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Activation and Regulation of the Lectin Pathway of the Complement System

Péter Gál

Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary

ABSTRACT

The complement system, a network of about 35 soluble and cell-surface proteins, is an ancient part of the innate immune system. It can recognize various danger signals provided by pathogens or altered host cells. The complement system can be activated through 3 different routes: the classical, the alternative, and the lectin pathways. Discovered at the end of the 20th century, the lectin pathway seems to be the most complex among the activation routes. Pattern-recognition molecules, such as mannan-binding lectin (MBL) and ficolins, recognize arrays of sugars or acetylated compounds on microorganisms and stimulate activation of MBL-associated serine proteases (MASPs), which results in the activation of the complement cascade. Three serine proteases (MASP-1, MASP-2, and MASP-3) and 2 nonenzymatic components (MAp19 and MAp44) are associated with the pattern-recognition molecules, although the stoichiometry is still unknown. The first enzymatic step of lectin pathway activation is the autoactivation of zymogen MASP-1. Active MASP-1 then cleaves zymogen MASP-2 and MASP-3. MASP-2 is able to generate C3 convertase (C4b2a) by cleaving C4 and C2. MASP-1 has substrates outside the complement system as well, such as high-molecular-weight kininogen, fibrinogen, factor XIII, and protease-activated receptors. By cleaving these substrates, MASP-1 can initiate various proinflammatory reactions to mount an even more powerful innate immune response. C1-inhibitor is a natural regulator of the lectin pathway, as it can inhibit the activity of both MASP-1 and MASP-2. Another serpin, antithrombin, also can efficiently inhibit the lectin pathway, but only in the presence of glycosaminoglycans such as heparin. When C1-inhibitor is absent or dysfunctional, uncontrolled activation of the lectin pathway may contribute to the worsening of symptoms of hereditary angioedema.

INTRODUCTION

The complement system is an ancient part of the innate immunity. This network of about 35 proteins (soluble and membrane bound) can recognize, label, and eliminate invading microorganisms (pathogen-associated molecular patterns), and dangerously altered self-structures (danger - or damage-associated molecular patterns) such as apoptotic or necrotic cells, cancer cells, and immune complexes.[1] Activation of the complement system results in a number of biological effects including opsonization and direct lysis of the pathogenic cells, and initiation of inflammatory reaction by triggering endothelial cells and leukocytes. The complement system, as an integral part of the innate immune system, serves as a first line of defense against invading pathogens, but it also bridges the innate and adaptive immunity in several ways.

The complement system can be activated by 3 different means: the classical, the lectin, and the alternative pathways (Figure 1). In light of the latest discoveries, this classification is somewhat artificial, because there are cross-talks between the different activation routes. In the case of the classical and lectin pathways, pattern-recognition molecules recognize the dangerous structures.[2] These molecules are similar in shape and domain organization. C1q, the pattern-recognition molecule of the classical pathway, consists of 6 C-terminal globular domains and 6 N-terminal collagen-like arms.[3] The 6 arms merge into a single stalk near the N-terminus at a point where the collagen sequence is interrupted, yielding a molecular architecture resembling a bunch of 6 tulips. The C-terminal globular domains are responsible for recognition of and binding to the target structures (mainly immune complexes and C-reactive protein). The recognition molecules of the lectin pathway are mannan-binding lectin (MBL),[4] ficolins (H-, L-, and M-ficolin),[5] and collectin-11.[6] The overall structure of these molecules resembles that of C1q, but they exist in different oligomeric forms, from dimer to hexamer. The C-terminal globular domains of MBL are C-type lectins recognizing carbohydrate arrays on the surface of bacteria. Ficolins bind to acetylated compounds. Because the natural targets of ficolins are acetylated sugars, these molecules behave like lectins, binding to the surface of the pathogens. The pattern-recognition molecules bind to the dangerous target structures. This recognition and binding event is converted into an enzymatic signal by the serine proteases associated with the pattern-recognition molecules.[7] Zymogen serine proteases are associated with the collagenous arms of the pattern-recognition molecules forming the initiation complexes of the classical and lectin pathways.

The C1 complex, the first component of the classical pathway, consists of a C1q molecule and a tetramer of 2 C1r and 2 C1s serine proteases.[8,9] When C1q binds to the target structure, the zymogen serine proteases become activated. Activation means a limited proteolysis of a single peptide bond at the N-terminal region of the serine protease domain. The first enzymatic event in the

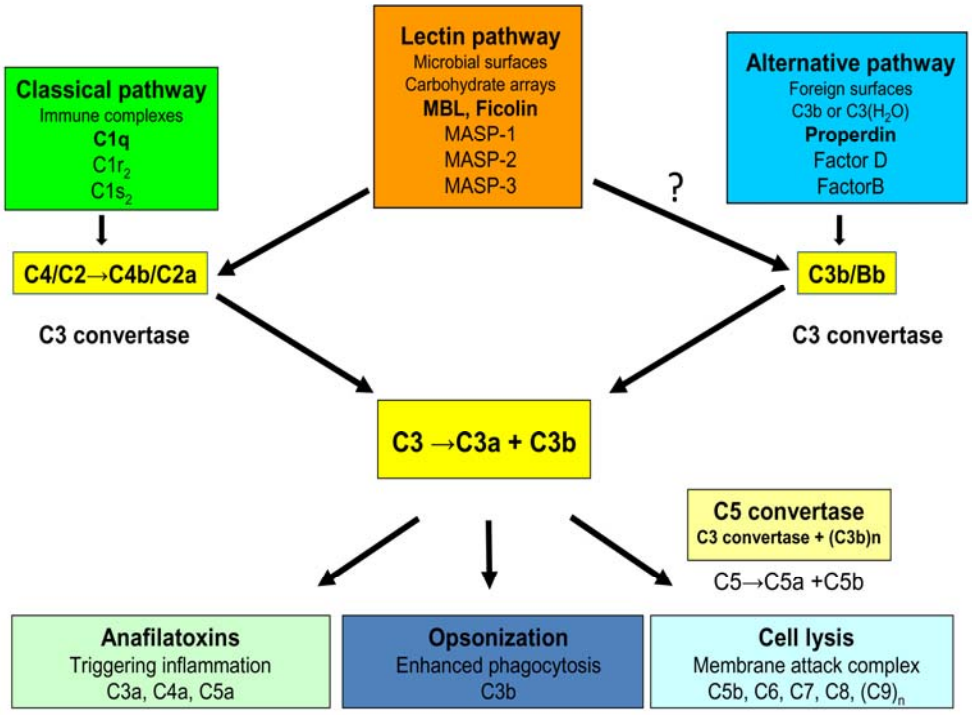

Figure 1. The complement system. The 3 activation routes of the complement system merge at the cleavage of C3, which initiates the common terminal pathway and elicits various biological effects. MASP, MBL-associated serine protease; MBL, mannan-binding lectin.

classical pathway is the autoactivation of C1r.[10] Activated C1r then cleaves and activates zymogen C1s. The initial enzymatic signal is amplified by a cascade reaction. C1s is the executive protease of the C1 complex. It can generate C3 convertase complex by cleaving C4 and C2. C4 is a thioester containing protein that can covalently bind to the surface of the cells after proteolytic cleavage. The deposited C4b binds the serine protease C2, which is cleaved by C1s, yielding the C4bC2a enzyme complex. The C4b2a complex is the classical C3 convertase. The proteolytic cleavage of C3 is the central event of the complement activation. It is the point where the 3 activation routes merge into a unified terminal complement pathway. C3 is cleaved into 2 fragments by the C3 convertase, and the bigger fragment (C3b) binds to the cell surface through a thioester bond. A C3 convertase complex cleaves a number of C3 molecules, further amplifying the initial signal. The activation of the lectin pathway results in the generation of the same C3 convertase complex. The mechanism of activation of the lectin pathway will be discussed later.

The appearance of C3b on the activation surface has 2 major consequences. First it serves as the initial molecule of the third activation route, the alternative pathway. C3b binds the serine protease factor B, which is cleaved by factor D. The resulting C3bBb complex is the alternative pathway C3 convertase. Because the appearance of C3b initiates the alternative pathway activation, which results in generation of more C3b, it is an extremely powerful amplifying mechanism (positive feedback). The alternative pathway is the major driving force behind the complement activation. It is estimated that regardless of the initial activation pathway (ie, classical or lectin), the alternative pathway is responsible for the generation of the majority (80%-90%) of C3b.[11] The alternative pathway can also be initiated independently of the other 2 pathways. C3, circulating in the blood, spontaneously hydrolyzes (tick over), which results in a molecule C3(H_2O) that can bind factor B. The bound factor B is cleaved by factor D, yielding C3(H_2O)Bb, which is a C3 convertase.[12] If this event occurs near the surface of a pathogenic cell, C3b will deposit on the cell, and the alternative pathway amplifies the initial signal. Self-tissues are protected from the alternative pathway, because the nascent C3 convertase will be destroyed and eliminated by soluble and surface-bound inhibitors. Properdin can stabilize the alternative pathway C3 convertase, and it is also suggested that properdin acts like a pattern-recognition molecule of the alternative pathway.[13]

As the concentration of the deposited C3b increases, the substrate specificity of the convertase complexes changes from C3 to C5. The cleavage of C5 by the C5 convertases [C4b2a($C3b)_n$ or C3bBb($C3b)_n$] is the last enzymatic event in the complement cascade. From this

point, protein-protein interactions lead to the formation of the membrane attack complex (MAC) ($C5b6789_n$) in a self -organizing manner. The MAC is capable of killing the bacterial cell through lysis, but this mechanism is not effective for all type of cells. The other effective way of killing the pathogens through complement activation is opsonization, in which the deposited complement components enhance the phagocytosis of the microorganisms by leukocytes.

The function of the intact complement system is essential to maintain immune homeostasis; however, improper uncontrolled activation can lead to serious self-tissue damage and development of disease conditions. Because complement is a potentially harmful system, its activation is tightly controlled by various soluble and surface-bound inhibitors.

MECHANISM OF LECTIN PATHWAY ACTIVATION

The lectin pathway was discovered in 1987, when the hemolytic activity of MBL was first demonstrated.[14] It was hypothesized that MBL was a pattern-recognition molecule for the classical pathway, as MBL can bind and activate the C1s-C1r-C1r-C1s tetramer. Later it became evident that MBL in the blood plasma copurified with a new protease, which was termed the MBL-associated serine protease, or MASP.[15] The sequence of the MASP proved that it belongs to the C1r/C1s protease family. The big surprise came when it was found that instead of MASP, another serine protease was responsible for C3 convertase generation.[16] The newly discovered serine protease was named MASP-2 and the old one MASP-1. It was very intriguing to learn that MASP-2 was only a minor component of the MBL-MASPs complex. According to the latest measurements, the concentration of MASP-1 in the plasma (143 nM) is more that 20 times higher than that of MASP-2 (6 nM).[17] Furthermore, it was hard to believe that MASP-2 "contamination" in the complexes was responsible for cleaving C4 and C2, while the much more abundant MASP-1 had only a supporting role. The key role of MASP-2 in lectin pathway activation was further supported by the fact that MASP-2 can autoactivate when MBL binds to the target.[18] It means that a MASP-2 dimer associated with an MBL or ficolin molecule can initiate the lectin pathway without the contribution of any other factor. MASP-1 knockout mice have lectin pathway activity, although its speed and efficiency do not reach that of wild-type mice.[19] Taken together, it seemed that MASP-2 was the autonomous activator of the lectin pathway. Regarding the substrate specificity, MASP-2 resembles C1s, as both proteases cleave C4 and C2. The only big difference is that MASP-2 can autoactivate whereas C1s cannot.

The substrate specificity and physiological function of MASP-1 have been some of the most debated issues in complement research in recent years. It was shown that MASP-1 can cleave C2 at a reasonably high efficiency, but it is inactive against C4.[20] Although MASP-1, like C1r, can autoactivate very efficiently, it cannot initiate the lectin pathway alone. It was therefore suggested that MASP-1 has a supporting role in the activation, because it can enhance the ability of MASP-2 to generate C3 convertase by C2 cleavage.[21] Later it was also suggested that MASP-1 can facilitate the autoactivation of MASP-2, which explains the slow lectin pathway response in MASP-1 knockout mice.[19] With the aim of clarifying the exact mechanism of the lectin pathway activation and the role of MASP-1 in normal human serum, we designed a new experimental approach. Using in vitro evolution technique (phage display), we developed selective inhibitors against MASP-1 and MASP-2.[22]

C1-inhibitor, the well-known natural inhibitor of MASP-1 and MASP-2, is a serpin with broad target specificity. It inhibits C1r and C1s of the classical pathway, MASP-1 and MASP-2 of the lectin pathway, plasma kallikrein and factor XII of the kallikrein-kinin pathway, and other proteases of the coagulation and fibrinolytic pathways. C1 -inhibitor is not suitable to selectively block the activity of the MASPs. We used naturally occurring canonical serine protease inhibitors to engineer selective serine protease inhibitors. We used the SGPI-2 serine protease inhibitor (isolated from *Schistocerca gregaria*), which belongs to the pacifastin family of canonical inhibitors.[23] During the in vitro evolution procedure, we optimized the sequence of the inhibitory loop to reach selective and efficient inhibition of the target protease. We selected 2 inhibitors: SGMI-1 selectively inhibits MASP-1 (K_i = 7 nM), whereas SGMI-2 selectively inhibits MASP-2 (K_i = 6 nM). Using SGMI-2 in the Wieslab (Euro Diagnostica AB) complement activation assay efficiently blocked the activity of the lectin pathway while leaving the classical and alternative pathways intact and fully functional.[24] This was not remarkable, because of the suggested central role of MASP-2. However, when we tested the MASP-1–specific SGMI-1, we had an unexpected result. Inhibition of MASP-1 completely and permanently inhibited the activation of the lectin pathway. This means that inhibition of MASP-1 somehow prevents the activation of MASP-2; in other words, without MASP-1 activity there is no MASP-2 activity. If we used serum preactivated on a mannan-coated surface, SGMI-2 could prevent C4 deposition while SGMI-1 could not. This was not surprising, because MASP-2 is the only lectin pathway protease that can cleave C4. If MASP-2 has been activated, the activation of the lectin pathway can be prevented only by using a MASP-2–specific inhibitor. However, in the case of unactivated (zymogen) serum, MASP-1–specific inhibitor is as efficient as the MASP-2–specific inhibitor in blocking C4 cleavage.

This phenomenon clearly shows that in normal human serum, MASP-1 activates MASP-2 during lectin pathway

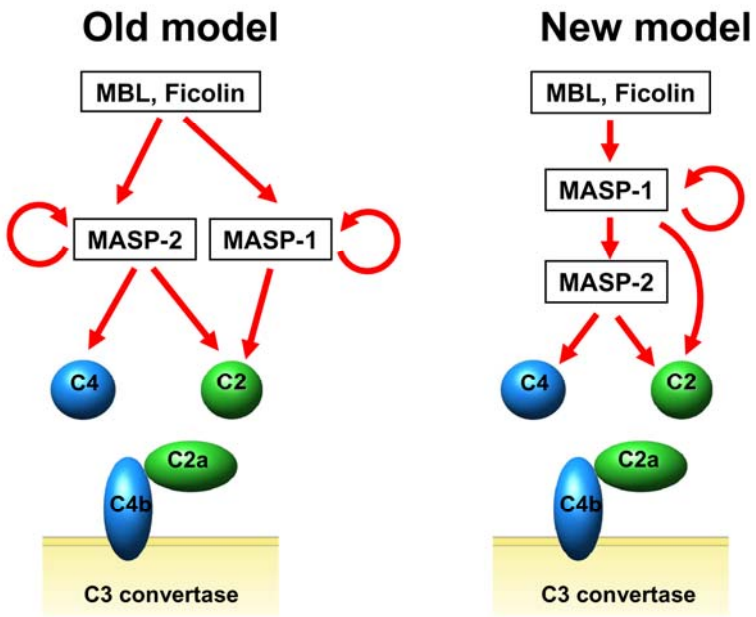

Figure 2. The mechanism of lectin pathway activation. According to the old model of lectin pathway activation, MASP-2 is the key protease because it can autoactivate and cleave C4 and C2, whereasMASP-1 has only a supportive role. In the new model, both MASP-1 and MASP-2 are equally important. MASP-1 controls the process, as it exclusively activates MASP-2. Then MASP-2 cleaves C4, while C2 is activated by both proteases. MASP, MBL-associated serine protease; MBL, mannan-binding lectin.

activation. These results were supported by kinetic measurements.[25] We found that MASP-1 autoactivates very efficiently and active MASP-1 cleaves zymogen MASP-2 much more efficiently than active MASP-2 cleaves its own zymogen. In normal human serum, in which every MASP-2 molecule is surrounded by MASP-1 molecules, the autoactivation of MASP-2 cannot take place and the exclusive activator of MASP-2 is MASP-1 (Figure 2). Our findings have been recently supported by Degn et al, who used MASP-1–deficient human serum from a patient.[26] The lack of lectin pathway activation in that case indicates that the autoactivation potential of MASP-2 is negligible in human serum in the absence of MASP-1. We can conclude that MASP-1 entirely controls lectin pathway activation in normal human serum. Moreover, MASP-1 enhances the C3 convertase–generating ability of MASP-2 by cleaving C2. Using our MASP-specific inhibitors, we showed that 60% of the C2a present in the C4b2a complex is the product of MASP-1 cleavage.[24]

After the discovery of MASP-2, other components of the lectin pathway have been discovered as well. It happens that there is a third protease, termed MASP-3,[27] and 2 nonproteolytic fragments: MAp44 (or MAP1)[28] and MAp19 (or sMAP).[29] MASP-3 and MAp44 are the alternative splice products of the *MASP-1* gene, while MAp19 is the alternative splice product of the *MASP-2* gene. MAp44 contains the first 4 domains of MASP-1, whereas MAp19 consists of the first 2 domains of MASP-2. These truncated MASPs have no enzymatic activity, but they can compete with the full-length MASPs for the pattern-recognition molecules. It has been suggested that these molecules play a role in the regulation of the lectin pathway. MASP-3 is different from MASP-1 and -2 because it cannot autoactivate. We have shown recently that MASP-1 can activate MASP-3.[25] MASP-3 cannot cleave either C4 or C2, so it seems that it does not participate in lectin pathway activation. Recently it has been suggested that MASP-3 can enhance alternative pathway activation by cleaving factor B.[30] Thus, MASP-3 might have similar role to factor D. However, factor D circulates in a proactivated form in the serum, whereas MASP-3 is a zymogen until MASP-1 cleaves it. We can assume that the autoactivation of MASP-1 has 2 consequences: it can generate the classical pathway C3 convertase (C4b2a) by activating MASP-2, and the alternative pathway convertase (C3bBb) by activating MASP-3. In this way, the 3 activation pathways are not totally independent; the lectin pathway bridges the classical and alternative pathways, while the alternative pathway amplifies the complement activation by positive feedback.

MASP-1 IS A PROMISCUOUS PROTEASE

Experimental evidence suggests that MASP-1 is a unique member of the C1r/C1s/MASP protease family. While C1r and C1s have only 1 or 2 substrates each, MASP-1 has much more relaxed substrate specificity. Many

characteristics of MASP-1 indicate that it has a thrombin-like specificity. MASP-1 can generate cross-linked fibrin polymers by cleaving fibrinogen and factor XIII (plasma transglutaminase).[31] The generation of a fibrin clot around an invading pathogen can prevent its spread in the body. This reflects an ancient mechanism of innate immunity. The fibrinopeptides that are liberated during fibrinogen cleavage have a proinflammatory effect on the immune cells. The other split products that are formed during complement activation (C3a, C5a) also stimulate cells through G-protein coupled receptors. Thrombin, however, can directly activate cells by cleaving protease-activated receptors (PARs).[32] Although PARs are G-protein coupled receptors that carry their own ligand at the N-terminal loop of the receptor, this ligand is cryptic until a protease cleaves the N-terminal loop. The tethered ligand then binds to the extracellular loop of the receptor and initiates signal transduction. According to current knowledge, there are 4 PARs. Thrombin cleaves PAR1 and PAR4, triggering endothelial cells and platelets. In vascular endothelium, thrombin elicits shape and permeability changes, mobilizes adhesive molecules, and stimulates cytokine production. Other proteases of the coagulation system (eg, factor X) are also suggested to be PAR agonists. It seemed reasonable that the complement system, which promotes inflammation, also activates PAR. Because MASP-1 is a thrombin-like enzyme, we presumed that it can directly activate cells. Human MASP-1 efficiently digested the peptide fragment representing the N-terminal loop of PAR4. Moreover, MASP-1 elicited calcium response in human umbilical vein endothelial cells, induced NF-κB nuclear translocation, and initiated p38 mitogen-activated protein kinase signaling.[33] Taken together, we can conclude that MASP-1 fundamentally contributes to the inherent vascular changes during inflammation through the cleavage of PAR. It was shown earlier that activation of PAR4 contributes to leukocyte rolling, adherence, and recruitment.[34] We are currently investigating whether MASP-1 is able to promote such effects.

There is another way through which MASP-1 can contribute to the development of inflammatory reactions. Using a proteomic approach, we discovered that MASP-1 cleaves high-molecular-weight kininogen and releases bradykinin (BK).[35] BK is a potent proinflammatory peptide that activates endothelial cells, resulting in vasodilatation, increased vascular permeability, and production of second-generation mediators. Although plasma kallikrein and tissue kallikrein are the serine proteases that are mainly responsible for BK generation, we cannot exclude that other proteases, especially the proteases of the immune system, also contribute to the elevated BK level under certain conditions. The BK-releasing ability of MASP-1 is rather small compared with that of plasma kallikrein, but because under normal circumstances complement acts locally, it can promote local inflammatory reaction. If the complement system is activated systemically owing to lack of proper control, for example, in the case of hereditary angioedema (HAE), the uncontrolled MASP-1 activity may contribute to the pathogenic BK production.

MASP-1, like thrombin, has a number of substrates. In the case of thrombin, all the substrates are related to the hemostatic response whereas in the case of MASP-1, all the substrates are related to the immune response. This means that thrombin and MASP-1 are at the same time selective and promiscuous (Figure 3). The substrate specificity of MASP-1 differs significantly from that of the other complement proteases. The other members of the

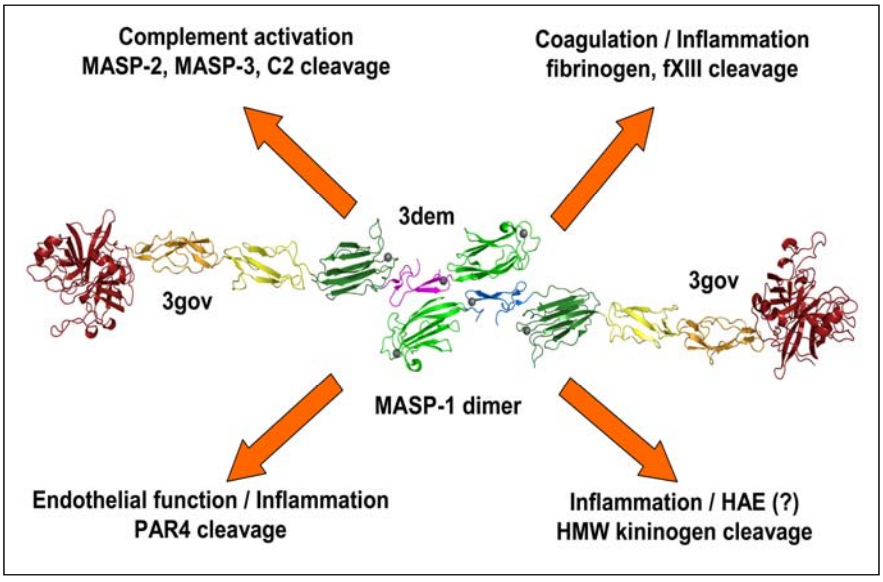

Figure 3. Diverse biological functions of MASP-1-
MASP-1, the most abundant protease of the lectin pathway, has broad substrate specificity and it participates in various biological processes. The structure of a MASP-1 dimer is depicted in the center of the illustration. This model used the crystal structures determined by Dobó et al[36] and Teillet et al.[37] fXII, factor XII; HAE, hereditary angioedema; HMW, high molecular weight; MASP, MBL-associated serine protease; MBL, mannan-binding lectin; PAR, protease-activated receptor.

C1r/C1s/MASP family have very tight substrate specificity. In recent years, we have determined the crystal structure of the catalytic fragment of C1r,[10] MASP-1,[36] and MASP-2.[38] Comparing the structure of the active site of these proteases, it was apparent that the substrate binding groove of MASP-1 is wide and accessible, while that of the other proteases is severely occluded by bulky regions that restrict substrate specificity. In this respect, MASP-1 resembles trypsin rather than early complement proteases. The gene structure and codon usage of MASP -1 and trypsin are also similar and differ from that of the other cognate complement proteases.[7] Whereas the serine protease domain of MASP-1 is encoded by 6 exons, the same domain of C1r, C1s, MASP-2, and MASP-3 is encoded by a single exon. The serine protease domain of MASP-1 contains the so-called histidine loop, which is absent in the other members of the family. These features support the ancient evolutionary status of MASP-1 among the early complement proteases.

CONTROL OF THE LECTIN PATHWAY

Although complement system is essential for fighting against infection, uncontrolled activation of the complement system can contribute to the development of disease conditions. Pathologic activation of the lectin pathway can cause severe self-tissue damage during ischemia reperfusion (eg, myocardial infarction, stroke). Because the complement system is a "double-edged sword," it is tightly regulated by different inhibitors. Inhibition of the serine proteases is a powerful method of preventing complement activation.[39,40] Interestingly, only the early serine proteases are inhibited directly. The convertase complexes are disassembled and degraded by different cofactors and the serine protease factor I. Serpins play a very important role in controlling the proteolytic cascades in the blood.[41] C1-inhibitor is a major regulator of the complement, kallikrein-kinin, blood coagulation, and fibrinolytic systems.[42] It makes a stable acyl-enzyme complex with active C1r, C1s, MASP-1, and MASP-2. C1-inhibitor is also able to prevent the autoactivation of zymogen C1r, MASP-1, and MASP-2. Notably, MASP-3 is not inhibited by C1-inhibitor.[43] Until now, no endogenous inhibitor of MASP-3 has been found. The bacterial inhibitor ecotin was shown to regulate MASP-3 activity. C1-inhibitor is an efficient inhibitor of the classical pathway. It binds covalently to activated C1r and C1s in the C1 complex, and the resulting C1-inh-C1s-C1r-C1-inh complex dissociates from C1q. No other inhibitors of C1r and C1s are known. C1-inhibitor also reacts with MASP-1 and MASP-2. Recently we determined the kinetic constants of these reactions.[44] The second-order association rate constants of the reactions between the MASPs and the inhibitor indicate that C1-inhibitor blocks the activity of MASP-2 more efficiently ($k_a = 1.6 \times 10^5$ $M^{-1}s^{-1}$) than that of MASP-1 ($k_a = 6.2 \times 10^3$ $M^{-1}s^{-1}$). Heparin, a negatively charged glycosaminoglycan, can usually potentiate protease/serpin reactions.[45] In the case of MASP-2, heparin accelerates the reaction ($k_a = 1.3 \times 10^6$ $M^{-1}s^{-1}$). In the case of MASP-1, however, there is no potentiation effect. As we previously showed that MASP-1 is a thrombin-like protease, we tested the effect of antithrombin, a natural serpin inhibitor of thrombin. Antithrombin alone inhibited the proteolytic activity of MASP-1 less efficiently than C1-inhibitor did ($k_a = 1.4 \times 10^3$ $M^{-1}s^{-1}$). Heparin exerted a strong potentiation effect: in the presence of heparin, antithrombin is the best natural inhibitor of MASP-1 ($k_a = 4.0 \times 10^4$ $M^{-1}s^{-1}$). Antithrombin also inhibited MASP-2: in the absence of heparin, the inhibitory effect was only weak ($k_a = 4.3 \times 10^2$ $M^{-1}s^{-1}$), but in the presence of heparin, it was similar to the inhibition of MASP-1 ($k_a = 4.7 \times 10^4$ $M^{-1}s^{-1}$). In the C3 and C4 deposition experiments, C1-inhibitor and antithrombin prevented lectin pathway activation very efficiently. It is probable that besides C1-inhibitor, antithrombin is also a physiological inhibitor of the lectin pathway.

The α_2-macroglobulin (α_2M) is a protease inhibitor that can inhibit proteases of all mechanistic classes.[46] Although α_2M is quite abundant in the blood plasma, its physiological role is not perfectly known—previously it was suggested that α_2M may inhibit lectin pathway activation. It has a unique mechanism of inhibition: after cleavage of the bait region by a protease, the overall conformation of α_2M changes in such a way that it engulfs the protease, preventing contact between the protease and the large protein substrates. The protease inside the cage formed by α_2M is still able to cleave low-molecular-weight substrates, but it cannot digest protein substrates such as C4 and C2. We showed that in the fluid phase, α_2M trapped the catalytic fragment of MASP-1 efficiently; however, in the C3 and C4 deposition assays, α_2M proved to be totally inefficient. The lack of inhibition in the latter case could be explained by steric hindrance: the large tetrameric inhibitor cannot gain access to the MASP-1 molecule immobilized on the activating surface though MBL. The rate of complex formation between α_2M and MASP-1 could be much slower than the MASP-1–mediated C4 and C2 cleavage. Taken together, we believe that α_2M can hardly be an efficient inhibitor of the lectin pathway in physiological conditions.

POSSIBLE ROLE OF COMPLEMENT IN HAE

Low level of functional C1-inhibitor in the blood results in the development of HAE.[47] Surprisingly, however, the symptoms of HAE are not caused by the uncontrolled complement activation, but rather by lack of both plasma kallikrein and factor XII regulation.[48] The unregulated kallikrein-kinin system overproduces the vasoactive peptide BK. Although the main problem in HAE is the elevated BK level, the disturbed complement activation also may have a detrimental effect. Because C1-inhibitor is the major inhibitor of the classical and lectin pathways,

it can be expected that the components of these pathways would be depleted. One of the most important functions of C1-inhibitor is to prevent spontaneous activation of both C1 and MBL-MASPs and ficolin-MASPs complexes. In the blood of patients with HAE, where spontaneous activation takes place, the consumption of the components of the classical and lectin pathways was observed. In some cases, the secondary deficiency of C2 and C4, caused by the low C1 -inhibitor level, led to the development of systemic lupus erythematosis–like disorder.[49] Interestingly, the spontaneous activation of C4 and C2 does not lead to C3 consumption. Probably the fluid phase–cleaved C4b and C2a do not readily associate to form C3 convertase, and the level of C4b-binding protein (a cofactor for factor I–mediated C4b destruction) is elevated in the blood of patients with HAE.[50] Regarding the lectin pathway, it was shown that the level of MASP-2 is decreased in patients with HAE, and depressed MBL-mediated activation was observed.[51] The limiting factor of the lectin pathway activation, however, was the reduced C4 level. The possible role of ficolin-mediated lectin pathway activation in HAE is suggested by the finding that disease severity and ficolin levels are inversely related.[52] We also cannot exclude the role of spontaneously activated MASP-1 in BK formation.[35] At a low C1-inhibitor level, the spontaneous activity of MASP-1 may contribute to the elevated BK baseline level in patients with HAE, or, activation of MASP-1 during infection, oxidative stress, or other conditions may initiate HAE attacks. Further studies are required to reveal the exact role of lectin pathway activation in HAE.

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ADDRESS CORRESPONDENCE TO:
Péter Gál
Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary
E-mail: gal.peter@ttk.mta.hu