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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

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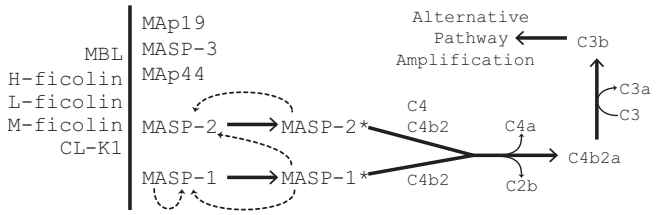

Fig. 1. The lectin pathway of complement activation. MBL, ficolins and CL-K1 are associated with MASP-1, MASP-2, MASP-3, MAp44 and MAp19. Upon recognition of distinct patterns on a surface, the associated zymogen MASP-1 and MASP-2 are activated (dashed arrows). Zymogen MASP-1 can auto-activate and activate 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.

into 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. *Cancida 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 apoptotic cells has been described and may facilitate clearance of such altered cells (Nauta et al., 2003; Stuart et al., 2005).

MBL serum concentrations range from a few ng/ml to more than 10 μg/ml, with a mean value of around 0.8 μg/ml and strongly correlates with the genotypes of the *MBL2* gene. Common single nucleotide polymorphism (SNPs) in the promoter region and exon 1 of the *MBL2* gene mirrors the variations in MBL serum concentrations, and MBL deficiency is quite common (Steffensen et al., 2000), and several studies indicate an association between disease and deficiency of MBL (Moller-Kristensen et al., 2009). In plasma MBL is found in complexes with the three serine proteases of the lectin pathway MASP-1 (Matsushita and Fujita, 1992), MASP-2 (Thiel et al., 1997) and MASP-3 (Dahl et al., 2001), and with the two associated proteins MAp19 (Stover et al., 1999; Thiel et al., 1997) and MAp44 (Degn et al., 2009). Apart from the complement activating abilities of the MBL/MASP complexes interactions of MBL with membrane bound proteins has been described as well, e.g. interaction with complement receptor 1 (CR1, CD35) (Ghiran et al., 2000; Jacquet et al., 2013; Tenner et al., 1995), with the chaperone calreticulin (Pagh et al., 2008) and with the low-density lipoprotein receptor-related protein CD91 (Duus et al., 2010). Such interactions may facilitate engulfment of apoptotic cells as seen via a calreticulin/CD91 complex by macrophages (Ogden et al., 2001).

2.2. Collectin-K1

CL-K1 (also named collectin sub-family member 11 or CL-11) resembles MBL in structure. The 34 kDa polypeptide chain of CL-K1 forms trimeric structural subunits via disulphide bridges between one cysteine in the N-terminal and two cysteines near the neck region and these homotrimers form larger oligomers (Hansen et al., 2010; Keshi et al., 2006). Human CL-K1 binds to mannose and fucose in a calcium dependent manner (Keshi et al., 2006), and to different microorganisms such as *E. coli*, *C. albicans* and *Pseudomonas aeruginosa* (Hansen et al., 2010), *Streptococcus pneumoniae* (Ali et al., 2012) and to LPS from *E. coli* and *Klebsiella pneumoniae* (Keshi et al., 2006). CL-K1 is able to interact with MASP-1 and MASP-3 (Hansen et al., 2010), MASP-2 and MAp44 (Ali et al., 2012; Ma et al., 2013) and is able to mediate complement activation on *C. albicans* (Ma et al., 2013). CL-K1 is localized in the kidney, adrenal gland and liver but is also present in plasma at 2 μg/ml (Hansen et al., 2010).

2.3. H-ficolin

H-ficolin was the first of the human ficolins to be discovered (Inaba et al., 1990) and present in human serum with a median concentration of around 20 μg/ml (Krarup et al., 2005). It is assembled from a 35 kDa polypeptide chain forming trimeric structural subunits that again forms larger oligomers of various sizes (Yae et al., 1991). H-ficolin binding toward acetylated structures has been observed (Garlatti et al., 2007a; Zacho et al., 2012), but despite this reactivity only very few microorganisms bind H-ficolin, emphasizing that although binding to simple molecules may be observed there is a demand for a correctly positioned pattern to allow for strong binding. Binding of H-ficolin to the capsular polysaccharides from *Aerococcus viridans* (Tsujimura et al., 2001) and lipopolysaccharides from *Hafnia alfvei* (Swierzko et al., 2012) or from other bacteria (*Salmonella typhimurium*, *Salmonella minnesota* and *E. coli*) (Sugimoto et al., 1998) has been observed. A neutralizing activity of Influenza A virus have been seen, but not though binding of the FBG domain (Verma et al., 2012). H-ficolin is also able to bind altered self-surfaces, e.g. on apoptotic, late apoptotic and necrotic cells and might subsequently mediate clearance via interactions with calreticulin (Honore et al., 2007; Kuraya et al., 2005). A number of studies have presented associations between patient cohorts and genotypes of the H-ficolin encoding gene *FCN3* and protein levels of H-ficolin. There are thus reports on patients lacking H-ficolin due to a frameshift mutation in the *FCN3* gene (Munthe-Fog et al., 2009; Schlapbach et al., 2011).

2.4. L-ficolin

The L-ficolin polypeptide of 35 kDa polypeptide chain forms trimeric structural subunits which oligomerizes primarily into complexes above 250 kDa (Krarup et al., 2004; Matsushita et al., 1996). The estimated serum and plasma L-ficolin concentration in normal individuals is around 5 μg/ml (Krarup et al., 2005). The FBG of L-ficolin has the broadest ligand specificity of the ficolins. It was found to bind to acetylated molecules, e.g. GlcNAc or GalNAc (Le et al., 1998) and various other acetylated glycans (Krarup et al., 2008) but also non-carbohydrate compounds, e.g., N-acetyl cysteine (CysNAc) and N-acetyl glycine (GlyNAc) (Krarup et al., 2004). L-ficolin binds to numerous pathogen associated molecular patterns, such as N-glycans of the hepatitis C virus envelope glycoproteins (Liu et al., 2009), and lipoteichoic acid (LTA) (Lynch et al., 2004). L-ficolin has a broad affinity for microorganisms and parasites, e.g. *S. typhimurium* TV119, *S. aureus*, *S. pneumoniae* (Krarup et al., 2005), *Trypanosom cruzi* (Cestari Idos et al., 2009), *Mycobacterium bovis* (Carroll et al., 2009), *Giardia intestinalis* (Evans-Osses