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Review

Mannan binding lectin and viral hepatitis

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Abstract

Mannan binding lectin (MBL) is a pattern recognition molecule of the innate immune system that binds to sugars on the surface of invading micro-organisms. Target binding, complement activation and other functions of MBL are dependent on the presence of multiple carbohydrate recognition domains. Several polymorphisms in the promoter and structural regions of *MBL2* adversely affect the plasma concentration and oligomeric state of MBL. The possession of mutant alleles has been linked to disease outcome for a variety of bacterial and viral infections. Viral hepatitis is caused by unrelated viruses referred to as hepatitis virus A–E. The disease usually has both acute and chronic phases, the latter leading to cirrhosis and hepatocellular carcinoma. Hepatitis viruses B and C (HBV and HCV, respectively) are a significant cause of morbidity worldwide. HBV encodes envelope glycoproteins termed large, middle, and small that may exist in glycosylated or unglycosylated forms on the virion. An interaction between HBV glycoproteins and MBL has been demonstrated *in vitro*. Significant associations between MBL levels, determined by *MBL2* haplotypes, and HBV persistence and disease progression have been described. HCV encodes two highly glycosylated envelope proteins, E1 and E2, which are potential targets for interaction with MBL. Mutant *MBL2* haplotypes have been linked to disease progression and response to therapy in HCV infection. Here we summarise the effect of *MBL2* polymorphisms on MBL function and how this may relate to disease outcome in HBV and HCV infection.

Keywords: Mannan binding lectin; Viral hepatitis; HCV; HBV; Complement

1. Introduction

Mannan binding lectin (MBL) is a plasma collectin which is synthesised in the liver and secreted into the bloodstream. The importance of this molecule in the innate immune system relates to its multimeric structure, to its functions as an opsonin and as an adaptor for activation of MBL associated serine proteases (MASPs). Hepatitis viruses are carried by approximately 500 million people, causing significant morbidity. Multiple factors are involved in the pathogenesis of viral hepatitis with both viral and host factors playing a role. In this review we summarise our present knowledge on MBL, viral hepatitis and the relationship between MBL single nucleotide polymorphisms (SNPs) and the outcome of viral hepatitis infection.

2. Mannan binding lectin

The MBL molecule is similar in quaternary structure to C1q, an innate immune effector which activates the classical complement pathway [1]. *MBL2*, the functional human gene for MBL, encodes a 25 kDa polypeptide chain with four main structural regions [2]. The C-terminal carbohydrate recognition domain (CRD) is responsible for interactions with carbohydrate patterns on non-self-surfaces. CRD ligand binding is dependent on the presence of at least two calcium ions which determines the classification of MBL as a C-type lectin [3]. Immediately next to

Abbreviations: CAH, chronic active hepatitis; CIH, chronic inactive hepatitis; CRD, carbohydrate recognition domain; FcR, Fc receptor; FHF, fulminant hepatic failure; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; LC, liver cirrhosis; MASP, MBL associated serine protease; MBL, mannan binding lectin; NR, non-responder; SR, sustained responder

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the CRD lies an alpha helical coiled-coil neck region which promotes trimerisation and sets the spatial orientation of CRDs within the context of the protein [4,5]. The collagenous region of each polypeptide is formed by Gly-Xaa-Yaa repeats and participates in the formation of a collagen triple helix, stabilising the trimeric collectin subunit [5]. The N-terminal cysteine-rich region facilitates the association of collectin subunits into higher order oligomers which range from dimers to hexamers *in vivo* [6–11]. Binding between each individual CRD and carbohydrate ligand is relatively weak; therefore interactions between MBL and its ligands are dependent on the presence of many sugar residues within repetitive arrays on pathogen surfaces and the presence of multiple CRDs within an MBL molecule. Biologically functional MBL molecules are those multimeric forms containing sufficient lectin domains for high avidity binding to pathogen surfaces [9,12–14].

On binding to micro-organisms, MBL may function directly as an opsonin or indirectly through activation of the complement system. MBL enhances phagocytosis of micro-organisms via a collectin receptor. Currently, calreticulin anchored to cell surfaces via CD91 or other molecules is the best candidate for an MBL receptor. Calreticulin bound to CD91 has been shown to mediate uptake of MBL-coated apoptotic cells [15,16]. Uptake of MBL by antigen presenting cells including dendritic cells may enable presentation of pathogen derived peptides and aid in priming the adaptive immune response.

MASPs interact with MBL via the collagenous regions of larger MBL oligomers [7,8,10,13,17]. Four related proteins derived from two genes have been described; namely MASP-1, its alternative splice product MASP-3, and MASP-2, with the alternative splice variant MAp19 [10,18–20]. MASP-2 activates the complement system by cleaving complement proteins C4 and C2. MASP-1 has some activity in cleaving C2, but it also cleaves fibrinogen and coagulation factor XIII [21]. MASP-3 has been reported to cleave insulin-like growth factor binding protein 5, though the significance of this finding is unknown [22]. The function of MAp19, which lacks a serine protease domain, is currently unknown. Currently it is thought that MBL exists in plasma mainly as trimeric and tetrameric forms, each of which can bind one MASP heterodimer. Thus there are separate populations of MBL-$(MASP\text{-}1)_2$, MBL-$(MASP\text{-}2)_2$, MBL-$(MASP\text{-}3)_2$ and MBL-$(MAp19)_2$ [23].

3. Single nucleotide polymorphisms in *MBL2*

The human collectin genes cluster to chromosome 10 (10q21–24 [24]). The single functional MBL gene (*MBL2*) comprises four exons [2]. Exon 1 encodes the signal peptide, the N-terminal cysteine-rich region and includes the first part of the collagenous domain containing the characteristic Gly-X-Y repeat. Exon 2 encodes the remainder of the collagen domain and exon 3 the alpha helical coiled-coil structure of the neck region. Exon 4 encodes the C-terminal CRD. Upstream of the coding region are a number of regulatory elements some of which may enhance MBL transcription during an acute phase response [2,25] or in response to thyroid hormones [26,27]. A second gene, *MBL1* has been characterised as a pseudo-gene expressing a truncated 51 amino acid peptide [28]. Both genes are thought to have arisen from a single evolutionarily ancient ancestor through a gene duplication event [29]. This is supported by the presence of two functional MBL genes producing higher oligomeric structures in mice and rhesus monkeys. MBL in chimpanzees and humans however is only represented by *MBL2* and both species possess an MBL1 pseudo-gene. The presence of the two genes and high frequencies of *MBL2* variants may indicate some dualism in function of MBL where in some circumstances there may be a selective advantage for low circulating levels or impaired function of the protein.

Polymorphisms in the promoter and structural regions of the *MBL2* gene have been shown to affect oligomer formation and circulating levels of protein [11,14,30–33]. Three single point mutations in codons 52 (Arg → Cys, allele *D*), codon 54 (Gly → Asp, allele *B*), and codon 57 (Gly → Glu, allele *C*) of exon 1 give rise to amino acid substitutions within the collagenous region of MBL. The wild type structural allele is referred to as allele A. The B and C alleles result in the replacement of critical axial glycines by dicarboxylic acids which distort the collagen triple helix of trimeric subunits [34]. Substitution of an arginine residue by a cysteine in allele D causes the formation of adventitious disulphide bonds disrupting interactions within the MBL subunit [13]. Both types of mutation reduce the proportion of higher order oligomers in circulation. This is assumed to decrease the amount of MBL able to bind targets through weak CRD–sugar interactions and to inhibit the association of MASPs resulting in a reduced capacity to activate complement [7,9,35,36]. Variant molecules with smaller numbers of subunits have also been shown to have a higher rate of turnover [7,35]. A potential mechanism of clearance is via proteolysis by metalloproteinases [31]. Low concentrations of MBL have also been linked with defects in opsonisation and phagocytosis [34,37].

Polymorphisms occur in the promoter region at −550 (H/L), −221 (X/Y) and +4 (P/Q) which result in varying levels of MBL expression. The −550 and −221 sites, giving rise to haplotypes HY, LY and LX, are within and adjacent to putative glucocorticoid response elements and may affect MBL regulation. The HY haplotype is associated with high levels of MBL, LY correlates with intermediate levels of MBL and LX is linked to low levels of MBL [32]. There is also a reduction of MBL level where a structural mutation on one chromosome is found with the LX haplotype on the other.

The different *MBL2* mutations giving rise to altered levels of functional MBL occur with varying frequencies within different populations (Table 1). The structural variant B allele occurs in 22–28% of Eurasian populations while the C variant is more characteristic of sub-Saharan African populations, reaching frequencies of 50–60%. The D mutation has been reported to reach 14% in European populations but can be lower in other ethnic groups. In a Korean population 34% had the B allele variation, compared with a Danish population where 39% possessed one of the three structural variants [32,38]. In an Australian population 42% were heterozygous for one of the three coding variants (A/O, where O is any of the three variants) and had higher mean levels of MBL than those in the Danish and Korean populations [36]. In Korean and Chinese populations MBL levels vary with