We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

The Study of MASPs Knockout Mice

Minoru Takahashi, Daisuke Iwaki, Yuichi Endo and Teizo Fujita

Additional information is available at the end of the chapter

1. Introduction

Plasma proteases, e.g. thrombin, factor X, complement factor D and C1s are responsible for the physiological activities, such as coagulation and complement system. These proteases circulate as their zymogen in blood and are activated by various stimulations. In this chapter, we focus on a family of plasma serine proteases, called MASP (MBL/ficolin-associated serine protease) that can activate the complement. Three distinct MASP, MASP-1, MASP-2 and MASP-3 have been identified in many species of vertebrates. Although the contribution of MASP-2 in activation of complement was well defined, the substrates for MASP-1 and MASP-3 were still obscure. We have generated MASP-1- and MASP-3-deficient mice (*Masp1/3$^{-/-}$*) to verify roles of MASP-1 and MASP-3 proteases *in vivo*. One major finding is that MASP-1, considered being a lectin pathway component—also acts as a pro-factor D (Df) convertase, the initiator of the alternative pathway. Our results emphasize a unique feature of MASP-1, participating two complement pathways. We also generated MASP-2 deficient mice. In here, we would like to summarize the results obtained from these knockout mice.

2. Complement system

The complement system is an important part of the innate immune system, mediating several major effector functions, such as directly killing pathogens, promoting phagocytosis, and clearance of immune complexes and apoptotic cells and modulating adaptive immune responses, as describing in some excellent reviews (Ricklin, et al., 2010) (Fujita, et al., 2004) (Carroll, 2004). On the other hand, inappropriate activation of complement affects the pathogenesis of inflammatory diseases (Holers, 2003). Therefore, well-understanding of the mechanisms of its activation is very important. More than 30 proteins in plasma consist of the complement system. The most abundant protein among them is the third component (C3). Once the complement system is activated, a chain of reactions involving restricted proteolysis and assembly occurs, resulting in cleavage of C3 into C3b and C3a. The cascade

up to C3 cleavage is called the activation pathway. There are three distinct activation pathways of the complement cascade; the classical, alternative, and lectin pathways, that all converge on factor C3 and lead to activation of complement effector functions as above (Walport, 2001a)(Fig. 1).
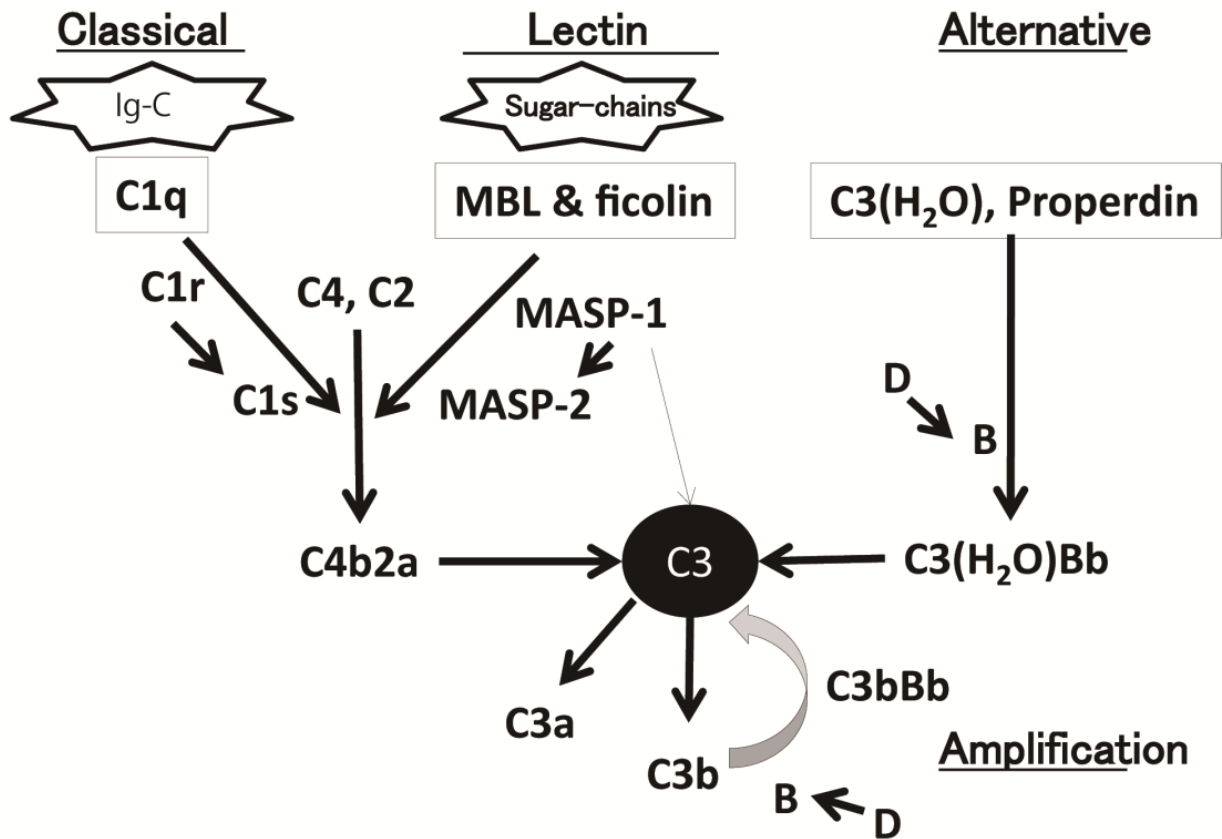

Figure 1. Activation pathways for complement system.

In the mammalian complement system, the pivotal molecule circulating C3 is cleaved into C3a and C3b by two different C3 convertases, C4b2a and C3bBb. C4b2a is generated by the classical and lectin pathway and C3bBb is generated by the alternative pathway.

2.1. The classical pathway

The classic pathway is initiated by recognition of the first C1 binding to a variety of targets, most prominently immune complexes (Walport, 2001a) (Walport, 2001b). C1 consists of a single C1q molecule associated with dimers of C1r and C1s(Lepow, et al., 1963). C1r and C1s are plasma serine proteases, normally existing in an inactive pro-enzyme form. The conformational exchange of C1q by binding to immune complexes results in the activation of C1r. C1r is thought to be cleaved in some autocatalytic manner and once C1r molecule is activated, it activate C1s, which in turn cleaves C4 and then C2(Arlaud, et al., 2002). The C4 cleavage products are C4a and C4b. The latter molecule may be bound to non-self surfaces on pathogens and is bound to C2 to form the classical pathway C3 convertase.

2.2. The alternative pathway

In the alternative pathway, spontaneous hydrolysis of C3, designated $C3(H_2O)$ results in triggering complement activation with complement factor B, making another C3 convertase, $C3(H_2O)Bb$ on foreign cells (Muller-Eberhard and Gotze, 1972, Pangburn, et al., 1981). This leads to the cleavage of factor B by factor D, giving rise to an active enzyme complex with the fragment Bb as the enzyme. The alternative pathway does not involve specific recognition molecules and also functions to amplify C3 activation (amplification loop) (Brouwer, et al., 2006).

2.3. The lectin pathway

Activation of the lectin pathway is similar with that of the classical pathway (Degn, et al., 2010). The lectin pathway is initiated by some serum lectins binding to pathogen-associated molecular patterns, mainly carbohydrate structures present on bacterial, fungal, or viral pathogens. In 1978, a serum lectin, designated mannose-binding lectin (MBL), which recognizes carbohydrates such as mannose and N-acetylglucosamine was first isolated from rabbit liver (Kawasaki, et al., 1978). MBL acts as the pattern recognition molecule, which recognizes sugar chains on some foreign pathogens. MBL is also found to have an avidity of complement activation (Ikeda, et al., 1987) (Holmskov, et al., 2003) (Turner, 1996). It has been thought that MBL activates complement by $C1r_2C1s_2$ protease complex that consists of classical pathway (Ohta, et al., 1990). However, in 1992, Matsushita and Fujita found a new plasma serum protease designated MBL-associated serine protease (MASP) that binds MBL (Matsushita and Fujita, 1992) (Matsushita, et al., 1998). Recent studies identified ficolins that are also plasma proteins with binding activity for carbohydrates to associate with MASP and to activate complement (Matsushita, et al., 2000, Matsushita, et al., 2001) (Cseh, et al., 2002). Ficolins has a collagen-like domain and a fibrinogen-like domain. Furthermore, CL-K1 (Keshi, et al., 2006) was also identified as a collectin that associates with MASP(Hansen, et al., 2010).

3. MBL-associated serine proteases

3.1. Three MASP proteins were associated with MBL and ficolins

MASP is homologue of C1r and C1s of the classical pathway, sharing the well-described domains structure in the order from N-terminus, CUB-I, EGF, CUB-II, CCP-I, CCP-II and SP (Sato, et al., 1994). The CUB (C1r/C1s, embryonic sea Urchin protein [Uefg], and Bone-morphogenetic protein 1 [Bmp1]) domain is approximately 110 aa, predicting a molecular structure of an antiparallel beta-barrel similar to those in immunoglobulins (Bork and Beckmann, 1993). The EGF (epidermal growth factor-like) domain of approximately 50 aa is also found in many proteins and is known to mediate protein-protein interactions via calcium ion. The N-terminal three domains consisting of CUB-I, EGF and CUB-II of the MASP are responsible for dimerization and for the calcium-dependent binding to MBL and ficolins(Feinberg, et al., 2003). The two contiguous CCPs (complement control protein) of

MASP, especially the second CCP domain, have been implicated in the binding of macromolecular substrates. The CCP domains of around 60 aa are found in a number of complement factors and other proteins(Chou and Heinrikson, 1997). The SP (serine protease) domain is the catalytically active unit of the proteases and defines them as part of the S1A family of chymotrypsin-like proteases (Yousef, et al., 2004). MASP is able to cleave C4 and C2 to generate a C3 convertase, C4b2a. Recent studies isolated two additional MASPs in human MBL complex (Thiel, et al., 1997) (Dahl, et al., 2001). These newly identified MASPs are called as MASP-2 and MASP-3 and the former one is MASP-1(Schwaeble, et al., 2002).

3.2. Substrates for MASP

It is apparently defined that MASP-2 cleaves C4 that is similar with C1s in the classical pathway (Vorup-Jensen, et al., 1998) (Ambrus, et al., 2003). However, substrates for MASP-1 and MASP-3 are still obscure. Several candidates were demonstrated by recent studies as shown in Table 1.

MASP	Substrates (reference)
MASP-1	C3 (Matsushita and Fujita, 1995), C2, fibrinogen, Factor XIII(Hajela, et al., 2002), PAR4 (Megyeri, et al., 2009), Df(Takahashi et al., 2010)
MASP-2	C4, C2(Ambrus, et al., 2003), prothrombin
MASP-3	IGFBP-5 (Cortesio and Jiang, 2006), Df(Iwaki, et al., 2011)
C1r	C1s
C1s	C4, C2

Table 1. Substrates for MASPs

3.3. *MASP* genes

3.3.1. MASP1

MASP1 is located on chromosome 3q27-q28 in human and chromosome 16 (B2-B3) in mouse (Takada, et al., 1995). Three gene products, MASP-1, MASP-3 and MAP44 are encoded from this gene by alternative splicing. MAP44 is a truncated protein of MASP-1/3 and lacks serine protease domain (Degn, et al., 2009) (Skjoedt, et al., 2010). MAP44 is thought to be a regulatory factor, attenuating activation of the lectin pathway. *MASP1* gene has a unique structure. A single exon, encoding whole MASP-3 light-chain and the six sprit exons, encoding MASP-1 are tandem located (Dahl, et al., 2001). Therefore, MASP-1 and MASP-3 consist of a common heavy-chain and the distinct light-chain.
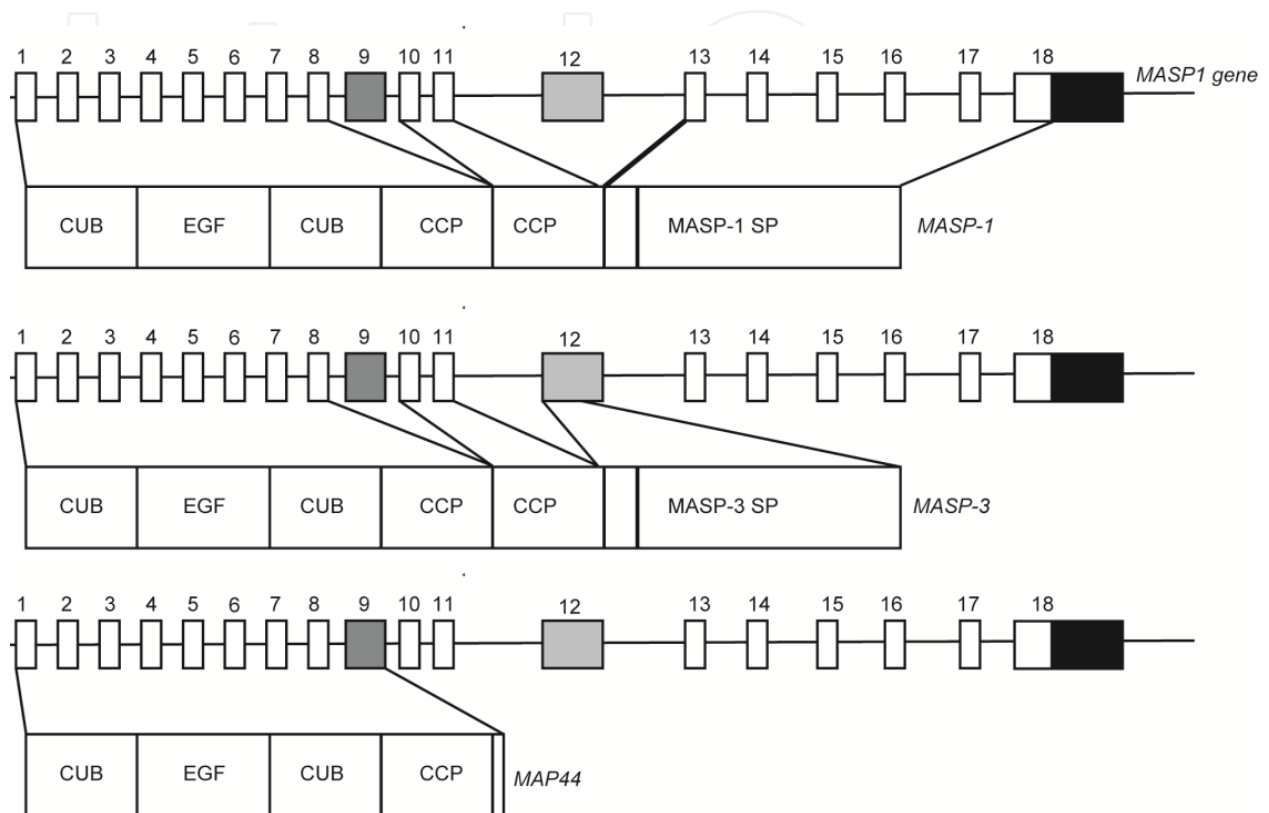
Figure 2. Schematic representation of *MASP1* gene

MASP1 gene consists of 18 exons, encoding three gene products, MASP-1, MASP-3 and MAP44 by alternative splicing.

3.3.2. MASP2

MASP2 gene is located on human chromosome 1p36.3-p36.2(Stover, et al., 1999a). And mouse Masp2 gene is located on chromosome 4(Lawson and Reid, 2000). It was shown that the MASP2 gene encodes two gene products, the 76 kDa MASP-2 serine protease and a plasma protein of 19 kDa, termed sMAP/MAp19 by alternative splicing (Takahashi, et al., 1999) (Stover, et al., 1999b). sMAP/MAp19 consist of only CUB-I and EGF-like domain of MASP-2, lacking catalytic domain.

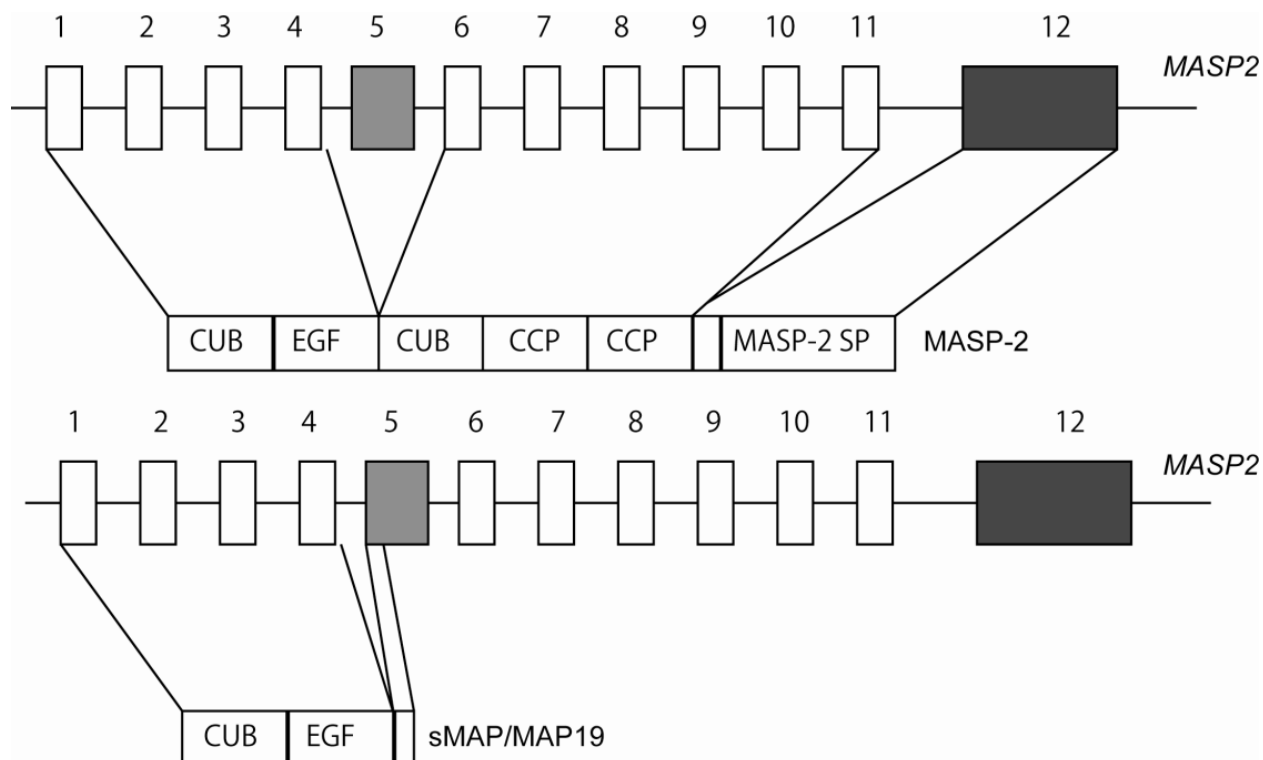**Figure 3.** Schematic representation of *MASP2* gene

MASP2 gene consists of 12 exons, encoding two gene products, MASP-2 and sMAP/Map19 by alternative splicing.

4. Studies for the Masp-knockout mice

Knockout mice	Mutant allele	chromosome	Targeted exon
Masp1/3$^{-/-}$	Masp1^{tm1Tefu}	16	2
sMAP/Masp2$^{-/-}$	Masp2^{tm1Tefu}	4	5
Masp2$^{-/-}$	Masp2^{tm1Wjsc}	4	11 &12

Table 2. Masps knockout mice

4.1. MASP-1 and MASP-3-deficient mice (*Masp1/3$^{-/-}$*)

To investigate the role of MASP-1 in complement activation, we planned to disrupt the second exon of Masp1 gene by a conventional gene targeting (Takahashi, et al., 2008). When this project was proceeding, MASP-3 was identified (Dahl, et al., 2001). Surprisingly, both gene products were produced from *MASP1* gene. Since the targeted second exon is at upstream of both transcripts, it was predicted that MASP-3 is also absent in this knockout mice. It was confirmed that not only MASP-1, but also MASP-3, is absent in *MASP1/3$^{-/-}$* mice (Takahashi, et al., 2008).

4.1.1. Masp1/3⁻/⁻ shows the abnormality of the lectin pathway activation

Serum from *Masp1/3⁻/⁻* shows the abnormality of both C4 and C3 activation on mannan and it is restored by adding recombinant MASP-1. This result supported that MASP-1 contributes the lectin pathway through C4 activation. Furthermore, MASP-2 activation is delayed in *Masp1/3⁻/⁻* to be compared with that of wild type. This result reveals that MASP-1 and/or MASP-3 may involve in the lectin pathway activation through the acceleration of MASP-2 activation (Takahashi, et al., 2008).

4.1.2. Masp1/3⁻/⁻ shows the abnormality of the alternative pathway activation

Further study noticed us that not only lectin pathway but also alternative pathway is abnormal in *Masp1/3⁻/⁻*. We found that complement factor D (Df) circulates as a zymogen in *Masp1/3⁻/⁻* (Takahashi, et al., 2010). Df was known to be active-form, but not a zymogen in circulation (Lesavre and Muller-Eberhard, 1978). However, it has become evident that most proteases in blood are secreted as zymogen. Df was thought to be an exception. We also found that Df is synthesized as zymogen from adipocytes (Takahashi, et al., 2010) (Fig. 4). This result supports the general consensus for Df. Interestingly, increasing evidence suggests that the alternative pathway is involved in human disease, such as inflammatory arthritis and ischemia/reperfusion injury (Thurman and Holers, 2006).
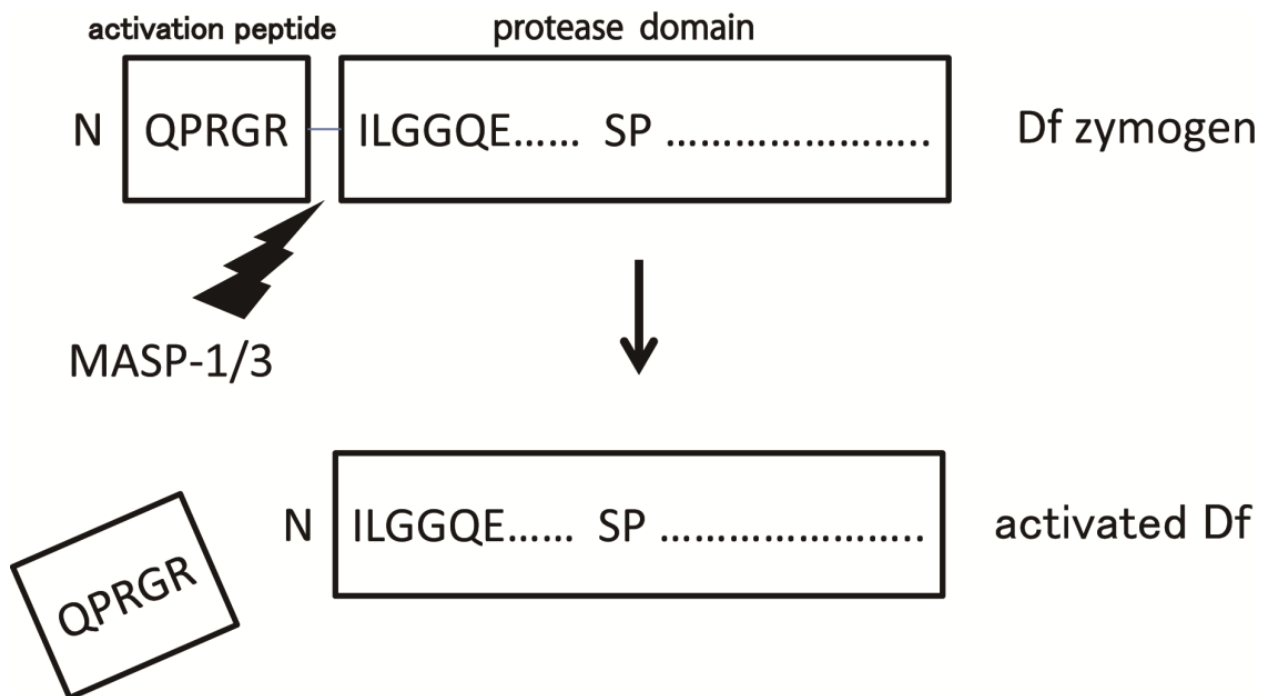

Figure 4. MASP-1 and/or MASP-3 involve in activating a zymogen of complement factor D

Complement factor D (Df) is synthesized as a zymogen (Pro-Df) from adipocytes. In serum of *Masp1/3⁻/⁻*, Pro-Df that has an activation peptide (QPRGR) at N-terminal of Df was observed.

4.1.3. MASP-1 and/or MASP-3 involve the fat metabolism through Df activation

It was also reported that the alternative pathway is involved in fat metabolism in adipose tissue (Paglialunga, et al., 2008). Recent studies have indicated that acylation-stimulating protein (ASP), which is identical to C3adesArg, stimulates fat storage in adipocytes (Yasruel, et al., 1991) (Maslowska, et al., 1997). ASP is a derivative of complement C3; thus, $C3^{-/-}$ mice are lean owing to ASP deficiency. Furthermore, plasma ASP levels are decreased in Bf-deficient and Df-deficient mice, indicating that the alternative pathway stimulates production of ASP. We found that $Masp1/3^{-/-}$ mice are also apparently lean (Takahashi, et al., 2008), strongly indicating a contribution of MASP-1 to fat metabolism via alternative pathway. We measured the plasma concentration of leptin and TNF-alpha (Fig. 5). Leptin plays a critical role in the regulation of body weight by inhibiting food intake and stimulating energy expenditure. Leptin appears to be a hormone secreted by adipocyte (Zhang, et al., 1994). It was shown that level of leptin in $Masp1/3^{-/-}$ significantly decrease. Furthermore, we determined that one of inflammatory factor, TNF-alpha increases in $Masp1/3^{-/-}$. As shown in Fig. 4, adipose tissues in Masp1/3-/- apparently show atrophy. Therefore, fat metabolisms in Masp1/3-/- adipose tissue might be reduced.
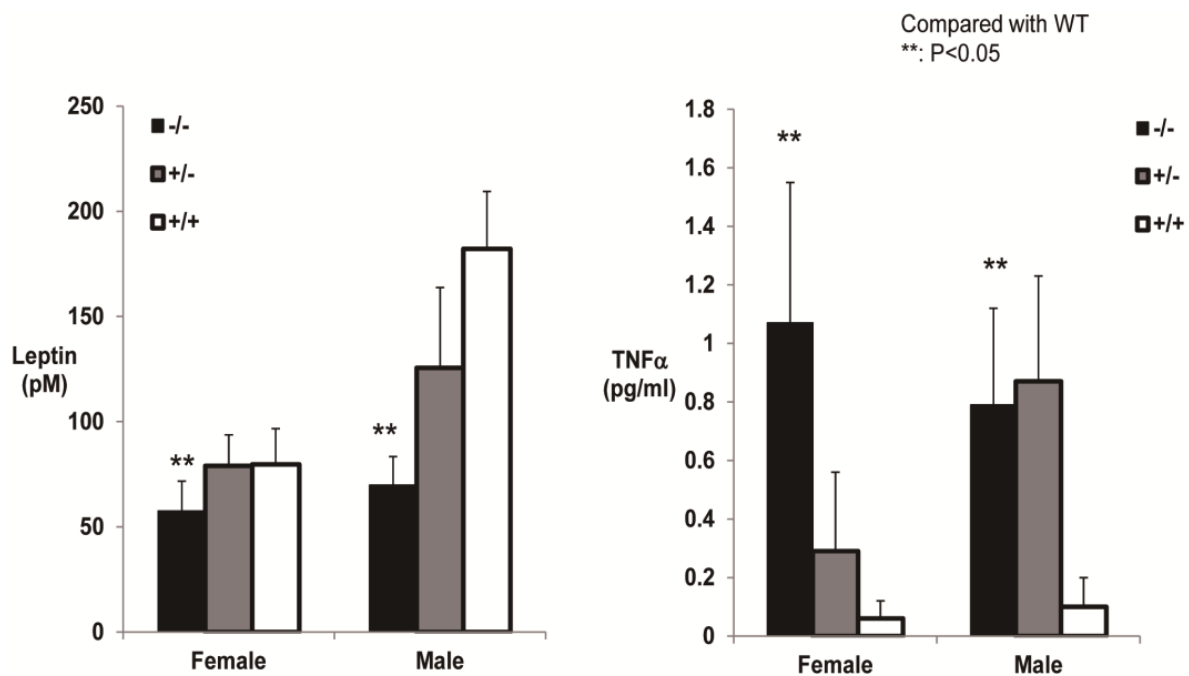

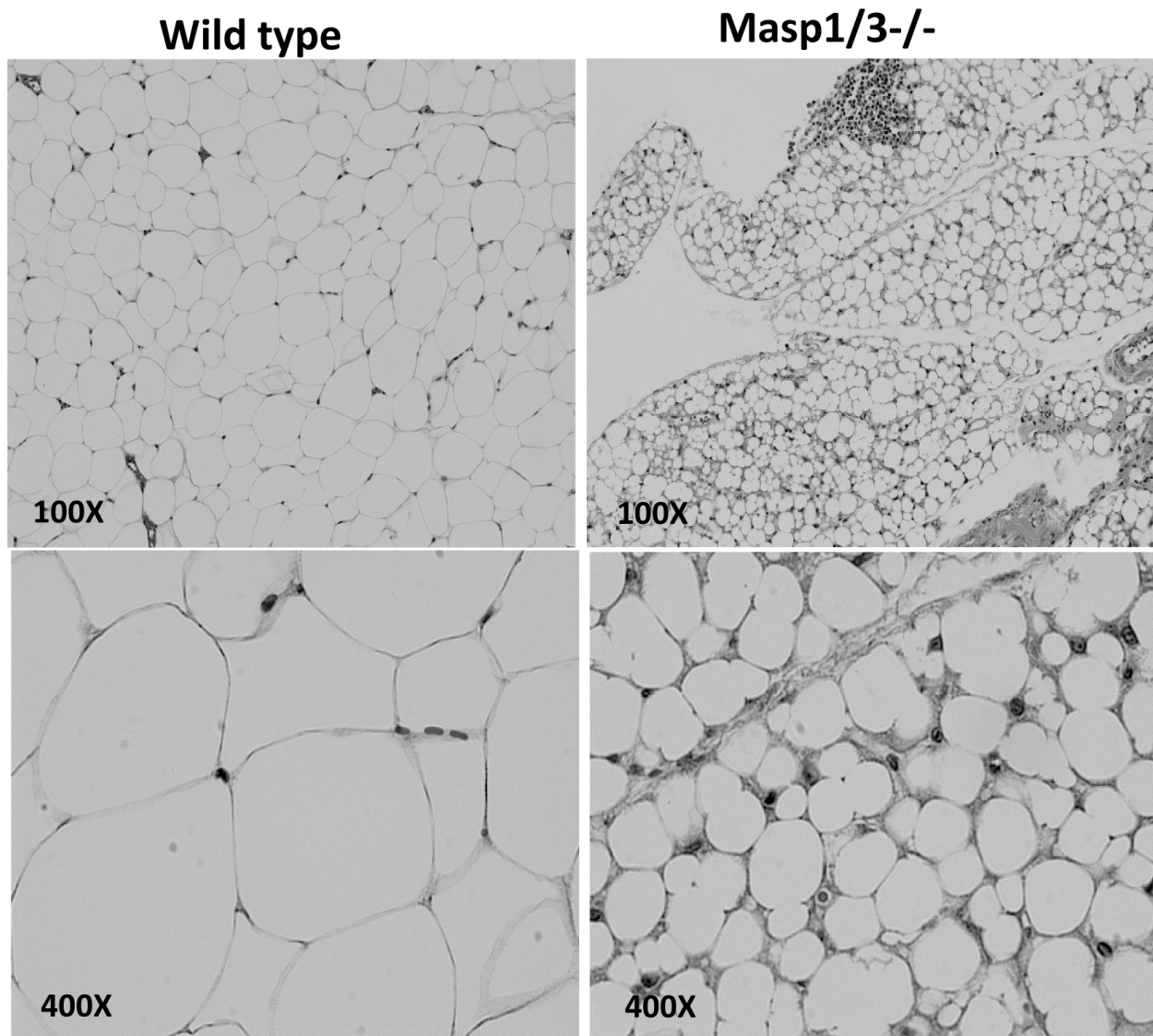**Figure 5.** Serum leptin and TNF-alpha level in $Masp1/3^{-/-}$

Wild type   **Masp1/3-/-**

Figure 6. H&E staining of mouse adipose tissue

4.1.4. Masp1/3$^{-/-}$ is resistant to AP-mediated joint damage

Banda et al. demonstrated that *Masp1/3$^{-/-}$* mice are highly resistant to CAIA as evidenced by a significant decrease in the histological scores as compared with WT mice (Banda, et al., 2010). Recent studies supported that the alternative pathway is both necessary and sufficient to induce disease in murine collagen Ab-induced arthritis (CAIA) (Banda, et al., 2006) (Banda, et al., 2007). This model mouse confirmed that *Masp1/3$^{-/-}$* shows the abnormality of the alternative pathway.

4.1.5. MASP3 mutation causes 3MC syndrome

3MC syndrome (Malpuech-Michels-Mingarelli-Carnevale syndrome) are four rare autosomal recessive disorders (Carnevale, et al., 1989) (Mingarelli, et al., 1996) (Malpuech, et al., 1983) (Michels, et al., 1978). This syndrome shows facial dysmorphic traits. Recent

observations for families, including patients who suffer from 3MC syndrome found the genetic mutations in *CL-K1* and *MASP1* genes (Rooryck, et al., 2011). This result was very interesting, since a possibility was raised that MASP-3 may be responsible to not only complement system, but also development system with a recognition molecule, CL-K1. In 2010, Sirmaci, et al. also found the mutations of *MASP1* gene in two Turkish famiies (Sirmaci, et al., 2010). Preliminary results was obtained that *Masp1/3* knockout mice have some developmental disorders (publication preparing).

4.2. sMAP and MASP-2-deficient mice (*sMAP/Masp2$^{-/-}$*)

To clarify the role of sMAP/Map19, we also generated another mutant mice, disrupting the fifth exon of *MASP2* gene by replacement with *neo^r*-gene (Iwaki, et al., 2006). Since this targeted region is the sMAP/Map19-specific exon, it was predicted that MASP-2 might be intact in this knockout mice. However, MASP-2 was not detected in their serum. Therefore, these mutant mice were named as *sMAP/Masp2$^{-/-}$*.

When recombinant sMAP 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. On the other hand, the addition of recombinant sMAP 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(Iwaki, et al., 2006).

4.3. MASP-2-deficient mice (*Masp2$^{-/-}$*)

An England group generated MASP-2-deficient mice(Schwaeble, et al., 2011). This strain lacks exon 11 and 12 of *Masp2* gene, encoding the C-terminal part of the CCPII and the SP domains. In their knockout mice, sMAP/Map19 is predicted to be intact. In vitro analysis of *MASP2$^{-/-}$* plasma showed a total absence of lectin pathway-dependent C4 cleavage on mannan- and zymosan-coated surfaces. They investigated whether MASP-2 affect the inflammatory process using a model of myocardial ischemia reperfusion injury (MIRI). It was observed that *MASP2$^{-/-}$* was protected from MIRI.

5. Conclusion

Here, we focus on analyses of three strains for *Masps* knockout mice, *Masp1/3$^{-/-}$*, *sMAP/Masp2$^{-/-}$* and *Masp2$^{-/-}$*. All strains show that activation of lectin pathway is deficient. We also detected the abnormality of the alternative pathway in *Masp1/3$^{-/-}$*. But *Masp2*-deficient phenotype does not affect the activity. MASPs are associated with MBL, ficolins and CL-K1. MBL-deficient mice were generated and analysed (Takahashi, et al., 2002, Shi, et al., 2004). Surprisingly, MBL-null mice show the comparable level of the alternative pathway with that of wild type. If MASP-1 and/or MASP-3 involve the activation of alternative pathway with MBL, MBL-null mice must be affected. Other recognition molecules, ficolin or CL-K1 might be involved in this phenomenon. This problem should be resolved in future study.

Recently MASP1 mutants were identified in human patients, suffering from 3MC syndrome. However, the mechanisms how MASP-1 and/or MASP-3 contribute the facial development are still unclear. Further study using Masp1/3-/- would provide a powerful tool to resolve this problem.

Author details

Minoru Takahashi, Daisuke Iwaki, Yuichi Endo and Teizo Fujita
Department of Immunology, Fukushima Medical University School of Medicine, Japan

6. References

Ambrus, G., Gal, P., Kojima, M., Szilagyi, K., Balczer, J., Antal, J., Graf, L., Laich, A., Moffatt, B. E., Schwaeble, W., Sim, R. B. and Zavodszky, P. (2003). Natural substrates and inhibitors of mannan-binding lectin-associated serine protease-1 and -2: a study on recombinant catalytic fragments. *J Immunol*, Vol. 170, No. 3, pp. 1374-1382

Arlaud, G. J., Gaboriaud, C., Thielens, N. M., Budayova-Spano, M., Rossi, V. and Fontecilla-Camps, J. C. (2002). Structural biology of the C1 complex of complement unveils the mechanisms of its activation and proteolytic activity. *Molecular Immunology*, Vol. 39, No. 7-8, pp. 383-394

Banda, N. K., Takahashi, K., Wood, A. K., Holers, V. M. and Arend, W. P. (2007). Pathogenic complement activation in collagen antibody-induced arthritis in mice requires amplification by the alternative pathway. *J Immunol*, Vol. 179, No. 6, pp. 4101-4109

Banda, N. K., Takahashi, M., Levitt, B., Glogowska, M., Nicholas, J., Takahashi, K., Stahl, G. L., Fujita, T., Arend, W. P. and Holers, V. M. (2010). Essential role of complement mannose-binding lectin-associated serine proteases-1/3 in the murine collagen antibody-induced model of inflammatory arthritis. *J Immunol*, Vol. 185, No. 9, pp. 5598-5606

Banda, Nirmal K., Thurman, Joshua M., Kraus, Damian, Wood, Allyson, Carroll, Michael C., Arend, William P. and Holers, V. Michael. (2006). Alternative Complement Pathway Activation Is Essential for Inflammation and Joint Destruction in the Passive Transfer Model of Collagen-Induced Arthritis. *The Journal of Immunology*, Vol. 177, No. 3, pp. 1904-1912

Bork, P. and Beckmann, G. (1993). The CUB domain. A widespread module in developmentally regulated proteins. *Journal of molecular biology*, Vol. 231, No. 2, pp. 539-545

Brouwer, N., Dolman, K. M., van Zwieten, R., Nieuwenhuys, E., Hart, M., Aarden, L. A., Roos, D. and Kuijpers, T. W. (2006). Mannan-binding lectin (MBL)-mediated opsonization is enhanced by the alternative pathway amplification loop. *Mol Immunol*, Vol. 43, No. 13, pp. 2051-2060

Carnevale, F., Krajewska, G., Fischetto, R., Greco, M. G. and Bonvino, A. (1989). Ptosis of eyelids, strabismus, diastasis recti, hip defect, cryptorchidism, and developmental delay in two sibs. *Am J Med Genet*, Vol. 33, No. 2, pp. 186-189

Carroll, M. C. (2004). The complement system in regulation of adaptive immunity. *Nature immunology,* Vol. 5, No. 10, pp. 981-986

Chou, K. C. and Heinrikson, R. L. (1997). Prediction of the tertiary structure of the complement control protein module. *Journal of protein chemistry,* Vol. 16, No. 8, pp. 765-773

Cortesio, C. L. and Jiang, W. (2006). Mannan-binding lectin-associated serine protease 3 cleaves synthetic peptides and insulin-like growth factor-binding protein 5. *Arch Biochem Biophys,* Vol. 449, No. 1-2, pp. 164-170

Cseh, S., Vera, L., Matsushita, M., Fujita, T., Arlaud, G. J. and Thielens, N. M. (2002). Characterization of the interaction between L-ficolin/p35 and mannan-binding lectin-associated serine proteases-1 and -2. *J Immunol,* Vol. 169, No. 10, pp. 5735-5743

Dahl, M. R., Thiel, S., Matsushita, M., Fujita, T., Willis, A. C., Christensen, T., Vorup-Jensen, T. and Jensenius, J. C. (2001). MASP-3 and its association with distinct complexes of the mannan-binding lectin complement activation pathway. *Immunity,* Vol. 15, No. 1, pp. 127-135

Degn, S. E., Hansen, A. G., Steffensen, R., Jacobsen, C., Jensenius, J. C. and Thiel, S. (2009). MAp44, a human protein associated with pattern recognition molecules of the complement system and regulating the lectin pathway of complement activation. *J Immunol,* Vol. 183, No. 11, pp. 7371-7378

Degn, S. E., Jensenius, J. C. and Bjerre, M. (2010). The lectin pathway and its implications in coagulation, infections and auto-immunity. *Current opinion in organ transplantation,* Vol. No. pp.

Feinberg, H., Uitdehaag, J. C., Davies, J. M., Wallis, R., Drickamer, K. and Weis, W. I. (2003). Crystal structure of the CUB1-EGF-CUB2 region of mannose-binding protein associated serine protease-2. *The EMBO journal,* Vol. 22, No. 10, pp. 2348-2359

Fujita, T., Matsushita, M. and Endo, Y. (2004). The lectin-complement pathway--its role in innate immunity and evolution. *Immunol Rev,* Vol. 198, No. pp. 185-202

Hajela, K., Kojima, M., Ambrus, G., Wong, K. H., Moffatt, B. E., Ferluga, J., Hajela, S., Gal, P. and Sim, R. B. (2002). The biological functions of MBL-associated serine proteases (MASPs). *Immunobiology,* Vol. 205, No. 4-5, pp. 467-475

Hansen, S., Selman, L., Palaniyar, N., Ziegler, K., Brandt, J., Kliem, A., Jonasson, M., Skjoedt, M. O., Nielsen, O., Hartshorn, K., Jorgensen, T. J., Skjodt, K. and Holmskov, U. (2010). Collectin 11 (CL-11, CL-K1) is a MASP-1/3-associated plasma collectin with microbial-binding activity. *Journal of immunology (Baltimore, Md. : 1950),* Vol. 185, No. 10, pp. 6096-6104

Holers, V. M. (2003). The complement system as a therapeutic target in autoimmunity. *Clin Immunol,* Vol. 107, No. 3, pp. 140-151

Holmskov, U., Thiel, S. and Jensenius, J. C. (2003). Collections and ficolins: humoral lectins of the innate immune defense. *Annu Rev Immunol,* Vol. 21, No. pp. 547-578

Ikeda, K., Sannoh, T., Kawasaki, N., Kawasaki, T. and Yamashina, I. (1987). Serum lectin with known structure activates complement through the classical pathway. *J Biol Chem,* Vol. 262, No. 16, pp. 7451-7454

Iwaki, D., Kanno, K., Takahashi, M., Endo, Y., Lynch, N. J., Schwaeble, W. J., Matsushita, M., Okabe, M. and Fujita, T. (2006). Small mannose-binding lectin-associated protein plays a regulatory role in the lectin complement pathway. *J Immunol,* Vol. 177, No. 12, pp. 8626-8632

Iwaki, Daisuke, Kanno, Kazuko, Takahashi, Minoru, Endo, Yuichi, Matsushita, Misao and Fujita, Teizo. (2011). The Role of Mannose-Binding Lectin-Associated Serine Protease-3 in Activation of the Alternative Complement Pathway. *The Journal of Immunology,* Vol. 187, No. 7, pp. 3751-3758

Kawasaki, T., Etoh, R. and Yamashina, I. (1978). Isolation and characterization of a mannan-binding protein from rabbit liver. *Biochem Biophys Res Commun,* Vol. 81, No. 3, pp. 1018-1024

Keshi, H., Sakamoto, T., Kawai, T., Ohtani, K., Katoh, T., Jang, S. J., Motomura, W., Yoshizaki, T., Fukuda, M., Koyama, S., Fukuzawa, J., Fukuoh, A., Yoshida, I., Suzuki, Y. and Wakamiya, N. (2006). Identification and characterization of a novel human collectin CL-K1. *Microbiol Immunol,* Vol. 50, No. 12, pp. 1001-1013

Lawson, P. R. and Reid, K. B. (2000). A novel PCR-based technique using expressed sequence tags and gene homology for murine genetic mapping: localization of the complement genes. *International immunology,* Vol. 12, No. 3, pp. 231-240

Lepow, I. H., Naff, G. B., Todd, E. W., Pensky, J. and Hinz, C. F. (1963). Chromatographic resolution of the first component of human complement into three activities. *The Journal of experimental medicine,* Vol. 117, No. pp. 983-1008

Lesavre, P. H. and Muller-Eberhard, H. J. (1978). Mechanism of action of factor D of the alternative complement pathway. *J Exp Med,* Vol. 148, No. 6, pp. 1498-1509

Malpuech, G., Demeocq, F., Palcoux, J. B. and Vanlieferinghen, P. (1983). A previously undescribed autosomal recessive multiple congenital anomalies/mental retardation (MCA/MR) syndrome with growth failure, lip/palate cleft(s), and urogenital anomalies. *Am J Med Genet,* Vol. 16, No. 4, pp. 475-480

Maslowska, M., Sniderman, A. D., Germinario, R. and Cianflone, K. (1997). ASP stimulates glucose transport in cultured human adipocytes. *Int J Obes Relat Metab Disord,* Vol. 21, No. 4, pp. 261-266

Matsushita, M., Endo, Y. and Fujita, T. (1998). MASP1 (MBL-associated serine protease 1). *Immunobiology,* Vol. 199, No. 2, pp. 340-347

Matsushita, M., Endo, Y. and Fujita, T. (2000). Cutting edge: complement-activating complex of ficolin and mannose-binding lectin-associated serine protease. *J Immunol,* Vol. 164, No. 5, pp. 2281-2284

Matsushita, M., Endo, Y., Hamasaki, N. and Fujita, T. (2001). Activation of the lectin complement pathway by ficolins. *Int Immunopharmacol,* Vol. 1, No. 3, pp. 359-363

Matsushita, M. and Fujita, T. (1992). Activation of the classical complement pathway by mannose-binding protein in association with a novel C1s-like serine protease. *J Exp Med,* Vol. 176, No. 6, pp. 1497-1502

Matsushita, M. and Fujita, T. (1995). Cleavage of the third component of complement (C3) by mannose-binding protein-associated serine protease (MASP) with subsequent complement activation. *Immunobiology,* Vol. 194, No. 4-5, pp. 443-448

Megyeri, M., Mako, V., Beinrohr, L., Doleschall, Z., Prohaszka, Z., Cervenak, L., Zavodszky, P. and Gal, P. (2009). Complement protease MASP-1 activates human endothelial cells: PAR4 activation is a link between complement and endothelial function. *J Immunol*, Vol. 183, No. 5, pp. 3409-3416

Michels, V. V., Hittner, H. M. and Beaudet, A. L. (1978). A clefting syndrome with ocular anterior chamber defect and lid anomalies. *J Pediatr*, Vol. 93, No. 3, pp. 444-446

Mingarelli, R., Castriota Scanderbeg, A. and Dallapiccola, B. (1996). Two sisters with a syndrome of ocular, skeletal, and abdominal abnormalities (OSA syndrome). *J Med Genet*, Vol. 33, No. 10, pp. 884-886

Muller-Eberhard, H. J. and Gotze, O. (1972). C3 proactivator convertase and its mode of action. *J Exp Med*, Vol. 135, No. 4, pp. 1003-1008

Ohta, M., Okada, M., Yamashina, I. and Kawasaki, T. (1990). The mechanism of carbohydrate-mediated complement activation by the serum mannan-binding protein. *J Biol Chem*, Vol. 265, No. 4, pp. 1980-1984

Paglialunga, S., Fisette, A., Yan, Y., Deshaies, Y., Brouillette, J. F., Pekna, M. and Cianflone, K. (2008). Acylation-stimulating protein deficiency and altered adipose tissue in alternative complement pathway knockout mice. *Am J Physiol Endocrinol Metab*, Vol. 294, No. 3, pp. E521-529

Pangburn, M. K., Schreiber, R. D. and Muller-Eberhard, H. J. (1981). Formation of the initial C3 convertase of the alternative complement pathway. Acquisition of C3b-like activities by spontaneous hydrolysis of the putative thioester in native C3. *J Exp Med*, Vol. 154, No. 3, pp. 856-867

Ricklin, D., Hajishengallis, G., Yang, K. and Lambris, J. D. (2010). Complement: a key system for immune surveillance and homeostasis. *Nature immunology*, Vol. 11, No. 9, pp. 785-797

Rooryck, C., Diaz-Font, A., Osborn, D. P., Chabchoub, E., Hernandez-Hernandez, V., Shamseldin, H., Kenny, J., Waters, A., Jenkins, D., Kaissi, A. A., Leal, G. F., Dallapiccola, B., Carnevale, F., Bitner-Glindzicz, M., Lees, M., Hennekam, R., Stanier, P., Burns, A. J., Peeters, H., Alkuraya, F. S. and Beales, P. L. (2011). Mutations in lectin complement pathway genes COLEC11 and MASP1 cause 3MC syndrome. *Nat Genet*, Vol. 43, No. 3, pp. 197-203

Sato, T., Endo, Y., Matsushita, M. and Fujita, T. (1994). Molecular characterization of a novel serine protease involved in activation of the complement system by mannose-binding protein. *Int Immunol*, Vol. 6, No. 4, pp. 665-669

Schwaeble, W., Dahl, M. R., Thiel, S., Stover, C. and Jensenius, J. C. (2002). The mannan-binding lectin-associated serine proteases (MASPs) and MAp19: four components of the lectin pathway activation complex encoded by two genes. *Immunobiology*, Vol. 205, No. 4-5, pp. 455-466

Schwaeble, W. J., Lynch, N. J., Clark, J. E., Marber, M., Samani, N. J., Ali, Y. M., Dudler, T., Parent, B., Lhotta, K., Wallis, R., Farrar, C. A., Sacks, S., Lee, H., Zhang, M., Iwaki, D., Takahashi, M., Fujita, T., Tedford, C. E. and Stover, C. M. (2011). Targeting of mannan-binding lectin-associated serine protease-2 confers protection from myocardial and

gastrointestinal ischemia/reperfusion injury. *Proc Natl Acad Sci U S A,* Vol. 108, No. 18, pp. 7523-7528

Shi, L., Takahashi, K., Dundee, J., Shahroor-Karni, S., Thiel, S., Jensenius, J. C., Gad, F., Hamblin, M. R., Sastry, K. N. and Ezekowitz, R. A. (2004). Mannose-binding lectin-deficient mice are susceptible to infection with Staphylococcus aureus. *J Exp Med,* Vol. 199, No. 10, pp. 1379-1390

Sirmaci, A., Walsh, T., Akay, H., Spiliopoulos, M., Sakalar, Y. B., Hasanefendioglu-Bayrak, A., Duman, D., Farooq, A., King, M. C. and Tekin, M. (2010). MASP1 mutations in patients with facial, umbilical, coccygeal, and auditory findings of Carnevale, Malpuech, OSA, and Michels syndromes. *Am J Hum Genet,* Vol. 87, No. 5, pp. 679-686

Skjoedt, M. O., Hummelshoj, T., Palarasah, Y., Honore, C., Koch, C., Skjodt, K. and Garred, P. (2010). A novel mannose-binding lectin/ficolin-associated protein is highly expressed in heart and skeletal muscle tissues and inhibits complement activation. *The Journal of biological chemistry,* Vol. 285, No. 11, pp. 8234-8243

Stover, C. M., Schwaeble, W. J., Lynch, N. J., Thiel, S. and Speicher, M. R. (1999a). Assignment of the gene encoding mannan-binding lectin-associated serine protease 2 (MASP2) to human chromosome 1p36.3-->p36.2 by in situ hybridization and somatic cell hybrid analysis. *Cytogenet Cell Genet,* Vol. 84, No. 3-4, pp. 148-149

Stover, C. M., Thiel, S., Thelen, M., Lynch, N. J., Vorup-Jensen, T., Jensenius, J. C. and Schwaeble, W. J. (1999b). Two constituents of the initiation complex of the mannan-binding lectin activation pathway of complement are encoded by a single structural gene. *J Immunol,* Vol. 162, No. 6, pp. 3481-3490

Takada, F., Seki, N., Matsuda, Y., Takayama, Y. and Kawakami, M. (1995). Localization of the genes for the 100-kDa complement-activating components of Ra-reactive factor (CRARF and Crarf) to human 3q27-q28 and mouse 16B2-B3. *Genomics,* Vol. 25, No. 3, pp. 757-759

Takahashi, K., Gordon, J., Liu, H., Sastry, K. N., Epstein, J. E., Motwani, M., Laursen, I., Thiel, S., Jensenius, J. C., Carroll, M. and Ezekowitz, R. A. (2002). Lack of mannose-binding lectin-A enhances survival in a mouse model of acute septic peritonitis. *Microbes Infect,* Vol. 4, No. 8, pp. 773-784

Takahashi, M., Endo, Y., Fujita, T. and Matsushita, M. (1999). A truncated form of mannose-binding lectin-associated serine protease (MASP)-2 expressed by alternative polyadenylation is a component of the lectin complement pathway. *Int Immunol,* Vol. 11, No. 5, pp. 859-863

Takahashi, M., Ishida, Y., Iwaki, D., Kanno, K., Suzuki, T., Endo, Y., Homma, Y. and Fujita, T. (2010). Essential role of mannose-binding lectin-associated serine protease-1 in activation of the complement factor D. *J Exp Med,* Vol. 207, No. 1, pp. 29-37

Takahashi, M., Iwaki, D., Kanno, K., Ishida, Y., Xiong, J., Matsushita, M., Endo, Y., Miura, S., Ishii, N., Sugamura, K. and Fujita, T. (2008). Mannose-binding lectin (MBL)-associated serine protease (MASP)-1 contributes to activation of the lectin complement pathway. *J Immunol,* Vol. 180, No. 9, pp. 6132-6138

Thiel, S., Vorup-Jensen, T., Stover, C. M., Schwaeble, W., Laursen, S. B., Poulsen, K., Willis, A. C., Eggleton, P., Hansen, S., Holmskov, U., Reid, K. B. and Jensenius, J. C. (1997). A

second serine protease associated with mannan-binding lectin that activates complement. *Nature*, Vol. 386, No. 6624, pp. 506-510

Thurman, J. M. and Holers, V. M. (2006). The central role of the alternative complement pathway in human disease. *J Immunol*, Vol. 176, No. 3, pp. 1305-1310

Turner, M. W. (1996). Mannose-binding lectin: the pluripotent molecule of the innate immune system. *Immunology today*, Vol. 17, No. 11, pp. 532-540

Vorup-Jensen, T., Jensenius, J. C. and Thiel, S. (1998). MASP-2, the C3 convertase generating protease of the MBLectin complement activating pathway. *Immunobiology*, Vol. 199, No. 2, pp. 348-357

Walport, M. J. (2001a). Complement. First of two parts. *N Engl J Med*, Vol. 344, No. 14, pp. 1058-1066

Walport, M. J. (2001b). Complement. Second of two parts. *N Engl J Med*, Vol. 344, No. 15, pp. 1140-1144

Yasruel, Z., Cianflone, K., Sniderman, A. D., Rosenbloom, M., Walsh, M. and Rodriguez, M. A. (1991). Effect of acylation stimulating protein on the triacylglycerol synthetic pathway of human adipose tissue. *Lipids*, Vol. 26, No. 7, pp. 495-499

Yousef, G. M., Elliott, M. B., Kopolovic, A. D., Serry, E. and Diamandis, E. P. (2004). Sequence and evolutionary analysis of the human trypsin subfamily of serine peptidases. *Biochimica et biophysica acta*, Vol. 1698, No. 1, pp. 77-86

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L. and Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature*, Vol. 372, No. 6505, pp. 425-432