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Serum MASP-1 in complex with MBL activates endothelial cells

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ABSTRACT

The complement system plays an important role in the induction of inflammation. In this study we demonstrate that the initiation complexes of the lectin pathway, consisting of mannose-binding lectin (MBL) and associated serine proteases (MASPs) elicit Ca^{2+} signaling in cultured endothelial cells (HUVECs). This is in agreement with our previous results showing that the recombinant catalytic fragment of MASP-1 activates endothelial cells by cleaving protease activated receptor 4. Two other proteases, MASP-2 and MASP-3 are also associated with MBL. Earlier we showed that recombinant catalytic fragment of MASP-2 cannot activate HUVECs, and in this study we demonstrate that the same fragment of MASP-3 has also no effect. We find the same to be the case if we use recombinant forms of the N-terminal parts of MASP-1 and MASP-2 which only contain non-enzymatic domains. Moreover, stable zymogen mutant form of MASP-1 was also ineffective to stimulate endothelial cells, which suggests that in vivo MASP-1 have the ability to activate endothelial cells directly as well as to activate the lectin pathway simultaneously. We show that among the components of the MBL–MASPs complexes only MASP-1 is able to trigger response in HUVECs and the proteolytic activity of MASP-1 is essential. Our results strengthen the view that MASP-1 plays a central role in the early innate immune response.

1. Introduction

The complement system is a major effector arm of innate immunity. Its main functions are labeling and eliminating invading pathogens and altered self-structures, triggering inflammation, and modulating the adaptive immune response (Ricklin et al., 2010). This network of about 40 proteins includes recognition molecules, serine proteases, thioester containing molecules, cell-surface-receptors and regulators. The danger signals presented by invading pathogens (PAMP: pathogen associated molecular pattern) or by altered host cells (DAMP: damage or danger associated molecular pattern) are recognized by pattern recognition molecules. The lectin pathway of the complement system has at least five different pattern recognition molecules: mannose-binding lectin (MBL), H-, L- and M-ficolin (also named ficolin-3, -2, -1, respectively) (Thiel, 2007) and collectin-11 (also named collectin kidney 1) (Hansen et al., 2010). These molecules may recognize specific patterns of carbohydrate arrays on the surface of microorganisms, hence the name "lectin pathway". The recognition event is converted into an enzymatic signal by serine proteases that are associated with the pattern recognition molecules. MBL and ficolins associate with three serine proteases: MASP (MBL-associated serine protease)-1, -2 and -3 (Matsushita and Fujita, 1992; Thiel et al., 1997; Dahl et al., 2001), and with two non-catalytic proteins MAp44 (Degn et al., 2009; Skjoedt et al., 2010) and MAp19 (Stover et al., 1999; Takahashi et al., 1999). The composition and the stoichiometry of the MBL–MASPs and ficolin–MASPs complexes are not known but it seems likely that a number of different complexes exist in the plasma. When the pattern recognition molecules bind to the target structure, the zymogen serine proteases become activated. An Arg-Ile bond is cleaved in the activation loop, which results in conformational change in the serine protease domain. Recently, we have shown that MASP-1, the most abundant protease component of the lectin pathway, controls lectin pathway activation (Degn et al., 2012; Heja et al., 2012a, 2012b;

Abbreviations: HUVEC, human umbilical vein endothelial cell; MAPK, mitogen-activated protein kinase; MASP, MBL-associated serine protease; MASP-1cf, MASP-1 catalytic fragment; MAp, MBL-associated protein; MBL, mannan-binding lectin; PAR, protease activated receptor.

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Kocsis et al., 2010; Megyeri et al., 2013). MASP-1 autoactivates and cleaves zymogen MASP-2, which then cleaves C4 and C2 components (Kidmose et al., 2012). The resulting C3 convertase (C4b2a) is responsible for maintenance and amplification of the complement cascade.

One of the most important functions of the complement cascade is to trigger inflammation. The split products released during complement activation (e.g. anaphylatoxins: C3a, C5a) activates leukocytes and endothelial cells through G protein-coupled receptors. Recently, we have discovered a new function of the complement system that may contribute to the pro-inflammatory effect of complement activation. MASP-1, like thrombin, is capable of activating endothelial cells directly through cleavage of protease activated receptor 4 (PAR4) (Megyeri et al., 2009).

Endothelial cells, besides their obvious barrier function, have significant role in several important physiological and pathophysiological processes. Among others, endothelial cells coordinate inflammatory response involving a range of receptors and the capability of producing pro-inflammatory cytokines and adhesion molecules. Endothelial cell activation through PARs involves intracellular Ca^{2+}-mobilization, which results in downstream cytoskeleton reorganization, mitogen-activated protein kinase (MAPK) activation and a shift in the pattern of gene expression. Thrombin and other agonist proteases, induce shape and permeability changes in the vascular endothelium and mobilize adhesive molecules to the cell surface promoting leukocyte rolling, adhesion and transendothelial migration. Previously we demonstrated that a recombinant catalytic fragment of MASP-1 (rMASP-1cf), which contains the serine protease (SP) domain and the two complement control protein (CCP) modules, cleaves PAR4 and elicits Ca^{2+} signaling, NF-κB nuclear translocation and p38 MAPK phosphorylation in human umbilical vein endothelial cells (HUVECs). Although the catalytic fragment is enzymatically equivalent to the full-length-molecule, it cannot bind to MBL or ficolins because the N-terminal three domains are missing. Under physiological conditions MASP-1 is a part of the MBL–MASPs complexes as well as part of ficolin–MASPs complexes. The complex formation between MASP-1 and MBL may change the accessibility of the active site of the protease for external substrates, like PARs. Moreover, it was shown earlier that MBL binds to endothelial cells (Oroszlán et al., 2007), and we cannot exclude the possibility that this binding modulates the effect of MASP-1 on the cells besides changing the local concentration of MASP-1 on the surface of the cells. It is therefore important to examine whether native MASPs in complexes with MBL are able to activate endothelial cells.

In the present study we analyzed the effect of MBL–MASPs complexes on endothelial cells. We isolated MBL–MASPs complexes from human plasma and studied their ability to stimulate endothelial cells. We also wanted to show unambiguously that proteolytically inactive MASP-1 and the N-terminal non-catalytic regions of MASP-1/3 and MASP-2 are unable to activate cells. Moreover, we asked whether zymogen MASP-1 is able to activate endothelial cells similarly to activated MASP-1. Finally, we also examined whether the catalytic activity of the third protease component of the lectin pathway, MASP-3, exerts any effect on HUVECs.

2. Materials and methods

2.1. Materials

CNBr-activated Sepharose 4B was from GE Healthcare. Coupling of mannan to CNBr-activated resin was performed according to the manufacturer's instructions. All the other reagents were purchased from Sigma–Aldrich, unless otherwise stated. Lyophilized plasma C1 inhibitor (C1-inh, Berinert P) was from ZLB Behring, and it was further purified by cation-exchange chromatography (Source 15S column; GE Healthcare) and gel filtration (Sephadex 75 HiLoad 16/60; GE Healthcare).

2.2. Recombinant MASP fragments

DNA constructs of recombinant human MASP-1, and -2 catalytic fragments encoding the CCP1-CCP2-SP region (rMASPcf) were used for expression in *E. coli* as described (Ambrus et al., 2003). rMASP-2cf was prepared according to Gál et al. (2005). The R448Q and S646A (precursor numbering) mutants of rMASP-1cf were made according to Megyeri et al. (2013). Wild-type (wt) rMASP-1cf was produced as described by Dobó et al. (2008, 2009), but in the absence of benzamidine. The two-step purification procedure was performed in a single day in order to minimize degradation.

The R448Q and the S646A variants of rMASP-1cf were prepared the same way as the wt enzyme, however in these cases the procedure yields the proenzymic (one-chain) form (Megyeri et al., 2013). The S646A mutant of rMASP-1cf at 100 μg/mL was cleaved by rMASP-2 catalytic fragment (2.5 μg/mL) by overnight incubation at room temperature to bring the enzyme into the two-chain form. After cleavage, the S646A mutant was further purified by repeating the anion exchange chromatographic step on a Source 30Q column to remove rMASP-2cf. We used rMASP-2cf to cleave rMASP-1cf S646A since the two can be separated due to their distinct chromatographic behavior. The procedure yielded an enzyme, which was in the active conformation, but due to the absence of the catalytic serine it had no catalytic activity. Since MASP-2 does not activate endothelial cells (Megyeri et al., 2009) traces of MASP-2 (if any) would not interfere with the cell activation assay.

Wild type proenzymic rMASP-3cf was produced as previously described (Megyeri et al., 2013). Purified rMASP-3cf (400 μg/mL) was activated by cleavage with rMASP-1cf (25 μg/mL) at 37 °C for 3 hours. In the next step rMASP-1cf were removed from the samples by cation exchange chromatography on a SP Sepharose High Performance (GE Healthcare) column. The N-terminal fragments (CUB1-EGF-CUB2) of MASP-1 and MASP-2 were prepared as described by Paréj et al., 2014.

2.3. Purification of MBL–MASPs complexes

MBL–MASPs complexes were purified by a modified method described by Matsushita et al. (2000b). Briefly, plasma samples were clotted by the addition 25 mM $CaCl_2$ and filtered through muslin and the resulting serum was kept at −80 °C until further usage. Serum samples were then thawed and incubated overnight at 4 °C with yeast mannan-Sepharose 4B resin using 10 mM imidazole buffer (pH 6.0) containing 0.2 M NaCl, 20 mM $CaCl_2$, 0.1 mg/ml Pefabloc, and 2% mannitol. After washing the resin with the same buffer, the MBL–MASPs complexes were eluted from the mannan-beads using the above buffer containing 0.3 M mannose. The MBL and MASP-1 concentrations in the eluted samples were determined by sandwich-type immune-assays (see below) as previously described (Thiel et al., 2002, 2012).

To increase the yield of the purified MBL–MASPs complexes we modified the protocol by adding 10 μg/ml recombinant MBL (Jensenius et al., 2003) to the serum samples before incubation with mannan-Sepharose 4B resin. After elution, the fractions were checked by TRIFMA and the appropriate fractions were pooled and concentrated on a spin concentrator and dialyzed against Hank's Balanced Salt Solution (HBSS) and kept at −80 °C until use on endothelial cells.