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Small Mannose-Binding Lectin-Associated Protein Plays a Regulatory Role in the Lectin Complement Pathway

Daisuke Iwaki,* Kazuko Kanno,* Minoru Takahashi,* Yuichi Endo,* Nicholas J. Lynch,† Wilhelm J. Schwaeble,‡ Misao Matsushita,§ Masaru Okabe,‡ and Teizo Fujita2*"  

Mannose-binding lectin (MBL) and ficolins are pattern recognition proteins acting in innate immunity, and they trigger the activation of the lectin complement pathway through MBL-associated serine proteases (MASPs). Upon activation of the lectin pathway, MASP-2 cleaves C4 and C2. A truncated form of MASP-2, named small MBL-associated protein (sMAP), is also associated with MBL/ficolin-MASP complexes. To clarify the role of sMAP, we have generated sMAP-deficient (sMAP−/−) mice by targeted disruption of the sMAP-specific exon. Because of the gene disruption, the expression level of MASP-2 was also decreased in sMAP−/− mice. When recombinant sMAP (rsMAP) and recombinant MASP-2 (rMASP-2) reconstituted the MBL-MASP-sMAP complex in deficient serum, the binding of these recombinant proteins to MBL was competitive, and the C4 cleavage activity of the MBL-MASP-sMAP complex was restored by the addition of rMASP-2, whereas the addition of rsMAP attenuated the activity. Therefore, MASP-2 is essential for the activation of C4 and sMAP plays a regulatory role in the activation of the lectin pathway. The Journal of Immunology, 2006, 177: 8626–8632.

The complement system mediates a chain reaction of proteolysis and assembly of protein complexes, playing a major role in biodefense as a part of both the innate and adaptive immune systems. The mammalian complement system consists of three activation pathways: the classical pathway, alternative pathway, and lectin pathway (1, 2). The lectin pathway provides the primary line of defense against invading pathogens. The pathogen recognition components of this pathway, mannose-binding lectin (MBL)3 and ficolins, bind to arrays of carbohydrates on the surfaces of bacteria, viruses, and parasites, and activate MBL-associated serine proteases (MASPs) to trigger a downstream reaction cascade. The importance of the lectin pathway for innate immune defense is underlined by a number of clinical studies linking a deficiency of MBL with increased susceptibility to a variety of infectious diseases, particularly in early childhood before the adaptive immune system is established (3–6). However, the lectin pathway also contributes to the undesired activation of complement, which is involved in inflammation and tissue damage in a number of pathological conditions, including ischemia or perfusion injury in the heart and kidneys (7–10).

As mentioned above, the lectin pathway involves carbohydrate recognition by MBL and ficolins (11–13), and these lectins form complexes with MASP-1 (14–16), MASP-2 (17), MASP-3 (18), and a truncated protein of MASP-2 (small MBL-associated protein; sMAP or MAP19) (19, 20). The MASP family consists of six domains: two C1r/C1s/Uegf/bone morphogenetic protein (CUB) domains, an epidermal growth factor (EGF)-like domain, two complement control protein (CCP) or short consensus repeat (SCR) domains, and a serine protease domain (21). MASP-2 and sMAP are generated by alternative splicing from a single structural gene, and sMAP consists of the first CUB (CUB1) domain, the EGF-like domain, and an extra 4 aas at the C-terminal end encoded by a sMAP-specific exon. MASP-1 and MASP-3 are also generated from a single gene by alternative splicing (22). When MBL and ficolins bind to carbohydrates on the surface of microbes, the proteolytic form of MASP is cleaved between the second CCP and the protease domain, resulting in the active form that consists of two polypeptides called H and L chains, and thus acquiring proteolytic activities against complement components. Accumulated evidence shows that MASP-2 cleaves C4 and C2 (23), which leads to the formation of the C3 convertase (C4b2a). We proposed that MASP-1 cleaves C3 directly and subsequently activates the amplification loop (24), but this function is controversial (25). Although MASP-3 also contains a serine protease domain in the L chain and exhibits its proteolytic activity against a synthetic substrate (26), its physiological substrates have not been identified. The function of sMAP lacking the serine protease domain remains unknown.

In this study, to clarify the role of sMAP in the activation of the lectin complement pathway, we have disrupted the sMAP-specific exon that encodes 4 aa residues (EQSL) at the C-terminal end of sMAP, and generated sMAP−/− mice. We report in this study for the first time the ability of sMAP to down-regulate activation of the lectin pathway.

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3 Abbreviations used in this paper: MBL, mannose-binding lectin; MASP, MBL-associated serine protease; sMAP, small MBL-associated protein; CUB, C1r/C1s/Uegf/bone morphogenetic protein; EGF, epidermal growth factor; CCP, complement control protein; SCR, short consensus repeat; ES, embryonic stem; WT, wild type; rsMAP, recombinant mouse sMAP; MASP-2i, inactive mouse MASP-2 mutant.
Materials and Methods

Mice

A targeting vector was constructed containing exons 1–4 and part of exon 6 of the 129/Sv mouse MASP-2 gene and a neomycin resistance gene cassette instead of exon 5 (Fig. 1A). A diphtheria toxin A (DT-A) gene was inserted into the 3' end of the vector, and three loxP sites were inserted to perform conditional targeting to remove the neomycin cassette and promoter region in the future. The targeting vector was electrophorated into 129/Sv embryonic stem (ES) cells. The targeted ES cells were screened by Southern blot analysis and PCR genotyping. The targeted mice were intercrossed to obtain double heterozygous offspring. The expression of sMAP and MASP-2 mRNAs was analyzed by Northern blot analysis and quantitative RT-PCR.

FIGURE 1. Targeted disruption of the sMAP gene. A, Partial restriction maps of the MASP-2/sMAP gene, the targeting vector, and the targeted allele. The sMAP-specific exon (exon 5) was replaced with a neo gene cassette. B, Southern blot analysis of genomic DNA from offspring derived from mating male chimeric mice with female C57BL/6J mice. Tail DNA was digested with BamHI and hybridized with the probe depicted in A. An 11-kbp band was derived from the WT allele, and a 6.5-kbp band was derived from the targeted allele. C, PCR genotyping analysis. Tail DNA was analyzed using a mixture of exon 4-specific and neo gene-specific sense primers and an exon 6-specific antisense primer. A 2.5-kbp band was obtained from the WT allele, and a 1.8-kbp band was obtained from the targeted allele.

FIGURE 2. The expression of sMAP and MASP-2 mRNAs in homozygous (−/−) mice. A, Northern blot analysis. Poly(A)+ RNAs from WT (+/+ and homozygous (−/−) mouse livers were electrophoresed, transferred to a nylon membrane, and hybridized with a 32P-labeled probe specific for sMAP, MASP-2 H-chain, MASP-2 L-chain, or the neo gene. A specific band for neo (2.2 kb) was observed in homozygous (−/−) mice. B, Quantitative RT-PCR. MASP-2 H and L chains and sMAP cDNA fragments were amplified by real-time PCR in a LightCycler instrument (Roche Diagnostic Systems). cDNAs synthesized from poly(A)+ RNAs from WT (+/+ and homozygous (−/−) mouse livers were used as templates. The data shown are the means of two experiments.
clones were microinjected into C57BL/6J blastocysts, which were implanted into uteri of foster ICR mice. Male chimeric mice were mated with female C57BL/6J mice to produce heterozygous (+/−) mice. Heterozygous (+/−) mice were screened by Southern blot analysis of tail DNA digested with BamH I using the probe indicated in Fig. 1A. Southern blot analysis showed 6.5-kbp and 11-kbp bands in DNA from heterozygous (+/−) mice (Fig. 1B). Heterozygous (+/−) mice were backcrossed with C57BL/6J mice. To obtain homozygous (−/−) mice, (+/−) mice were intercrossed. Homozygous (−/−) mice (C57BL/6J background) were identified by PCR-based genotyping of tail DNA. PCR analysis was performed using a mixture of exon 4-specific and neo gene-specific sense primers and an exon 6-specific antisense primer. DNA from homozygous (−/−) mice yielded a single 1.8-kbp band (Fig. 1C). In all experiments, 8- to 12-wk-old mice were used according to the guidelines for animal experimentation of Fuku-shima Medical University.

Northern blot analysis
Poly(A)+ RNA (1 μg) from wild-type (WT; +/+ ) and homozygous (−/−) mouse livers was separated by electrophoresis, transferred to a nylon membrane, and hybridized with a 32P-labeled cDNA probe specific for sMAP, MASP-2 H chain, MASP-2 L chain, or the neo gene. The same membrane was stripped and rehybridized with a probe specific for GAPDH.

Quantitative RT-PCR
RT-PCR was performed with the LightCycler System (Roche Diagnostic Systems). cDNAs synthesized from 60 ng of poly(A)+ RNA from WT (+/+) and homozygous (−/−) mouse livers were used as templates for RT-PCR, and cDNA fragments of MASP-2 H and L chains and sMAP were amplified and monitored.

Immunoblotting
The sample was electrophoresed on 10 or 12% SDS-polyacrylamide gels under reducing conditions, and proteins were transferred to polyvinylidene difluoride membranes. Proteins on the membranes were detected with anti-MASP-1 antiserum raised against the L chain of MASP-1 or with anti-MASP-2/sMAP antiserum raised against the peptide from the H chain of MASP-2.

Detection of MASPs and sMAP in the MBL-MASP-sMAP complex
Mouse serum (2 μl) was added to 480 μl of TBS-Ca2+ buffer (20 mM Tris-HCl (pH 7.4), 0.15 M NaCl, and 5 mM CaCl2) containing 0.1% weight to volume BSA (TBS-Ca2+/BSA) and incubated with 40 μl of 50% mannan-agarose gel slurry (Sigma-Aldrich) in TBS-Ca2+/BSA buffer at 4°C for 30 min. After incubation, each gel was washed with TBS-Ca2+ buffer and the washing buffer for SDS-PAGE was added to the gel. The gel was boiled and the supernatant was subjected to SDS-PAGE, followed by immunoblotting to detect MASP-1, MASP-2, and sMAP in the MBL complex.

C4 deposition assay
Mouse serum was diluted with TBS-Ca2+/BSA buffer up to 100 μl. The diluted sample was added to mannan-coated microtiter wells and incubated at room temperature for 30 min. The wells were washed with the chilled washing buffer (TBS-Ca2+ buffer containing 0.05% (v/v) Tween 20). After the washing, 100 μl of human C4 dilutions (17 μg/ml in TBS-Ca2+/BSA) were added to each well and incubated on ice for 30 min. Human C4 was isolated as previously described (14). The wells were washed with the chilled washing buffer, and HRP-conjugated anti-human C4 polyclonal Ab (Biogenesis) was added to each well. Following incubation at 37°C for 30 min, the wells were washed with the washing buffer and 3,3′,5,5′-tetramethylbenzidine solution was added to each well. After developing, 1 M H3PO4 was added and the absorbance was measured at 450 nm.

C3 deposition assay
Mouse serum was diluted with BBS buffer (4 mM barbital, 145 mM NaCl, 2 mM CaCl2, and 1 mM MgCl2 (pH 7.4)) containing 0.1% weight to volume BSA up to 100 μl. The diluted sample was added to mannan-coated microtiter wells and incubated at room temperature for 30 min. The wells were washed with the chilled washing buffer (TBS-Ca2+ buffer containing 0.05% (v/v) Tween 20). After the washing, 100 μl of human C4 dilutions (17 μg/ml in TBS-Ca2+/BSA) were added to each well and incubated on ice for 30 min. Human C4 was isolated as previously described (14). The wells were washed with the chilled washing buffer, and HRP-conjugated anti-human C4 polyclonal Ab (Biogenesis) was added to each well. Following incubation at 37°C for 30 min, the wells were washed with the washing buffer and 3,3′,5,5′-tetramethylbenzidine solution was added to each well. After developing, 1 M H3PO4 was added and the absorbance was measured at 450 nm.

FIGURE 3. Deficiency of MASP-2 in homozygous (−/−) mouse serum. A, Immunoblotting of MASP-2 and sMAP in mouse serum. WT (+/+ ) or homozygous (−/−) mouse serum (2 μl) was subjected to immunoblotting and detected with anti-MASP-2/sMAP antiserum. B, Detection of MASPs and sMAP in the MBL-MASP-sMAP complex. Mouse serum was incubated with mannan-agarose gel, and sMAP, MASP-1, and MASP-2 in the MBL complex bound to the gel were detected as described in Materials and Methods.

FIGURE 4. Decreased cleavage of C4 and C3 in homozygous (−/−) mouse serum. A, Deposition of C4 on mannan-coated wells. Mouse serum was diluted 2-fold and incubated in mannan-coated wells at room temperature for 30 min. After the washing of the wells, human C4 was added to each well and incubated on ice for 30 min. The amount of human C4 deposited on the wells was measured using HRP-conjugated anti-human C4 polyclonal Ab. B, Deposition of C3 on mannan-coated wells. Diluted mouse serum was added to mannan-coated wells and incubated at 37°C for 1 h. The deposition of endogenous C3 on the wells was detected with HRP-conjugated anti-human C3c polyclonal Ab.
volume human serum albumin up to 100 μl. The diluted sample was added to mannan-coated microtiter wells and incubated at 37°C for 1 h. The wells were washed with the washing buffer. After the washing, HRP-conjugated anti-human C3c polyclonal Ab (DakoCytomation), which reacts with C3c was added to each well. Following incubation at room temperature for 1 h, the wells were washed with the washing buffer. After the washing, HRP-conjugated anti-human C3c polyclonal Ab (DakoCytomation), which reacts with C3c was added to each well. Following incubation at room temperature for 1 h, the wells were washed with the washing buffer and 3,3',5,5'-tetramethylbenzidine solution was added to each well. The color was measured as described above.

Recombinants
Recombinant mouse sMAP (rsMAP), rMASP-2, and the inactive mouse MASP-2 mutant (MASP-2i), whose active-site serine residue in the serine protease domain was substituted for the alanine residue, were prepared as described previously (27).

Reconstitution of the MBL-MASP-sMAP complex
Homozygous (−/−) mouse serum (20 μl) and various amounts of MASP-2i and/or rsMAP were incubated in a total volume of 40 μl in TBS-Ca2+/BSA buffer and added to mannan-agarose gel slurry, and MASP-2i and rsMAP in the MBL-MASP complex bound to the gel were detected as described in Detection of MASPs and sMAP in the MBL-MASP-sMAP complex. In another experiment to restore the C4 deposition activity, homozygous (−/−) mouse serum (0.5 μl) and various amounts of MASP-2 and/or rsMAP were incubated in a total volume of 20 μl in TBS-Ca2+/BSA buffer and added to mannan-coated wells. All subsequent procedures were performed as described in C4 deposition assay.

Results
Expression of sMAP and MASP-2 in homozygous (−/−) mice
To clarify the role of sMAP in vivo, we established a gene-targeted mouse that lacked sMAP. A targeting vector was constructed to replace the specific exon for sMAP (exon 5) with a neomycin resistance gene cassette (Fig. 1A). Positive ES clones were injected into C57BL/6 blastocysts, and the founder chimeras bred with C57BL/6J females. Southern blot analysis of tail DNA from agouti-color pups showed a germinal transmission of the targeted allele. Heterozygous (+/−) mice were screened by Southern blot analysis of tail DNA. Southern blot analysis showed 6.5-kbp and 11-kbp bands in DNA from heterozygous (+/−) mice (Fig. 1B). Heterozygous (+/−) mice were backcrossed with C57BL/6J mice. To obtain homozygous (−/−) mice, heterozygous (+/−) mice were intercrossed. Homozygous (−/−) mice (C57BL/6J background) were identified by PCR-based genotyping of tail DNA, yielding a single 1.8-kbp band (Fig. 1C).

Homozygous (−/−) mice developed normally and showed no significant difference in body weight from WT (+/+) mice. There were no morphological differences between them either. In a Northern blot analysis, the probe specific for sMAP detected a single 0.9-kb band in WT (+/+) mice, whereas no specific bands were detected in homozygous (−/−) mice (Fig. 2A). When the probe specific for MASP-2 H or L chain was used, several specific bands were detected in WT (+/+) mice as reported previously (20) and the H chain-specific probe also detected the sMAP specific-band. However, in homozygous (−/−) mice the corresponding bands were very weak and several extra bands were detected. We also performed a quantitative RT-PCR analysis to check the expression levels of sMAP and MASP-2 mRNAs. In homozygous (−/−) mice, the expression of sMAP mRNA was completely abolished and that of MASP-2 was also decreased markedly; it was quantitated as ~2% of that of WT (+/+) mice in both H and L

FIGURE 5. Competitive binding of sMAP and MASP-2 to MBL. A, Reconstitution of the MBL-MASP-sMAP complex in homozygous (−/−) mouse serum. MASP-2i and/or rsMAP (4 μg) were incubated with homozygous (−/−) mouse serum (20 μl). The mixture was further incubated with mannan-agarose gel, and rsMAP and MASP-2i in the fraction bound to the gel were detected by immunoblotting. B, Various amounts of MASP-2i (0 –5 μg) and a constant amount of rsMAP (5 μg) were incubated with homozygous (−/−) mouse serum (20 μl) and further incubated with mannan-agarose gel. C, A constant amount of MASP-2i (0.5 μg) and various amounts of rsMAP (0 –20 μg) were incubated with homozygous (−/−) mouse serum (20 μl). D, Various amounts of rsMAP (0 –20 μg) were incubated with WT (+/+) mouse serum (20 μl).
FIGURE 6. Restoration of the C4 deposition activity by addition of rMASP-2 and down-regulation of the activity by rsMAP. Various amounts of rsMAP (0–5 μg) (A) or rMASP-2 (0–1.5 μg) (B) were incubated with 0.5 μl of homozygous (−/−) mouse serum in a total volume of 20 μl in TBS-Ca²⁺/Mg²⁺ buffer on ice overnight. Then, the mixture was diluted with 80 μl of TBS-Ca²⁺/Mg²⁺/BSA buffer and added to mannan-coated wells, and the amount of C4 deposited on the wells was measured. C, rMASP-2 (1 μg) and various amounts of rsMAP (0–0.5 μg) were incubated with 0.5 μl of homozygous (−/−) mouse serum. The mixture was added to mannan-coated wells, and the amount of C4 deposited on the wells was measured. D, rsMAP (0–0.7 μg) was incubated with WT serum (0.5 μl), and the amount of C4 deposited on mannan-coated wells was measured.

chains by real-time PCR (Fig. 2B). Furthermore, we examined the expression of MASP-2 at the protein level. Both sMAP and MASP-2 were undetectable in homozygous (−/−) mouse serum by immunoblotting (Fig. 3A). After the incubation of homozygous (−/−) mouse serum with mannan-agarose gels, both sMAP and MASP-2 were not detectable in the fraction bound to the gels, although MASP-1 was detected in the complex (Fig. 3B).

Cleaving activities of C4 and C3 through the lectin pathway in homozygous (−/−) mouse serum

When homozygous (−/−) mouse serum was incubated in mannan-coated wells, the amount of human C4 deposited on the wells was ~20% of that in normal serum at dilutions ranging from 1/400 to 1/50 (Fig. 4A). We also examined the C3 deposition activity of the lectin pathway in homozygous (−/−) mouse serum. The mouse serum was added to mannan-coated wells, and the amount of endogenous C3 deposited on the wells was measured. The amount was decreased in the deficient serum and was 21% of that in normal serum at a dilution of 1/10 (Fig. 4B).

Reconstitution of the MBL-MASP-sMAP complex in homozygous (−/−) mouse serum

When rsMAP or MASP-2i was added to homozygous (−/−) mouse serum, both recombinants were able to bind to MBL (Fig. 5A, lanes 3 and 4). When rsMAP and MASP-2i were simultaneously incubated with the serum (Fig. 5A, lane 5), both recombinants were detected in the MBL-MAST-sMAP complex. However, the amount of sMAP bound to the complex was less than that when only rsMAP was incubated with the serum. We further investigated the competitive binding of sMAP and MASP-2 to MBL. A constant amount of rsMAP and various amounts of MASP-2i were added to the deficient serum. The binding of rsMAP decreased in a dose-dependent manner with increasing amounts of MASP-2i (Fig. 5B). Inversely, the amount of MASP-2i bound to MBL decreased by the addition of rsMAP (Fig. 5C). When rsMAP was added to WT serum, the binding both of endogenous sMAP and of MASP-2 to MBL decreased in a dose-dependent manner (Fig. 5D).

Restoration of C4 deposition activity in homozygous (−/−) mouse serum

We performed a reconstitution experiment of the deposition of C4 on mannan-coated wells using recombinants. When rsMAP was added to the deficient serum, the amount of C4 deposited actually decreased to basal levels in a dose-dependent manner (Fig. 6A). When rMASP-2 was added to the serum, the amount of C4 was restored by up to 80% of that of WT serum in a dose-dependent manner and reached a plateau (Fig. 6B). Next, we investigated the effect of sMAP on the deposition of C4. When a constant amount of rMASP-2 and various amounts of rsMAP were added to the deficient serum, the amount of C4 deposited decreased with the addition of rsMAP in a dose-dependent manner (Fig. 6C), and the addition of rsMAP to WT serum also decreased the amount of C4 deposited (Fig. 6D), suggesting that sMAP plays a regulatory role in the activation of the lectin pathway.

Discussion

We have generated sMAP−/− mice through targeted disruption of the sMAP-specific exon. The expression level of MASP-2 was also extremely decreased at both the mRNA and protein levels in these mice (Figs. 2 and 3). A Northern blot analysis with a MASP-2 probe showed only extra bands in poly(A)⁺ RNA from sMAP−/− mice, suggesting that the normal splicing of the MASP-2 gene was altered by the targeting of the sMAP gene and, therefore, the expression level of MASP-2 was markedly decreased. As a result, the cleavage of C4 by the MBL-MAST complex in the deficient serum was decreased by ~80% compared with that in the normal serum (Fig. 4A). In the reconstitution experiments, the C4 cleavage activity was restored by addition of rMASP-2 but not rsMAP (Fig. 6, A and B). The reduction in the deposition of C4 observed in the
deficient serum should be caused by the deficiency of MASP-2 in the MBL-MASP complex (Fig. 3B). Therefore, it is clear that MASP-2 is essential for the activation of C4 by the MBL-MASP complex. However, addition of rMASP-2 did not completely restore the cleavage activity, and the deposition of C4 reached a plateau. As reported previously (27, 28), most rMASP-2 was converted to the active form by autoactivation during the purification procedures and some lost its protease activity. Because the active or inactive state of MASP-2 has no significant influence on its association with MBL (26), it is possible that rMASP-2 that has lost its protease activity binds to MBL and competitively prevents the association of the active form, thereby resulting in an incomplete restoration of C4 deposition. The C3 cleavage activity of the lectin pathway was also attenuated in the deficient serum (Fig. 4B). The decline in the amount of C3 deposited is probably due to the very low level of activity of the C3 convertase, which consists of C4b and C2a fragments generated by MASP-2.

MASP and sMAP each associated as homodimers and formed complexes with MBL or L-ficolin through their N-terminal CUB and EGF-like domains (26, 28, 29, 30). The crystal structures of sMAP and the CUB1-EGF-CUB2 segment of MASP-2 reveal their homodimeric structure (31, 32). The collagen-like domain of MBL is involved in associating with MASPs (33, 34), and some mutations introduced into the domain have decreased the binding of MBL to the CUB1-EGF-CUB2 segments of MASP-1 and MASP-2 (35). The binding sites for MASP-2 and for MASP-1/3 overlap but are not identical (36). Although the sMAP binding site of MBL has not been identified yet, the binding sites for sMAP and MASP-2 are probably identical, because the CUB1-EGF region is the same in sMAP and MASP-2. Thus, it is reasonable that sMAP and MASP-2 compete with each other to bind MBL in the reconstitution of the MBL-MASP-sMAP complex (Fig. 5). The affinity of sMAP for MBL is lower than that of MASP-2 (28, 29). The concentration of sMAP in mouse serum has not been determined. As shown in Fig. 3A, however, the amount of sMAP in the WT serum is much greater than that of MASP-2. Therefore, sMAP is able to occupy the MASP-2/sMAP binding site and prevent MASP-2 from binding to MBL, and consequently the C4 cleavage activity of the MBL-MASP complex is reduced.

The regulatory mechanism of sMAP in the lectin pathway remains to be investigated. It is still unknown whether sMAP plays its regulatory role before or after complement activation. sMAP may prevent inadvertent activation of the MBL-MASP complex before microbial infection or suppress overactivation of the lectin pathway once activated. There is another potential regulator in the lectin pathway, MASP-3 is also a competitor of MASP-2 in binding to MBL and down-regulates the C4 and C2 cleavage activity of MASP-2 (18). Although the interaction between sMAP and MASP-3 has not been investigated, it is possible that they are able to down-regulate activation of the lectin pathway cooperatively.

In this study, we have demonstrated that sMAP and MASP-2 compete to bind MBL, and that sMAP has the ability to down-regulate the lectin pathway, which is activated by the MBL-MASP complex. It is reasonable that sMAP also regulates another route of the lectin pathway activated by the ficolin-MASP complex. MASP-2 and sMAP also competed to bind mouse ficolin A and down-regulate the C4 cleavage activity of the ficolin A-MASP complex (37). A study of MBL null mice was recently reported (38). MBL null mice have no C4 cleavage activity in the MBL lectin pathway and are susceptible to Staphylococcus aureus infections. In this study, sMAP-/- mice, which are also deficient in MASP-2, showed reductions in C3 cleavage activity besides C4 cleavage activity in the lectin pathway. Because of their impaired opsonizing activity, the sMAP-deficient mice may be susceptible to bacterial infections. Further investigation of the sMAP-deficient mice will clarify the function of the lectin pathway in the protection against infectious diseases.

Another important finding is that the addition of rSMAp to normal serum results in a reduction in the activation of C4 (Fig. 6D). The lectin pathway has been also demonstrated to regulate inflammation and tissue damage in several organs (7–10). In MBL-deficient patients undergoing treatment for a thoracic abdominal aortic aneurysm, complement was not activated and levels of proinflammatory markers were reduced following surgery (8). Accumulated evidence has demonstrated the potential pathophysiologic role of MBL during conditions of ischemia and reperfusion in a variety of vascular beds. Therefore, the specific blockade of MBL or inhibition of the lectin complement pathway may represent a therapeutically relevant strategy for the prevention of ischemia or perfusion-associated damage. Thus, it is possible that sMAP is one of the candidates for such an inhibitor, because it acts as an attenuator of the lectin pathway’s activation.

Disclosures
The authors have no financial conflict of interest.

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