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REVIEW

Mannose-binding lectin and its genetic variants

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Mannose-binding lectin (MBL) is a collagen-like serum protein that mediates activation of the complement system and is of importance for host defence. Common variant alleles situated both in the promoter and structural region of the human MBL gene (MBL2) influence the stability and the serum concentration of the protein. Epidemiological studies have suggested that genetically determined variation in MBL serum concentration influences the susceptibility to and the course of different types of infections, autoimmune, metabolic and cardiovascular diseases, but this is still a subject of debate. The fact that these genetic variations are very frequent indicates a dual role for MBL in host defence. In this survey, we summarize the current molecular understanding of human MBL genetics.
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Introduction

Glycine-Xaa-Yaa repeating sequences (Xaa-Yaa indicate any amino acid) are characteristic for collagen triple helices and a common feature of proteins of importance in innate immune defence including complement component C1q, the lung surfactant proteins A (SP-A) and D (SP-D), CL-L1, CL-P1, Ficolin-1, -2 and -3, as well as mannose-binding lectin (MBL), which is also named mannan-binding protein or lectin.[1] These proteins play a role in first-line host defence and are therefore known as defence collagens and those that are lectins are also named collectins (collagen and lectin). Over the past 10 to 15 years MBL has been the focus of interest for several research groups around the world owing to its putative clinical role, especially based on epidemiological studies that have suggested that genetically determined variation in MBL serum concentration influences the susceptibility to and course of different types of infections as well as autoimmune, metabolic and cardiovascular diseases (for recent reviews see Turner and Hamvas[2], Kilpatrick[3], Eisen and Minchinton[4], and Nuytinck and Shapiro[5]). In particular, MBL may have some importance during the period from the time at which the maternal-derived antibodies disappear to the time at which the child's own immune system is being matured and in the lag phase before a primary immune response is generated following microbial challenges. MBL may also function as a disease modifier in some accompanying diseases and immunodeficiency states. However, the clinical significance of MBL and MBL deficiency states in human disease is debated particularly in the general population.[6] In this review we will not focus on the clinical aspects of MBL, but rather on the molecular basis behind MBL deficiency states.

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Historical background

In 1968, a patient with a serum-dependent defect in phagocytosis of yeast particles was described.[7] The defect was subsequently linked to the complement system, because C3 was deposited in lower amounts on yeast surfaces incubated in sera from affected individuals.[8] However, no abnormality in complement function as such could be shown, suggesting that a hitherto unknown serum component was the key mediator of the impaired complement deposition.

Independent of these efforts, a protein that could be extracted from rabbit liver using mannan particles from *Saccharomyces cerevisiae* as probe was discovered. This protein was initially given the name mannan-binding protein that we today know as MBL.[9] It was shown that MBL was present in human serum and that it could activate the complement system when bound to a mannan surface.[10,11] Subsequently it was shown that the originally described phagocytic defect was due to deficiency of MBL.[12] Further studies revealed that MBL in serum was associated with a serine protease named MBL-associated serine protease or MASP (MASP-1).[13] However, further studies showed that two other proteases, MASP-2 and MASP-3, and a protein with no protease activity named sMAP or MAP19 were also associated with MBL.[14–17] It is generally believed that MASP-2 is the initiator of the so-called lectin complement pathway, which is illustrated in Figure 1, while the physiological role of the other MASPs is still uncertain. The MASPs were originally named after MBL, but it has been shown that they additionally form proteolytically

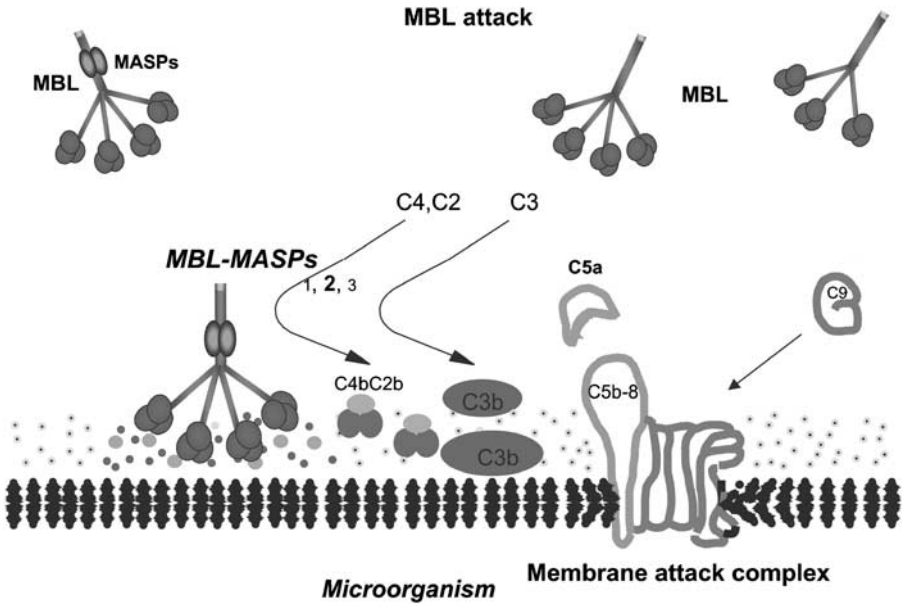

Figure 1 MBL-mediated complement attack. Mannose-binding lectin (MBL) complexed with the MASPs binds to sugar arrays on a microorganism and mediates a complement attack through MASP2. MASPs denote MBL-associated serine proteases.

active complexes with Ficolin-1 (M-ficolin), Ficolin-2 (L-ficolin) and Ficolin-3 (H-ficolin or Hakata antigen), which are also defence collagens.[18–20]

The organization of the human *MBL2* gene

The molecular structure of the human *MBL2* gene was resolved in 1989.[21,22] However, an expressed *MBL1* pseudogene has also been found (*MBL1P1*).[23] Ancient *MBL1* and *MBL2* are believed to have arisen by gene duplication from a common ancestral *MBL* gene.[24] In mice, two different forms of MBL are encoded by two distinct functional genes known as *mbl-a* and *mbl-c*, which are positioned at different chromosomes, 14 and 19, respectively,[25] whereas the two human analogues *MBL1PI* and *MBL2* are closely positioned on chromosome 10 (10q11.2–q21).[22,23] MBL-1 and MBL-2 have been detected in sera from the rhesus monkey, whereas in chimpanzee and in man MBL is only represented by MBL-2.[26]

The protein-encoding region of *MBL2* consists of four exons interrupted by three introns of 600, 1350 and 800 base pairs in size[21] (Figure 2). Exon 1 encodes the signal peptide, a cysteine-rich domain and seven copies of a repeated Glycine-Xaa-Yaa motif typical for the triple helix formation of collagen structures. This pattern is disrupted by a Glycine-Glutamine duplex and continued by additional 12 Glycine-Xaa-Yaa repeats in exon 2. Exon 3 encodes a neck region and exon 4 a carbohydrate-binding (CRD) domain. This region recognizes not only microbial carbohydrates as mannose and *N*-acetylglucosamine sugar motifs, but also nucleic acids in a calcium-dependent manner.[27] The promoter sequence of the *MLB2* gene contains several consensus elements indicating that MBL is an acute phase reactant. Of particular interest is the rather unnoticed fact that approximately 1 kb upstream of exon 1 the *MBL2*gene contains an extra alternative exon named exon 0 and that transcription of the *MBL2* gene also is initiated at exon 0.[28] This exon is not translated into protein; thus, the alternative transcript encodes a polypeptide identical to that of the predominant transcript. The vast majority of MBL produced by the liver originates from transcripts initiated at exon 1, but roughly 10–15% may originate from exon 0 initiated transcripts.[29]

The structure of the human MBL protein

The translated MBL protein is a complex of triple helix structures and each polypeptide chain contains four domains: (1) a 21 amino-acid *N*-terminal cysteine-rich region involved in oligomerization by formation of intra- and inter-subunit disulphide bonds, (2) a 59 amino-acid collagen-like domain consisting of 20 tandem repeats of Glycine-Xaa-Yaa (except repeat 8, which consists of only Glycine-Glutamine) that account for the long stalk of the molecule, (3) a 30 amino-acid α-helical, hydrophobic coil–coil neck domain, which is crucial for initiating the oligomerization and (4) a 188 amino-acid C-terminal carbohydrate-recognition domain (Figure 2). In the literature, the common notion has been that the mature MBL protein consists of oligomers, each with three identical polypeptide chains of 32 kD as evaluated on reduced SDS–PAGE.[30,31] However, under non-reducing conditions, the two major oligomeric forms of immunopurified MBL migrate in SDS as bands with an apparent molecular weight just above and below 250 kD, whereas the lowest band observed has a molecular weight compatible with 50 kD[32] (Figure 3). However, based on the calculated amino-acid sequence the molecular weight should be around 25 kD. This assumption is confirmed by the finding that when recombinant and purified MBL are subjected to mass spectrometry the molecular weight of the human MBL polypeptide is found to be around 25.3–25.5 kD.[33,34] Thus, MBL seems to consist of multiple triple helices of polypeptide chains of 25 kD chains built up to a complex structure of different types of oligomers in which the lowest covalent bound form of MBL chains

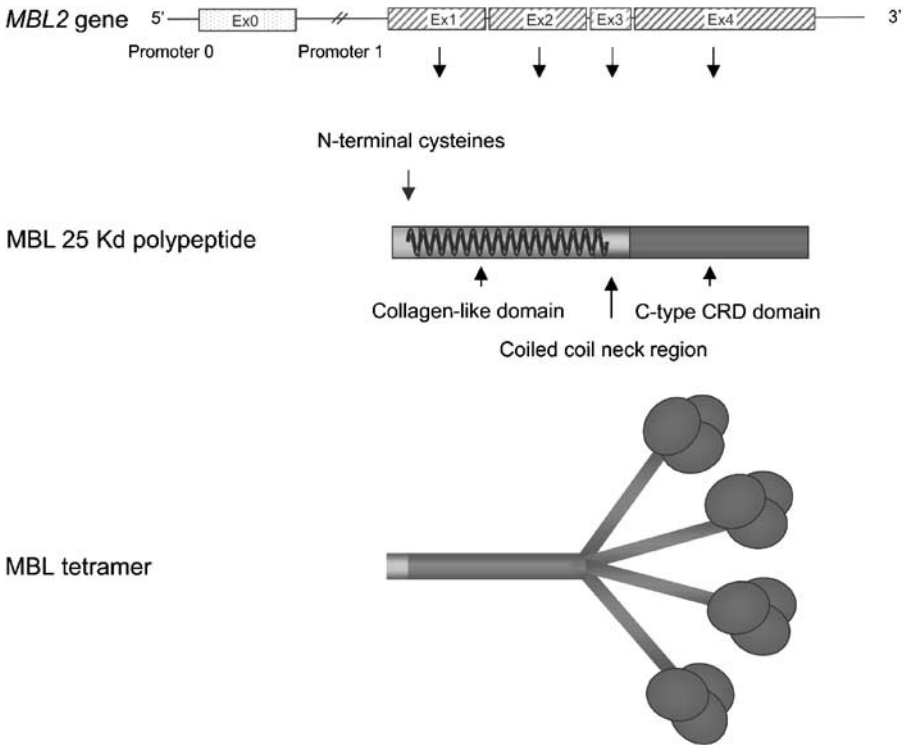

Figure 2 *MBL2* gene structure, MBL polypeptide structure and MBL oligomeric structure. Upper panel shows the exon structure of the human *MBL2* gene. Exon 0 (Ex0) is not translated into protein. Middle panel shows the organization of the MBL polypeptide. The MBL polypeptide consists of four domains, including an *N*-terminal cysteine-rich region cross linking the polypeptides, a collagen-like stalk region, an α-helic neck region and a C-type carbohydrate recognition domain. The lower panel illustrates one of the predominant forms of MBL found in serum, which consists of four subunits of triple helices of MBL polypeptides.

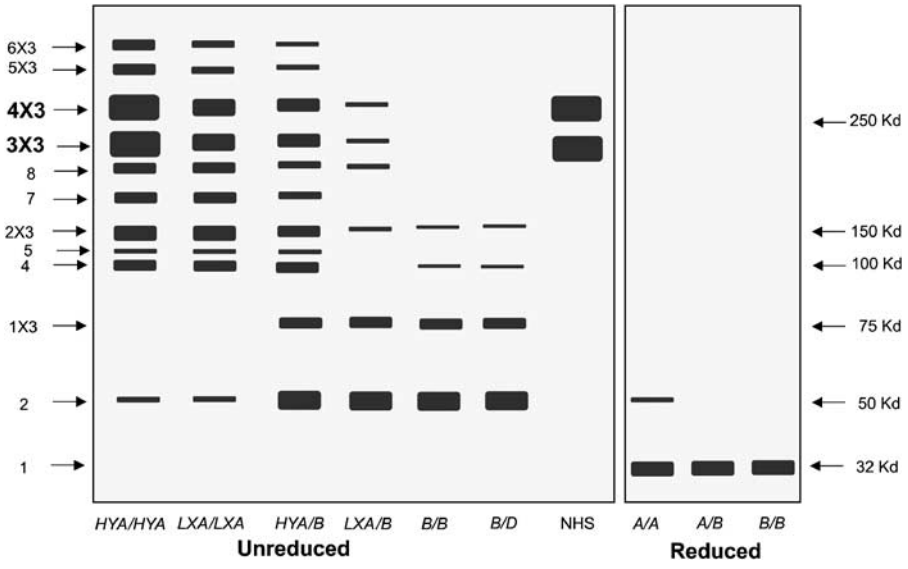

Figure 3 MBL chain and oligomer structure. Cartoon of Western blot after SDS–PAGE gradient electrophoresis from individuals with different *MBL2* genotypes. The figure is based on data from Garred *et al*.[32] and Larsen *et al*.[33] The NHS lane shows the MBL pattern observed when normal human serum is affinity purified on a mannan matrix. The other lanes illustrate the MBL banding pattern when anti-MBL antibodies are used for purification of sera containing different MBL genotypes. On the left the positions of the different MBL chains and triple helix oligomeric forms are indicated. As all these bands do not sum up with the view of triple helices suggests that MBL polypeptides consist of both covalent and non-covalent structures, which are disrupted in SDS–PAGE. On the right molecular weights are indicated.

as evaluated in SDS–PAGE comprise of two polypeptide chains. Consistent with this notion is recombinant MBL in which the three *N*-terminally situated cysteines have been replaced with serine mutants exhibit only one band in SDS–PAGE, whereas the maintenance of one or more cysteines form polypeptide dimers of 50 kD.[35] The reason

for the discrepancy between 25 and 32 kD when analysed by SDS–PAGE is not known. However, it may arise from two characteristics of MBL: (i) the high content of glycine in MBL compared with other proteins, that is, when run in SDS–PAGE, the molecular weight will be overestimated because SDS–PAGE determines the length of a protein rather than the mass, and (ii) the fact that collagenous proteins can be difficult to denature in SDS owing to their rigid triple helix structure and thus bind fewer SDS molecules per amino acid. In both cases, the true molecular weight is overestimated. Another factor which could contribute is an addition of 1–2 kD owing to post-translational modifications, such as O-linked glycosylation of lysine residues and hydroxylation of proline residues. Although serum MBL is characterized by a complex mixture of oligomers, it is now generally accepted that the main portion of MBL in serum consists of trimers and tetramers of three and four triple helices of functional MBL subunits, but that both higher (pentamers and hexamers) and lower oligomeric forms may also be found (Figure 3).[34,36]

Genetic variation in the *MBL2* gene

The discovery that the phagocytic defect was due to the deficiency of MBL initiated an effort to resolve the molecular mechanism behind MBL deficiency.[12] Sequencing the exons of the *MBL2* gene from three children who had low MBL serum concentrations and phagocytic deficiency and were suffering from recurrent infections revealed that each child carried a point mutation at codon 54 in exon 1 that caused a substitution of glycine with aspartic acid (GGC to GAC) (allele *B*, the normal allele is given the name *A*) (Figure 4).[37] Investigation of family members of the probands suggested an autosomal dominant inheritance of low MBL concentration because the median protein concentration decreased about 10 times in individuals with the heterozygous genotype. It was suggested that the decreasing effect of the *B* allele on the MBL serum concentration was due to the incorrect assembly of the MBL triple helix structure. If the variant chain took part in the assembly only one in eight $(1/2)^3$ of the basic triplet structure would statistically avoid the presence of a variant polypeptide, whereas the remaining seven would contain one or more defective polypeptides and therefore give rise to an unstable molecule. However, the *B* allele was surprisingly frequent in healthy Caucasians (0.13) and Inuits from East Greenland, whereas it was rare in East Africa.[38,39] Soon after an additional structural MBL substitution in codon 57 (GGA to GAA) causing a glycine to be substituted with glutamic acid (allele *C*) was found in individuals from West Africa (Figure 4).[40] The *C* allele was very frequent and found with a remarkable high frequency (0.29). However, the *C* allele was found with very low frequency in Caucasians and it was absent in Asians and pure Inuits. As observed for the *B* allele, the *C* allele also had a dominant effect on the MBL serum concentration. Subsequently, a third MBL substitution was found in codon 52 (CGT to TGT) causing an arginine to be substituted with a cysteine (allele *D*) (Figure 4).[41] This allele had a frequency of 0.05 in both East Africans and Caucasians, but as for the *C* allele it was virtually absent in Asians and in Inuits and even in West Africans. The *D* allele also had a decreasing effect on the median MBL concentration in *A*/*D* heterozygotes, but the effect was less dramatic and the range in serum concentration was wider compared to the other two alleles. Nevertheless in *D*/*D* homozygotes or in the compound heterozygous combinations (*B*/*D* and *C*/*D*) MBL could not be detected analogous to the observation for the *B*/*B*, *C*/*C* and *B*/*C* genotypes. As the effect on serum MBL is rather similar, in order to improve power in statistical analyses the structural variants *B*, *C* and *D* are often pooled and given the common designation *O*.

Still a rather huge variation in the MBL serum concentration remained, which in most part could be explained by polymorphisms found in the promoter 1 region of the *MBL2* gene (Figure 4).[42,43] Particularly two polymorphisms situated at positions −550 (H/L variant) and −221 (X/Y variant), which both are G to C nucleotide substitutions were shown to be of importance.[42] Taking their chromosomal location into account

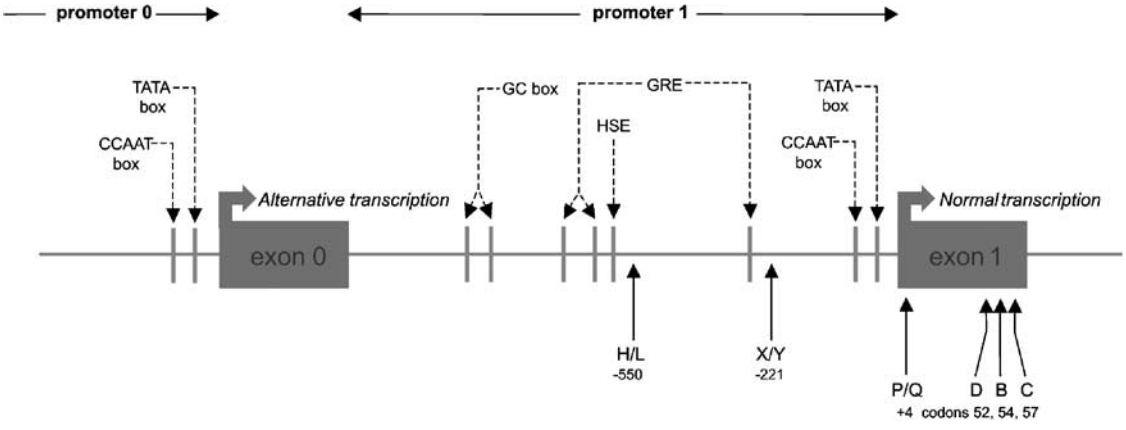

Figure 4 MBL2 polymorphisms. Two promoters, promoter 1 and promoter 0 regulate the transcription of the human *MBL2* gene. Similar to promoter 1 promoter 0 also includes a TATA box for transcription initiation, and transcription factor binding DNA sequences. Six DNA polymorphisms in the *MBL2* gene are known to be associated with variation in quantity and/or function of MBL in serum. Three variants affect the expression of the *MBL2* gene. They are localized in the promoter 1 (position −550, *H*/*L* variant and –221, *X*/*Y* variant) and in the 5′-untranslated region (position + 4, *P*/*Q* variant) of the *MBL2* gene. Three base substitutions in exon 1 in codons 52 (*D*), 54 (*B*) and 57 (*C*) result in amino-acid changes (arginine to cysteine, glycine to aspartic acid and glycine to glutamic acid, respectively) and decreased level and function of MBL. The normal allele is named *A*.

the following common haplotypes can be identified: *HYA*, *LYA* and *LXA* on a normal *A* chromosomal background and *HYD*, *LYB* and *LYC* on chromosomes carrying structural variant alleles. The haplotypes *HYA*, *LYA*, *LXA* correlates rather nicely with high, intermediate and low MBL serum levels, respectively. However, the *LYA* haplotype could be further subdivided into additional haplotypes when a polymorphism (*P*/*Q*) located in the 5′-untranslated portion of exon 1 (position +4) is taken into consideration.[41,43] Thus, the MBL *LY* type consists of the following haplotypes: *LYPA*, *LYPB*, *LYQA* and *LYQC*. A difference in the serum concentration between the *LYPA* and *LYQA* haplotypes can also be observed showing that each of the identified promoter haplotypes is associated with different MBL serum levels.[43] In a functional promoter analysis using reporter gene assays it was found that the three haplotypes *HY*, *LY* and *LX* correlated with high, medium and low promoter activity, which is in agreement with the serum measurements.[28] The *MBL2* haplotypes are extremely well preserved in all population studied and differences in frequencies may explain inter-racial variation in serum concentration. However, for instance in the Czech population a low frequent *LYPD* haplotype (allele frequency, 0.01) has been observed.[44] Sporadic rare haplotypes such as *HXA* have also been reported.[45] Although most of the relevant inter-racial variation in MBL serum concentration can be explained by these haplotypes still some variation in MBL serum concentration cannot be accounted for, which suggests that additional genetic factors may influence the MBL serum concentration. Recently, sequencing of a 10 kb region that includes the whole *MBL2* gene (with introns) was performed in individuals representing four major American ethnic groups as well as a follow-up in Dutch Caucasians.[46,47] In total, 87 polymorphic sites were detected. These studies confirmed the strong preservation of the classical seven *MBL2* haplotypes. However, estimates of linkage disequilibrium across the *MBL2* gene indicated that the gene is divided into two blocks with a probable recombination hot spot in the 3′-end. Thus, the presence of additional polymorphisms in the vicinity of the *MBL2* gene or in introns, which can be present on different classical haplotypes, is apparent. So far no conclusive functional data have been reported. Thus, the phenotypic relevance of these findings awaits further studies.

Sequencing of the *MBL2* gene from individuals carrying different genotypes has shown the existence of five additional base substitutions/deletions in the promoter region of the LYQA and LYQC haplotypes that differ from the *LYPA* and *LYPB* haplotypes.[43] These polymorphisms distinguished the human *LYP* haplotype from the *LYQ* haplotype. Intermediate haplotypes have not been described in humans but sequencing of primates has revealed intermediate forms.[48] These results indicate that the intermediate forms may have been lost through history and that the ancestral human *MBL2* haplotype may have arisen in the evolutionary gap between *LYQA* and *LYPA* haplotypes (Figure 5). It has been shown that the exon structure in the *MBL2* gene is extremely well preserved between human and higher primates.[49] In particular, the collagen-like domain is identical in humans, chimpanzees and gorillas at the amino-acid level and the difference in the whole protein

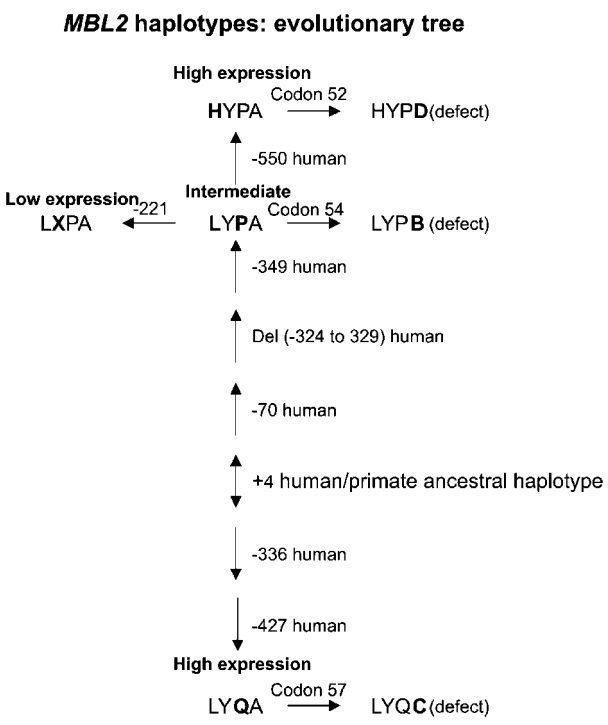

Figure 5 MBL2 evolutionary tree. Model of a possible evolutionary tree of the *MBL2* gene generated from the knowledge of *MBL2* in primates obtained from data in Seyfarth *et al*.[48] The positions of the mutational steps in the *MBL2* promoter 1 are indicated.

is less than 1%. These few amino-acid differences do not result in changes of the structure or function of MBL. More important, the *MBL2* gene investigated in higher primates has not been shown to carry the exon 1 variants *B*, *C* and *D* or analogues, which are so prevalent in humans.

Consequences of *MBL2* gene variations on MBL serum levels

Most current systems to measure MBL serum concentration could theoretically underestimate the amount of MBL antigen in heterozygotes and homozygotes for the variant alleles because if lower oligomers were present in the blood they could be underestimated in assays depending on the repetitive structure of MBL as in some double antibody sandwich-based ELISA systems, which preferentially detect higher MBL oligomeric forms.[32] In addition, functional assays based on MBL binding to, for example, mannan preferentially reflect higher oligomers because of lower avidity towards mannan for higher compared to lower-order oligomerized MBL. Biochemical analyses have indicated that the most important quantitative effect of these structural variant alleles *in vivo* in man is a reduction of the protein concentration; however, trace amounts of low molecular weight material from carriers of these variant alleles have been observed.[36] Thus, the existence of variant MBL in the blood not picked up by the common current detection techniques is apparent.

By using different types of antibodies, alternative methods, to measure and purify MBL it has

recently become clear that MBL obtained from different genotypes may be present in larger amounts than previously anticipated and that they represent different oligomerization patterns in serum[32,50–53] (Figure 3 and 6). Variant MBL binds bacteria and mannan with lower avidity than normal MBL without activating the complement system.[33,54] However, when the different forms of MBL are artificially bound via an antibody to a polystyrene surface, MASP2 interacts with variant MBL forms and complement activation may be detected even though markedly reduced compared to normal MBL.[33] Thus, reduced serum concentration, disruption of MBL-MASP2 interactions as well as changes in the oligomeric structure and reduced binding to carbohydrate ligands in variant MBL compared with normal MBL probably accounts for the biological phenotype in MBL-deficient individuals.[55] Variant recombinant MBL has been shown to retain full potential to kill tumour cells in a cellular-dependent cytotoxicity assay, indicating that some avidity may be retained in the variant molecule.[56] Nevertheless, the biological significance of variant MBL remains to be established.

Maintenance of high frequencies of *MBL2* variant alleles in different populations

The MBL variants encoding low MBL serum concentrations with few exceptions have been found with high frequencies all around the globe (Table 1); this leads to the almost inevitable question that there must be selective forces favouring survival of carriers of these alleles, that is, a low MBL concentration would not be just disadvantageous, but would also confer some relative benefits for the host.[38–40,57] Thus, MBL polymorphisms may be maintained by heterosis (advantage for heterozygous individuals) in analogy with the classical example of the sickle cell trait (HbS allele) in Sub-Saharan Africa.

In certain South American Indian groups like the Quechua and Aymara islanders of the lake of Titicaca in Peru, the *B* allele has almost replaced the normal *A* allele, with a *B* allele frequency as high as 0.80 and with more than 60% of the population being homozygous *B*/*B* defective (Table 1). It could be argued that these extreme frequencies have arisen due to founder effects alone or that MBL is completely without importance (genetic

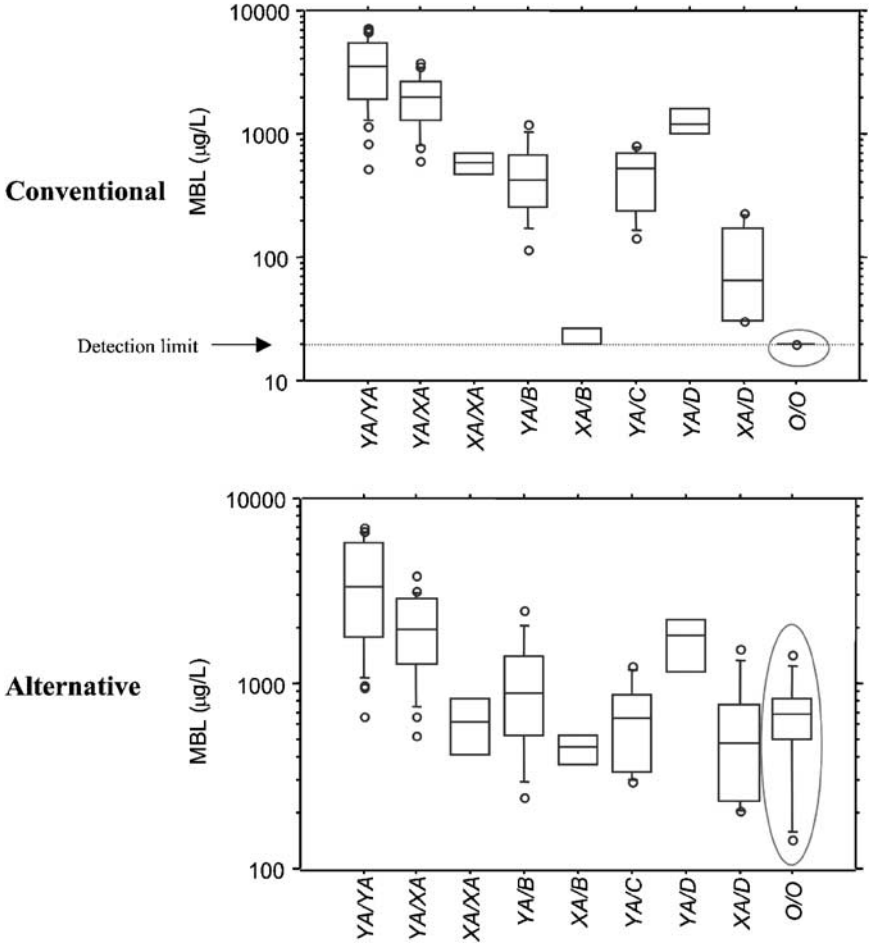

Figure 6 Detection of variant MBL forms in serum. Upper panel shows distribution in an MBL ELISA using the same monoclonal anti-MBL antibody to capture and detect as described.[39] The lower panel shows the distribution in an alternative MBL ELISA replacing the capture antibody as used in the upper panel with another anti-MBL monoclonal antibody keeping the same detector antibody in the assay.[32] The individual serum concentrations were stratified according to the truncated haplotypes *YA* and *XA* taking only the variants in position −221 into consideration and the different structural alleles *B*, *C* and *D*. Variant alleles homozygosity or compound heterozygosity are illustrated pooled as *O*/*O*. The figure is redrawn from reference Garred *et al*.[32]

Table 1 Structural *MBL2* genotype and allele frequencies in different populations representing various ethnic groups worldwide

Ethnic groups	*Genotype frequencies in %*					*Allele frequencies*		
	A/A	*A/B*	*A/C*	*A/D*	*O/O*	*pB*	*pC*	*pD*
Europeans								
Danish Caucasians	60	21	5	10	4	0.12	0.03	0.06
British Caucasians	60	23	3	10	4	0.14	0.02	0.07
Sub-Saharan Africans								
East Africans (Kenya)	51	6	23	6	14	0.03	0.24	0.05
West Africans (Ghana)	47	1	42	NF	10	0.004	0.32	0
San Bushmen (Namibia)	79	6	14	NF	1	0.03	0.07	0
Xhosa (South Africa)	51	NF	44	NF	5	0	0.27	0
Asians								
Chinese (Hong Kong)	78	20	NF	NF	2	0.11	0	0
Japanese (Kyoto)*	59	36	NF	NF	5	0.23	0	0
Australia and Oceana								
Papua people (New Guinea)	97	3	NF	NF	NF	0.01	0	0
Aboriginals (Australia)	100	NF	NF	NF	NF	0	0	0
Americans								
Eskimos (East Greenland)	78	18	NF	NF	4	0.12	0	0
Chiriguanos Amerindians (Argentina)	30	56	NF	NF	14	0.42	0	0
Quechua Amerindians (Peru)*	7	28	NF	NF	65	0.80	0	0

A denotes the normal *MBL2* allele, *B* the codon 54 allele, *C* the codon 57 allele and *D* the codon 52 allele. O/O indicates any combination of the structural variant alleles, that is, *B/B*, *B/D*, etc. The numbers are adapted from the studies by Garred *et al*.,[39,61] Madsen *et al*.,[42,43] Mead *et al*.,[60] Lipscombe *et al*.,[62] Juliger *et al*.,[63] Turner *et al*.[64] and *own unpublished results.
NF = Not found.

drift), whose functions are replaced by other proteins. However, the fact that disease associations are found and that the frequent deficiency state in Africa (*C* allele) and in South America (*B* allele) is caused by independent molecular events support the idea that they at least in part have reached such a high frequency owing to positive selection pressure.[43] Thus, they may represent balanced polymorphisms. Although it is not certain whether these putative selective and counter balancing forces are operating in present day populations, sequence analysis of the extended *MBL2* haplotypes of different US ethnic groups indicates a higher degree of heterozygosity than expected across the *MBL2* gene, which indeed supports the notion that such forces are working even today.[46] So far, the putative selective forces have not been determined and may at this stage be regarded as hypotheses. Two assumptions are prevailing and both would confer survival advantages in response to infections in the reproductive age particularly in small children when innate immune mechanisms are relatively more important than adaptive immune mechanisms. The first suggests that low levels of functional MBL would reduce possible deleterious consequences owing to complement activation and subsequent release of inflammatory mediators that may lead to tissue damage.[38,40] Recent animal studies using MBL knockout mice indirectly support such a notion as mice partially defective for MBL may be protected in a sepsis model, whereas mice with complete MBL deficiency may have increased susceptibility in another model.[58,59] These conceptual findings could be relevant for the outcome of certain infectious diseases that could influence the frequency of the *MBL2* gene pool. The other and not necessarily mutually exclusive theory suggests that MBL and its complement may contribute to enhanced uptake and virulence of certain intracellular microbes, whereas low MBL levels may be protective.[39,57]

The molecular mechanisms behind the silencing of the *MBL1* (*MBL1P1*) gene

As *MBL2* polymorphisms are so frequent thus making dysfunctional MBL very prevalent, an obvious question would be whether similar molecular mechanisms could be responsible for the silencing of the highly homologous *MBL1* gene (*MBL1P1*).[48] Originally the human *MBL1P1* pseudogene, which is an expressed pseudogene was described with an intron 1 splicing defect and two nonsense mutations (stop codons) positioned in exons 3 and 4.[23] However, it also possesses in codon 53 a glycine to arginine substitution. This substitution most likely disrupts the collagenous backbone structure of the protein because of its analogy with the human *MBL2* codon 54 glycine to aspartic acid substitution. The *MBL1P1* codon 53 substitution was also found among the most closely related primates.[48] In addition, the higher primates contained other glycine substitutions. None of the more distant primates including the baboon, rhesus monkey and African green monkey carry any of these glycine substitutions (Figure 7). This type of variation is the same as that observed in the human *MBL2* gene. The mutations leading to the substitution of glycine residues are probably introduced by specific but independent events. The most likely introduction of an analogue mutation in two homologous genes is through

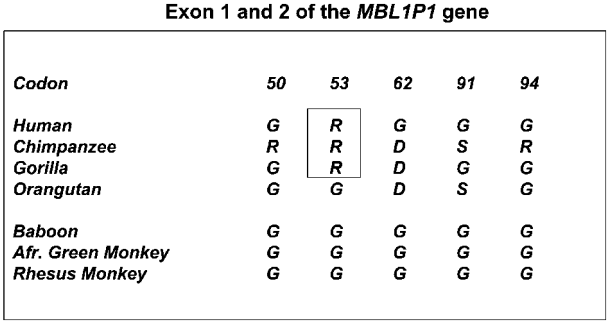

Figure 7 Substitutions in the glycine-coding sequence of the *MBL1P1* gene. The substitution in codon 53 homologous to codon 54 (*B* allele) in human *MBL2* is indicated in the rectangle. Letters indicate corresponding amino acids (G = glycine, R = arginine, D = aspartic acid and S = serine). No glycine substitutions were observed in lower primates. Comparison of codon 53 in *MBL1P1* and codon 54 in *MBL2* indicates that it is unlikely that the mutation disrupting the repetitive glycine structure has occurred through accidental gene conversion between the *MBL1P1* and *MBL2* genes as the nucleotides differ in all three codon positions between the genes. The figure is redrawn from reference Seyfarth *et al*.[48]

a gene-conversion event. However, in the case of the human *MBL1PI* and *MBL2* genes, the sequences of the mutated homologous codons (codon 53 and 54, respectively) differ in all three nucleotide positions, which makes it very unlikely that gene conversion is the responsible event.[48] Taken together these data indicate that both the *MBL1P1* and *MBL2* genes selectively have been silenced by the same molecular mechanisms, but skewed in time ultimately downregulating MBL levels in the present human population. This may be consistent with the notion that selective forces have promoted low MBL serum levels or the creation of variant MBL forms during the course of evolution. Obviously, it has not been necessary to maintain the functions derived from *MBL1P1* in higher primates, probably owing to redundancy with MBL-2.

Concluding remarks

It is quite clear that the *MBL2* gene harbours a complex genetic system, which in several studies have been shown to be associated with certain infectious conditions, particularly in small children or as a disease modifier in patients with an accompanying disease; however, the importance for the general population is debated. However, the nature and prevalence of these variations and discovery of a similar mechanism used for downregulating the *MBL1P1* gene is even more intriguing suggesting a dual role for MBL in innate immune defence and may be an example of how species over time may adapt to the environment.

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