Restoration of MBL-deficiency: Redefining the safety, efficacy and viability of MBL-substitution therapy☆

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ABSTRACT

MBL-deficiency is a commonly occurring deficiency of the innate immune system, affecting a substantial part of the population and has been extensively studied. MBL appears to function as a disease modifier. The role of MBL in different conditions is context-dependent. Many clinical studies show conflicting results, which can be partially explained by different definitions of MBL-deficiency, including phenotype- and genotype-based approaches. In this review we give an overview of literature of MBL, its role in different pathologies, diseases and patient populations. We review MBL replacement studies, and discuss the potential of MBL substitution therapy. We finally suggest that new MBL substitution trials should be conducted within a predefined patient population. MBL-deficiency should be based on serum levels and confirmed by genotyping.

1. Introduction

A report in 1968 by Miller et al. (1968) described how fresh frozen plasma infusion could be used to restore an opsonization defect in a young child with recurrent infections. Opsonophagocytosis of pathogens is dependent on opsonization by the complement system. Miller et al. (1968) found that this patient lacked one or more specific opsonic plasma component(s) which resulted in recurrent infections and an impaired *in vitro* phagocytosis of yeast by phagocytes. Investigating children hospitalized for recurrent infections, Super et al. (1989) found that 25% of these children had an opsonic defect. The missing plasma component in these children was identified as mannan-binding protein (now mannan or mannose-binding lectin, MBL), a protein already discovered in 1978 by Kawasaki et al. (1978) and the observed opsonic function defect could be restored by MBL purified from plasma. This

eventually led to speculations on the therapeutic potential of MBL substitution.

Activation of the complement system occurs *via* three pathways; the classical pathway (CP), the alternative pathway (AP) and the lectin pathway (LP). All pathways converge into a common terminal pathway (TP) *via* generation of a C3-convertase (Forneris et al., 2012). As a result, the complement system can opsonize and lyse invading pathogens and altered self-structures and attract inflammatory cells. The CP is activated *via* binding of C1q to immune complexes. Binding of MBL, the collectins CL-L1 and CL-K1, or one of the three ficolins, to its ligand will activate the MBL-associated serine proteases (MASPs). MASPs will convert C2 and C4 into a C3-convertase, the C4b2a-complex, similar to the complex generated by the CP. The AP acts as an amplification loop for the CP and LP. The AP can also be activated through spontaneous conversion of C3 (Nilsson and Nilsson Ekdahl, 2012). The structurally related lung surfactant collectins, SP-A and SP-D, do not activate complement.

In contrast to classical primary immunodeficiencies, which are rare, single nucleotide polymorphisms (SNPs) within the innate immune system, including the lectin pathway, are frequent. It has been postulated that specific SNPs in innate immunity pathways may explain the large inter-individual variation in vulnerability to invasive infections (Brouwer et al., 2009; Casanova and Abel, 2007).

Indeed, a substantial part of the population is MBL deficient and MBL-deficiency, often defined as serum levels below 500 ng/ml, has been associated with increased frequency and severity of infections in different patient groups, including neonates (Dzwonek et al., 2008; Wahab Mohamed and Saeed, 2012), pediatric oncology patients (Dommett et al., 2013; Neth et al., 2001) and patients with cystic fibrosis (CF) (Haerynck et al., 2012). In addition, studies have shown that MBL-deficiency leads to an increased susceptibility to sepsis and to a higher mortality in critically ill patients (Garred et al., 2003a). These associations with susceptibility to infections led to the first case reports of MBL replacement therapy in selected patients (Valdimarsson et al., 1998). Following the relative success of these first infusions of plasma-derived MBL (pdMBL), subsequent phase I and II studies with pdMBL were conducted (Brouwer et al., 2009; Frakking et al., 2009; Valdimarsson et al., 2004).

Despite the fact that MBL substitution resulted in normal plasma levels and was safely given to patients and adult volunteers without any adverse events, no further clinical studies were conducted. The most likely explanation may be in the plethora of associations with a variety of diseases and the number of reports regarding the positive or negative role of MBL in some of these diseases. As long as the role of MBL in various settings remains unsettled, so does the potential benefit of MBL infusion in these patient groups.

In this review, we will give a comprehensive overview of literature on MBL and discuss the role of MBL in different pathologies, disease processes and in specific patient populations. We will focus on the different MBL replacement studies which have been conducted, and discuss the potential of MBL substitution.

2. Structure and function of MBL

MBL is an oligomer of identical polypeptides consisting of four regions: the C-terminal carbohydrate recognition domain (CRD), an alpha helical neck, a collagenous region with 19 glycine-X–Y repeats and a cross-linking N-terminal region with two cysteine residues (Fig. 1). The collagenous regions of three polypeptides form a triple helix stabilized by disulfide bonds between cysteine residues and hydrophobic interactions in the N-terminal region thereby generating the subunit of MBL (Lu et al., 1990). Similar to the formation of a single subunit of MBL, inter-subunit disulfide bridges in the N-terminal region are responsible for the association of the monomeric subunits into higher oligomeric forms (Lipscombe et al., 1992; Super et al., 1992). In serum, MBL consists of oligomers ranging from dimers to hexamers of subunits, each displaying 6–18 CRDs empowering MBL with high avidity toward targets with suitable displayed ligands (Dahl et al., 2001; Jensen et al., 2007). Recent imaging studies have revealed that MBL has a sertiform structure quite different from the famous

"bunch of tulips"-like form of C1q (Dommett et al., 2006; Jensenius et al., 2009). Formation of the complex between MBL and MASPs takes place at the collagenous region of MBL which interacts in a calcium-dependent manner with the CUB1-EGT-CUB2 domains of the MASPs (Thielens et al., 2001). However, the exact composition of MBL-MASP complexes remains unclear. The ligand specificity of MBL is determined by the CRD, which is able to bind a range of oligosaccharides including mannose, N-acetylglucosamine (GlcNAc) and L-fucose (Turner, 1996). MBL is able to distinguish between self, non-self and altered-self based on the presence and availability of specific carbohydrates. MBL is able to bind to apoptotic and necrotic cells (Nauta et al., 2003). The ligand specificities for the ficolins and MBL differ slightly. All ficolins react with a wide-range of N-acetylated molecules, including GlcNAc, but not with mannose and can, like MBL, activate the complement system upon binding to their ligand (Frederiksen et al., 2005; Krarup et al., 2004; Sugimoto et al., 1998; Teh et al., 2000).

3. MBL-associated serine proteases

MBL circulates in complex with zymogens of different MASPs. Three functional serine proteases have been described; MASP-1, MASP-2 and MASP-3. They are generated from two genes, the *MASP1* gene located on chromosome 3q27-q28, and the *MASP-2* gene on chromosome 1p36.2-3 (Stover et al., 1999a). MASP-1, MASP-3 and a 44-kDa truncated MBL-associated protein (MAp)44, also called MAP-1, are generated from the *MASP1/3* gene (Degn et al., 2009; Skjoedt et al., 2010), whereas MASP-2 and a 19-kDa truncated MAp19 (also called sMAP) are the alternative splice products from the *MASP-2* gene (Stover et al., 1999b; Takahashi et al., 1999) (Fig. 2). The two truncated proteins are able to inhibit the LP function, most likely due to competitive inhibition with the MASPs (Degn et al., 2009; Skjoedt et al., 2010). Pavlov et al. (2012) found that infusion of MAp44, when given at pharmacological doses, could alleviate I/R reactions, and also reported that MAp44 inhibited the binding of MBL to mannan. Ligand-binding of MBL or any of the other collectins is able to induce the activation of MASPs through proteolytic cleavage in the linker region, producing two polypeptide chains which are connected through a disulfide bridge (Sato et al., 1994).

MASP-1 activation will result in activation of MASP-2 (Degn et al., 2012), which will convert C2 and C4 into a C4b2a-complex. Although MASP-2 appears to be able to auto-activate, the efficiency of the LP is greatly increased in the presence of MASP-1 (Degn et al., 2012; Heja et al., 2012). Since the role of MASP-1 has recently been realized, current research is focusing on the actual structure and composition of MBL-MASP complexes circulating in human plasma. If MASP-1 is necessary for activation of MASP-2, a heterocomplex
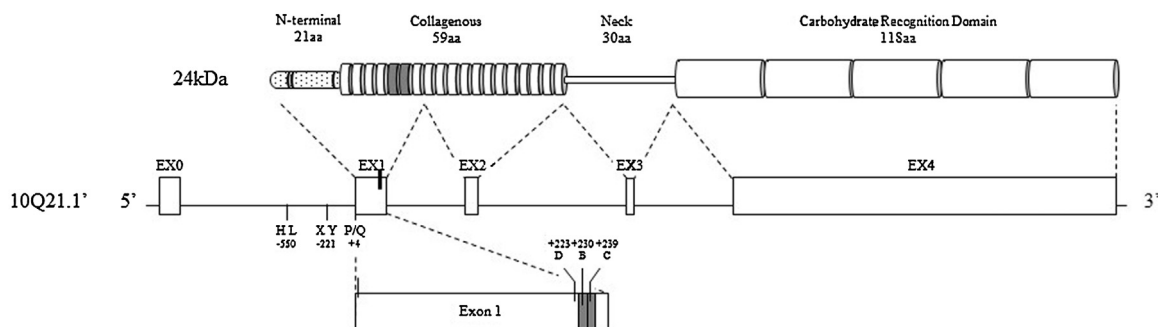

Fig. 1. Overview of the *MBL2* gene and protein structure. The encoded regions of a single MBL peptide are aligned with the exon organization of the *MBL2* gene. Exons and intron are drawn to scale. The position of different gene polymorphisms in the promoter region and within exon 1 are shown. The black lines in the N-terminal region show the location of cysteine, responsible for forming disulfide bridging within and between different monomers of MBL. The gray discs within the collagenous region show the mutated glycine-repeats affected by polymorphisms B and C.