DEFICIENCY OF MANNAN BINDING PROTEIN- A NEW COMPLEMENT DEFICIENCY SYNDROME

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The concept of a common opsonic deficiency arose following work which was initiated by Miller et al in 1968 (1). These authors described a girl with severe dermatitis, diarrhoea, repeated infections and failure to thrive who, on investigation, was found to have a plasma associated defect of phagocytosis. This was demonstrable by the failure of plasma from the child to opsonize heat killed baker's yeast (*Saccharomyces cerevisiae*). Subsequently the same yeast opsonization defect was identified in the sera of 11 of 43 children with frequent unexplained infections (2), was associated with chronic diarrhoea in infancy (3) and, in a prospective study, was significantly linked with the development of otitis media during the first 12 months of life (4). In addition to these clinical associations various groups using assays with phagocytosis as an end point have found the defect to be surprisingly frequent in the general population (5-7% for groups which include children aged 11-12 (5) and elderly adults aged 65-88 years (6)).

The opsonic deficiency was linked to the complement system following the demonstration that sera with the deficiency deposited less C3b /C3bi on yeast surfaces (7,8). However, there was no consistent demonstrable abnormality of the complement system in the sera with the deficiency and evidence was adduced for the existence of an additional co-factor closely associated with C3b deposition on yeast surfaces by normal sera but either absent or inactive in the sera of individuals with this defect (8). In an attempt to further define the molecular mechanisms underlying the defect the yeast cell wall component mannan was selected as a substrate for coating microtitre plates for subsequent capture of opsonic proteins. When the deposition of C3b opsonic fragments was studied using 5% serum from 179 healthy blood donors a bimodal distribution was observed and similar population distributions were noted for properdin, Factor B, C4b and mannan binding protein (MBP) (9). Moreover, there was a highly significant correlation between the binding patterns of all these proteins. These observations, together with those of Lu et al (10), suggest that at low serum concentrations (e.g. 5-15%) MBP binds to its ligand e.g. mannan, initiates activation of the classical pathway of complement and subsequently regulates the degree of C4 and C3 cleavage. The surface bound C3b fragments would then act as foci for properdin and Factor B binding in a 1:1:1 stoichiometric fashion (11). In each of the bimodal distributions there was a minor population of individuals essentially defined as those with an MBP of less than 30mg/litre. When MBP was measured in serum samples from 10 individuals with the yeast opsonization defect who had been referred to Great Ormond Street Hospital for Sick Children, all were found to have low levels (<30mg/litre). The link between low MBP levels and the common opsonic defect was further confirmed by in vitro dose dependent correction of the functional defect using MBP prepared by affinity chromatography (12).

MBP is a C type (Ca^{++} dependent) serum lectin secreted by the liver of several mammalian species. Although the circulating protein has a molecular weight of 400-700 kDa it is based on a single type of peptide chain of 32kDa. Each chain consists of a cysteine rich N-terminal region, a collagenous region and a carboxyterminal domain. It seems probable that following translation and release into the rough endoplasmic reticulum of the hepatocyte three such chains become closely associated through the creation of disulphide bridges in the N-terminal region and the subsequent-formation of a triple helix by the collagenous regions. The three carboxyterminal regions associate to form a globular region presumed to have three carbohydrate binding sites. This three chain subunit then assembles into higher polymers (ranging from dimers to hexamers) which are again linked through their cysteine rich N-terminal regions. It is elieved that the multiple globular domains (each with carbohydrate recognition sites) permit the protein to make multipoint attachments to mannose and N-acetylglucosamine groups which are widely distributed in the cell walls of Gram negative bacteria, mycobacteria and yeasts. Although most of the earlier work on the opsonic defect made use of either baker's yeast (*S.cerevisiae*), or its cell wall extract zymosan, a similar discrimination between sera having either poor or normal opsonic potential was also reported using *Candida albicans, Staphylococcus aureus* and *Escherichia coli* (13). This suggested that the defect was relevant to a range of organisms and in the light of recent findings may now be interpreted as a reflection of the widespread occurrence of mannose and N-acetylglucosamine sugar groups.

We have previously proposed that MBP activation of the classical pathway of complement may be an accessory immune mechanism, the absence of which may become important when there is some other

co-existing deficiency (14,15). In infants of 6-18 months of age there is a well documented period of vulnerability to infection. Passively transferred maternal antibody has largely disappeared and the infant's own repertoire of available antibodies is restricted. This is reflected in both a low level of IgG opsonins and a reduced capacity of classical pathway activation of complement via antibody. MBP may be critically important in responses to mannose rich microorganisms during this window of vulnerability and low levels of the of the lectin would constitute a significant risk factor. It is noteworthy that many of the patients described have initially presented between the ages of 6 months and 2 years. Nevertheless, susceptibility would be transient since the normal maturation of the immune response would begin to provide efficient antigen-specific opsonic mechanisms. Many infants with the opsonic deficiency probably pass through this transient physiological window without serious infection and it is entirely plausible that another co-existing deficiency is required for pathology to become manifest. There are numerous potential candidates and there is already evidence that some of these occur at high frequency in the general population. One such example involves the complement component C4. It is well established that approximately 8% of Caucasians lack two of four possible functioning C4 genes (16) and as a consequence would be expected to manifest a reduced capacity for classical pathway activation. It has been claimed that there is an increased susceptibility to bacterial meningitis in infants with a homozygous deficiency of the protein products of the C4B loci which interact preferentially with carbohydrate-rich surfaces (17). In view of the evidence suggesting that MBP activates complement through the classical pathway it follows that co-existing (albeit partial) deficiencies of both MBP and C4 could predispose to episodes of infections and would occur in about 1 in 300 individuals. Other candidates for co-existing second defects are various immunoglobulin abnormalities, some of which also appear to occur relatively frequently. These include an affinity maturation defect, an isotype switching defect, a V-region restriction or possession of a Gm-allotype associated with poor responses to polysaccharide antigens (15).

The gene encoding human MBP, which is on the long arm of chromosome 10, has been cloned and sequenced in two laboratories (18,19). There are four exons: exon 1 encodes the cysteine rich N-terminus and the first part of the collagenous region, exon 2 encodes the remainder of the collagenous domain, exon 3 encodes a short "neck region" and exon 4 encodes the carbohydrate recognition domain. In addition exons 1 and 4 also encode extensive untranslated regions characterized by consensus sequences which may be involved in controlling the expression of human serum MBP. These consensus sequences suggest that MBP is regulated as an acute phase protein synthesized by the liver as originally proposed by Ezekowitz et al (20).

Our recent studies of three families with children having the opsonic defect suggest that a mutation leading to a single base change in the MBP gene is responsible for this immunodeficiency (21). The complete nucleotide sequence of all four MBP exons was determined in two probands. The nucleotide sequence of exon 1 only was determined in a further sixteen members of the three families. In all three probands the sequence showed a point mutation at base 230 of exon 1 causing a change in codon 54 from GGC to GAC which results in the substitution of aspartic acid for glycine in the translated protein. In the two probands for whom the complete nucleotide sequence was available for all four MBP exons, this mutation was the only difference found. The DNA sequences showed that family members could be classified into one of three genotypes, homozygous Gly/Gly, heterozygous Gly/Asp or homozygous Asp/Asp. The mutation in codon 54 co-inherited with low serum MBP levels in an autosomal dominant fashion. Thus all family members with low serum MBP levels were either heterozygous or homozygous for the aspartic acid substitution at codon 54 with only one of nine Gly/Asp heterozygotes having a serum MBP level approaching that seen in Gly/Gly homozygotes.

The Gly --> Asp mutation in codon 54 would disrupt the fifth Gly-Xaa-Yaa repeat in exon 1 of the MBP gene (see Figure). In the formation of the collagenous triple helix only the regularly occurring small glycine residues can be accommodated along the axis of the helix and the presence of aspartic acid would lead to a profound distortion of the secondary structure of the collagenous domain as reported in some variants the well studied inherited collagen disorder osteogenesis imperfecta (22). We predict that the abnormal unstable MBP subunits fail to polymerise and are probably degraded intracellularly.

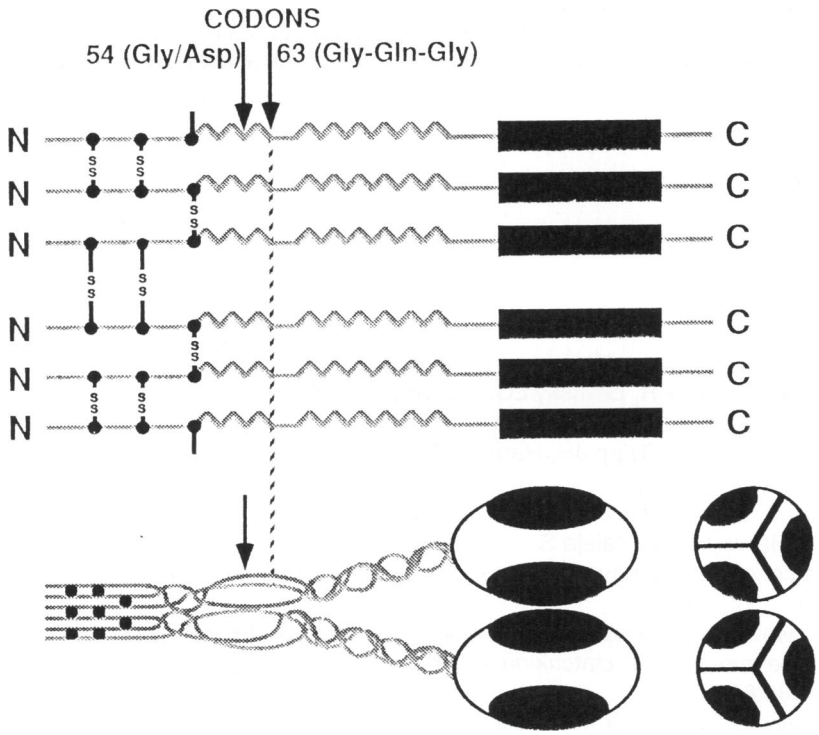

Figure 1.

Normal MBP is assembled from identical 32kDa polypeptide chains. Three such chains become disulphide bonded at their N-termini and associate into a collagenous triple helix terminating with a globular region containing three of the carbohydrate recognition domains. There are probably three sugar specific sites in each globular head (shown in black - bottom right). In normal MBP 7 repeats of the Gly-Xaa-Yaa motif are followed by the sequence Gly-Gln-Gly encoded by codon 63 and this appears to be associated with the "bend" observed on electron microscopy (23) and characteristic also of Clq.

In the mutant form of the MBP gene there is a point mutation at base 230 of exon 1 causing a change of codon 54 from GGC to GAC. This translates into the substitution of aspartic acid for glycine in the fifth Gly-Xaa-Yaa repeat. The bulky aspartate group cannot be accommodated along the axis of the triple helix which, it is predicted, would be severely disrupted (bottom arrow) before reforming at a more distal point. By analogy with the extensively studied collagen abnormalities associated with osteogenesis imperfecta it is further predicted that the protein would be unstable and probably degraded intracellularly before secretion.

The heterozygous state is of particular interest because their levels are also profoundly reduced (15g/litre compared to 168g/litre for Gly/Gly normal individuals and 1.9mg/litre for Asp/Asp mutants). However, if the biosynthetic mechanisms for the production and incorporation of the mutant chains are unimpaired, then in a heterozygote synthesizing both type of chain, only 1 in 8 of the trimers would be spared the disruptive influence of mutant polypeptide chains. This prediction appears to agree closely with the above levels.

The high frequency of the mutation suggests that it may be associated with some selective advantage. We have recently shown that opsonic dysfunction and low MBP levels are found in 8% of a healthy population of Chinese children (Lipscombe, Lau, Levinsky & Turner, unpublished observation). This is almost identical to the frequency in Caucasians and it will be of interest to establish whether the same point mutation is involved and also investigate other racial groups. Other points which need to be addressed include the fate of the mutant protein, whether or not homozygous mutants are more likely than heterozygotes to suffer from infections and the whole range of clinical associations and possible synergistic risk factors (15).

Acknowledgement

This work has been generously supported by Action Research.

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