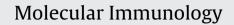
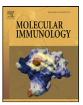
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Mechanisms of mannose-binding lectin-associated serine proteases-1/3 activation of the alternative pathway of complement

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ABSTRACT

Mannose-binding lectin-associated serine proteases-1/3 (MASP-1/3) are essential in activating the alternative pathway (AP) of complement through cleaving pro-factor D (pro-Df) into mature Df. MASP are believed to require binding to mannose binding lectins (MBL) or ficolins (FCN) to carry out their biological activities. Murine sera have been reported to contain MBL-A. MBL-C. and FCN-A. but not FCN-B that exists endogenously in monocytes and is thought not to bind MASP-1. We examined some possible mechanisms whereby MASP-1/3 might activate the AP. Collagen antibody-induced arthritis, a murine model of inflammatory arthritis dependent on the AP, was unchanged in mice lacking MBL-A, MBL-C, and FCN-A (*MBL*^{-/-}/*FCN* A^{-/-} mice) in comparison to wild-type mice. The in vitro induction of the AP by adherent mAb to collagen II was intact using sera from MBL-/-/FCN A-/- mice. Furthermore, sera from MBL-/-/FCN $A^{-/-}$ mice lacked pro-Df and possessed only mature Df. Gel filtration of sera from $MBL^{-/-}/FCN A^{-/-}$ mice showed the presence of MASP-1 protein in fractions containing proteins smaller than the migration of MBL-A and MBL-C in sera from $C4^{-/-}$ mice, suggesting possible binding of MASP-1 to an unknown protein. Lastly, we show that FCN-B was present in the sera of $MBL^{-/-}/FCN A^{-/-}$ mice and that it was bound to MASP-1. We conclude that MASP-1 does not require binding to MBL-A, MBL-C, or FCN-A to activate the AP. MASP-1 may cleave pro-Df into mature Df through binding to FCN-B or to an unknown protein, or may function as an unbound soluble protein.

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1. Introduction

The complement system is part of the innate immune system and plays important roles in host resistance to infection. However, the complement system may mediate tissue damage in autoimmune and inflammatory diseases that involve many different organs. Three complement activation pathways have been

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described in the fluid phase and on cell surfaces: the classical pathway (CP), the alternative pathway (AP), and the lectin pathway (LP) (Ricklin et al., 2010). All 3 pathways result in formation of C3 convertases and C5 convertases, leading to the release of the biologically active fragments C3a and C5a and to generation of the membrane attack complex.

The CP is initiated by IgG or IgM engagement of the C1 complex consisting of C1q, C1s, and C1r. This is followed by the local cleavage of C4 and C2 leading to formation of the CP C3 convertase C4b2a. The LP is activated by the binding of a complex of mannan binding lectin (MBL), ficolin (FCN), and MBL-associated proteases (MASPs) to carbohydrates and acetylated residues on cell surfaces (Fujita et al., 2004; Thiel, 2007). C2 and C4 are then cleaved, generating the CP C3 convertase. The AP is activated by low-grade spontaneous hydrolysis of C3 in plasma to form C3(H₂O) with generation of C3b and interaction with factor B to form a C3bB complex. Complement factor D (Df) then cleaves factor B into fragments Ba and Bb with formation of the AP C3 convertase C3bBb (Harboe and Mollnes, 2008; Pangburn et al., 1981). C3b generated by the CP and AP convertases

Abbreviations: AP, alternative pathway; CAIA, collagen antibody-induced arthritis; CP, classical pathway; EGTA, ethylene glycol tetraacetic acid; FCN, ficolin(s); Df, factor D; IC, immune complex; LP, lectin pathway; MBL, mannose binding lectin(s); MASP-1, 2 and 3, mannose-binding lectin-associated serine proteases 1, 2 and 3; MAp19 or sMAP, mannose-binding lectin-associated protease of 19 kDa; NBF, neutral buffered formalin; pro-Df, pro-factor D; RA, rheumatoid arthritis; T-blue, Toluidine blue.

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binds covalently to cell surfaces. The AP may greatly enhance the local generation of C3b, initially formed by any of the 3 complement activation pathways, by a process of amplification.

The serine proteases associated with MBL or FCN vary considerably in structure and function. MASP-1 and MASP-3 are formed from one gene by alternative splicing. Similarly, MASP-2, and its truncated form MBL-associated protease of 19kDa (MAp19), are formed by alternative splicing from a different single gene (Takahashi et al., 1999). The MASPs circulate as biologically inactive pro-enzymes with MASP-1 and MASP-2 activated from zymogen forms by autocatalysis (Ambrus et al., 2003). MBL enhances MASP-2 activity after binding of the MBL/MASP-2 complex to a cell surface substrate by the MBL moiety. MBL then increases the rate of MASP-2 autocatalysis leading to cleavage of nearby covalently attached C4 and C2. It is not known whether MASP-1 requires a similar process of MBL- and substrate-dependent activation in vivo. However, recombinant MASP-1K is able to cleave pro-factor D (pro-Df) into mature factor D in the fluid phase in vitro in the absence of serum as a source of MBL or FCN (Takahashi et al., 2010).

MASP-1 has evolutionary and structural differences from the other early complement proteases, and may function as a promiscuous protease (Dobo et al., 2009). MASP-1 may augment initiation of the LP through direct cleavage of C2, but not of C4, and by promoting the activation of MASP-2 (Matsushita et al., 2000; Moller-Kristensen et al., 2007; Rossi et al., 2001; Takahashi et al., 2008). MASP-1 weakly cleaves C3(H₂O), but this activity is probably not biologically relevant (Hajela et al., 2002; Matsushita et al., 2000). However, MASP-1 exhibits activity on substrates outside of the complement system including fibrinogen, factor XIII (transglutaminase), and protease-activated receptor 4 (PAR4) (Hajela et al., 2002; Megyeri et al., 2009). In addition, MASP-1 exhibits thrombinlike properties in vitro by cleaving synthetic substrates after an Arg or Lys residue and being inhibited by antithrombin (Megyeri et al., 2009; Takahashi et al., 1999, 2010). Although most MASP-1 may be present in plasma in complex with MBL or FCN, some unbound or free MASP-1 may also exist (Takahashi et al., 2010; Terai et al., 1997; Thiel et al., 2000). The major, if not sole, function of MASP-2 is to cleave C2 and C4, leading to formation of the CP C3 convertase. The function of MASP-3 is largely unknown; MASP-3 is present primarily in complex with FCN-3 where it down-regulates FCN-3-mediated activation of the LP (Skjoedt et al., 2010).

The generation of mice genetically deficient in the MASP-1/3 gene has led to new information about the unique role of MASP-1/3 in the AP. No activation of the AP in vitro was present in sera from $MASP1/3^{-/-}$ mice, with a lack of cleavage of factor B into fragments Ba and Bb. The circulating Df in these mice was present solely in the zymogen form (pro-Df) (Takahashi et al., 2010). Additional studies show that collagen antibody-induced arthritis (CAIA), an experimental model of inflammatory arthritis that is dependent on the AP, is markedly inhibited in $MASP1/3^{-/-}$ mice (Banda et al., 2010a,b, 2007, 2006). The addition of recombinant human Df restored the ability of sera from $MASP1/3^{-/-}$ mice to generate C3 deposition and C5a generation in vitro by the AP after stimulation by adherent anti-collagen type II (CII) mAb (Banda et al., 2010b).

The structure of FCN and MBL are similar with each possessing an N-terminal collagen-like domain (Fujita et al., 2004). MBL possess a C-terminal carbohydrate recognition domain and FCN have a C-terminal fibrinogen-like domain responsible for carbohydrate binding. Humans express one form of MBL whereas mice exhibit two forms, MBL-A and MBL-C. MBL are primarily synthesized in the liver and are found in the circulation. Humans express 3 forms of FCN: FCN-1 or M-ficolin, FCN-2 or L-ficolin, and FCN-3, or H-ficolin (Endo et al., 2007). However, mice only possess 2 forms of FCN, FCN-A and FCN-B. The serum types of FCN, such as human FCN-2 and FCN-3 and murine FCN-A, are synthesized in the liver and are present in the circulation. However, human FCN-1 and murine FCN-B are thought to be absent in serum but to be present both in secretory granules and on the surface of monocytes and macrophages (Endo et al., 2007; Runza et al., 2008). Human FCN-1 binds both MASP-1 and MASP-2 with cleavage of C4 by the bound MASP-2; human MASP-1 also binds to human FCN-1 but a function has not been described (Liu et al., 2005). Although mouse FCN-A binds MASP-2 and MAp19 with activation of the LP, mouse FCN-B binds to neither protease (Endo et al., 2005).

The objectives of these studies were to examine CAIA in mice lacking MBL-A, MBL-C, and FCN-A, and to determine the possible mechanisms of MASP-1 cleavage of pro-Df into mature Df resulting in activation of the AP.

2. Materials and methods

2.1. Mice

Eight to ten-week-old homozygous $MBL^{-/-}/FCN A^{-/-} C57BL/6$ male mice were used for this study of Arthrogen-induced CAIA. *MBL*^{-/-}/*FCN A*^{-/-} mice lacking MBL-A, MBL-C, and FCN-A, or mice lacking only FCN-A, were obtained from Dr. K. Takahashi. The *MBL*^{-/-}/*FCN A*^{-/-} mice were generated by Drs. Stahl and Takahashi by cross breeding MBL $A/C^{-/-}$ mice with FCN $A^{-/-}$ mice obtained from Dr. Fujita. The identity of the $MBL^{-/-}/FCN A^{-/-}$ mice was determined by RT-PCR on DNA obtained from tail cuttings. Studies on these mice have not previously been described. Sera for in vitro studies were obtained from $C3^{-/-}$, $C4^{-/-}$, $Bf^{-/-}$, $Df^{-/-}$, and MASP1/3^{-/-} mice. Our laboratory has maintained colonies of $C3^{-/-}$, $C4^{-/-}$, $Bf^{-/-}$, $Df^{-/-}$, and $MASP1/3^{-/-}$ C57BL/6 homozygous mice with the F10 progeny used for this study. Age-matched and sex-matched C57BL/6 mice were used as wild type (WT) controls (Jackson Laboratories). All animals were kept in a barrier animal facility with a climate-controlled environment having 12-h light/dark cycles. Filter top cages were used with 3 mice in each cage. During the course of this study, all experimental mice were fed breeder's chow provided by the Center for Laboratory Animal Care, University of Colorado School of Medicine.

2.2. Induction of collagen antibody-induced arthritis

CAIA was induced in $MBL^{-/-}/FCN A^{-/-}$ and WT mice by using a cocktail of 5 mAb to CII (Arthrogen-CIA, Chondrex) suspended in sterile Dulbecco's PBS. Age and sex-matched WT C57BL/6 mice were used as controls for these studies. All 5 mAb (3 IgG2a and 2 IgG2b) in this cocktail recognize conserved epitopes within the CB11 fragment, whose recognition sequences are shared by CII in many species. All mice received i.p. injections of 8 mg/mouse of Arthrogen on day 0 and 50 µg/mouse of LPS from *E. coli* strain 0111B4 on day 3 to synchronize the development of arthritis. All mice started to develop arthritis at day 4 and were sacrified at day 10, including 3 age-matched $MBL^{-/-}/FCN A^{-/-}$ and WT mice that were not treated with Arthrogen-CIA.

2.3. Examination for clinical disease activity

The prevalence of disease and severity of clinical disease activity (CDA) in all groups of $MBL^{-/-}/FCN A^{-/-}$ and WT mice were determined every day by a trained individual blinded to the experimental treatment group. The CDA score is based on a 3 point scale per paw: 0 = normal joint; 1 = slight inflammation and redness; 2 = severe erythema and swelling affecting the entire paw with inhibition of use; and 3 = deformed paw or joint with ankylosis, joint rigidity and loss of function. The total CDA score is based on all 4 paws with a maximum score of 12 for each mouse.

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