The role of human lectins in host defence

The Linacre Lecture 1991

Lectins are proteins that bind sugars. Their ligands are glycoproteins terminating in the appropriate sugar. Lectins have long been known to occur in plants, especially their seeds. Concanavalin A is a well known example. Over the past two decades lectins have been described in mammals, including man. Examples of mammalian lectins are the galactose receptor, a cell surface receptor on hepatocytes, and the mannose receptor, a cell surface receptor on macrophages.

Here I will describe the work that has shown that a common immunodeficiency is caused by a point mutation in the gene encoding a mammalian lectin. This immunodeficiency, due to an opsonic defect which presents as recurrent infections in infants, is due to low levels of a serum lectin termed (because of its sugar specificity) mannose-binding protein (MBP).

Immunodeficiency caused by defective opsonisation

Infants with this immunodeficiency syndrome suffer repeated bacterial and fungal infections between six and 18 months of age [1,2]. It is common, its estimated frequency being 5–7% [2–4]. The immunodeficiency is found in about 25% of children with frequent unexplained infections [2], and children with the defect suffer about twice as many severe infections as matched control children [5]. The infections may be severe, and five deaths have been reported. In these families there are usually relatives who also suffered repeated infections in infancy. There is also a high frequency of atopy in the children and their families [5].

The sites of infection are varied. Otitis media, chronic diarrhoea and meningitis are most common but infected eczema, septicaemia, pneumonia, osteomyelitis and oral infections are reported. Similarly, the microorganisms cultured from these infections are diverse. They include *Staphylococcus aureus, Neisseria meningitidis, Escherichia coli, streptococci, haemophilus, klebsiella, proteus, pseudomonas* and *candida.*

The laboratory findings in this immunodeficiency syndrome are characteristic. *In vitro,* normal polymorphonuclear leukocytes, when incubated with serum from patients, do not phagocytose bacteria or yeasts. The defect can be corrected by the addition of heterologous serum, indicating that the patient's serum lacks an opsonin which is essential to prepare the microorganisms for phagocytosis. Furthermore, intravenous infusions of fresh frozen plasma correct the opsonisation defect in affected infants [2], and in one child plasma infusions were reported to lead to clinical improvement [1]. However, complement levels are normal in these patients. In the first report of the syndrome, Miller *et al* [1] showed that the serum opsonisation defect in the affected infant was also present in the mother but not the father. Furthermore, they showed that both maternal grandparents were defective opsonisers. These reports clearly showed that the immunodeficiency was due to the inherited lack of some serum opsonic factor.

Mannose-binding protein

Human serum contains a lectin, termed mannose-binding protein (MBP), which binds glycoproteins terminating in mannose residues. We found MBP while studying the uptake of glycoproteins by liver cells when we discovered that serum contained a lectin which inhibited the uptake of mannose-terminated glycoproteins by these cells [6]. MBP is a calcium-dependent lectin secreted by hepatocytes which binds glycoproteins terminating in mannose or *N*-acetylglucosamine. MBP occurs in serum as a mixture of oligomers of between nine and 18 identical polypeptide chains of 32 kDa [7–10]. On binding to a mannose-rich surface, MBP activates complement through the classical pathway [11] (Fig. 1). MBP binds C1r and C1s to form C1 esterase. This enzyme cleaves C2 and C4, and their split products in turn complex on the mannose rich surface to form C3 convertase. That enzyme then cleaves C3 to form C3b which then binds to the surface and opsonises the ligand. The C3b coated surface, usually a microorganism, can then be bound by C3b receptors on polymorphonuclear leukocytes and phagocytosed by the cell.

The gene encoding MBP has been cloned [12,13]. MBP is encoded by four exons, each coding for different functional domains of the molecule (Fig. 2).

- Exon 1 encodes the signal sequence of a secreted protein, a cysteine-rich domain and seven copies of the motif Gly-Xaa-Yaa, which is typical of a collagen domain.
- The junction between exon 1 and exon 2 encodes the sequence Gly-Gln-Gly.
- Exon 2 encodes a further 12 Gly-Xaa-Yaa collagen repeats.
- Exon 3 encodes a short 'neck' domain.

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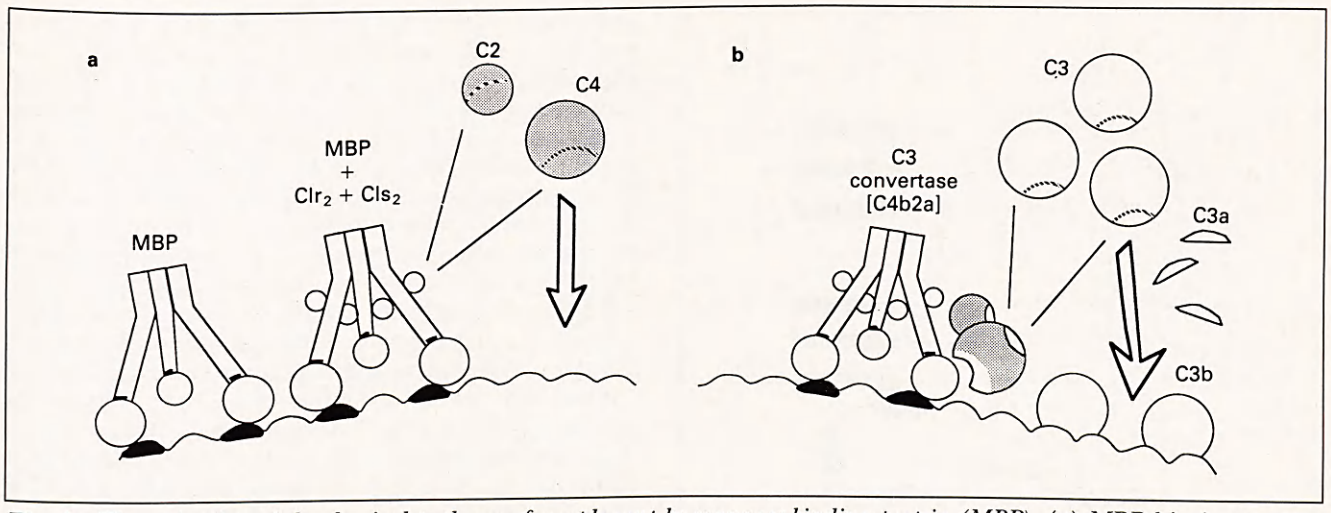

Fig. 1. *The activation of the classical pathway of complement by mannose-binding protein (MBP).* (a) MBP binds to sugar residues on the surface of a microorganism through the carbohydrate recognition domains. MBP binds Clr_2 and Cls_2 to form C1 esterase which cleaves C2 and C4 which in turn complex on a mannose-rich surface to form C3 convertase (C4b2a). (b) C3 convertase cleaves C3 to form C3b which binds to the surface and opsonises the ligand. The C3b coated surface can then be bound by C3b receptors on polymorphonuclear leukocytes and phagocytosed. (By courtesy of Professor M. W. Turner, reproduced with permission)

- Exon 4, the largest exon, encodes the carbohydrate binding domain.

The structure of the high-molecular-weight oligomers of MBP found in serum can be inferred from the organisation of the MBP gene. Trimers of the MBP subunit, translated by the MBP gene, associate by the formation of a triple helix between their collagen domains. The interruption of the collagen motif by the sequence Gly-Gln-Gly between exons 1 and 2, by analogy with C1q, is probably the site where the triple helical chains of MBP appear to bend on electron microscopy [14]. The triple helices are stabilised by disulphide bridges between the cysteine-rich domains. These trimers then associate, again by disulphide bridges, into oligomers of between nine and 18 identical MBP polypeptide chains (Fig. 3). This gives the final MBP oligomer the appearance of a bunch of flowers in which the flower heads are the carbohydrate binding domains and the stalks are the collagen domains.

When the MBP was cloned it became apparent that MBP was an acute-phase protein [12, 15]. The 5' flanking sequence of the MBP gene contains common regulatory elements: a TATA box, a CAT box, and two GC boxes. These consensus sequences mediate the constitutive expression of the gene by specifying the binding of RNA polymerase II to the beginning of the gene's coding region (transcription initiation site). However, the 5' flanking sequence of the MBP gene also contains a heat-shock promoter sequence and two glucocorticoid-responsive promoter sequences. These pro-

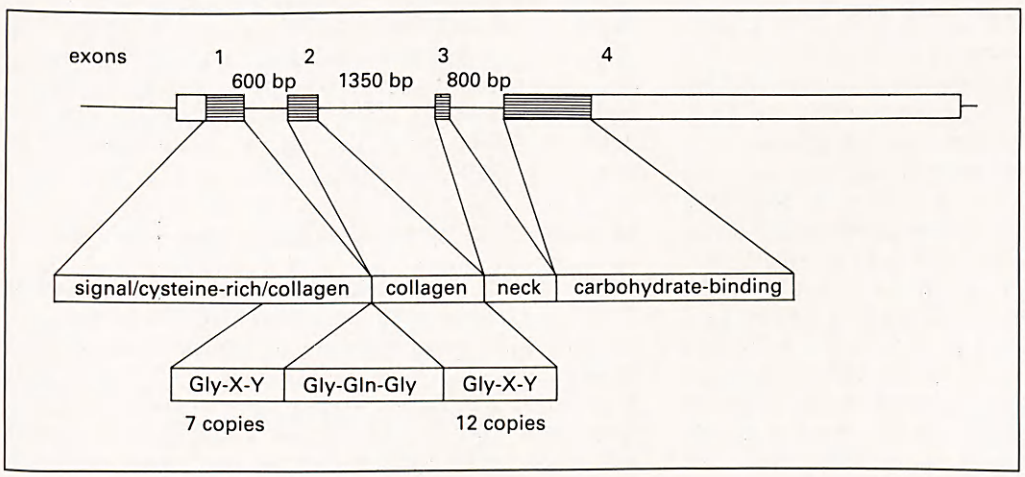

Fig. 2. *Organisation of the MBP gene. The MBP subunit is encoded by four exons. Each exon encodes a different functional domain.*

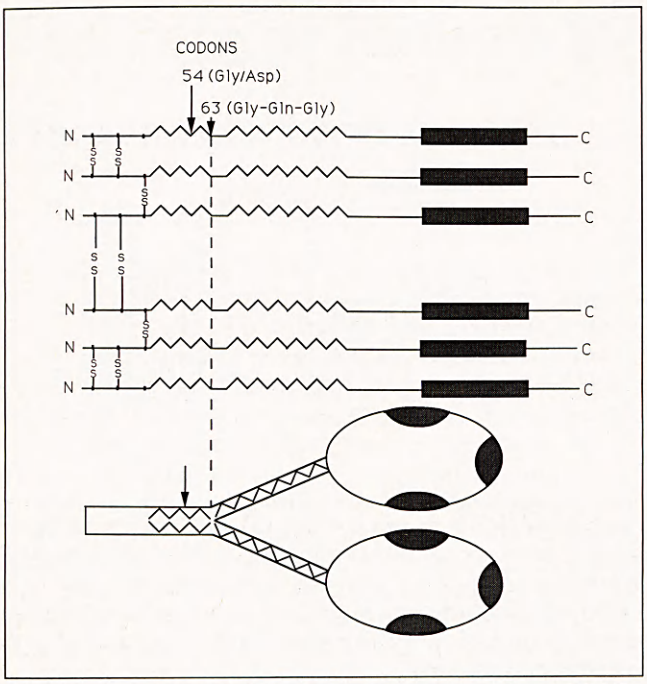

Fig. 3. *Assembly of MBP oligomers from constituent 32 kDa peptide chains.* (Reproduced from Sumiya *et al* [20], with permission)

moters are typical of acute-phase protein genes and can greatly increase gene expression when bound by their transcription factors (eg a steroid-receptor complex).

We have studied expression of the MBP gene *in vitro* using Huh-7 hepatoma cells (T. Arai, J. A. Summerfield, unpublished). Because constitutive MBP mRNA expression by these cells is low, mRNA was detected by reverse transcription to cDNA followed by quantitative amplification by the polymerase chain reaction (PCR) and Southern blot hybridisation. Interleukin-6 (IL-6), dexamethasone and heat shock (42° for 1h) significantly increased MBP mRNA levels. In contrast, IL-1 suppressed MBP mRNA expression. IFNγ, TNFα and TGFβ had no effect on MBP mRNA levels. These data are consistent with MBP being an acute-phase protein and indicate that MBP gene expression is regulated by the interleukins. This also appears to be the first demonstration of a functional heat-shock promoter in a mammalian acute-phase protein gene.

In further experiments we have used gel shift and DNAse1 footprinting assays to detect the appearance of transcription factors (which bind to the promoter sequences) in response to agents which modulate MBP gene expression. Nuclear protein extracts of Huh-7 cells were incubated with radiolabelled probes derived from the 5' sequences of the MBP gene, and the mixtures were resolved by electrophoresis. Nuclear extracts from IL-1 and IL-6 treated cells formed specific complexes with 5' sequences of the MBP gene. The data indicate that, following exposure to these interleukins, the hepatoma cells synthesise specific transcription factors which, by binding the 5' flanking sequence of the MBP gene, modulate gene expression.

We have produced a transgenic mouse lineage bearing the human MBP gene (P. Tabona, A. Mellor, J. A. Summerfield, unpublished). These mice carry a 15 kb *Sal I* to *Cla I* restriction fragment derived from our human genomic MBP clone. The transgenic mice constitutively express large amounts of human MBP mRNA and have high serum levels of human MBP (about 10 times normal). Intravenous injections of the yeast *candida* cause a massive but transient fall in human MBP levels in the transgenic mice followed by increased MBP mRNA expression and MBP secretion. This finding indicates that circulating MBP is consumed by binding to *candida* and that following MBP consumption there is increased hepatic production of MBP. The signal mediating increased MBP production has not been determined but clearly release of IL-6 by macrophages following their phagocytosis of *candida* must be a prime candidate.

To summarise, the MBP gene encodes polypeptide chains of 32 kDa. These polypeptides associate into trimers and MBP is composed of oligomers of three to six trimers. The collagen domain of the polypeptides mediates MBP assembly. The MBP gene is regulated as an acute-phase protein.

Association of defective opsonisation with deficiency of mannose-binding protein

Following initial reports that sera from these children lacked a factor which opsonised a variety of bacteria and yeasts, experiments were performed to characterise the opsonin. Turner and colleagues [16] first showed an association between the opsonic defect and the deposition of low amounts of the complement components C3b/C3bi on the surface of yeast. They proposed that this was due to absence of an unidentified opsonic cofactor [17]. They then showed that antibody-independent cleavage of C4 occurred when serum was incubated with mannan, a component of the yeast cell wall, and that the opsonin mediating this complement cleavage was MBP [18]. Furthermore, they found that ten children with the opsonic defect had very low serum MBP levels, and that the opsonic defect could be corrected *in vitro* by the addition of purified MBP to their serum [19].

Molecular basis of the opsonisation defect

To determine the molecular basis of the opsonisation defect associated with low serum MBP levels, we analysed the MBP gene structure in families with affected children [20]. Three families were studied. Each proband had suffered from recurrent bacterial infections during the first 14 months of life. These included recurrent upper respiratory tract infections and otitis

media (two patients) and meningococcal septicaemia (one patient). All had previously been shown to have the opsonic defect and low serum MBP levels. Serum MBP concentrations were measured by an enzyme-linked immunosorbent assay (ELISA) [19]. Genomic DNA was isolated from whole blood and each of the four exons which encode MBP were amplified using primers derived from the genomic sequence [12] by asymmetric PCR [21] and sequenced directly using a complementary primer. The complete nucleotide sequence of the four exons was determined in two probands. Analysis of the nucleotide sequence of exon 1 was performed in a further 16 family members of the three families.

Figure 4 shows that the transmission of low serum MBP levels in the three families fits best with autosomal dominant inheritance. In all three probands the sequence showed a point mutation at base 230 of exon 1, causing a change of codon 54 from GGC to GAC; this results in the substitution of aspartic acid for glycine in the translated protein. The DNA sequences showed that the family members could be classified into one of three genotypes: homozygous Gly/Gly, heterozygous Gly/Asp, or homozygous Asp/Asp. All 14 family members with low serum MBP levels were either heterozygous or homozygous for the aspartic acid substitution at codon 54 (Fig. 4), with only one of nine Gly/Asp heterozygotes having a serum MBP level approaching that seen in Gly/Gly homozygotes. Since the frequency of the opsonic defect is 5–7%, the frequency of the mutant allele in the general population is probably of the order of 3%. This contrasts with the high frequency of the mutant allele in these three families with a clinically affected child.

The correlation between the genotype and the serum MBP level is shown in Fig. 5. The mean serum MBP concentration in the 5 Gly/Gly homozygotes was 168±25μg/l (mean ± SEM), in the 9 Gly/Asp heterozygotes it was 15±8μg/l, and in the 5 Asp/Asp homozygotes it was 1.9±0.8μg/l. It is clear that the presence of this mutation profoundly lowers serum MBP levels even in heterozygotes.

The mechanism whereby this mutation in the collagen domain reduces serum MBP levels is probably analogous to that in osteogenesis imperfecta. The collagen-like domain of MBP is encoded by exons 1 and 2 [12]. Exon 1 encodes seven copies of the Gly-Xaa-Yaa motif typical of the triple helix-forming region of collagen. This pattern is interrupted at the site of the first intron by the sequence Gly-Gln-Gly which may be the site where the triple helical chains of MBP appear to 'bend' when viewed by electron microscopy (Fig. 3). Exon 2 then encodes a further 12 Gly-Xaa-Yaa repeats. This mutation in codon 54 encodes aspartic acid instead of glycine and disrupts the fifth Gly-Xaa-Yaa repeat in exon 1 of the MBP gene. Computer analysis confirms that the mutation profoundly distorts the secondary structure of the collagen-like domain. In osteogenesis imperfecta, mutations of homologous axial

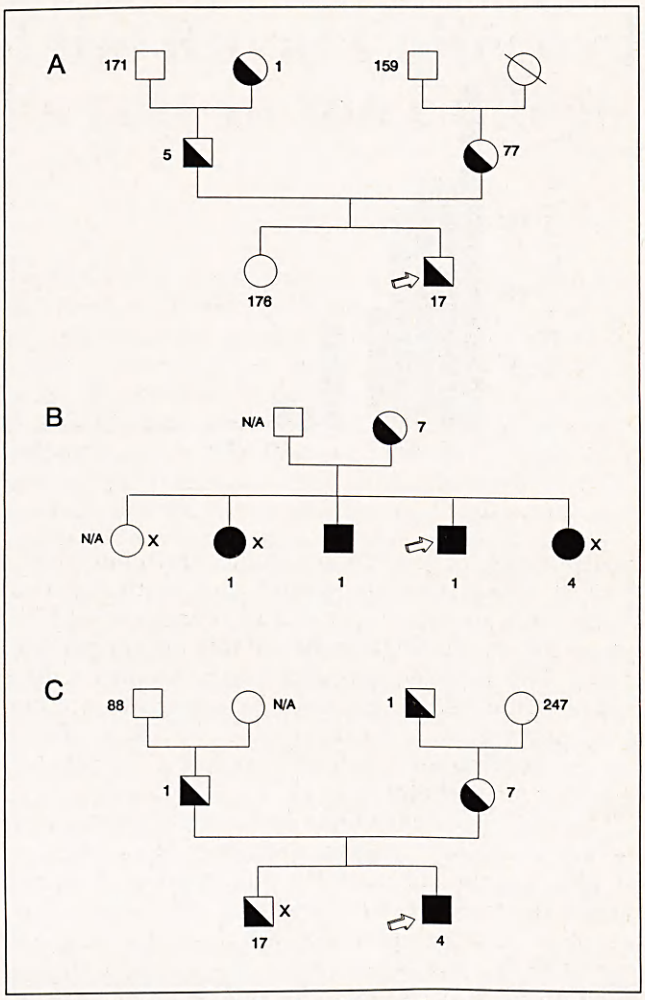

Fig. 4. *Pedigrees of the families with immunodeficiency caused by an opsonic defect.* Open symbols = Gly/Gly wild type; closed symbols = Asp/Asp; half symbols = Gly/Asp. Probands shown by arrows. Numbers = serum MBP concentrations in μg/l. The four children marked X also suffered recurrent severe infections. N/A = not available. (Reproduced from Sumiya *et al* [20], with permission)

glycine residues result in failure of collagen polymerisation [22]. This is because in the helical coils of collagen only the glycine residues are small enough to pack into the axial positions which occur every third amino acid. Of the many mutations found in osteogenesis imperfecta, substitution of glycine with aspartic acid (the mutation found in MBP) is the most disruptive of polymerisation and causes the most severe clinical syndrome. This is because aspartic acid is not only a large amino acid but is also electrically charged. Thus the mutation in the MBP gene appears to result in low serum MBP levels because of failure of the abnormal MBP subunits to polymerise into functional oligomers. If the biosynthetic mechanisms for the production and

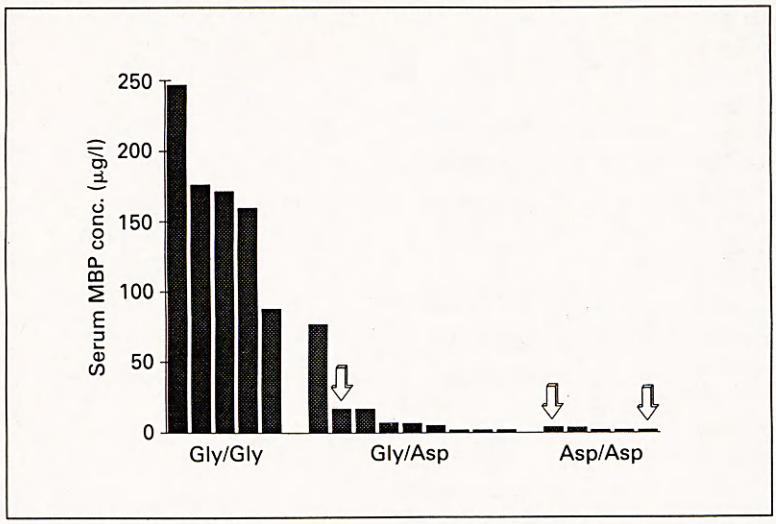

Fig. 5. *Effect of genotype on serum MBP concentration. Probands shown by arrows.* (Reproduced from Sumiya *et al* [20], with permission)

incorporation of the mutant chains are unimpaired, then in a heterozygote synthesising both types of chain, only one in eight of the trimers would be spared the disruptive influence of mutant polypeptide chains. This probably accounts for the marked reduction of serum MBP levels in the heterozygotes and the dominant pattern of inheritance. The fate of the mutant MBP subunit is unknown but it is probably degraded intracellularly.

The MBP deficiency described here appears to be the first example of a lectin deficiency being associated with human disease. No doubt other diseases caused by defective lectins will be discovered. The pathology observed in infants with this defect suggests that MBP is a major antigen non-specific defence mechanism in early life. The relevance of MBP in adult infections remains to be determined.

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