EVOLUTION OF THE LECTIN–COMPLEMENT PATHWAY AND ITS ROLE IN INNATE IMMUNITY

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Discrimination between self and non-self by lectins (carbohydrate-binding proteins) is a strategy of innate immunity that is found in both vertebrates and invertebrates. In vertebrates, immune recognition mediated by ficolins (lectins that consist of a fibrinogen-like and a collagen-like domain), as well as by mannose-binding lectins, triggers the activation of the complement system, which results in the activation of novel serine proteases. The presence of a similar lectin-based complement system in ascidians, our closest invertebrate relatives, indicates that the complement system probably had a pivotal role in innate immunity before the evolution of an adaptive immune system in jawed vertebrates.

Immunity to infection is mediated by two systems — the acquired (or adaptive) immune system and the innate (or natural) immune system. Acquired immunity arose early in vertebrate evolution, at some point between the divergence of the cyclostomes (lamprey) and cartilaginous fishes (sharks). The innate immune system is an evolutionarily ancient form of immunity and offers the main resistance to microbial pathogens within the first minutes, hours or days of an infection[1]. Innate immunity was thought originally to be a non-specific immune response that was characterized by phagocytosis. However, innate immunity has considerable specificity and is able to discriminate between pathogens and self, as well as between classes of pathogen. The recognition of pathogens is mediated by a set of pattern-recognition receptors that bind conserved pathogen-associated molecular patterns (PAMPs) that are shared by broad classes of microorganism. This recognition successfully defends invertebrates and vertebrates against infection[2].

Complement was first described in the 1890s as a heat-labile protein in serum that 'complemented' heat-stable antibodies in the killing of bacteria. Now, the complement system, which consists of more than 30 plasma and cell-surface proteins, is known to be a highly sophisticated host-defence system that is engaged both by innate immunity and as one of the main effector mechanisms of antibody-mediated immunity. Once the complement system is activated, a chain of reactions that involves proteolysis and assembly occurs, which results in cleavage of the third complement component (C3). The cascade that leads to the cleavage of C3 is called the activation pathway. It is followed by the lytic pathway, during which the membrane-attack complex (MAC) is formed. There are three types of activation pathway: the classical, lectin and alternative pathways (FIG. 1). The classical pathway is activated by antibody–antigen complexes, whereas the other two pathways — the lectin and alternative pathways — function in innate immune defence. The lectin pathway involves carbohydrate recognition by pattern-recognition receptors, such as mannose-binding lectin (MBL) and ficolins, and the subsequent activation of associated unique enzymes that are known as MBL-associated serine proteases (MASPs). The alternative pathway is initiated by the covalent binding of a small amount of C3 to hydroxyl or amine groups on the cell-surface molecules of microorganisms, and it does not involve specific recognition molecules. This pathway also functions to amplify the activation of C3 (amplification loop). Activation of the complement system promotes three main biological activities: opsonization of pathogens; chemotaxis and activation of leukocytes; and direct killing of pathogens. Recently, accumulating

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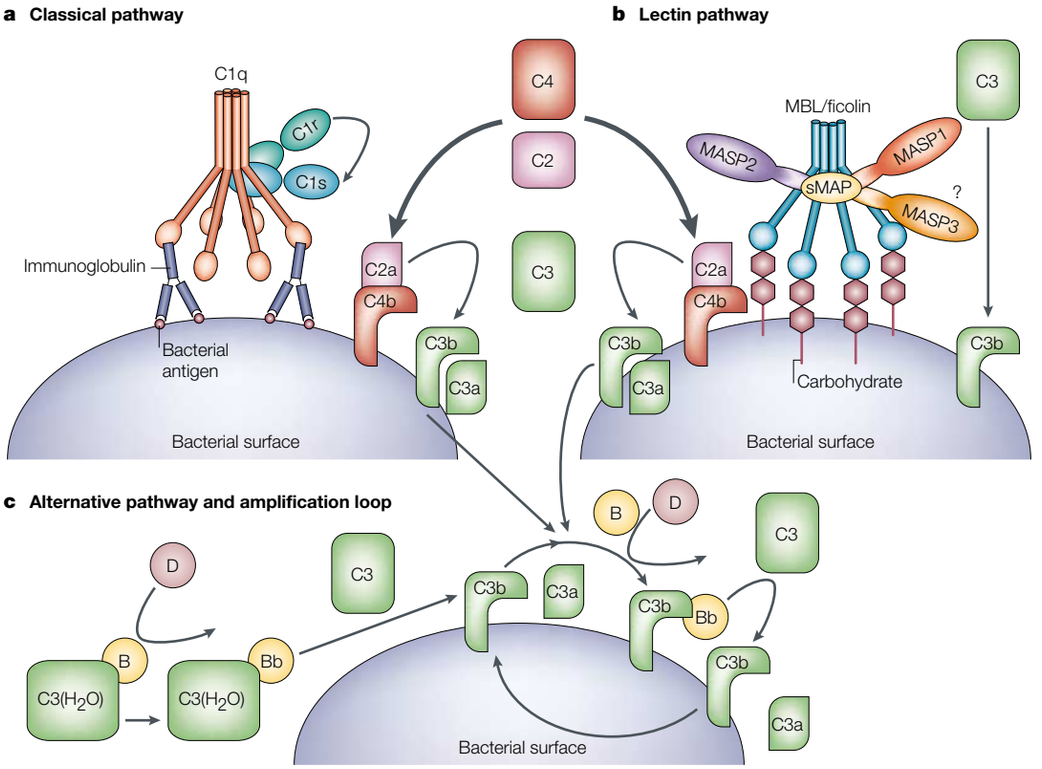

Figure 1 | **Activation of the classical, lectin and alternative pathways. a** | The classical pathway is initiated by the binding of the C1 complex to antibodies that are bound to antigens on the surface of bacteria. The C1 complex consists of C1q and two molecules each of C1r and C1s. The binding of the recognition subcomponent C1q to the Fc portion of immunoglobulins results in autoactivation of the serine protease C1r. C1r then cleaves and activates C1s, which translates the activation of the C1 complex into complement activation through the cleavage of C4 and C2 to form a C4bC2a enzyme complex. C4bC2a acts as a C3 convertase and cleaves C3, which results in products that bind to and cause the destruction of invading bacteria. **b** | The lectin pathway is initiated by the binding of either mannose-binding lectin (MBL) or ficolin — associated with MBL-associated serine protease 1 (MASP1), MASP2, MASP3 and small MBL-associated protein (sMAP) — to an array of carbohydrate groups on the surface of a bacterial cell. Similar to C1s, MASP2 is responsible for the activation of C4 and C2, which leads to the generation of the same C3 convertase (C4bC2a) as in the classical pathway. MASP1 is able to cleave C3 directly. **c** | The alternative pathway is initiated by the low-grade activation of C3 by hydrolysed C3 ($C3(H_2O)$) and activated factor B (Bb). The activated C3b binds factor B (B), which is then cleaved into Bb by factor D (D) to form the alternative pathway C3 convertase, C3bBb. Once C3b is attached to the cell surface, the amplification loop consisting of the alternative-pathway components is activated, and the C3-convertase enzymes cleave many molecules of C3 to C3b, which bind covalently around the site of complement activation.

C-TYPE LECTIN
A calcium-dependent animal lectin that is a carbohydrate-binding protein. The binding activity of a C-type lectin is based on the structure of the carbohydrate-recognition domain, which is highly conserved among this family. Calcium is essential not only for the carbohydrate binding itself, but also for the structural maintenance of this domain.

COLLECTIN
A C-type lectin that has a collagen-like domain. One group, the secreted lectins, consists of mannose-binding lectin, bovine conglutinin and collectin 43 in blood, and the two mucosal-associated proteins surfactant proteins A and D. The other group consists of the newly discovered, non-secreted-type collectin liver-1 and membrane-type collectin placenta-1.

evidence has shown that the complement system also acts as an adjuvant by enhancing and directing the adaptive immune response, and can function in the disposal of apoptotic cells[3,4].

In this review, I focus on the elucidation of the lectin pathway, the discovery of which was one of the most outstanding in recent complement research. Lectins are now simply defined as proteins that specifically bind carbohydrates. In animals, soluble lectins function as weapons against pathogens by aggregating and opsonizing them. These are primitive strategies of innate immunity that are found in both invertebrates and vertebrates. Evolutionary pressure, however, has afforded lectins the more powerful ability to activate the complement system, thereby effectively eliminating pathogens from the host. The identification of several components of the lectin pathway in ascidians, our closest invertebrate relatives, should provide new insights into the complement system, particularly in terms of its evolution.

Recognition molecules in complement activation

In the classical pathway, C1q, a subcomponent of the first complement component (C1), recognizes the Fc region of immunoglobulins that are bound to antigen[5]. C1q has an unusual modular structure that consists of six globular heads, each of which is connected by a strand to a central fibril-like region that is composed of collagen-like, triple-helical structures. Mannose-binding lectin and ficolins, which are the moieties that mediate recognition in the lectin pathway, have a similar overall structure to C1q (FIG. 1).

Mannose-binding lectin is a C-TYPE LECTIN[6–8] that has a crucial role in the first line of host defence[9]. The importance of this molecule is underlined by several clinical studies that link MBL-deficiency with increased susceptibility to various infectious diseases[10–13]. MBL belongs to the COLLECTIN family of proteins, which consist of a collagen-like domain and carbohydrate-recognition domain (CRD)[14]. Three polypeptides fold together to

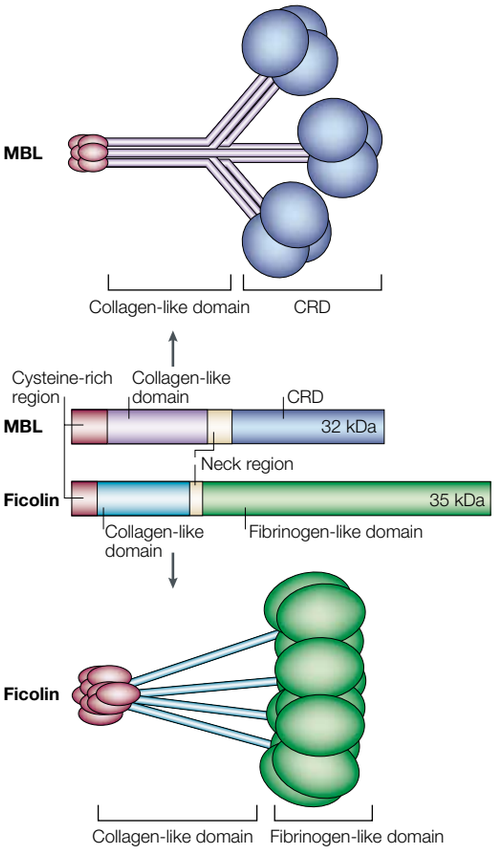

Figure 2 | **Domain and oligomeric structure of mannose-binding lectin and ficolins.** Mannose-binding lectin (MBL) and ficolins are oligomers of structural subunits, each of which is composed of three identical 32-kDa and 35-kDa polypeptides, respectively. Each subunit contains: an amino-terminal, cysteine-rich region; a collagen-like domain that consists of tandem repeats of Gly-Xaa-Yaa triplet sequences (where Xaa and Yaa represent any amino acid); a neck region; and a carboxy-terminal carbohydrate-recognition domain (CRD) in MBL and fibrinogen-like domain in ficolins. MBL forms several sizes of oligomers[15] and the trimeric form is shown. The tetrameric form of L-ficolin/P35 that is shown here was indicated by electron microscopy studies.

form the structural subunit and 3–6 of these subunits join to form human MBL, which has an apparent molecular mass of ~300–650 kDa (REF. 15) (FIG. 2). Through its CRD, MBL binds carbohydrates with 3- and 4-hydroxyl groups in the pyranose ring in the presence of calcium[16]. So, prominent ligands for MBL are mannose and *N*-acetyl-glucosamine (GlcNAc), whereas carbohydrates that do not fit this steric requirement — for example, galactose and sialic acid, which usually decorate the mammalian glycoproteins — have undetectable affinity for MBL[17]. This steric specificity of MBL, along with differences in the spatial organization of its ligands, enables the specific recognition of carbohydrates on pathogenic microorganisms, including bacteria, fungi, parasitic protozoans and viruses, and avoids the recognition of non-infectious self[18]. MBL has been characterized in mammals, chickens[19] and carp[20] (FIG. 3).

Ficolins, like MBL, are a group of proteins that contain a collagen-like stem structure. Unlike MBL, however, they have a fibrinogen-like domain, which is similar to fibrinogen β- and γ-chains[21] (FIG. 2). They were identified originally as transforming growth factor-β1 (TGF-β1)-binding proteins on pig uterus membranes[22]. Since then, ficolins have been identified in mammals, including human[23–28], rodents[29], pig[22,30] and hedgehog[31], and, recently, in ascidians[32] (see below and FIG. 3). Serum ficolins are lectins that have a common binding specificity for GlcNAc[24,28–30]. In human serum, two types of ficolin, known as L-ficolin/P35 (ficolin L)[24,25] and H-ficolin (Hakata antigen)[29,33], have been identified, and both of them have lectin activity. Another ficolin, known as M-ficolin[25,34] or P35-related protein[26] — the messenger RNA of which is found in leukocytes and lung — is not considered to be a serum protein. Recently, it has been reported that L-ficolin/P35 and H-ficolin activate the lectin–complement pathway in association with MASPs[21,35]. The functions of the fibrinogen-like domain of the ficolins are not fully understood; however, recent work has shown that the fibrinogen-like domain of several lectins has a similar function to the CRD of C-type lectins, which indicates that ficolins might also function as pattern-recognition receptors to discriminate pathogens from self[36,37].

Serine proteases of the lectin pathway

MASPs, a new member of the serine-protease superfamily, are proteolytic enzymes that are responsible for activation of the lectin pathway[38–41]. The MBL–MASPs complex was first described as a complement-dependent bactericidal factor in mouse[42,43]. MBL and ficolins have been found to be associated with MASP1 and MASP2, and a non-protease, small MBL-associated protein (sMAP or MAp19; a truncated form of MASP2)[44,45]. Recently, a third MASP (MASP3), which is generated by alternative splicing of MASP1, was reported to be associated with MBL[15]. The overall structure of MASPs resembles that of the two proteolytic components of the first factor in the classical complement pathway, C1r and C1s[39–41] (FIG. 4).

As illustrated in FIGURE. 1, activation of the classical pathway is triggered by binding of the recognition subcomponent C1q to an antibody, which, in turn, is translated into activation of the serine proteases C1r and C1s. Likewise, binding of the lectin-pathway recognition molecules (that is, MBL or ficolins) to microbial carbohydrates activates the lectin-pathway-specific serine proteases, MASPs[46,47]. The detailed molecular events, however, remain to be clarified. MASP2 is the enzyme component that — like C1s in the classical pathway — cleaves the complement components C4 and C2 to form the C3 convertase C4bC2a, which is common to both the lectin- and classical-pathway activation routes[41,48]. This result has been confirmed by the functional analysis of recombinant MASP2 (REFS 49–52). By contrast, MASP1 is able to cleave C3 directly[15,53,54], which results in activation of the alternative pathway[53]. The functions of MASP3 and sMAP are presently unknown. MASP1, MASP2, MASP3 and sMAP are

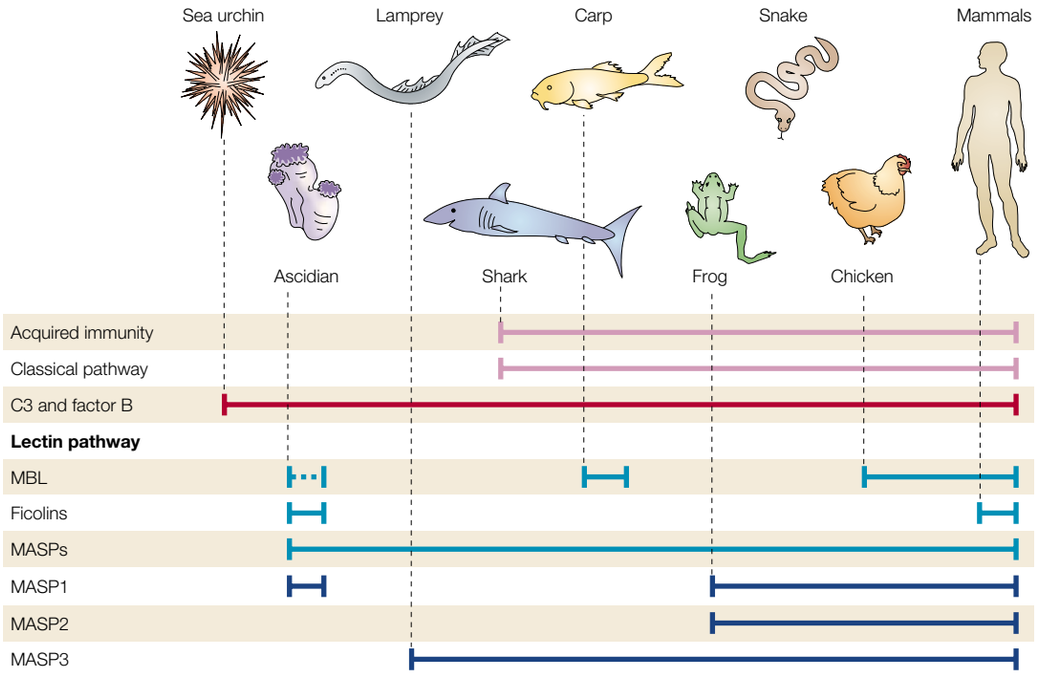

Figure 3 | **The complement system from an evolutionary perspective.** Acquired immunity was established at an early stage in the evolution of the jawed vertebrates, as illustrated by a cartilaginous fish (shark) in this figure. Evolutionary studies have revealed that shark and higher vertebrates have a well-developed complement system that contains all three activation pathways, although not all components of each pathway have been identified. C3 — the central component of the complement system — and a C2/factor-B-like sequence have been identified in a marine invertebrate, the sea urchin. In ascidians, several pivotal molecules, such as GBL (which is homologous to MBL), ficolins, MASPs, C3 and the C3 receptor have been identified. The evolutionary development of each type of MASP is also shown. GBL, glucose-binding lectin; MASP, MBL-associated serine protease; MBL, mannose-binding lectin.

encoded by two genes; sMAP is a truncated form of MASP2 (REFS 44,45), and MASP3 is produced from the *MASP1* gene by alternative splicing[15]. The *MASP1* gene has an H-chain-encoding region that is common to MASP1 and MASP3, which is followed by tandem repeats of protease-domain-encoding regions that are specific to MASP3 and MASP1 (FIG. 5).

Molecular evolution of the MASP family

Homologues of the MASP family have been cloned from various vertebrate and invertebrate species. On the basis of the genomic organization of the serine-protease domains[55] and their deduced protein structure, the MASP family can be divided into two phylogenetic lineages — TCN-type and AGY-type lineages[56]. The TCN-type lineage, which includes MASP1, has a TCN codon (where N denotes A, G, C or T) that encodes the active-site serine, the presence of a histidine-loop disulphide bridge and split exons. By contrast, the AGY-type lineage, which includes MASP2, MASP3, C1r and C1s, is characterized by an AGY codon (where Y denotes C or T) that encodes the active-site serine, the absence of a histidine loop and a single exon[56]. The TCN-type MASPs have been observed in ascidians, *Xenopus* and mammals, whereas AGY-type MASPs have been observed in lamprey, shark, carp, *Xenopus* and mammals (FIG. 3).

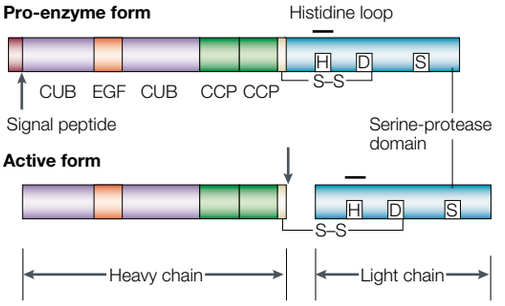

Figure 4 | **Domain structure of the MASP family.** Mannose-binding lectin (MBL)-associated serine protease 1 (MASP1), MASP2, MASP3, complement component 1r (C1r) and C1s consist of six domains: two C1r/C1s/Uegf/bone morphogenetic protein 1 (CUB) domains, an epidermal growth factor (EGF)-like domain, two complement control protein (CCP) domains or short consensus repeats (SCRs), and a serine-protease domain. Histidine (H), aspartic acid (D) and serine (S) residues are essential for the formation of the active centre in the serine-protease domain. Only MASP1 has two additional cysteine residues in the light chain, which form a histidine-loop disulphide bridge (S–S), as is found in trypsin and chymotrypsin. On binding of MBL and ficolins to carbohydrate on the surface of a pathogen, the pro-enzyme form of a MASP is cleaved between the second CCP and the protease domain, which results in an active form that consists of two polypeptides — heavy and light chains (also known as A and B chains[39,41]).

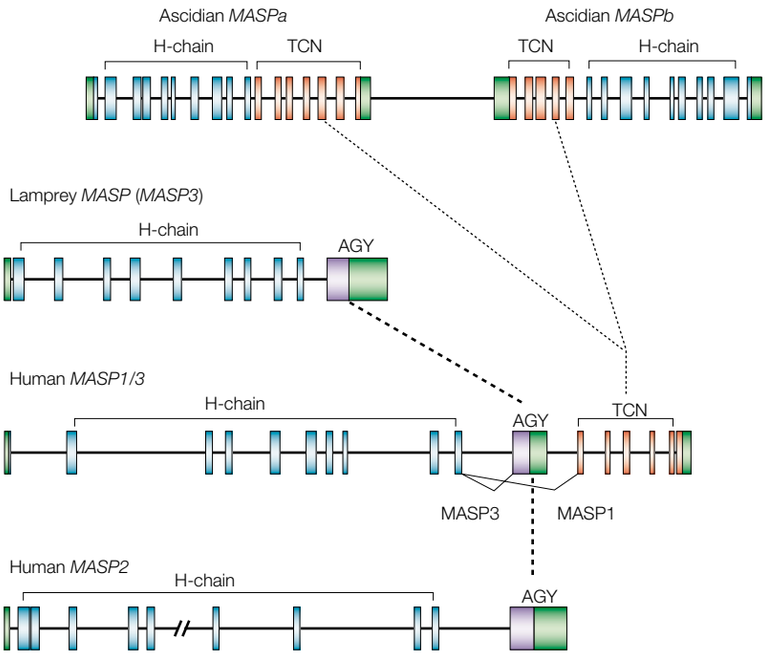

Figure 5 | **Gene organization of the MASP family.** The ascidian *MASPa* and *MASPb* genes each consist of heavy (H)-chain-encoding and TCN-type light (L)-chain-encoding regions. By contrast, vertebrate *MASP* genes, for example in lamprey, have the H-chain-encoding region and an AGY-type L-chain-encoding region. The human *MASP1/3* gene has a unique structure, in that it also has the downstream TCN-type L-chain-encoding region. Human MASP1 (TCN-type) and MASP3 (AGY-type) have a common H-chain and distinct L-chains, which are produced from the *MASP1/3* gene by alternative splicing. A comparison of gene structure between the ascidian genes and vertebrate genes indicates that the exon that encodes AGY-type L-chain might have inserted into the prototype gene by retroposition before the emergence of primitive vertebrates. The MASP-family genes, including *MASP2*, which is shown in this figure, and *C1r* and *C1s*, have similar organization, and are believed to be derived from a common ancestral *MASP1/3*-like gene by gene-duplication events. MASP, mannose-binding-lectin-associated serine protease.

From an evolutionary point of view, it has been suggested that the AGY-type MASPs diverged from the TCN-type MASPs before the emergence of primitive vertebrates. It is possible that an ancient TCN-type gene was converted to the *MASP1/3* gene by the insertion of a processed, intron-less serine-protease domain[56] (AGY-type) (FIG. 5). The MASP-family genes, including those that encode MASP2, C1r and C1s, are believed to originate from a common ancestral *MASP1/3*-like gene. The recent identification of a C1r/C1s-like sequence in the bony fish carp, in conjunction with phylogenetic analysis, implies that the divergence of the gene that encodes C1r/C1s might precede that of *MASP2*. The absence of the phylogenetically older TCN-type MASPs in primitive vertebrates, such as lamprey, cartilaginous fishes and bony fishes, can only be explained by the loss of TCN-type encoding exons in each lineage[57].

Accumulating data confirm that MASP3 is present in all of the vertebrates that have been analysed so far. This indicates that MASP3 might have a fundamental physiological role, although no definitive substrate for MASP3 has been identified among the complement components. The presence of the three types of MASP indicates that the lectin pathway is present and similarly composed in higher vertebrates, including amphibians and humans.

Putative original complement system

The origin of the complement system can be traced back at least as far as echinoderms, because C3, the key component of the complement system, and a C2/factor-B-like sequence have been identified in sea urchin[58–61]. Sea squirts — ascidians that were previously known as tunicates (phylum, Chordata; subphylum, Urochordata) — occupy a pivotal intermediary position between invertebrates and vertebrates. Therefore, studies of the host-defence mechanisms of ascidians could provide us with important information about the evolution of a primitive innate immune system in vertebrates. *Halocynthia roretzi* is a large solitary ascidian that is native to the coastal waters of Japan. So far, two lectins that correspond to mammalian MBL[62] and ficolins[32], two MASPs[63], C3 (REF. 64), a C2/factor-B-like sequence[61] and C3 receptor[65] have been identified in ascidians (FIG. 3).

Although several lectins have been reported in ascidians — including collectin-type lectin in *Stylea plicata*[66], C-type lectin in the colonial ascidian *Clavelina picta*[67] and MBL-like lectin in sea cucumber (the holothurian *Cucumaria japonica*)[68] — and are thought to represent MBL homologues in invertebrates, their complete structure has not been elucidated. Recently, we have purified an MBL-like 36-kDa lectin from ascidian plasma[62]. The purified protein binds specifically to glucose, but not to mannose or GlcNAc, and so was designated glucose-binding lectin (GBL). Sequence analysis of GBL complementary DNA revealed that the carboxy-terminal half of the ascidian lectin contains a CRD that is homologous to that of C-type lectin, but it lacks a collagen-like domain, which is present in mammalian MBLs. Although the structure and binding specificity of GBL is different from that of mammalian MBL, GBL associates with two ascidian MASPs, and GBL–MASPs complexes activate ascidian C3 in the same manner as the human MBL–MASP1 complex activates human C3. So, it seems probable that GBL might have evolved as an early prototype of MBL and has acquired the broad binding specificity for carbohydrates and the collagen structure that is characteristic of MBL during evolution. In addition, we have isolated ascidian ficolins that have short collagen-like sequences and fibrinogen-like domains[32]. Although it is unknown, at present, whether these ficolins associate with MASPs and activate complement, these observations indicate that ficolins, as well as GBL, act as the recognition molecules of the primitive ascidian complement system in a similar manner to the mammalian lectin pathway.

C3 has been identified as the main opsonic factor in ascidian plasma[64], and a C3 receptor has also been identified on ascidian haemocytes as the homologue of mammalian complement receptor type 3 or type 4 (CR3 or CR4)[65]. Antibodies that are specific for GBL[62], C3 (REF. 64) and C3 receptor[65] completely inhibited the phagocytosis of yeast in ascidians, which indicates that complement-mediated phagocytosis is a central part of the physiological function of their primitive complement system. In addition, yeast that were treated with purified GBL–MASPs complex and C3 were phagocytosed to a greater extent than untreated yeast by haemocytes[62].

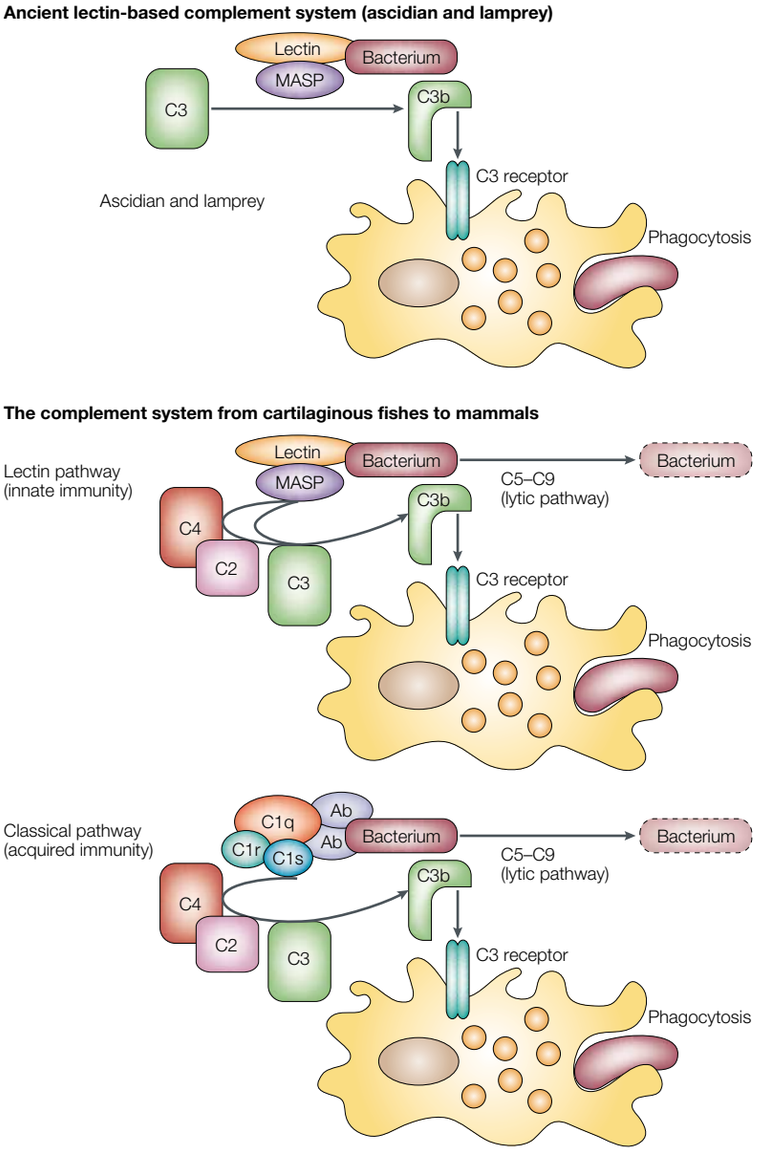

Figure 6 | **Putative model of an ancient lectin-based complement system and its evolution.** The lectin–protease (lectin–MASP) complex, C3 and C3 receptor are probably the minimal ancestral components of the primordial complement system, which functioned in an opsonic manner and appeared in the ascidian lineage. The complement system of lamprey (the most primitive vertebrate) lacks the classical and lytic pathways, and so, lamprey seem to have a similar complement system to ascidians. Therefore, the complement system developed dramatically at an early stage of vertebrate evolution into a sophisticated, multifunctional system. Gene-duplication events seem to have been important in this process and several sets of homologous complement components are noted, such as MBL and C1q, MASPs and C1r/C1s, C2 and factor B, and C4 and C3. Ab, antibody; MASP, MBL-associated serine protease; MBL, mannose-binding lectin.

These observations indicate that a lectin–protease (lectin–MASP) complex, C3 and its receptor might have developed as the minimal ancestral components of a primordial complement system in the ascidian lineage, as shown in FIGURE 6. Therefore, the ascidian complement system, which has similar mechanisms of activation and function to the mammalian system, has remained unchanged since its appearance at least 600 million years ago, well before the emergence of adaptive immunity.

The classical and lytic pathways of the complement system seem to have emerged at the cartilaginous-fishes stage, coincident with the emergence of adaptive immunity[61]. The complement system of lamprey — the most primitive vertebrate — also lacks the classical and lytic pathways, which indicates that lamprey probably have a similar complement system to ascidians. Although the molecular composition of the lectin pathway in cartilaginous and bony fishes has not been fully clarified, the C1r and C1s components of C1 are clearly derived from the MASP lineage, and C1q is closely related to MBL or ficolins by the substitution of antibody-recognition domains for the CRDs or fibrinogen-like domain. From an evolutionary point of view, the primitive lectin pathway in innate immunity seems to have developed into the more sophisticated, multifunctional complement system of the classical pathway through gene duplication, to serve as an effector system of acquired immunity (FIG. 6). A strong link between the innate immune systems of invertebrates and acquired immunity in vertebrates is, therefore, established.

As no opsonization was observed in the absence of GBL[62] in ascidians, and the alternative pathway was found to be responsible for the activation of serum C3 by yeast cell wall (zymosan), without the involvement of any recognition molecules[69], the alternative pathway might not have emerged in the ascidian lineage. However, the possibility of a simple role for C2/factor-B-like protein as an amplifier of C3 deposition can not be excluded completely. The sophisticated mechanisms of the alternative pathway to recognize a broad spectrum of pathogens developed more recently.

Perspectives

The identification and functional characterization of the lectin-based activation mechanisms of the complement system have provided new insights into the role of complement in innate immunity, which enables molecular patterns that specifically characterize microorganisms to be detected. Two human ficolins, L-ficolin/P35 and H-ficolin, as well as MBL, associate with MASPs and activate the lectin–complement pathway. However, the role of ficolins in innate immune defence remains to be clarified, by carrying out pathogen-binding analyses and constructing gene-targeted animal models. Although a new member of the MASP family, MASP3, and the molecular composition of the MBL–MASPs complex have been identified[15], the activation mechanisms of the lectin pathway — in particular those that are specific to each MASP — remain to be elucidated. MBL and L-ficolin/P35, as well as C1q, act as opsonins and enhance phagocytosis[24,70], but, so far, no opsonic receptor that recognizes the collagenous region of these molecules has been identified[47].

An ancient lectin-based complement system in ascidians reveals that the primitive complement system is one of the most highly organized innate immune systems in invertebrates. The origin of the complement system is much more ancient than that of adaptive immunity, which is only found in jawed vertebrates. In this respect, it will be of particular interest to solve the molecular

architecture of complement in the jawless fish, lamprey. In addition, as the alternative pathway was thought previously to be an ancient mechanism that is characterized by non-specific activation, elucidation of the function of C2/factor-B-like protein in sea urchin, ascidians and lamprey will provide interesting data.

1. Hoffmann, J. A., Kafatos, F. C., Janeway, C. A. & Ezekowitz, R. A. Phylogenetic perspectives in innate immunity. *Science* **284**, 1313–1318 (1999).
2. Medzhitov, R. & Janeway, C. Jr. Innate immune recognition: mechanisms and pathways. *Immunol. Rev.* **173**, 89–97 (2000).
3. Walport, M. J. Complement. First of two parts. *N. Engl. J. Med.* **344**, 1058–1066 (2001).
4. Walport, M. J. Complement. Second of two parts. *N. Engl. J. Med.* **344**, 1140–1144 (2001).
References 3 and 4 provide an excellent review of the complement system. They focus on recent advances in the role of complement in disease, and the interaction between innate and adaptive immunity.
5. Kishore, U. & Reid, K. B. C1q: structure, function and receptors. *Immunopharmacology* **49**, 159–170 (2000).
6. Mizuno, Y., Kozutsumi, Y., Kawasaki, T. & Yamashina, I. Isolation and characterization of a mannan-binding protein from rat liver. *J. Biol. Chem.* **256**, 4247–4252 (1981).
7. Drickamer, K., Dordal, M. S. & Reynolds, L. Mannose-binding proteins isolated from rat liver contain carbohydrate-recognition domains linked to collagenous tails. Complete primary structures and homology with pulmonary surfactant apoprotein. *J. Biol. Chem.* **261**, 6878–6887 (1986).
8. Ezekowitz, R. A., Day, L. E. & Herman, G. A. A human mannose-binding protein is an acute-phase reactant that shares sequence homology with other vertebrate lectins. *J. Exp. Med.* **167**, 1034–1046 (1988).
9. Turner, M. W. Mannose-binding lectin: the pluripotent molecule of the innate immune system. *Immunol. Today* **17**, 532–540 (1996).
10. Super, M., Thiel, S., Lu, J., Levinsky, R. J. & Turner, M. W. Association of low levels of mannan-binding protein with a common defect of opsonisation. *Lancet* **2**, 1236–1239 (1989).
11. Summerfield, J. A. *et al*. Mannose-binding protein gene mutations associated with unusual and severe infections in adults. *Lancet* **345**, 886–889 (1995).
12. Neth, O. *et al*. Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition. *Infect. Immun.* **68**, 688–693 (2000).
13. Jack, D. L., Klein, N. J. & Turner, M. W. Mannose-binding lectin: targeting the microbial world for complement attack and opsonophagocytosis. *Immunol. Rev.* **180**, 86–99 (2001).
14. Holmskov, U., Malhotra, R., Sim, R. B. & Jensenius, J. C. Collectins: collagenous C-type lectins of the innate immune defense system. *Immunol. Today* **15**, 67–74 (1994).
15. Dahl, M. R. *et al*. MASP-3 and its association with distinct complexes of the mannan-binding lectin complement-activation pathway. *Immunity* **15**, 127–315 (2001).
This study shows that human MBL is associated with a third serine protease termed MASP3, which is generated through alternative splicing of the *MASP1/3* gene. Another important finding is that different MBL oligomers have distinct MASP composition and biological activities, as shown by the association of MASP1, sMAP and C3 activation with smaller MBL oligomers.
16. Weis, W. I., Drickamer, K. & Hendrickson, W. A. Structure of a C-type mannose-binding protein complexed with an oligosaccharide. *Nature* **360**, 127–134 (1992).
17. Drickamer, K. Engineering galactose-binding activity into a C-type mannose-binding protein. *Nature* **360**, 183–186 (1992).
18. Sheriff, S., Chang, C. Y. & Ezekowitz, R. A. Human mannose-binding protein carbohydrate-recognition domain trimerizes through a triple α-helical coiled-coil. *Nature Struct. Biol.* **1**, 789–794 (1994).
19. Laursen, S. B. & Nielsen, O. L. Mannan-binding lectin (MBL) in chickens: molecular and functional aspects. *Dev. Comp. Immunol.* **24**, 85–101 (2000).
20. Vitved, L. *et al*. The homologue of mannose-binding lectin in the carp family *Cyprinidae* is expressed at high level in spleen, and the deduced primary structure predicts affinity for galactose. *Immunogenetics* **51**, 955–964 (2000).
21. Matsushita, M. & Fujita, T. Ficolins and the lectin complement pathway. *Immunol. Rev.* **180**, 78–85 (2001).
22. Ichijo, H. *et al*. Molecular cloning and characterization of ficolin, a multimeric protein with fibrinogen- and collagen-like domains. *J. Biol. Chem.* **268**, 14505–14513 (1993).
23. Edgar, P. F. Hucolin, a new corticosteroid-binding protein from human plasma with structural similarities to ficolins, transforming growth factor-β-1-binding proteins. *FEBS Lett.* **375**, 159–161 (1995).
24. Matsushita, M. *et al*. A novel human serum lectin with collagen- and fibrinogen-like domains that functions as an opsonin. *J. Biol. Chem.* **271**, 2448–2454 (1996).
25. Lu, J., Tay, P. N., Kon, O. L. & Reid, K. B. Human ficolin: cDNA cloning, demonstration of peripheral-blood leucocytes as the major site of synthesis and assignment of the gene to chromosome 9. *Biochem. J.* **313**, 473–478 (1996).
26. Endo, Y., Sato, Y., Matsushita, M. & Fujita, T. Cloning and characterization of the human lectin *P35* gene and its related gene. *Genomics* **36**, 515–521 (1996).
27. Harumiya, S. *et al*. Characterization of ficolins as novel elastin-binding proteins and molecular cloning of human ficolin-1. *J. Biochem. (Tokyo)* **120**, 745–751 (1996).
28. Sugimoto, R. *et al*. Cloning and characterization of the Hakata antigen, a member of the ficolin/opsonin p35 lectin family. *J. Biol. Chem.* **273**, 20721–20727 (1998).
29. Fujimori, Y. *et al*. Molecular cloning and characterization of mouse ficolin-A. *Biochem. Biophys. Res. Commun.* **244**, 796–800 (1998).
30. Ohashi, T. & Erickson, H. P. Two oligomeric forms of plasma ficolin have differential lectin activity. *J. Biol. Chem.* **272**, 14220–14226 (1997).
31. Omori-Satoh, T., Yamakawa, Y. & Mebs, D. The antihemorrhagic factor, erinacin, from the European hedgehog (*Erinaceus europaeus*), a metalloprotease inhibitor of large molecular size possessing ficolin/opsonin P35 lectin domains. *Toxicon* **38**, 1561–1580 (2000).
32. Kenjo, A. *et al*. Cloning and characterization of novel ficolins from the solitary ascidian, *Halocynthia roretzi*. *J. Biol. Chem.* **276**, 19959–19965 (2001).
The first description of ficolins in invertebrates as GlcNAc-binding lectins, which are homologues of mammalian ficolins. The presence of ficolins in invertebrates indicates their crucial role in innate immunity.
33. Akaiwa, M. *et al*. Hakata antigen, a new member of the ficolin/opsonin p35 family, is a novel human lectin secreted into bronchus/alveolus and bile. *J. Histochem. Cytochem.* **47**, 777–786 (1999).
34. Teh, C., Le, Y., Lee, S. H. & Lu, J. M-ficolin is expressed on monocytes and is a lectin binding to *N*-acetyl-D-glucosamine and mediates monocyte adhesion and phagocytosis of *Escherichia coli*. *Immunology* **101**, 225–232 (2000).
35. Matsushita, M., Endo, Y. & Fujita, T. Cutting edge: complement-activating complex of ficolin and mannose-binding-lectin-associated serine protease. *J. Immunol.* **164**, 2281–2284 (2000).
This paper describes L-ficolin/P35 as another lectin that is able to mediate complement activation through association with MASPs.
36. Gokudan, S. *et al*. Horseshoe crab acetyl-group-recognizing lectins involved in innate immunity are structurally related to fibrinogen. *Proc. Natl Acad. Sci. USA* **96**, 10086–10091 (1999).
37. Kairies, N. *et al*. The 2.0 Å crystal structure of tachylectin 5A provides evidence for the common origin of the innate immunity and the blood coagulation systems. *Proc. Natl Acad. Sci. USA* **98**, 13519–13524 (2001).
38. Matsushita, M. & Fujita, T. Activation of the classical complement pathway by mannose-binding protein in association with a novel C1s-like serine protease. *J. Exp. Med.* **176**, 1497–1502 (1992).
39. Sato, T., Endo, Y., Matsushita, M. & Fujita, T. Molecular characterization of a novel serine protease involved in activation of the complement system by mannose-binding protein. *Int. Immunol.* **6**, 665–669 (1994).
40. Takada, F., Takayama, Y., Hatsuse, H. & Kawakami, M. A new member of the C1s family of complement proteins found in a bactericidal factor, Ra-reactive factor, in human serum. *Biochem. Biophys. Res. Commun.* **196**, 1003–1009 (1993).
41. Thiel, S. *et al*. A second serine protease associated with mannan-binding lectin that activates complement. *Nature* **386**, 506–510 (1997).
42. Ji, Y. H. *et al*. Activation of the C4 and C2 components of complement by a proteinase in serum bactericidal factor, Ra-reactive factor. *J. Immunol.* **150**, 571–578 (1993).
43. Matsushita, M., Takahashi, A., Hatsuse, H., Kawakami, M. & Fujita, T. Human mannose-binding protein is identical to a component of Ra-reactive factor. *Biochem. Biophys. Res. Commun.* **183**, 645–651 (1992).
44. Takahashi, M., Endo, Y., Fujita, T. & Matsushita, M. 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.* **11**, 859–863 (1999).
45. Stover, C. M., Schwaeble, W. J., Lynch, N. J., Thiel, S. & Speicher, M. R. 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.* **84**, 148–149 (1999).
46. Thiel, S. *et al*. Interaction of C1q and mannan-binding lectin (MBL) with C1r, C1s, MBL-associated serine proteases 1 and 2, and the MBL-associated protein MAp19. *J. Immunol.* **165**, 878–887 (2000).
47. Gadjeva, M., Thiel, S. & Jensenius, J. C. The mannan-binding-lectin pathway of the innate immune response. *Curr. Opin. Immunol.* **13**, 74–78 (2001).
48. Matsushita, M., Thiel, S., Jensenius, J. C., Terai, I. & Fujita, T. Proteolytic activities of two types of mannose-binding-lectin-associated serine protease. *J. Immunol.* **165**, 2637–2642 (2000).
49. Vorup-Jensen, T. *et al*. Distinct pathways of mannan-binding lectin (MBL)- and C1-complex autoactivation revealed by reconstitution of MBL with recombinant MBL-associated serine protease 2. *J. Immunol.* **165**, 2093–2100 (2000).
50. Wallis, R. & Dodd, R. B. Interaction of mannose-binding protein with associated serine proteases: effects of naturally occurring mutations. *J. Biol. Chem.* **275**, 30962–30969 (2000).
51. Chen, C. B. & Wallis, R. Stoichiometry of complexes between mannose-binding protein and its associated serine proteases. Defining functional units for complement activation. *J. Biol. Chem.* **276**, 25894–25902 (2001).
52. Thielens, N. M. *et al*. Interaction properties of human mannan-binding lectin (MBL)-associated serine proteases 1 and 2, MBL-associated protein 19 and MBL. *J. Immunol.* **166**, 5068–5077 (2001).
References 49–52 describe the molecular interactions between MASP1, MASP2 and sMAP/MAp19 in association with MBL.
53. Matsushita, M. & Fujita, T. Cleavage of the third component of complement (C3) by mannose-binding-protein-associated serine protease (MASP) with subsequent complement activation. *Immunobiology* **194**, 443–448 (1995).
54. Rossi, V. *et al*. Substrate specificities of recombinant mannan-binding-lectin-associated serine proteases 1 and 2. *J. Biol. Chem.* **276**, 40880–40887 (2001).
55. Endo, Y., Sato, T., Matsushita, M. & Fujita, T. Exon structure of the gene encoding the human mannose-binding-protein-associated serine protease light chain: comparison with complement C1r and C1s genes. *Int. Immunol.* **8**, 1355–1358 (1996).
56. Endo, Y. *et al*. Two lineages of mannose-binding-lectin-associated serine protease (MASP) in vertebrates. *J. Immunol.* **161**, 4924–4930 (1998).
57. Matsushita, M., Endo, Y., Nonaka, M. & Fujita, T. Complement-related serine proteases in tunicates and vertebrates. *Curr. Opin. Immunol.* **10**, 29–35 (1998).
58. Smith, L. C., Chang, L., Britten, R. J. & Davidson, E. H. Sea urchin genes expressed in activated coelomocytes are identified by expressed sequence tags. Complement homologues and other putative immune response genes suggest immune system homology within the deuterostomes. *J. Immunol.* **156**, 593–602 (1996).
59. Smith, L. C., Shih, C. S. & Dachenhausen, S. G. Coelomocytes express SpBf, a homologue of factor B, the second component in the sea urchin complement system. *J. Immunol.* **161**, 6784–6793 (1998).

60. Smith, L. C., Clow, L. A. & Terwilliger, D. P. The ancestral complement system in sea urchins. *Immunol. Rev.* **180**, 16–34 (2001).
61. Nonaka, M. Evolution of the complement system. *Curr. Opin. Immunol.* **13**, 69–73 (2001).
An excellent, recent review of the molecular evolution of the complement system.
62. Sekine, H. *et al*. An ancient lectin-dependent complement system in an ascidian: novel lectin isolated from the plasma of the solitary ascidian, *Halocynthia roretzi*. *J. Immunol.* **167**, 4504–4510 (2001).
This study describes the presence of glucose-binding lectin (GBL) in the plasma of the solitary ascidian, which has a carbohydrate-recognition domain but lacks a collagen-like domain. The important finding is that the GBL–MASPs complex activates C3, leading to C3-dependent phagocytosis, which indicates that the primitive complement system consists of the lectin–proteases complex and C3.
63. Ji, X., Azumi, K., Sasaki, M. & Nonaka, M. Ancient origin of the complement lectin pathway revealed by molecular cloning of mannan-binding-protein-associated serine protease from a urochordate, the Japanese ascidian, *Halocynthia roretzi*. *Proc. Natl Acad. Sci. USA* **94**, 6340–6345 (1997).
64. Nonaka, M. *et al*. Opsonic complement component C3 in the solitary ascidian, *Halocynthia roretzi*. *J. Immunol.* **162**, 387–391 (1999).
65. Miyazawa, S., Azumi, K. & Nonaka, M. Cloning and characterization of integrin α-subunits from the solitary ascidian, *Halocynthia roretzi*. *J. Immunol.* **166**, 1710–1715 (2001).
This study describes the presence of C3 receptor in the ascidian — which is homologous to human C3 receptor, CR3 or CR4 — and that this receptor is involved in the phagocytosis of yeast by ascidian haemocytes.
66. Nair, S. V. *et al*. A collectin-like protein from tunicates. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **125**, 279–289 (2000).
67. Vasta, G. R., Quesenberry, M., Ahmed, H. & O'Leary, N. C-type lectins and galectins mediate innate and adaptive immune functions: their roles in the complement activation pathway. *Dev. Comp. Immunol.* **23**, 401–420 (1999).
68. Bulgakov, A. A. *et al*. Isolation and properties of a mannan-binding lectin from the coelomic fluid of the holothurian *Cucumaria japonica*. *Biochemistry (Moscow)* **65**, 933–939 (2000).
69. Horstmann, R. D., Pangburn, M. K. & Muller-Eberhard, H. J. Species specificity of recognition by the alternative pathway of complement. *J. Immunol.* **134**, 1101–1104 (1985).
70. Kuhlman, M., Joiner, K. & Ezekowitz, R. A. The human mannose-binding protein functions as an opsonin. *J. Exp. Med.* **169**, 1733–1745 (1989).

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