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Research paper

MAp19, the alternative splice product of the *MASP2* gene

Søren E. Degn [a,*], Steffen Thiel [a], Ole Nielsen [b], Annette G. Hansen [a], Rudi Steffensen [c], Jens C. Jensenius [a]

[a] Department of Biomedicine, Aarhus University, Wilhelm Meyers Allé 4, DK-8000 Aarhus C, Denmark
[b] Department of Clinical Pathology, Odense University Hospital, Winsløwparken 15, 5000 Odense C, Denmark
[c] Regional Centre for Blood Transfusion and Clinical Immunology, Aalborg Hospital, Urbansgade 36, DK-9000 Aalborg, Denmark

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ABSTRACT

The lectin pathway of complement is a central part of innate immunity, but as a powerful inducer of inflammation it needs to be tightly controlled. The *MASP2* gene encodes two proteins, MASP-2 and MAp19. MASP-2 is the serine protease responsible for lectin pathway activation. The smaller alternative splice product, MAp19, lacks a catalytic domain but retains two of three domains involved in association with the pattern-recognition molecules (PRMs): mannan-binding lectin (MBL), H-ficolin, L-ficolin and M-ficolin. MAp19 reportedly acts as a competitive inhibitor of MASP-2-mediated complement activation. In light of a ten times lower affinity of MAp19, versus MASP-2, for association with the PRMs, much higher serum concentrations of MAp19 than MASP-2 would be required for MAp19 to exert such an inhibitory activity. Just four amino acid residues distinguish MAp19 from MASP-2, and these are conserved between man, mouse and rat. Nonetheless we generated monoclonal rat anti-MAp19 antibodies and established a quantitative assay. We found the concentration of MAp19 in serum to be 217 ng/ml, i.e., 11 nM, comparable to the 7 nM of MASP-2. In serum all MASP-2, but only a minor fraction of MAp19, was associated with PRMs. In contrast to previous reports we found that MAp19 could not compete with MASP-2 for binding to MBL, nor could it inhibit MASP-2-mediated complement activation. Immunohistochemical analyses combined with qRT-PCR revealed that both MAp19 and MASP-2 were mainly expressed in hepatocytes. High levels of MAp19 were found in urine, where MASP-2 was absent.

1. Introduction

The recognition of non-self by the innate immune system is mediated by cellular and humoral pattern-recognition molecules (PRMs), targeting conserved pathogen-associated molecular patterns (PAMPs). A group of humoral PRMs, mannan-binding lectin (MBL), H-ficolin, L-ficolin, and M-ficolin, can activate the lectin pathway of complement by means of associated serine proteases, MASP-1, MASP-2 and MASP-3 (Thiel, 2007). Two additional proteins, MAp19 and MAp44, are found associated with these PRMs.

MAp19, also known as sMAP, is one of two proteins arising by alternative splicing of the primary transcript of the *MASP-2* gene (Stover et al., 1999b; Takahashi et al., 1999). The other product is the pro-enzyme MASP-2, which is composed of a CUB domain, an EGF domain, a second CUB domain, two CCP domains and the activation peptide, followed by the serine protease domain (Thiel et al., 1997). Mature MASP-2 is generated by cleavage of the activation peptide, resulting in two disulfide-linked fragments, the larger known as the A-

Abbreviations: MBL, mannan-binding lectin;MASP, MBL-associated serine protease;MAp, MBL-associated protein;pAb, polyclonal antibody;MBS, m-maleimidobenzoyl-N-hydroxysuccinimid;DVS, divinylsulfone;PPD, purified protein derivative;HRP, horseradish peroxidase;KLH, keyhole limpet hemocyanin;BCG, Bacillus Calmette-Guérin;C1-Inh, C1 inhibitor;o.n., overnight;pMBL/MASP, plasma-derived MBL/MASP complexes;PAMP, pathogen-associated molecular pattern;PRM, pattern-recognition molecule;hIgG, normal human IgG;NHS, normal human serum;TRIFMA, time-resolved immunofluorometric assay;RT, room temperature.

* Corresponding author. Tel.: +45 89 42 17 78; fax: +45 86 19 61 28.
E-mail address: sdegn@microbiology.au.dk (S.E. Degn).

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chain and the smaller as the B-chain (Schwaeble et al., 2002; Thiel, 2007). MAp19 consists of only the first CUB domain and the EGF domain, with an additional small C-terminal tail of four unique amino acid residues (EQSL) (Fig. 1). These four amino acid residues are encoded by exon 5 of the gene, which also harbors the 3′UTR. Like MASP-2, MAp19 is known to be expressed in the liver and is found in plasma (Stover et al., 1999a). It forms a head-to-tail dimer, the structure of which has been solved by X-ray crystallography to a resolution of 2.5 Å (Gregory et al., 2004), and associates with MBL and the ficolins in a calcium-dependent manner. The dissociation constant for the interaction with MBL has been determined to be 13 nM, identical to the similar CUB-EGF fragment of MASP-2, but around 16 times higher than that of full-length MASP-2 (Thielens et al., 2001). MAp19 has been suggested to act as an inhibitor of calcium oxalate crystal growth in human urine (Kang et al., 1999). More recently, MAp19 has been reported to compete with MASP-2 for binding to MBL, leading to attenuation of C4-cleaving activity and thus down-regulation of complement activation (Iwaki et al., 2006). MAp19 is relatively well conserved, the amino acid sequence for human MAp19 being 80% and 77% identical with mouse and rat MAp19, respectively, and the unique 4 C-terminal amino acids are conserved between these species (Fig. 1A).

We present the generation of monoclonal antibodies specific for MAp19 and the construction of a solid-phase assay for quantification of MAp19 under dissociating conditions. Using this assay we determine the mean concentration of MAp19 in blood and urine. We compare the mRNA expression profiles of MAp19 and MASP-2, as well as the tissue distribution of the proteins by immunohistochemical analyses. Finally, we characterize the interaction of MAp19 with MBL and ficolins under native conditions and examine the reported inhibitory activity of MAp19 on activation of the complement system.

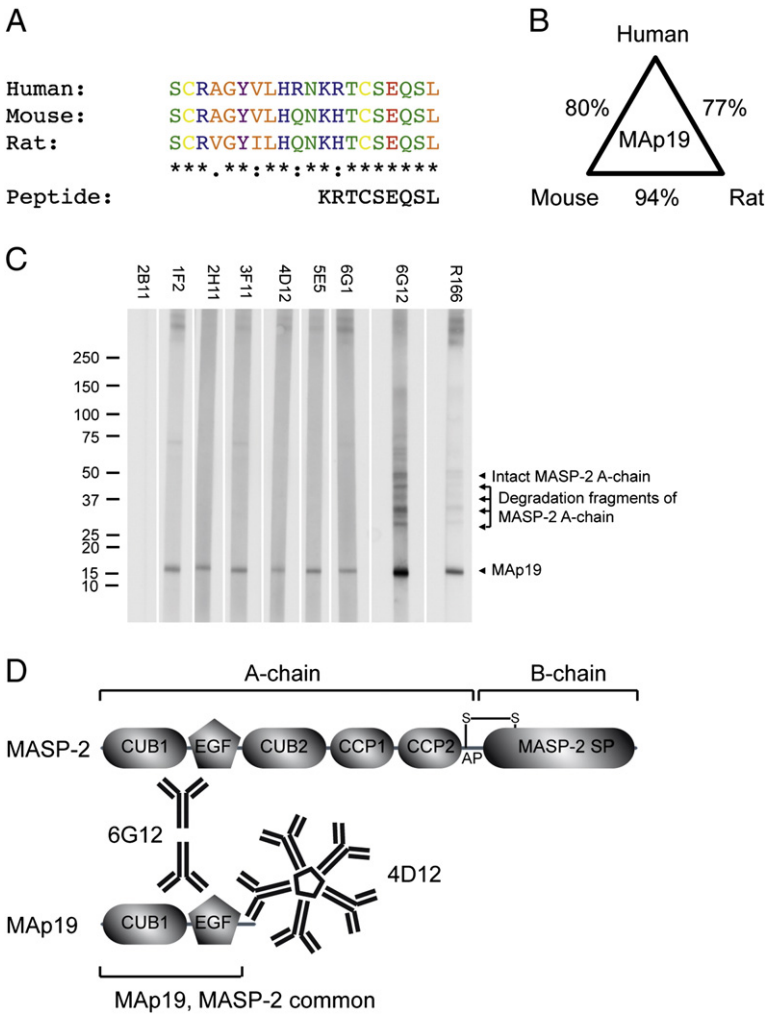

Fig. 1. MAp19 and anti-MAp19 antibodies. A) Alignment of the C-terminal ends of human, mouse and rat MAp19, demonstrating a high degree of conservation with total identity of the 4 ultimate amino acids. The peptide used for immunizations is shown. B) Percent identity between full-length human, mouse and rat MAp19. C) Western blot reactivity of anti-MAp19 antibodies, demonstrating specificity for MAp19. Strips of a Western blot containing purified MBL/MASP complexes from human plasma were developed using hybridoma supernatants as primary antibodies. Negative control is a non-reactive hybridoma (2B11) and positive control is a monoclonal antibody toward MASP-2 and MAp19 (6G12). The parental rat serum (R166) is included, showing similar reactivity to that of the hybridoma supernatants. D) Overview of the domain structure of MAp19 and MASP-2, with indication of approximate antibody epitopes.