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Lessons learned from mice deficient in lectin complement pathway molecules☆

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ABSTRACT

The lectin pathway of the complement system is initiated when the pattern-recognition molecules, mannose-binding lectin (MBL), ficolins or collectin-11, bind to invading pathogens or damaged host cells. This leads to activation of MBL/ficolin/collectin-11 associated serine proteases (MASPs), which in turn activate downstream complement components, ultimately leading to elimination of the pathogen. Mice deficient in the key molecules of lectin pathway of complement have been generated in order to build knowledge of the molecular mechanisms of the lectin pathway in health and disease. Despite differences in the genetic arrangements of murine and human orthologues of lectin pathway molecules, the knockout mice have proven to be valuable models to explore the effect of deficiency states in humans. In addition, new insight and unexpected findings on the diverse roles of lectin pathway molecules in complement activation, pathogen infection, coagulation, host tissue injury and developmental biology have been revealed by *in vivo* investigations. This review provides an overview of the mice deficient in lectin pathway molecules and highlights some of the most important findings that have resulted from studies of these.

1. Introduction

The complement system is a part of the innate immune system, responsible for initiation of inflammation and elimination of invading pathogens or altered host cells. Three pathways initiate the complement cascade; the classical, alternative and lectin pathways. All three pathways fuse at formation of the C3 convertase and cleavage of C3 into C3a and C3b, followed by the formation of the C5 convertase and cleavage of C5 into C5a and C5b. C5b can interact with C6, C7, C8 and multiple C9 units to form the terminal complement complex C5b-9. This cascade of events leads to the formation of cleavage products that function in opsonization, cell lysis and generation of the inflammatory response. The classical pathway is initiated by C1q binding to antibody bearing immune complexes to initiate the activation of the associated serine proteases C1r and C1s, resulting in C4 and C2 cleavage and generation of the C3 convertase. The alternative pathway is activated by spontaneous hydrolysis of C3 (C3(H_2O)). This pathway also functions as an amplification loop for the cleavage of C3 initially triggered by other mechanisms. C3(H_2O) or C3b bound to target surfaces, *i.e.* foreign cells, are bound by factor B (FB). Additionally, but still not completely resolved, properdin the positive regulator of alternative pathway activation, may in some instances function as a recognition molecule initiating direct activation of the alternative pathway. Factor D is a serine protease that cleaves C3(H_2O) or C3b-bound FB resulting in the generation of the Bb and formation of the alternative pathway C3 and C5 convertases. Many soluble or surface bound regulatory molecules protect the host from collateral damage mediated *via* complement activation (Ricklin et al., 2010).

The lectin pathway (LP) is initiated when different pattern recognition molecules bind to pathogen-associated molecular patterns on the surface of microorganisms or altered host cells,

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Table 1
Lectin pathway recognition molecule knockout mice.

Gene knockout	Mutant allele	Chromosome	Targeted exon(s)
Mbl-a	Mbl1^{tm1Kata}	14	5
Mbl-c	Mbl2^{tm1Kata}	19	6
Fcna	Fcna^{tm1Tefu}	2	Promotor + 1–3
Fcnb	Fcnb^{tm1Yend}	2	Promotor + 1–4

promoting the activation of associated serine proteases, which lead to subsequent cleavage of C4 and C2 to form the C3 convertase (Endo et al., 2011). This review includes an overview of mice deficient of LP key molecules and highlights some of the important findings and lessons obtained from *in vivo* studies using these animals.

2. The pattern recognition molecules

In 1946 MBL was originally discovered in serum as β-inhibitor that was able to inactivate influenza virus (Burnet and McCrea, 1946). In 1978 the protein, initially named mannan-binding protein, was extracted from rabbit liver and characterized (Kawasaki et al., 1978). Subsequently, the same group showed that MBL was present in human serum and activated complement (Kawasaki et al., 1983; Ikeda et al., 1987). As early as 1973, a ficolin was shown to exist as a thermolabile macro protein in human serum (Epstein and Tan, 1973). This protein later turned out to be ficolin-3 (Hakata antigen) (Yae et al., 1991; Sugimoto et al., 1998). In 1991 a porcine ficolin known as transforming growth factor-β1-binding protein was isolated from pig uterus membranes (Ichijo et al., 1991), and subsequently a human homologue of the protein (ficolin-2) was isolated from plasma (Matsushita et al., 1996). In addition, a third human ficolin (ficolin-1) was identified (Lu et al., 1996). Recently, collectin-11 (CL-11, CL-K1) was also identified as a molecule that associates with lectin pathway associated serine proteases (MASPs) enabling activation of complement *via* LP (Keshi et al., 2006; Hansen et al., 2010; Ma et al., 2013). A mouse line deficient in CL-11 is not yet described in the literature, thus CL-11 is not included in the current review. An overview of the knockout mice deficient LP recognition molecules is shown in Table 1.

2.1. Genetics and structure of lectin pathway recognition molecules

2.1.1. MBL

The human MBL gene is named *MBL2* and located on chromosome 10q11.2-21(Sastry et al., 1989). Closely positioned to *MBL2* is *MBL1P1* which is a pseudogene (Guo et al., 1998), and it is believed that the two genes arise from a common ancestral MBL gene (Sastry et al., 1995). Mice, however, express two distinct functional genes, *Mbl-a* and *Mbl-c*, that are positioned at different chromosomes (14 and 19, respectively), encoding two different forms of MBL (White et al., 1994). The liver is the major site of expression for both mouse MBL-genes as well as for the human *MBL2*-gene. *Mbl-c* appears to be the mouse orthologue of *MBL2*, while *Mbl-a* is the orthologue of *MBL1P1*. The overall structure of the two murine gene products, MBL-A and MBL-C, is similar and the homology between them is about 50%, whereas human MBL is about 60% identical to the murine forms. It is believed that an ancient MBL gene duplicated prior to human-murine divergence, and that the functionality of the human orthologue to *Mbl-a*, *MBL1P1*, was lost during evolution at the transition from lower to higher primates (Sastry et al., 1995; Seyfarth et al., 2005). The human *MBL2* gene harbors multiple polymorphisms, some of which affect the function or level of MBL (for reviews see (Garred et al., 2003, 2006). Functional MBL deficiency is very frequent (5–10%) in healthy individuals (Garred et al., 2003)

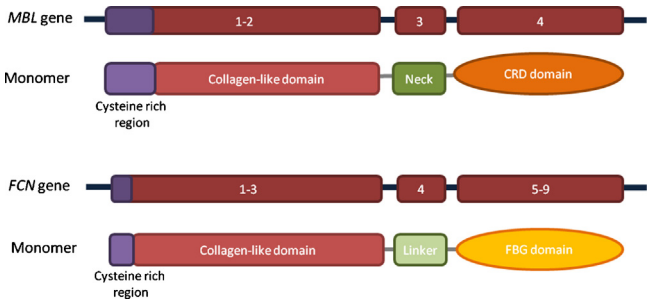

Fig. 1. Prototypic structures of mannose-binding lectin (MBL) (upper panels) and ficolin (lower panels) genes and monomers. Each monomeric polypeptide contains: an N-terminal cysteine-rich region cross linking the polypeptides; a collagen-like domain; a neck/linker region; and a C-terminal carbohydrate-recognition domain (CRD) in MBLs and a fibrinogen-like (FBG) domain in ficolins.

and the level of MBL has been associated with infectious disease in several studies (Takahashi and Ezekowitz, 2005). MBL belongs to the collectin family of proteins, which are characterized by a collagen-like region and a C-type carbohydrate recognition domain (CRD) in their C-terminal end (Fig. 1). MBL has an oligomeric structure, built of triple helical structures that contain three identical polypeptides. The protein-encoding region of *MBL2* consists of four exons interrupted by three introns. Exon 1 encodes the signal peptide, a cysteine rich domain and seven copies of a repeated Glycine motif (Gly-Xaa-Yaa). Exon 2 contains twelve additional Gly-Xaa-Yaa repeats, exon 3 contains a neck region and exon 4 a CRD (Garred et al., 2009).

2.1.2. Ficolins

Three human ficolin genes have been identified; *FCN1*, *FCN2* and *FCN3*, encoding ficolin-1, ficolin-2 and ficolin-3, respectively. Ficolin-2 is predominantly expressed in the liver; ficolin-3 is expressed in the liver and the lungs, whereas ficolin-1 is expressed by the bone marrow, human peripheral blood monocytes and neutrophils (Matsushita et al., 1996; Hummelshoj et al., 2008; Lu et al., 1996; Liu et al., 2005b). All three human ficolins are present in serum (Honore et al., 2008; Munthe-Fog et al., 2007, 2008). Two ficolins, termed ficolin-A and ficolin-B have been identified in mice. Ficolin-A mRNA is expressed in the liver and spleen and the protein is present in plasma (Ohashi and Erickson, 1998; Fujimori et al., 1998). Ficolin-B is expressed in bone marrow (myeloid cells) and spleen, and has also recently been detected in serum in low concentrations (Liu et al., 2005a; Weber-Steffens et al., 2013; Endo et al., 2012). The primary structure of ficolin-A is approximately 80% identical to human ficolin-2, ficolin-1 and mouse ficolin-B, suggesting that these four ficolins are closely related. Detailed analysis, based on gene loci, gene organization and phylogenetic trees have indicated that ficolin-B is the mouse orthologue of human ficolin-1, and that the genes encoding ficolin-A and ficolin-2 evolved independently from a common ficolin-B/1 gene linage (Fujita et al., 2004; Garred et al., 2010). Nevertheless, in terms of distribution and function it appears that ficolin-A and ficolin-2 resemble each other and may be regarded as functional equivalents. The same apply to ficolin-B and ficolin-1 (Garred et al., 2010). Ficolin-3 has been identified only in humans and primates, and the mouse orthologue exists as a pseudogene (Endo et al., 2004).

The ficolin genes encode similar polypeptides containing a collagen-like domain and a C-terminal fibrinogen-like (FBG) domain (Fig. 1). The *FCN3* gene is located on chromosome 1p36, whereas *FCN1* and *FCN2* are located on chromosome 9q34. Exon 1 of the *FCN*-genes encodes a signal peptide and the N-terminal region. Exons 2 and 3 encode the collagen-like domain, exon 4 encodes a linker region and exons 5–9 encode the FBG-domain. The ficolins exist as multimeric proteins consisting of 34–35 kDa subunits.