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Mannan-binding lectin associated serine protease 2 (MASP-2) activates prothrombin directly and initiates low-level clotting

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The complement system is involved as a recognition and effector mechanism in both the innate and the adaptive immune systems. It is activated by three different pathways: the classical which is initiated by binding of C1q, the alternative which relies on spontaneous hydrolysis of C3 and the lectin pathway which is activated when Mannan-binding lectin (MBL), L-ficolin, H-ficolin or M-ficolin bind to a target and the MBL-associated serine proteases 1, 2, 3 (MASP-1, MASP-2, MASP-3) become activated. Of these MASP-2 has been identified as the enzyme responsible for cleavage of C4 and C2 to form the C3 convertase C4b2a. The coagulation system has many similarities to the complement system, but here the critical step is the generation of thrombin. It is generated by a double cleavage of prothrombin by factor Xa found in the prothrombinase complex (factor Va–Xa complex). Thrombin has a wide range of substrates including fibrinogen and FXIII, activation of which result in fibrin clot formation.

We have demonstrated that rMASP-2, both as a truncated activated form and in complex with rMBL cleaves prothrombin in a similar fashion to factor Xa, and generates active thrombin, as assessed by cleavage of the thrombin substrates fibrinogen and VPR-AMC. The cleavages (at 2 sites) of prothrombin by factor Xa and MASP-2 were identical when compared by prothrombin fragmentation and N-terminal sequencing. Additionally mannan-bound rMBL/rMASP-2 has been shown to be capable of generating fibrin clots via activation of prothrombin. Further work is required to integrate these findings with the fibrinogen and FXIII cleavage activity of MASP-1. Clotting may be a relevant immune defense in vertebrates as it is in some invertebrates. Fibrinopeptides A and B are chemotactic for neutrophils, and fibrin deposition may influence adhesion. Prothrombin activation by MASP-2 may also be relevant to a recently described "C3-bypass" activation of C5.

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CSMD1 is expressed as a membrane protein on neuronal growth cones that colocalizes with F-actin and alpha-3 integrin

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We previously described the identification and *in vitro* complement inhibitory activity of the rat homologue of the human protein known as Cub and sushi multiple domains 1 (CSMD1). CSMD1 consists of 14 N-terminal CUB domains that are separated from each other by a SCR followed by 15 tandem SCRs, a transmembrane domain, and a short cytoplasmic tail that is most highly expressed in neurons of the adult and developing CNS. We found that a recombinant fragment containing two CUB-SCR domains and 12 tandem SCRs inhibits the rat classical complement pathway. To further characterize CSMD1, we wished to determine the location of CSMD1 and verify that it is expressed as a membrane protein in developing neurons. Double immunolabeling experiments of CSMD1 with filamentous actin (F-actin); alpha-3 integrin, CD81, or MARKS were performed to examine the cellular distribution and localization of CSMD1 in cultured neurons of the developing E17 rat dorsal root ganglia. Neurons double-labeled with Alexafluor 488-conjugated phalloidin to reveal F-actin and anti-CSMD1 Ab followed by Alexafluor 594-conjugated anti-rabbit IgG indicated that CSMD1 colocalizes with F-actin at the amoeboid leading edge of growing DRG neurons. CSMD1 did not localize with CD81, a protein that colocalizes with CR2 in B cells. Staining of neurons for alpha-3 integrin, a putative neuronal adhesion molecule, and CSMD1 revealed that CSMD1 colocalizes with alpha-3 integrin at the leading edge of DRG growth cones. However, CSMD1 did not localize with MARKS, an important signal transduction molecule that is highly enriched in neuronal growth cones. These results indicate that CSMD1 is a membrane protein of growing neurons that colocalizes with F-actin and alpha-3 integrin especially in the filopodia of neuronal growth cones. This expression pattern is consistent with a transmembrane protein with the capacity to regulate complement activation or serve as an important receptor in neuronal growth function.

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