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Ficolins: Novel pattern recognition molecules of the innate immune response

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Abstract

Ficolins are members of the collectin family of proteins which are able to recognize pathogen-associated molecular pattern (PAMP) on microbial surfaces. Upon binding to their specific PAMP, ficolins may trigger activation of the immune system by either binding to cellular receptors for collectins or by initiating activation of complement via the lectin pathway. For the latter, the human ficolins (i.e. L-, H- and M-ficolin) and murine ficolin-A were shown to associate with the lectin pathway-specific serine protease MBL-associated serine protease-2 (MASP-2) and catalyse its activation which in turn activates C4 and C4b-bound C2 to generate the C3 convertase C4b2a. There is mounting evidence underlining the lectin nature of ficolins with a wide range of carbohydrate moieties recognized on microbial surfaces. However, not all members of the ficolin family appear to act as lectin pathway recognition components. For example, murine ficolin-B does not associate with MASP-2 and appears to be absent in plasma and other humoral fluids. Its stringent cellular localization points to other functions within the immune response, possibly acting as an intracellular scavenger to target and facilitate clearance of PAMP-bearing debris. When comparing ficolin orthologues from different species, it appears evident that human, murine, and porcine ficolins differ in many aspects, a specific point that we aim to address in this review.

Keywords: Complement system; Ficolin; Innate immunity; Lectin pathway

Introduction

Innate immunity uses a variety of induced effector mechanisms to fight infections and control them until the causative pathogens are eventually recognized by the adaptive immune system. The complement system represents one of the major humoral systems of the innate host defense and is composed of a cascade of activation events that occur on the surface of pathogens or infected cells and generate active products with various effector functions including opsonization and

Abbreviations: CRD, carbohydrate recognition domain; GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; LTA, lipoteichoic acid; LPS, lipopolysaccharide; MASP, MBL-associated serine protease; MBL, mannan-binding lectin; PAMP, pathogen-associated molecular pattern; PCR, polymerase chain reaction; UT, untranslated region.

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pathogen lysis, chemotaxis, and proinflammatory activation of the cellular immune system.

There are three distinct pathways by which the complement system can be activated: the classical, the lectin, and the alternative pathway, all of which converge to generate the same set of activation products. The lectin pathway is initiated when mannan-binding lectin (MBL) or ficolins bind to carbohydrate moieties on bacterial surfaces. This binding promotes the activation of the MBL-associated serine proteases (MASPs) which lead to subsequent cleavage of the C4 and C4b-bound C2. Activation of the lectin pathway leads to the generation of the C3 converting enzyme complex C4b2a which – upon accumulation of the C3 cleavage product C3b – can develop C5 convertase activity. With the cleavage of C5, all enzymatically mediated activation steps are completed, while C5b – the major cleavage fragment of C5 – initiates the assembly of the terminal activation steps of C6–C9, leading to the formation of the membrane attack complex (MAC) through a cascade of intermolecular rearrangements.

Ficolins were first documented as transforming growth factor-β1 (TGF-β1)-binding proteins on pig uterus membranes by Ichijo et al. (1991). Their primary structure revealed that they are mainly composed of fibrinogen- and collagen-like domains and, this unique feature gave them their name *ficolins* (Ichijo et al., 1993). Since the first description of porcine ficolins, other homologous proteins with very similar structural features have been identified at the cDNA and/or protein level in human (Endo et al., 1996; Lu et al., 1996; Sugimoto et al., 1998), rodents (Fujimori et al., 1998; Ohashi and Erickson, 1998), *Xenopus* (Kakinuma et al., 2003), and invertebrates (Kenjo et al., 2001), showing different locations of biosynthesis suggestive of different local functions. Moreover, it has been shown that ficolins present in human, mouse, and pig are lectins with a common binding specificity for *N*-acetylglucosamine (GlcNAc) (Matsushita et al., 1996).

Since the time of their first descriptions, human ficolins have been intensively investigated and numerous reports have been published on their molecular structure, their expression, their involvement as carbohydrate recognition subcomponents of the lectin pathway, and their gene polymorphisms. The biological functions of murine ficolin, however, are less well understood than those of their human orthologues and gene-targeted ficolin-deficient mouse strains may help to define their roles in the antimicrobial immune defense.

Sites of ficolin expression

The published data on ficolins show that these lectins are present in two forms: a serum type (predominantly synthesized in the liver) and a cell-associated type of ficolin (predominantly synthesized in phagocytic cells).

Pigs have two distinct but closely related ficolin genes, named α and β (Ichijo et al., 1993). Ficolin-α is expressed in liver, bone marrow, spleen, lung (Ohashi and Erickson, 1998) and very weakly in the uterus (Ichijo et al., 1993), whereas ficolin-β is expressed in bone marrow and neutrophils (Brooks et al., 2003b). Ficolin-α and -β share 81–84% identity at the amino acid level. Ficolin-α is the major plasma ficolin and consists of *N*-glycosylated subunits of 35 kDa (Ohashi and Erickson, 1998) while ficolin-β has an apparent molecular weight of 39 kDa and was found to be synthesized, stored, and secreted by porcine neutrophils but not by peripheral blood monocytes or platelets (Brooks et al., 2003b). Ficolin-β is present in both cytoplasmatic and membrane fractions of neutrophil preparations but its subcellular distribution has not been shown.

Amongst human ficolins, L- and H- (Hakata antigen) are the plasma ficolins. The hepatocytes are the primary source of synthesis for L-ficolin and its concentration in sera from 181 blood donors was found to range from 1.1 to 12.8 (median 3.7) μg/ml (Kilpatrick et al., 1999). H-ficolin is also synthesized by hepatocytes as well as by bile duct epithelial cells, and in the lung by ciliated bronchial and type II alveolar epithelial cells (Akaiwa et al., 1999). H-ficolin is found in circulation at a median concentration of 18.4 μg/ml (Krarup et al., 2005). On the other hand, the non-serum-type M-ficolin is expressed in peripheral blood leukocytes. Even though its primary structure lacks an apparent transmembrane domain, M-ficolin was found on the surface of PBMs (Teh et al., 2000). Recently, M-ficolin protein was localized in secretory granules in the cytoplasm of neutrophils, monocytes, and type II alveolar epithelial cells in the lung (Liu et al., 2005b). These observations led to the hypothesis that M-ficolin might act as an acute phase protein that is temporarily stored in the secretory granules of the leukocytes to be released locally and to execute its functions in host defense upon the right stimuli, similar to ficolin-β in pigs.

Mice, as well as rats, have two ficolin forms, termed ficolin-A and -B. The ficolin-A gene (*Fcna*) was first isolated by Fujimori and co-workers in 1998 from a mouse liver library (Fujimori et al., 1998). The protein encoded by this gene located on chromosome 2 is 60%, 59.3%, 59.1%, and 59% identical to those of porcine ficolin-α, -β, human M-ficolin, and L-ficolin, respectively (Ohashi and Erickson, 1998). Ficolin-A is the plasma protein with a molecular mass of 37 kDa, highly expressed in liver and spleen (Fujimori et al., 1998). In a recent report, Liu and co-workers showed that ficolin-A mRNA is expressed during ontogenesis as early as on embryonic day (E) 12.5, displaying an increase in abundance during development, peaking around