentrations of fibrinolytic and antifibrinolytic proteins, genetic and environmental factors (Ajjan and Grant, 2006; Weisel and Litvinov, 2008). Efficiency of clot lysis on the other hand can determine thrombotic risk and also the success of pharmacological

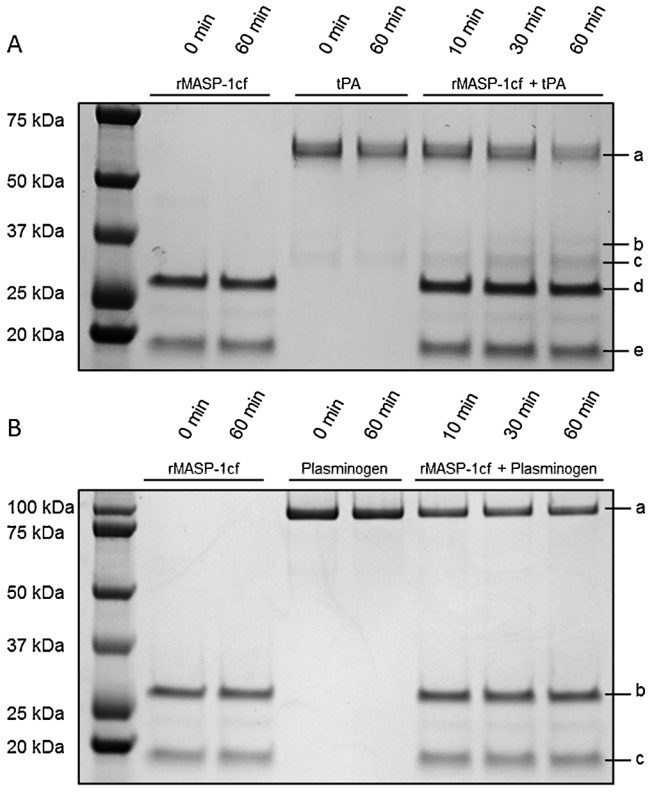

Fig. 5. Cleavage of tPA and plasminogen by MASP-1. A) rMASP-1cf and single-chain tPA are shown individually at 0 and 60 min incubation at 37 °C, and also incubated together for 10, 30 and 60 min. The bands indicated on the right are: a) single-chain tPA, b) and c) two-chain tPA, d) and e) MASP-1. B) rMASP-1cf and plasminogen are shown individually at 0 and 60 min incubation at 37 °C, and also incubated together for 10, 30 and 60 min. The bands indicated on the right are: a) plasminogen, b) and c) MASP-1.

Table 3
Turbidimetric clot lysis assay in presence/absence of SGMI-1 in normal plasma and plasma from sepsis patients.

		No SGMI-1	SGMI-1	p-value
Normal plasma	Peak absorbance (AU)	0.42 (0.40-0.43)	0.41 (0.40-0.43)	n.s.
	AUC (AU*sec)	37 (36-39)	39 (36-43)	0.006
	Lysis time to 50% lysis (sec)	632 (587-721)	721 (632-766)	0.003
Sepsis plasma	Peak absorbance (AU)	0.69 (0.59-0.87)	0.81 (0.64-0.90)	< 0.01
	AUC (AU*sec)	71 (52-133)	79 (58-163)	0.001
	Lysis time to 50% lysis (sec)	1420 (907-3396)	1624 (1303-4293)	< 0.01

AU: aborbance units; n.s.: not significant. Data are shown as median (interquartile range). p-value from pairwise comparison using the Wilcoxon signed rank test.

thrombolysis (Okafor and Gorog, 2015). It is therefore important to understand whether MASP-1 may alter clot properties and/or fibrinolytic activity itself.

In this study we designed experiments under different conditions to investigate possible effects of MASP-1 on clot lysis. The combination of these different experimental settings allowed us to draw several conclusions which shed light on the role of MASP-1 in clot lysis but also raised further questions. A limitation of our study is the use of the recombinant active fragment rMASP-1cf instead of full-length MASP-1. We have shown before that the effect of rMASP-1cf and full-length MASP-1 on clot formation is similar, and therefore conclude that their effect on fibrinolysis may also be similar, but due to the poor availability of full-length MASP-1 we were not able to prove this in our various fibrinolysis assays. Another limitation of the experiments carried out in plasma or with purified proteins is the lack of cell membranes to enable complex formation. We therefore always aim to include experiments in whole blood to make our settings physiologically more relevant.

MASP-1 significantly accelerated the lysis of whole blood clots in thrombelastometry. This effect seemed to be independent of clot formation kinetics and viscoelastic clot properties since the experiment was designed to standardise and control these parameters. The Chandler loop experiment was designed to pick up effects of MASP-1 on whole blood clot structure which may later affect the lysis rate of the clots without direct interference of MASP-1 with fibrinolytic proteins. Overall, the presence of MASP-1 during clot formation did not show a significant effect on later lysis, however, there seemed substantial interindividual differences, and in half of the donors the clots formed in presence of MASP-1 were more resistant to lysis. The observed interindividual differences could, at least in part, be due to interindividual differences in plasma levels of serpins known to inhibit MASP-1 such as C1-inhibitor and antithrombin. Finally, the experiment of fibrinolysis of plasma clots observed in real-time by confocal microscopy also allowed us to separate the processes of fibrin formation and lysis. Interestingly, fibrinolysis was significantly prolonged when MASP-1 was directly added to the lysis mix of tPA and plasminogen. A direct interaction between MASP-1 and the fibrinolytic proteins was also supported by the results from the cleavage gels.

Besides adding exogenous rMASP-1cf in our experiments, we also used the specific MASP-1 inhibitor SGMI-1 to investigate possible effects of endogenous MASP-1. In the whole blood experiments using ROTEM, lysis parameters in samples with SGMI-1 were not different from the control samples. This result can be interpreted in two ways: either endogenous MASP-1 becomes activated during coagulation activation but is quickly inhibited by endogenous serpins, or endogenous MASP-1 does not become activated in sufficient amounts to promote clot formation and lysis. We have previously shown that SGMI-1 prolonged clot formation in whole blood in a microvascular flow model (Jenny et al., 2018) suggesting that endogenous MASP-1 does in fact become activated during clot formation. In the turbidimetric clot lysis assay, SGMI-1 prolonged lysis in both normal plasma and in plasma from patients with sepsis, suggesting that activated endogenous MASP-1 was possibly less inhibited by serpins (due to consumption of serpins in a hypercoagulable state) and accelerated lysis.

These apparent contradictory findings emphasise the complexity of the interactions of MASP-1 with plasma components, blood cells, and also the clot itself. In plasma, the action of MASP-1 may be focused on the activation of coagulation factors, making the fibrin clot more stable, the activation of TAFI, and possibly cleavage of tPA and plasminogen, and taken together this then results in a more pronounced procoagulant and antifibrinolytic effect. However, as soon as fibrin is formed and deposited in a clot, fibrin and also activated platelets can activate MASP-1 and MASP-2 (Kozarcanin et al., 2016). Elevated concentrations of active MASP-1 present around the clot could then influence clot lysis. In whole blood, MASP-1 can also interact with blood cells, in particular platelets, which may in turn exhibit a profibrinolytic phenotype. The complex role of platelets in fibrinolysis is not yet fully understood either, but it has been shown that platelets may also exhibit profibrinolytic properties (Colucci et al., 2017; Whyte et al., 2015). Also, platelet activation via PAR4 has been shown to affect clot elasticity (Vretenbrant et al., 2007) and MASP-1 is able to cleave PAR4 (Megyeri et al., 2009).

Furthermore, our results indicate that the effect of MASP-1 on clot lysis strongly depends on the temporal and spatial balance between procoagulant and profibrinolytic processes. It seems to matter at what time during clot formation and lysis MASP-1 is present to interact with other plasma proteins or blood cells.

Taken together, we could speculate that MASP-1 may be considered as a procoagulant protease with profibrinolytic properties, it contributes to clot formation when needed but also controls its own action by accelerating fibrinolysis in order to prevent clot overgrowth and complete vessel obstruction. It shares these characteristics with the coagulation system with its negative feedback loops for regulation and thus may represent an important redundant back-up system for the coagulation system.

Finally, we observed large interindividual differences even among healthy blood donors. These differences may be based on genetic and environmental factors affecting the current state of the haemostatic and fibrinolytic balance which we could not further explore in our study. Further studies in disease models and clinical studies are needed to clarify under which conditions MASP-1 may promote or impair clot lysis and whether it may also affect pharmacological thrombolysis.

In conclusion, we have demonstrated that MASP-1 is able to alter the susceptibility of blood clots to the fibrinolytic system, underpinning the close interactions between this complement serine protease and the fibrinolytic system. MASP-1 has complex, mostly promoting effects on fibrinolysis with high inter-individual variation. These interactions may be relevant in the development and therapy of cardiovascular and thrombotic diseases.

Funding

This work was supported by the Swiss National Science Foundation [grant numbers310030_140925, 310030_166413]; the OPO Foundation, Zurich, Switzerland; and the National Research, Development and Innovation Office of Hungary [grants K119374, K119386 and KH130376].

References

Ajjan, R., Grant, P.J., 2006. Coagulation and atherothrombotic disease. Atherosclerosis 186, 240–259. https://doi.org/10.1016/j.atherosclerosis.2005.10.042.

Amara, U., Rittirsch, D., Flierl, M., Bruckner, U., Klos, A., Gebhard, F., Lambris, J.D., Huber-Lang, M., 2008. Interaction between the coagulation and complement system. Adv. Exp. Med. Biol. 632, 71–79.

Barthel, D., Schindler, S., Zipfel, P.F., 2012. Plasminogen is a complement inhibitor. J. Biol. Chem. 287, 18831–18842. https://doi.org/10.1074/jbc.M111.323287.

Campbell, R.A., Aleman, M.M., Gray, L.D., Falvo, M.R., Wolberg, A.S., 2010. Flow profoundly influences fibrin network structure: implications for fibrin formation and clot stability in haemostasis. Thromb. Haemost. 104, 1281–1284. https://doi.org/10.1160/TH10-07-0442.

Carter, A.M., 2012. Complement activation: an emerging player in the pathogenesis of cardiovascular disease. Scientifica (Cairo), 402783. https://doi.org/10.6064/2012/402783.

Chapin, J.C., Hajjar, K.A., 2015. Fibrinolysis and the control of blood coagulation. Blood Rev. 29, 17–24. https://doi.org/10.1016/j.blre.2014.09.003.

Colucci, M., Semeraro, N., Semeraro, F., 2017. . Platelets and Fibrinolysis, in: Platelets in Thrombotic and Non-Thrombotic Disorders. Springer International Publishing, Cham, pp. 463–487. https://doi.org/10.1007/978-3-319-47462-5_33.

Conway, E.M., 2015. Reincarnation of ancient links between coagulation and complement. J. Thromb. Haemost. 13 (Suppl 1), S121–32. https://doi.org/10.1111/jth.12950.

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. https://doi.org/10.4049/jimmunol.0901141.

Dobó, J., Schroeder, V., Jenny, L., Cervenak, L., Závodszky, P., Gál, P., 2014. Multiple roles of complement MASP-1 at the interface of innate immune response and coagulation. Mol. Immunol. 61, 69–78. https://doi.org/10.1016/j.molimm.2014.05.013.

Ekdahl, K.N., Teramura, Y., Hamad, O.A., Asif, S., Duehrkop, C., Fromell, K., Gustafson, E., Hong, J., Kozarcanin, H., Magnusson, P.U., Huber-Lang, M., Garred, P., Nilsson, B., 2016. Dangerous liaisons: complement, coagulation, and kallikrein/kinin cross-talk act as a linchpin in the events leading to thromboinflammation. Immunol. Rev. 274, 245–269. https://doi.org/10.1111/imr.12471.

Foley, J.H., 2017. Plasmin(ogen) at the Nexus of Fibrinolysis, inflammation, and complement. Semin. Thromb. Hemost. 43, 135–142. https://doi.org/10.1055/s-0036-1592302.

Héja, D., Harmat, V., Fodor, K., Wilmanns, M., Dobó, J., Kékesi, K.A., Závodszky, P., Gál, P., 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. J. Biol. Chem. 287, 20290–20300. https://doi.org/10.1074/jbc.M112.354332.

Héja, D., Kocsis, A., Dobó, J., Szilágyi, K., Szász, R., Závodszky, P., Pál, G., 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. U S A. 109, 10498–10503. https://doi.org/10.1073/pnas.

Hertle, E., Stehouwer, C.D.A., van Greevenbroek, M.M.J., 2014. The complement system in human cardiometabolic disease. Mol. Immunol. 61, 135–148. https://doi.org/10.1016/j.molimm.2014.06.031.

Hess, K., Ajjan, R., Phoenix, F., Dobó, J., Gál, P., Schroeder, V., 2012a. Effects of MASP-1 of the complement system on activation of coagulation factors and plasma clot formation. PLoS One 7, e35690. https://doi.org/10.1371/journal.pone.0035690.

Hess, K., Alzahrani, S.H., Mathai, M., Schroeder, V., Carter, A.M., Howell, G., Koko, T., Strachan, M.W.J., Price, J.F., Smith, K.A., Grant, P.J., Ajjan, R.A., 2012b. A novel mechanism for hypofibrinolysis in diabetes: the role of complement C3. Diabetologia 55, 1103–1113. https://doi.org/10.1007/s00125-011-2301-7.

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. Diabetes Vasc. Dis. Res. Off. J. Int. Soc. Diabetes Vasc. Dis. 9, 216–225. https://doi.org/10.1177/1479164111432788.

Jenny, L., Dobó, J., Gál, P., Schroeder, V., 2015a. MASP-1 of the complement system promotes clotting via prothrombin activation. Mol. Immunol. 65, 398–405. https://doi.org/10.1016/j.molimm.2015.02.014.

Jenny, L., Dobó, J., Gál, P., Schroeder, V., 2015b. MASP-1 induced clotting–The first model of prothrombin activation by MASP-1. PLoS One 10, e0144633. https://doi.org/10.1371/journal.pone.0144633.

Jenny, L., Dobó, J., Gál, P., Pál, G., Lam, W.A., Schroeder, V., 2018. MASP-1 of the complement system enhances clot formation in a microvascular whole blood flow model. PLoS One 13, e0191292. https://doi.org/10.1371/journal.pone.0191292.

Kastl, S.P., Speidl, W.S., Kaun, C., Rega, G., Assadian, A., Weiss, T.W., Valent, P., Hagmueller, G.W., Maurer, G., Huber, K., Wojta, J., 2006. The complement component C5a induces the expression of plasminogen activator inhibitor-1 in human macrophages via NF-kappaB activation. J. Thromb. Haemost. 4, 1790–1797. https://doi.org/10.1111/j.1538-7836.2006.02046.x.

Kozarcanin, H., Lood, C., Munthe-Fog, L., Sandholm, K., Hamad, O.A., Bengtsson, A.A., Skjoedt, M.-O., Huber-Lang, M., Garred, P., Ekdahl, K.N., Nilsson, B., 2016. The lectin complement pathway serine proteases (MASPs) represent a possible crossroad between the coagulation and complement systems in thromboinflammation. J. Thromb. Haemost. 14, 531–545. https://doi.org/10.1111/jth.13208.

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. https://doi.org/10.1016/j.bbapap.2008.03.020.

Kurosawa, S., Stearns-Kurosawa, D.J., 2014. Complement, thrombotic microangiopathy and disseminated intravascular coagulation. J. Intensive Care 2, 65. https://doi.org/10.1186/s40560-014-0061-4.

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.

Larsen, J.B., Laursen, M.A., Hvas, C.L., Larsen, K.M., Thiel, S., Hvas, A.M., 2019. Reduced mannose-binding lectin-associated serine protease (MASP)-1 is associated with disturbed coagulation in septic shock. Thromb. Haemost. 119, 952–961.

Lisman, T., Leebeek, F.W., Mosnier, L.O., Bouma, B.N., Meijers, J.C., Janssen, H.L., Nieuwenhuis, H.K., De Groot, P.G., 2001. Thrombin-activatable fibrinolysis inhibitor deficiency in cirrhosis is not associated with increased plasma fibrinolysis. Gastroenterology 121, 131–139.

Longstaff, C., Subcommittee on Fibrinolysis, 2017. Development of Shiny app tools to simplify and standardize the analysis of hemostasis assay data: communication from the SSC of the ISTH. J. Thromb. Haemost. 15, 1044–1046.

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. https://doi.org/10.4049/jimmunol.0900879.

Mutch, N.J., Koikkalainen, J.S., Fraser, S.R., Duthie, K.M., Griffin, M., Mitchell, J., Watson, H.G., Booth, N.A., 2010. Model thrombi formed under flow reveal the role of factor XIII-mediated cross-linking in resistance to fibrinolysis. J. Thromb. Haemost. 8, 2017–2024.

Neergaard-Petersen, S., Mogensen, V.B., Veirup, M.S., Grove, E.L., Kristensen, S.D., Hvas, A.M., 2018. Fibrin clot lysis assay: establishment of a reference interval. Thromb. Res. 167, 9–11.

Okafor, O.N., Gorog, D.A., 2015. Endogenous fibrinolysis. J. Am. Coll. Cardiol. 65, 1683–1699. https://doi.org/10.1016/j.jacc.2015.02.040.

Pillemer, L., Ratnoff, O.D., Blum, L., Lepow, I.H., 1953. The inactivation of complement and its components by plasmin. J. Exp. Med. 97, 573–589.

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.

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.
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.
Vretenbrant, K., Ramström, S., Bjerke, M., Lindahl, T.L., 2007. Platelet activation via PAR4 is involved in the initiation of thrombin generation and in clot elasticity development. Thromb. Haemost. 97, 417–424.
Weisel, J.W., Litvinov, R.I., 2008. The biochemical and physical process of fibrinolysis and effects of clot structure and stability on the lysis rate. Cardiovasc. Hematol. Agents Med. Chem. 6, 161–180.
Whyte, C.S., Swieringa, F., Mastenbroek, T.G., Lionikiene, A.S., Lancé, M.D., van der Meijden, P.E.J., Heemskerk, J.W.M., Mutch, N.J., 2015. Plasminogen associates with phosphatidylserine-exposing platelets and contributes to thrombus lysis under flow. Blood 125, 2568–2578. https://doi.org/10.1182/blood-2014-09-599480.
Wojta, J., Huber, K., Valent, P., 2003. New aspects in thrombotic research: complement induced switch in mast cells from a profibrinolytic to a prothrombotic phenotype. Pathophysiol. Haemost. Thromb. 33, 438–441. https://doi.org/10.1159/000083842.