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Review

Toward a structure-based comprehension of the lectin pathway of complement

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ABSTRACT

To initiate the lectin pathway of complement pattern recognition molecules bind to surface-linked carbohydrates or acetyl groups on pathogens or damaged self-tissue. This leads to activation of the serine proteases MASP-1 and MASP-2 resulting in deposition of C4 on the activator and assembly of the C3 convertase. In addition MASP-3 and the non-catalytic MAp19 and MAp44 presumably play regulatory functions, but the exact function of the MASP-3 protease remains to be established. Recent functional studies have significantly advanced our understanding of the molecular events occurring as activation progresses from pattern recognition to convertase assembly. Furthermore, atomic structures derived by crystallography or solution scattering of most proteins acting in the lectin pathway and two key complexes have become available. Here we integrate the current functional and structural knowledge concerning the lectin pathway proteins and derive overall models for their glycan bound complexes. These models are used to discuss *cis*- versus *trans*-activation of MASP proteases and the geometry of C4 deposition occurring on glycans in the lectin pathway.

1. Introduction

Many immunological mechanisms have evolved to defend the body toward infections and for maintenance of homeostasis in the body. Thus many cells and molecules are taking part in the anti-microbial defence systems and at the same time are involved in the removal of apoptotic or necrotic cells and tissue components. The complement system is an integral part of the innate immune system formed by more than 50 proteins. Its activation triggers a proteolytic cascade eliciting a number of immunological effector functions including the enhancement of phagocytosis, the recruitment of inflammatory cells, the formation of pores in membranes and further an instructive role on a following adaptive immune response (Ricklin et al., 2010). Complement may be activated through the alternative, the classical, and the lectin pathways; here we focus on activation through the lectin pathway (LP). The principal players of the LP are the recognition molecules: The collectins mannan-binding lectin (MBL), collectin K-1 (CL-K1), and the three ficolins (H-ficolin, L-ficolin and M-ficolin) (Fig. 1). Associated with these are three proteases: the MBL associated serine proteases (MASPs) MASP-1, MASP-2 and MASP-3 and the two MBL associated proteins MAp19 (also known as sMAP) and MAp44 (also called MAP-1) (Yongqing et al., 2012).

2. The pattern recognition molecules

MBL and CL-K1 belongs to the collectin family, a family also encompassing the surfactant proteins of the lung (SP-A and SP-D), collectin L-1 (CL-L1, also named collectin 11) and the membrane bound long placental collectin-P1 (CL-P1) (Veldhuizen et al., 2011). Collectins are characterized by a collagen-like region and a C-type carbohydrate recognition domain (CRD) in their C-terminal end (Fig. 2A). Such a C-type CRD specifically recognizes a monosaccharide exposing horizontal 3′- and 4′-OH groups, i.e. as in glucose and mannose and in N-acetyl-glucosamine. The affinity for the monosaccharide is very weak (mM range) and only when ligands are organized in a pattern fitting with simultaneous binding of several CRDs will strong binding (nM range) of the collectins to the pattern occur.

In humans three ficolins exist, H-ficolin (also named Hakata-antigen or ficolin-3), L-ficolin (also named p35 or ficolin-2) and M-ficolin (also named p35-related protein or ficolin-1) whereas only two, equivalent to L- and M-ficolin, are found in mice and rats. Similar to the collectins the polypeptide chain of ficolins contains a short N-terminal and a collagen-like domain, but in this case the C-terminal recognition domain is a fibrinogen-like domain (FBG) instead of the CRD domain. The FBG has affinity for N-acetylated carbohydrate structures in general, e.g. as in GlcNAc, but will also bind other acetylated molecules, e.g. acetylated-albumin or acetylated glycine. As for the collectins a strong binding only occurs when several FBG domains simultaneously interact with acetyl groups in a fitting pattern. Both MBL and the ficolins associate into

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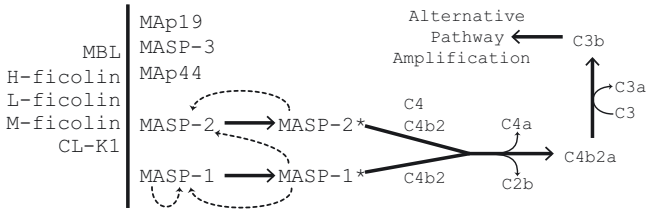

Fig. 1. The lectin pathway of complement activation. MBL, ficolins and CL-K1 are associated with MASP-1, MASP-2, MASP-3, MAp44 and MAp19. Upon recognition of distinct patterns on a surface, the associated zymogen MASP-1 and MASP-2 are activated (dashed arrows). Zymogen MASP-1 can auto-activate and activate zymogen MASP-2 resulting in active forms, i.e. MASP-1* and MASP-2*. The activated forms can activate more zymogens. Activated MASP-2 cleaves C4 and C2 in association with C4b (C4b2), whereas MASP-1 only cleaves C2 in the C4b2 complex. These activities result in the assembly of the C3-convertase C4b2a on the surface, which cleaves C3. The anaphylotoxin C3a is released, and C3b is bound to the surface where it participates in the alternative pathway amplification loop. MASP-3 and MAp44 competes with MASP-1 and MASP-2 for the same binding sites on e.g. MBL and may thus inhibit lectin pathway activation.

homotrimers that further oligomerize as described in details below for MBL. The ficolins and MBL and CL-K1 associate with the MASPs and are thus able to activate the lectin pathway complement cascade upon recognition of a suitable pattern (Yongqing et al., 2012).

2.1. Mannan-binding lectin

MBL was the first protein described to be able to initiate the lectin pathway, in this case on surfaces composed of constituents of *Saccharomyces cerevisiae*, i.e. mannan. The composition and structural organization of MBL will be discussed in detail below, but the overall organization means that many CRDs are present in the fully assembled MBL molecule (Fig. 2C). Several of these recognition domains must simultaneously bind to a pattern ligand to obtain strong binding of MBL and lectin pathway activation. Such patterns are presented by bacteria, e.g. *Escherichia coli* and *Staphylococcus aureus*, on viruses, e.g. Influenza A virus and HIV and on fungi, e.g. *Candida albicans* and *S. cerevisiae*, as previously reviewed (Thiel and Gadjeva, 2009). Recognition of altered self or damaged self is an important function of the immune system, and binding of MBL to

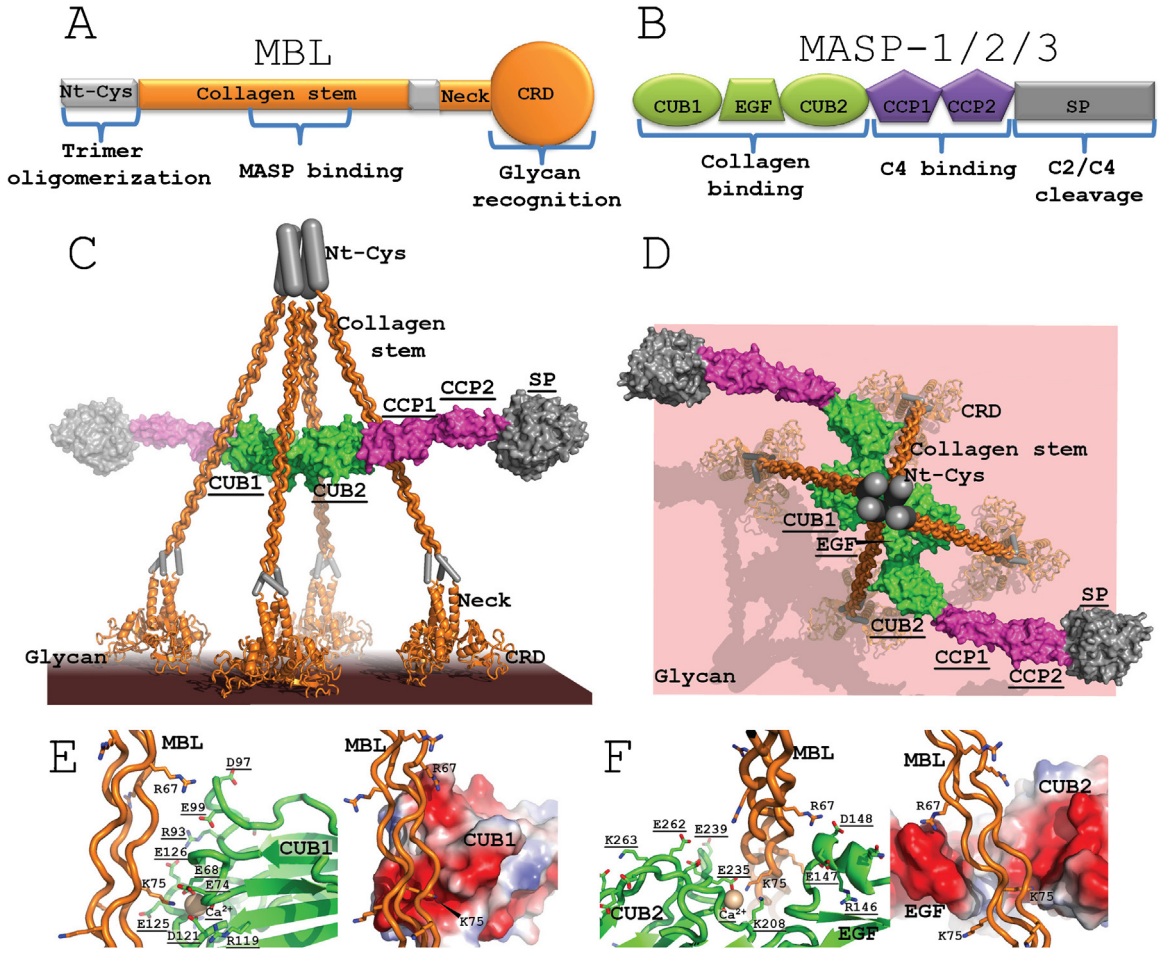

Fig. 2. A model for the MBL:MASP complexes. A) Schematic representation of MBL. Regions of unknown structure (N-terminal ends and the transition from the collagen-like region and the neck) are colored gray, whereas the collagen stem and the C-terminal carbohydrate recognition domain are orange. B) Domain structure of the MASP proteinases. The known functions of the domains are indicated below. C) The assembled model of a MASP protease in complex with a tetramer of MBL trimers, see the supplementary text for description of procedure. The plane at the bottom approximates the glycan layer. Labels for MASP proteases are underlined. D) as panel C, but with in a perpendicular view. E) Docking of the MBL collagen triple-helix onto the Ca^{2+} site of CUB1 (left) in MAp44 to satisfy the principle of calcium dependent ligand interaction with CUB domains (Andersen et al., 2010). Side chains of all aspartates, glutamates, lysines and arginines in the vicinity of the proposed interaction interface are shown in sticks. To the right the electrostatic potential of the CUB1 domain supports the suggested interaction between MBL Arg67 and a negatively charged (red) surface area on CUB1. F) As in panel E, but displaying docking of the MBL collagen stem onto the MASP-1 CUB2 and EGF domain (left) in a manner very similar to that observed in the complex between MASP-1 CUB1 domain and a MBL collagen mimicking peptide (Gingras et al., 2011). To the right, the electrostatic potential of the EGF-CUB2 domains reveals a very acidic environment for accommodation of MBL Arg67 and possibly a Lys75 not involved in CUB2 interaction.