The Pro-Factor D Cleaving Activity of MASP-1/-3 Is Not Required for Alternative Pathway Function

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The Pro-Factor D Cleaving Activity of MASP-1/-3 Is Not Required for Alternative Pathway Function

We recently reported in The Journal of Immunology (1) the presence of a functional alternative pathway in a human patient with Malpueh–Michels–Mingarelli–Carnevale (3MC) syndrome caused by a genetic defect in the MASPI gene. This defect abolished the production of all three splice products of this gene: mannan-binding lectin (MBL)–associated serine proteases (MASP)-1 and MASP-3, and MBL-associated protein of 44 kDa (MAP44) (human complement).

Our paper generated debate because the findings directly conflicted with results obtained in a mouse model of complete MASPI deficiency (2, 3). In 2010 Takahashi and colleagues (4) reported in the Journal of Experimental Medicine that MASPI was the protease responsible for cleaving profactor D (profD) and thus essential for the presence of mature, active profD in the blood [(4); murine complement]. Thus, in a mouse model of genetic deficiency of all three Map1 gene products, Takahashi et al. (4) reported complete deficiency of the alternative pathway. They determined this to be caused by a complete absence of mature, active profD in the Map1 knockout serum, and demonstrated that recombinant murine MASPI-1 could cleave profD, rescuing alternative pathway activation in vitro. The group followed up on these findings (5) reporting that MASPI-3 could also cleave profD, and in addition, at low efficiency, factor B to Bb [(5); murine complement].

The suggestion of an essential role of MASPI-1/-3 in alternative pathway function was corroborated by a study using the murine anti-collagen Ab-induced model of inflammatory arthritis, which is known to be sensitive to alterations in the alternative pathway [(6); murine complement].

Surprisingly, our results (1), based on the 3MC syndrome patient and in vitro studies with recombinant human MASPI-1, indicated that in humans neither MASPI-1 nor MASPI-3 are required for alternative pathway function [(1); human complement]. It was suggested that the activity we observed was due to backup pathways, with thrombin, kallikrein, and plasmin being able to cleave profD (3). Because of natural variation in alternative pathway hemolytic activity and the availability of only a single deficient human, we could not determine whether MASPI-1/-3 deficiency caused a quantitative deficiency, but simply noted the existence of significant alternative pathway activity, indicating that mature profD was indeed present in the absence of MASPI-1/-3 (2).

The issue remained that the complete absence of alternative pathway activity in the MASPI-1/-3 deficient mouse was not reconcilable with the existence of the proposed backup pathways.

Furthermore, our observation (1) of marked alternative pathway activity in the MASPI-1/-3 deficient human suggested that any such backup pathways were highly functional, calling into question the purported essential role of MASPI-1/-3.

Recently, fresh observations have shed new light on the basis of this controversy, prompting us to readdress the issue in this letter. In their recent article, Ruseva and colleagues (7) demonstrated that MASPI-1 and MASPI-3 are not essential for activation of the complement system via the alternative pathway in vivo [(7); murine complement]. However, they noted that this occurred in the absence of detectable mature profD [(7); murine complement]. This reconciles the previous seemingly disparate findings, as it confirms both the observation of Takahashi et al. (4) on the role of MASPI-1/-3 in cleaving profD and our observation of a functioning alternative pathway in the absence of MASPI-1/-3. Two possible scenarios present themselves: either undetectable but catalytically functional levels of mature profD are generated during alternative pathway activation via the bypass pathways suggested by Takahashi and colleagues; or, in contrast to what was previously reported [(8); human complement], profD has significant activity toward the fBC3(H2O) substrate. Further studies are needed to answer this question.

Meanwhile, the discrepancy between results of Ruseva et al. [(7); murine complement] and Takahashi et al. [(4); murine complement] regarding alternative pathway activity in the Map1 knockout mouse serum prompted us to reexamine this activity. We report in this letter that the difference arises from the use of different buffer systems. At physiological ionic strength, alternative pathway activity is observed, albeit at a lower level than in wild type mice, thus confirming the recent results of Ruseva et al. [(7); murine complement] (Fig. 1A, 1B). Conversely, the high ionic strength buffer used by Takahashi et al. [(4); murine complement] suppresses the weaker alternative pathway activity of the Map1 knockout mouse to background levels (Fig. 1C). This suggests that either the interaction of backup enzymes with profD or the profD interaction with fBC3(H2O) substrate is sensitive to high ionic strength.

Regarding the discrepancy between the role of MASPI-1/-3 in vivo in the anti-collagen Ab-induced arthritis model of Banda et al. [(6); murine complement] and in the fH deficient model of Ruseva et al. [(7); murine complement], we also attempt to offer some insight. The former model is dependent on the alternative pathway for initiation and propagation of injury, and due to the weaker alternative pathway activation in the Map1 knockout, significant protection is observed. The latter model is based on the absence of a crucial regulator of the alternative pathway: factor H. In this scenario, the lower level alternative pathway activity in Map1 knockouts is sufficient to initiate formation of C3 convertase, which in the absence of factor H forms an unregulated positive amplification loop. Hence, the two scenarios are mechanistically fundamentally different, explaining the different outcomes.
measurements. Experiment repeated with two different dilution series. GVB/EGTA/MgCl2 (GB/EGTA/Mg: 5 mM barbital, 145 mM NaCl, pH 7.4) with 5 mM CaCl2 series of C57BL/6 serum in various buffers. Mean and SD of duplicates, experiment repeated twice. VBS/EGTA/Mg, Veronal-buffered saline (5 mM barbital, 145 mM NaCl, pH 7.4) with 5 mM CaCl2 = all complement pathways are active. VBS/EGTA/Mg, VBS with 10 mM EGTA and 5 mM MgCl2 = the alternative pathway is active. B, C3 deposition in microtitre wells coated with the alternative pathway activator zymosan, as a function of a 2-fold dilution series of C57BL/6 serum in various buffers. Mean and SD of duplicates, experiment repeated twice. VBS/ECa, Veronal-buffered saline (5 mM barbital, 145 mM NaCl, pH 7.4) with 5 mM CaCl2 = all complement pathways are active. VBS/EGTA/Mg, VBS with 10 mM EGTA and 5 mM MgCl2 = only the alternative pathway is active. Boric acid/EGTA/Mg, boric acid-buffered saline (200 mM boric acid, 140 mM NaCl, pH 8.0) with 30 mM EGTA and 35 mM MgCl2 = alternative pathway in presence of high ionic strength (as in Ref. 4). VBS/EDTA, VBS with 10 mM EDTA = no pathways active (i.e., background signal). C As in (B), but for Masp1 knockout serum. Note the influence of high ionic strength on alternative pathway activity, especially in the Masp1 knockout mouse serum.

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Abbreviations used in this article: MAp44, MBL-associated protein of 44 kDa; MASP, mannose-binding lectin–associated serine protease; MBL, mannan-binding lectin; 3MC, Mannan–Malpuech–Michels–Mingarelli–Carnevale; prof D, profactor D.

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Comment on “The Pro-Factor D Cleaving Activity of MASP-1/-3 Is Not Required for Alternative Pathway Function”

W e would like to express our deepest gratitude to the editor for giving us the opportunity to discuss Degn and colleagues’ argument. We previously claimed (1) in the Journal of Experimental Medicine that mannan-binding lectin (MBL)–associated serine protease (MASP)-1 has an essential role in converting profactor D (profD) into its active form, because the vast majority of D in the blood of MASP-1/-3 knockout mice was identified as profD by Western blot. We also observed an absence of alternative pathway activity in the knockout sera by zymosan assay. However, Degn et al. (2) reported contradictory results...
obtained using samples derived from human patients with Malpuech–Michels–Mingarelli–Carnevale (3MC) syndrome with a genetic deficiency in the MASP1 gene.

Therefore, it was crucial to determine whether profD exists in the sera of 3MC patients. Degn and colleagues’ group kindly provided us with their samples derived from 3MC patients, as well as from healthy controls, in a blinded fashion. We compared the m.w. of the fD by Western blot of each of the four samples. As shown in Fig. 1, two out of the four samples showed a slightly larger m.w. of fD compared with the others. After we sent our results to Degn et al., they informed us that the two samples that showed a larger m.w. of fD were indeed derived from the 3MC patients. Thus, we successfully distinguished the difference in m.w. of fD between normal and 3MC patients with 100% accuracy. Coinciding with our previous results (1) using MASP-1/-3 knockout mice, 3MC patients with 100% accuracy. Coinciding with our previous results (1) using MASP-1/-3 knockout mice, 3MC patients with a genetic deficiency in the MASP1 gene have profD in their sera. This fact strongly advocates that MASP-1 and MASP-3 are the main enzymes for converting profD into active form in both species.

It has been reported (3, 4) that thrombin, kallikrein, and plasmin have the ability to cleave profD, suggesting another possibility, which is that they could act as backup enzymes, producing an undetectable amount of active fD in both MASP-1/-3 knockout mice and 3MC patients. In a recent paper, Ruseva and colleagues (5) demonstrated in vivo activation of the alternative pathway in sera of MASP-1/-3 and factor H doubleknockout mice. The data suggested that MASP-1 and MASP-3 are not essential for the activation of the alternative pathway (5). Their results (5) indicated that uncontrolled activation of the alternative pathway occurs in the absence of factor H. In this condition, an undetectable amount of active fD in MASP-1/-3 knockout mice might be sufficient to trigger activation of the alternative pathway.

Degn and colleagues observed that the ionic strength in rabbit erythrocyte lysis assay affects the outcome of the results (figure 1B, 1C in their letter). Formerly, Dijk and colleagues (6) investigated the optimal reaction conditions for hemolytic assay of alternative pathway activity in mouse serum. We followed their method because it avoids all nonspecific erythrocyte lysis other than by fD. Degn et al. also showed that samples that have high concentrations of serum from MASP-1/-3-deficient mice still have the ability to activate alternative pathway (figure 1A in their letter). However, it should be noted that MASP-1/-3-deficient serum had a lower ability to lyse rabbit erythrocytes compared with MASP-1/-3-sufficient serum under a low serum concentration, suggesting that the total quantity of active fD that is required for full alternative pathway function is not equivalent between MASP-1/-3-deficient and MASP-1/-3-sufficient serum. MASP-1-deficient human serum also had lower hemolytic ability compared with MASP1+/− and MASP1+/+ human serum under low serum concentrations (figure 2A in Ref. 2). However, these important findings were not discussed in the reports from Degn and colleagues.

In conclusion, MASP-1 and MASP-3 are crucial enzymes for alternative pathway activation through the activation of profD into its active form, and this is similar to murine results. The conclusion proposed by Degn et al., “the pro-factor D cleaving activity of MASP-1/-3 is not required for alternative pathway function,” is liable to cause a profound misunderstanding in that neither MASP-1/-3 nor active fD has a role for the alternative pathway function. Therefore, it is reasonable to propose that the pro-factor D cleaving activity of MASP-1/-3 is required for the full alternative pathway function.

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Abbreviations used in this article: MASP, mannan-binding lectin–associated serine proteases; MBL, mannan-binding lectin; 3MC, Malpuech–Michels–Mingarelli–Carnevale; profD, pro-factor D.

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FIGURE 1. ProfD was found in human MASP-1/-3–deficient sera. Degn’s group kindly provided us with their samples derived from 3MC patients and healthy controls in a blinded fashion (lanes 2–5). The sample in lane 1 is a freshly isolated healthy control. We performed Western blotting by using anti-fD Ab to compare the m.w. of fD. From the results, we noticed that the m.w. of fD from the samples in lanes 3 and 5 is slightly different from the others. After we sent this figure to Degn’s group, they informed us that the samples in lanes 3 and 5 were derived from the 3MC patients, whereas the samples in lanes 2 and 4 were from healthy controls.