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Review

Mannan-binding lectin: clinical significance and applications

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Abstract

Mannan-binding lectin (MBL) is a collectin (protein with both collagen-like and C-type lectin domains) synthesised in the liver and secreted into the bloodstream. Its plasma concentration is for the most part genetically determined by a series of allelic dimorphisms located both in the structural gene and in the promoter region. Genotypes made up of combinations of seven haplotypes are mainly responsible for a 1000-fold concentration variation found in human beings. MBL is a pattern recognition molecule able to bind repeating sugar arrays on many microbial surfaces, and can activate complement via associated serine proteases. A poorly defined proportion (roughly 10%) of the population with the lowest MBL concentrations is thought to be MBL insufficient and more vulnerable to a variety of infectious and noninfectious disorders. The evidence that MBL makes an important contribution to innate immunity, by increasing susceptibility to disease and/or affecting the course of disease, is discussed in detail. Preliminary results from MBL replacement therapy are encouraging, and extension of this approach to large-scale randomised clinical trials would provide solid evidence concerning the physiological significance of this protein.

Keywords: Mannan-binding lectin; Mannose-binding lectin; Collectin; Opsonization; Innate immunity

1. Introduction

1.1. Discovery of mannan-binding lectin

An opsonic activity we now attribute to mannan (or mannose)-binding lectin (MBL) was first described in relation to immune deficiency in 1968 [1]. A baby girl suffering from atopic dematitis and recurrent bacterial infections in association with a defect of phagocytosis was found to lack an inherited plasma factor detected by the opsonization of baker's yeast. This opsonic activity was later found to be lacking in 5–10% of apparently healthy adults, but the association of its absence with infections (and the development of atopy) in infancy was confirmed by a small but plausible prospective study [2].

An unrelated series of biochemical investigations began in the early 1970s with the chance discovery of a mannose-binding protein contaminating a preparation of α-mannosidase from human liver [3]. Similar lectins were subsequently isolated from both liver homogenates and serum from various mammals including humans [4–8].

After the yeast opsonin and the mannose-binding protein were known to share complement-activating properties, the identity of the two was demonstrated by the strong correlation between the opsonic defect and low serum MBL concentrations in both blood donors and a group of 10 children with the opsonic defect; purified MBL corrected the defect in vitro [9]. Later, several families were described in which the co-inheritance of MBL and the opsonic defect through three generations could be demonstrated [10].

The previously used name of mannan-binding protein has now fallen out of favour, since the abbreviation (MBP) can be confused with that of myelin-binding protein. Mannan-binding lectin or mannose-binding lectin is largely preferred. However, MBL is not specific for, nor particularly sensitive to, mannose, unlike the family of mannose-specific lectins found in monocotyledonous plants of the family Amaryllidaceae [11]. MBL is often detected by binding to solid-phase mannan, and therefore "mannan-binding lectin" is perhaps the most appropriate and specific name.

1.2. Biochemistry of MBL

MBL is a C-type lectin [12] with a typical soluble collectin structure [13]. After reduction in the presence of SDS, MBL yields a single band on electrophoresis with an apparent molecular weight of 28 to 31K, consisting of a 24,000 kDa polypeptide [14] and approximately 5% carbo-

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Table 1
Variant alleles of MBL in different populations

Population	Wild type (A)	Codon 52m (D)	Codon 54m (B)	Codon 57m (C)
European	0.79	0.06	0.14	0.01
African	0.75	0.02	0.01	0.22
Chinese	0.88	0.01	0.11	0.00
Japanese	0.75	0.00	0.25	0.00
Eskimo	0.87	0.00	0.13	0.00
Aboriginal Australian	1.00	0.00	0.00	0.00
Argentine Indian	0.54	0.00	0.44	0.02

hydrate [15]. This fundamental subunit consists principally of a long collagen-like tail domain and a globular head domain, although it also has a discrete cysteine-rich N-terminal domain and a link or neck region between the collagenous domain and the C-type carbohydrate recognition domain that forms the globular head of the chain. The basic structural unit, however, consists of three of those polypeptide chains joined at the N-terminal end, the collagenous regions intertwined, and the globular C-terminal domains forming a head cluster. These trimers themselves associate into oligomers of two to six units, producing species with molecular weights ranging up to 500,000, and forming a quaternary structure resembling a "bowl of tulips" shape similar to C1q [16,17]. It is believed that the predominant forms in humans are dimers, trimers and tetramers [18,19].

The carbohydrate-binding sites of each triplet are separated by a distance of approximately 54 Å, permitting easy interaction with the saccharide repeats that are typical of microbial surfaces but rarely associated with mammalian high-mannose structures [20]. The binding affinity for ligands is greatly increased by oligomerization; the basic triplet does not bind solid phase mannan and cannot activate complement [21,22]. The triplets are joined at the N-terminal region of the molecule by a reaction that is genetically determined, as discussed below.

MBL interacts with several proteases to initiate the lectin pathway of complement. MBL-associated serine proteases (MASP)-1, -2 and -3 plus two related proteins combine with MBL to form a complex, the biochemistry of which has not been fully elucidated [22,23].

1.3. Genetics of MBL

From around the time of the earliest investigations, the opsonic activity for baker's yeast was known to be inherited in a dominant manner. The molecular basis is now known to be mutations in the first of four exons of the human MBL gene on chromosome 10 resulting in a failure of the basic triplet unit to oligomerize [9,22]. The most frequent mutation in European populations is a point mutation at codon 54, resulting in the replacement of a glycine with an aspartic acid residue [10]. Two similar point mutations in the first exon of the structural gene have been identified at codons 52 and 57 [8,22]. These are relatively rare in the European population, but may occur more frequently in other ethnic groups (Table 1). The alleles containing the codon 52, 54 and 57 mutations are designated D, B and C, respectively, in contrast to the wild type (A). Each mutation results in a rather similar phenotype: individuals who are homozygous for a mutant allele (or are compound heterozygotes) produce MBL that is virtually undetectable by ELISA, cannot bind MASPs and cannot activate complement [21,22]. Heterozygotes (wild type/mutant) typically have about 10% of the ELISA-detectable MBL of wild-type homozygotes, and the molecule consists mainly of monomeric triplet units. MBL of wild-type structural gene homozygotes, however, includes not only dimers, trimers and tetramers but a minority of higher oligomers (which are particularly effective at complement activation) [18,24,25].

Mutations in the promoter region of the gene are also relevant. The dimorphic H/L and X/Y loci allow modulation at the transcriptional level, with associated production H > L and Y>X. Indeed, haplotypes (with the wild-type structural gene, A) containing X produce levels similar to those with the B, C or D structural variants. Yet another dimorphism is found in an untranslated region of the first exon and designated P or Q. Because of linkage disequilibrium, these various dimorphisms combine to form a limited number of only seven or eight extended haplotypes [22].

The concentration of MBL possessed by an individual is therefore genetically determined by the two haplotypes inherited from his parents (Table 2). Variation in an individual over time is influenced by response to environment (infection, etc.), with an acute phase response causing an increase of up to threefold [26]. An interesting recent finding is that growth hormone can significantly upregulate the plasma concentration of MBL [27].

Table 2
Genotypes and phenotypes

Genotype	Frequency (%)[a]	Typical concentration (μg/ml)
HYPA/HYPA	12	2.5
HYPA/LXPA	8	1.4
HYPA/LYQA	8	2.4
LXPA/LXPA	7	0.2
LYQA/LXPA	6	1.0
LYQA/LYQA	6	1.9
HYPA/LYPB	5	0.4
LXPA/LYPB	4	0.03
LYQA/LYPB	3	0.3
HYPA/HYPD	3	0.7
LXPA/HYPD	2	0.02
LYQA/HYPD	2	0.8
LYPB/LYPB	2	0.02
HYPA/LYPA	2	1.9
LYPA/LYPB	1	0.3
LYPA/HYPD	1	0.6

[a] The genotypes are ranked according to estimates of prevalence based on published data for haplotype frequencies in Europeans.

Genotype is a reasonably good predictor of circulating MBL concentration at the population level, but cannot predict plasma MBL accurately in individuals. For example, 90% of individuals who are homozygous for the wild-type (A) structural gene have MBL >0.6 μg/ml, and about 85% of heterozygotes (A/O) have MBL <0.6 μg/ml [28]. Homozygotes for the structural gene mutants always have very little ELISA-detectable MBL, and genotypes with two copies of the LXPA haplotype are also often very low in concentration. Within most genotypes, however, a wide concentration range is found and it must particularly be stressed that in some individuals, very low concentrations may be found that cannot be accounted for by the genotype [29,30].

2. MBL and general immune deficiency

2.1. Paediatric populations

The early studies in which MBL was measured functionally and semi-quantitatively by opsonizing yeast suggested an association between MBL deficiency and susceptibility to infections in infancy [2]. This relationship was supported many years later by a large hospital series of children (0 to 18 years of age) classified according to whether the presenting illness was an infection or not. Variant structural MBL genes known to confer lower MBL concentrations were twice as prevalent in children with infections than in children presenting without infections [31]. The statistical significance of this finding (P<0.0001) was highly convincing. This increased susceptibility to infection was found in both heterozygotes and homozygotic children, but the minority who were homozygotic for variant alleles had some of the most severe problems. Perhaps the most convincing data comes from a prospective population-based study on young children (<2 years old) from Greenland [32]. A two-fold increased relative risk of acute respiratory tract infection was found in MBL-insufficient subjects compared to MBL-sufficient subjects, based on MBL genotypes. The size of the study (n=252) and the statistical significance achieved (P<0.0001) seem most satisfactory.

Several other reports provide conflicting information [33–36]. Most surprisingly, there was an inverse association of MBL variant alleles with severe infections in the context of sickle cell disease [35].

It is popular to surmise that MBL-insufficiency is only clinically important when found in conjunction with another deficiency of the immune response. This view is supported by a strong association between symptomatic children with combined MBL and IgG subclass deficiencies [34], and, in another series, MBL deficiency and a defect of chemotaxis [37]. The observation of Koch et al. [32] that MBL-related infection risk was greater in children aged 6–17 months (the time of transient hypogammaglobulinaemia of infancy) than in children under 6 months or between 18 and 23 months of age is also consistent with that view. In contrast, Summerfield et al. [31] found an association between MBL deficiency and infection risk at all ages throughout childhood.

Another area of uncertainty concerns the precise level of MBL that confers protection, and below which children are at risk. Early studies using the functional opsonization assay identified 5–10% of the population as deficient and therefore at risk; this corresponds to a gravimetric level of <100 ng/ml. Garred et al. [33] found only homozygotes for variant structural alleles to be at risk; the corresponding concentration would perhaps be <20 ng/ml. Summerfield et al. [31], however, found heterozygotes to be at risk as well. Heterozygotes possess concentrations ranging from the virtually undetectable to as high as 1.2 μg/ml, and therefore some such individuals may be much more at risk than others. These studies based on genotyping have been of little value, therefore, in establishing the clinically significant cut-off point.

2.2. Adult populations

Several of the series referred to in the previous section contained children well beyond the age of infancy, including some in their teenage years. However, a rather anecdotal report concerning only five patients (aged 15–56 years) was the first indication that MBL insufficiency might increase susceptibility to infections during adulthood [38]. More convincing evidence was obtained from a study of both adults and children: low MBL concentration was found to be a significant risk factor of comparable magnitude for both age ranges [39]. However, this relationship was not apparent in a smaller group of pyrexial patients with suspected or proven infections of various kinds [40].

2.3. Chemotherapy patients

Chemotherapy causes neutropenia and a greatly increased susceptibility to infection. Neth et al. [41] recently related MBL to febrile neutropenic episodes in 100 children receiving chemotherapy for (mainly haematological) malignancy. MBL concentration was found to be inversely related to the duration of febrile neutropenia over a 6-month follow-up period. An independent study by Peterlund et al. [42] provided results consistent with those of Neth et al. The latter group investigated 54 adult patients with a variety of haematological malignancies, and related serum MBL to severity of infections contracted within three weeks of starting chemotherapy. The 16 patients deemed to have experienced major infections (bacteraemia and/or pneumonia) were compared to the 38 without such infections. Most (94%) of the first group had MBL concentrations less than 0.55 μg/ml, but only 37% of the infection-free group had concentrations below that level (P<0.0001).

The post-chemotherapy period was a unique context for studying the role of MBL because a coexisting secondary immune deficiency (cytopenia) is known to occur. It is

noteworthy that in this setting, MBL insufficiency was relevant to both juvenile and adult patients and that the clinically significant cut-off concentration was as high as 0.55 μg/ml. This value or thereabouts roughly distinguishes individuals homozygous for the wild-type structural gene from the heterozygotes; virtually all wild-type homozygous individuals have concentrations in excess of 0.55 μg/ml, while the majority of heterozygotes have less than 0.55 μg/ml [28].

3. MBL and specific infections

3.1. Viral diseases

The role of MBL in human immune deficiency virus (HIV) disease has attracted much attention, but has resulted in somewhat conflicting findings. It was first demonstrated in vitro that HIV infection of $CD4^+$ lymphocytes was impaired by physiological concentrations of MBL, but that a superphysiological concentration (50 μg/ml) was required for 100% inhibition in the model system used [43]. Later, primary isolates of HIV were shown to bind both soluble and immobilised MBL, apparently via its carbohydrate recognition domain since the interaction could be inhibited by EDTA or mannan [44]. In principle, MBL insufficiency might (a) increase susceptibility to HIV after exposure, (b) affect the course of HIV disease, or (c) have an influence on secondary infections associated with HIV disease.

Several studies support the concept that MBL insufficiency increases susceptibility to primary HIV infection [45–48], and two others are consistent with it [49,50], but another two [51,52] provide evidence for the contrary. Nevertheless, a clear majority of reports indicates that MBL insufficiency is overrepresented in HIV infected individuals, so a tentative conclusion that MBL deficiency is a risk factor for HIV infection seems reasonable. Whether MBL concentration influences HIV disease progression, survival, and so on is less clear-cut. A strong prognostic value was found in relation to progression to AIDS in infancy [50], and MBL status was associated with survival time after diagnosis of AIDS in Danish homosexuals [46]. However, other studies have failed to find any prognostic value in MBL status, and one actually reported a weak protective effect of MBL mutant alleles on progression to AIDS and death [52]. Regarding specific secondary infections in HIV-infected subjects, a strong association between cryptosporidiosis and MBL insufficiency was found [53], as was a less convincing relationship between low MBL concentrations and bacterial pneumonias [54].

The role of MBL in relation to viral hepatitis is even more controversial and unclear. Thomas et al. [55] reported a positive association between the codon 52 mutation (but not other structural gene mutations) and chronic hepatitis B infection in Europeans (but not Asians); this unconvincing study could not be confirmed in two independent and larger series [56,57] so must be considered a chance finding. However, Yuen et al. [58], studying Chinese patients, found the codon 54 mutation to be increased in hepatitis B carriers whose disease progressed to symptomatic cirrhosis or to spontaneous bacterial peritonitis; chronic infection with either hepatitis B or hepatitis C virus was also associated with lowered MBL levels. Matsushita et al. [59] found that hepatitis C patients with MBL haplotypes conferring low concentrations were less likely to respond to interferon-α treatment; however, it was odd that as many as 53% of the patients and 57% of their healthy controls had "low" MBL genotypes as defined in the study. Also, the proportion of sustained interferon responders (33%) was perhaps surprisingly high. In a separate investigation of Japanese patients infected with hepatitis C virus [60], possession of the codon 52 mutation was positively associated with disease progression (active vs. inactive hepatitis, liver cirrhosis). It has also been suggested that MBL may promote membrano-proliferative glomerulonephritis associated with HCV infection [61].

Influenza A virus is also recognised and antagonised by human MBL [62,63]. Although complement-dependent neutralisation of the virus by guinea pig MBL has been shown [64], a reversible, complement-independent neutralisation has also been demonstrated using recombinant human MBL [65]. MBL blocked primary viral attachment to host cells and also viral spreading from infected cells to contiguous uninfected cells. These neutralising activities were mediated by the carbohydrate recognition domain of MBL, which was shown to bind to the viral haemagglutinin and neuraminidase glycoproteins. Both native and recombinant MBL possessed potent neutralising activity in vitro at physiological concentrations [65]. Other collectins possess anti-influenza virus activities, but exhibit some differences in the mechanisms involved [66].

3.2. Bacterial diseases

It has been demonstrated using flow cytometry that MBL can bind to clinical isolates and other sources of many bacterial species [67]. *Staphylococcus aureus* and β-haemolytic group A streptococci typically exhibited strong MBL binding, but for several species (*Escherichia coli*, *Haemophilus influenzae*, etc.) only a single isolate of several tested exhibited significant binding. Not only was there a marked variation in binding within and between species, but in some analyses, it was observed that a minority of organisms was responsible for the binding while the morphologically similar majority did not bind at all. In a similar study of obligate anaerobes [68], it was found that pathogenic species (*Clostridia*, *Bacteroides*, etc.) did not bind MBL, while (in general) non-pathogenic bacteria (*Bifidobacteria*, etc.) did bind MBL, and it was suggested there may be an inverse relationship between pathogenicity and the degree of MBL binding. Certainly, these investigations indicate a potential role for MBL in the innate response to bacterial

infections, and the within-species variation provides an explanation for any inconsistencies arising from different studies of the same bacterial disorder.

One such bacterial disorder that has received much attention in this context is meningococcal disease (meningitis and/or septicaemia) caused by *Neisseria meningitidis*. For both group B and C serotypes, there is evidence that MBL binding is inhibited by encapsulated organisms and that the degree of MBL binding is dependent on the extent of sialylation [69,70]. However, MBL did not opsonize group A or B meningococci irrespective of binding [71]. This was at least consistent with a Norwegian study that found no association between MBL insufficiency and serogroups B or C meningococcal disease [72]. On the other hand, meningococcal meningitis was reported in association with MBL insufficiency in three generations of a single family [73], and meningococcal disease was associated with MBL gene variants (homozygous and heterozygous) in both hospital-based and community-based cohorts [74]. Although the association was much stronger with MBL variant homozygotes, such patients tended to have less severe disease than heterozygotes and wild-type homozygotes. Jack et al. [75,76] have attempted to explain this apparent paradox by adducing data indicating that MBL concentration can regulate inflammatory cytokine release by monocytes, and thereby might influence meningococcal disease severity. The role of MBL in meningococcal disease may well be complex, varying with time and concentration, and at present remains to be fully elucidated.

The role of MBL in ischaemic heart disease is even less clear, but the importance of the latter is such that careful consideration is warranted. *Chlamydia pneumoniae* has been suspected, though by no means proved to be, of etiological importance in atherosclerosis. Human recombinant MBL binds the chlamydial 40 kDa glycoprotein (the major cell surface protein) and can inhibit infection of HeLa cells by *C. pneumoniae* (and *C. trachomatis* and *C. psittaci*) [77]. This could be an explanation for a moderately strong association between possession of MBL mutant alleles and severe atherosclerosis in Norwegian patients [78]. Mutant MBL alleles were also significantly associated with increased carotid plaque area in Canadian subjects [79]. These two independent investigations are at least consistent with a role for an infectious factor in ischaemic heart disease (whether or not *C. pneumoniae* is involved), and suggest further investigation might be fruitful.

The possible protective influence of MBL on bacterial pneumonia in the context of AIDS (Section 3.1) has already been mentioned. The possible roles of MBL in bacterial pneumonia in systemic lupus erythematosus (SLE) patients (Section 4.1) and in relation to *Burkholderia cepacia* infection in cystic fibrosis (CF) (Section 4.3) are discussed later. The possible relationship of MBL to mycobacterial infection has been left until the final (Section 6) section.

3.3. Fungal and protozoal diseases

MBL was first discovered as an opsonin for baker's yeast, and its relevance to *Crytosporidium parvum* diarrhoea in the context of AIDS has been mentioned in an earlier section (Section 3.1). MBL is certainly capable of binding to *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*, including clinical isolates [67], but few disease association studies have been carried out with non-viral, non-bacterial pathogens.

The exceptions are studies of young Gabonese children with malaria [80] and adult British patients with chronic necrotizing pulmonary aspergillosis (CNPA) [81]. The former study compared plasma MBL concentrations and MBL gene mutations between severe and mild *Plasmodium falciparum* malaria patients. The latter study compared MBL genotypes (not plasma protein) in a small group of CNPA patients with normal controls. Both studies found that MBL genetic variants known to result in low circulating concentrations of the collectin were positively and significantly associated with the respective diseases under investigation. Both therefore support a role for MBL in protection from the parasitic organisms involved. However, variant alleles of MBL were not associated with clinical malaria in another series of Gambian children [57], and MBL failed to inhibit growth of *P. falciparum* in vitro [82].

4. MBL and non-infectious diseases

4.1. Systemic lupus erythematosus

Four independent reports [83–86] involving ethnically different populations provided evidence for an increased frequency of mutant MBL genes in SLE. One of those studies also provided consistent data on serum MBL [84]. The genotyping data are impressively similar, and it can be calculated that the presence of a variant allele approximately doubles the relative risk of developing the syndrome [87]. Consistent findings have since been reported in three further series [88–90], and the only conflicting data has come from analysis of the B allele frequency in Japanese subjects [91]. The putative association of SLE with low MBL levels is relatively weak, and therefore a failure to demonstrate it at the DNA level in one series out of seven is not so surprising. This association is probably real and should not be unexpected, since deficiencies of other complement components (especially C1q) are associated with lupus-like disease [92]. The lupus-like symptoms experienced by patients with these various complement deficiencies presumably arise from a similar mechanism related to inadequate complement activation and possibly defective immune complex formation.

The complementary finding of Garred et al. [90] that homozygotes for variant MBL alleles were overrepresented in Danish SLE patients was not the most interesting part of that study. Of 91 lupus patients, the group of 7 who were

homozygous for MBL variant alleles was strongly and significantly associated with renal involvement, infections requiring hospitalisation, and frequency of infectious events, compared with other patients (wild-type homozygotes and heterozygotes). MBL variant allele homozygosity was also associated with a much shorter time interval between diagnosis and first infectious event. When the nature of the severe infections was investigated, a dramatic difference in the proportion of patients experiencing pneumonia was apparent between the variant allele homozygotes (5/7=71%) and the others (3/84=3.6%). Unfortunately, little information was obtained concerning the causes of the pneumonias, all of which were community-acquired. These findings may be considered further evidence that MBL insufficiency is more likely to be clinically relevant in the context of coexisting primary or secondary immune deficiency.

Finally, two other reports are relevant to this section. Foster et al. [93], studying patients with chronic granulomatous disease, found an association between MBL variant alleles and lupus-like disorders (discoid lupus, etc.), but not with colitis, granuloma or perirectal abscess, in this clinical context. Mullighan et al. [94], studying patients with common variable immunodeficiency, found an association between the MBL Q allele/LYQA haplotype and ''autoimmunity'', although low producing MBL alleles generally were strongly associated with early age of disease onset. The data presented certainly suggest MBL insufficiency may compound the IgG deficiency in this disease, but the association of a single haplotype (conferring intermediate MBL concentrations) with a miscellany of autoimmune disorders (idiopathic thrombocytopenia, pernicious anaemia, vitiligo, etc.) is unclear and impossible to explain at present.

In summary, the literature firmly supports the view that low MBL concentrations confer a small but real increased risk of developing SLE. There is also a strong hint that in patients with diseases (particularly those involving immune dysregulation), as opposed to healthy subjects, MBL insufficiency may have a much stronger association both with infectious and with autoimmune diseases.

4.2. Rheumatoid arthritis

Before any direct MBL data in rheumatoid arthritis (RA) were reported, an elegant hypothesis of relevance was constructed by a group from Oxford. Glycoproteins like IgG are often underglycosylated to some degree, producing molecular species lacking terminal sialic acids and subterminal galactose; this agalactosyl IgG (G_0 isoforms) occurs in some patients with inflammatory diseases, most commonly RA, and has been implicated in the pathogenesis of that disease in an animal model [95]. Malhotra et al. [96] observed that G_0 glycoforms could bind to MBL and thereby activate complement. It follows that MBL would promote excessive complement activation and release of inflammatory mediators in diseases like RA, and conversely, that MBL deficiency should limit tissue damage and associated clinical symptoms under those circumstances. Therefore, it was to be expected that patients with RA and related complaints would have above-average MBL concentrations, and conversely (and most importantly) that lower MBL concentrations would be underrepresented or absent.

This expectation was first tested in a series of 99 rheumatoid factor-positive patients, about half of whom had definite RA [97]. However, low levels (however defined) were not found to be less common in RA patients and those with related inflammatory conditions, and therefore this small study provided no support for the hypothesis under consideration. Consistent with these negative results was a subsequent investigation of the frequency of the codon 54 mutation in 181 British RA patients; the mutant gene frequency was similar to that for controls [98]. Similar findings were subsequently obtained from a Japanese series [91].

Several other studies in different populations have addressed this question and have provided somewhat variable results. Graudal et al. [99], in a longitudinal study of Danish RA patients, actually found that more patients (11%) than controls (3%) had undetectable MBL, and also that low MBL concentrations were associated with poor prognostic signs including radiographic scores. However, two subsequent papers by the same research group [100,101] reported no significant association between variant MBL alleles and RA, although low MBL-conferring genotypes were associated with early-onset RA and with disease severity including progression of radiographically detected joint destruction.

Ip et al. [102] analysed a large (n=211) series of Chinese RA patients for codon 54, H/L and X/Y mutations, as well as serum MBL protein. An association of RA with the codon 54 mutation and with low serum MBL was found, but it was curious that only 1% of the control group had undetectable MBL levels. Low serum MBL and the codon 54 mutation were also associated with erosive disease and serious extra-articular manifestations in this series.

Saevarsdottir et al. [103] included two separate studies on Icelandic RA patients in a single paper. The first was a prospective study of 65 patients with early RA followed up for 6 months or more: low serum MBL was associated with less improvement (including radiological joint erosions at recruitment and follow-up). The second series consisted of 63 women with advanced RA: low serum MBL was associated with radiological damage. It was noteworthy, moreover, that neither series provided any evidence of increased MBL insufficiency per se in RA.

Most recently, the Danish investigators have focused on patients presenting with polyarthritis observed for a year. In this cohort, variant alleles of the MBL gene conferring low plasma concentrations were associated with the development of definite RA and with radiographic evidence for erosive disease [104].

What conclusion(s) can be drawn from this group of largely independent studies? Although there are some incon-

sistencies, it is quite clear that low MBL concentrations are not under-represented in RA, since not one out of the nine series cited found any evidence for that. Therefore, the attractive Oxford hypothesis that first stimulated this area of research is almost certainly false. On the other hand, six out of six series that addressed prognostic considerations found that low MBL was associated with a poor prognosis [99–104]. The opposite contention to the Oxford hypothesis, that low MBL increases susceptibility to RA (like SLE), is supported by three out of eight studies [99,102,104]. Disease susceptibility and severity/progression can only be confidently distinguished by comprehensive, community-based prospective studies of long duration. My own interpretation of the existing data is that MBL insufficiency probably influences the course of RA, but any influence on disease susceptibility is unproven and rather doubtful.

4.3. Cystic fibrosis

Garred et al. [105] studied 149 Danish patients with CF with regard to MBL genotypes. Lung function was significantly impaired in patients with low MBL-producing genotypes compared to high MBL-producing genotypes (Table 3). Moreover, poor lung function was largely confined to patients with *Pseudomonas aeruginosa* infection, although *B. cepacia* infection was more strongly associated with MBL insufficiency. The risk of end-stage CF increased three-fold amongst carriers of variant MBL alleles, and survival time decreased over a 10-year follow-up period. It was estimated that the predicted age of survival was 8 years less in variant MBL allele carriers compared to wild-type homozygotes.

Remarkably similar results concerning lung function were obtained from a small (n=22) group of French CF patients [106; Table 3]. The authors concerned also reported a higher frequency of colonisation by *P. aeruginosa* in patients homozygous for variant MBL alleles than in patients homozygous for the wild-type structural gene, although this relationship failed to achieve statistical significance (P=0.08) with the small numbers involved.

Table 3
MBL and lung function in cystic fibrosis patients

	Lung function index	
	Mean FEV_1 (%)	Mean FVC (%)
Danish high [MBL] genotypes	65	90
French MBL wild type (A) homozygotes	76	89
Danish low [MBL] genotypes	50	72
French 2-variant MBL gene patients	47	63

The Danish patients [105] were divided into MBL-sufficient (A/A and YA/0) and MBL-insufficient (XA/O and O/O) categories, while the French patients [106] were either homozygous wild type (A/A, n=11) or homozygous/compound heterozygous for mutant structural alleles (O/O, n=11). The Danish values are typical of measurements made over an 8-year period. All appropriate comparisons were statistically significant.
FEV_1—forced expiratory volume in one second.
FVC—forced vital capacity.

The data from those two independent studies (summarised in Table 3) clearly indicate that MBL concentration can influence lung function in CF patients, but the mechanism involved is less obvious. *Pseudomonas* infection was implicated in both studies, but MBL failed to bind appreciably to most isolates of *P. aeruginosa* from CF patients [107]. MBL did bind to *B. cepacia* leading to complement activation, but *B. cepacia*, although causing severe disease, is a much less common pathogen in CF patients. The role of MBL in maintaining good lung function, therefore, may lie in combating intercurrent infections with organisms other than *P. aeruginosa*, or by influencing some aspect of the recently discovered process by which *P. aeruginosa* assumes a mucoid, nonmotile phenotype within anaerobic airway mucus [108]. However, since MBL insufficiency appears to be a very strong and significant risk factor for *B. cepacia* infection [105], and the latter is such a serious infection in CF patients both pre- and post-lung transplantation [109], such MBL-deficient CF patients might be a most suitable target group for MBL substitution therapy.

Finally, it should be mentioned that liver disease in the context of CF was reportedly more severe in patients homozygous or compound heterozygous for MBL structural gene mutants [110]. Such patients had a relative risk of over 6.0 for developing cirrhosis compared to homozygotes for wild-type alleles. MBL insufficiency therefore may influence both hepatic and respiratory disease in CF, both of which have major relevance for life (or organ) expectancy in these patients.

4.4. Recurrent miscarriage

An Edinburgh group first claimed that low circulating MBL concentrations were over-represented in both male and female partners of couples experiencing recurrent miscarriage when compared to blood donors and (especially) obstetrically normal women [111]. Recurrent miscarriage women with MBL deficiency were also less likely to have a successful next pregnancy after presentation than those with normal MBL concentrations (47% vs. 59%), although this difference was not statistically significant with only 15 patients in the former category. MBL was detected in first trimester placentas by immunohistochemistry, and it was conjectured that MBL within the fetoplacental unit conferred protection against early fetal loss.

This relationship was re-examined in another series of (mainly) Danish patients [112]. The association between relative MBL deficiency and recurrent miscarriage was confirmed for the female patients, but was not apparent in the small number of Danish male partners available. There was also a significant correlation between the frequency of MBL deficiency in the women and their number of previous miscarriages, but no such correlation was found with their husbands.

The Edinburgh series was extended for a subsequent publication, and odds ratios were examined as a function of cut-off levels [113]. It was concluded that MBL concentrations ≤0.1 μg/ml were clinically significant in that context, a value roughly corresponding to negative for the original yeast opsonization assay. A corollary of that finding was that genotyping for structural gene mutants would be a much less sensitive means of identifying couples at risk of experiencing recurrent miscarriage.

Nevertheless, two genotyping studies have been carried out. The smaller study involved 76 recurrent miscarriage couples attending a London clinic and 69 control couples; all were genotyped for nine structural and promoter allelic variants [114]. No difference was found between patients and controls with regard to low and high-level MBL haplotypes.

A larger and more instructive study of Danish RM patients comprised 106 RM women whose serum MBL had previously been reported plus 111 couples (41 of whom had previously been included in the serum MBL study). The control groups were 104 couples with good obstetric histories and 210 blood donors [30]. The RM association with low serum MBL was confirmed in women patients but not in their male partners, and a much weaker association with low MBL-producing genotypes was found, which achieved statistical significance only when women who had had ≥4 previous miscarriages were considered. Moreover, a prospective study within this series revealed a significant association of serum MBL with future pregnancy outcome: the abortion rates were 64% vs. 44%, respectively, for women with low vs. normal MBL concentrations. This trend was apparent in the original Edinburgh data, and is a clinically useful finding.

In conclusion, it seems there is a genuine association between low serum MBL in women and spontaneous abortion, and this has predictive value. The primary association is with plasma protein, not with genotype. (The association therefore cannot be explained by linkage to an abortion gene on chromosome 10.) The putative association between RM and paternal serum MBL must be considered uncertain. It is apparent in the Scottish data, but does not exist in the Danish data. It is impossible to explain this difference other than by chance or the inherent limitations of statistical analysis.

Finally, it should be noted that pregnancy itself does not have a major influence on circulating MBL levels, at least during the first trimester, although a modest increase may occur in a minority of women [115].

4.5. Other non-infectious diseases

Posttransplant revascularisation can lead to reperfusion injury, a process mediated by oxygen radicals, complement activation and local recruitment of granulocytes. P-selectin has been implicated in this mechanism [116], and the lectin pathway of complement activation could also be involved, as endothelial cells subjected to oxidative stress bind MBL [117,118]. Endothelial iC3b deposition was MBL-dependent in this system. Furthermore, anti-MBL antibodies protected the myocardium from ischaemia–reperfusion injury in a rat disease model [119].

Neoplastic diseases are often associated with altered patterns of glycosylation, so in principle, surfaces of malignant cells might be recognised by MBL as non-self. No clinical studies seem to have been carried out on the possible role of MBL in human cancers, but human MBL does appear to have anti-tumour activity in an animal model [120]. Human colorectal carcinoma cells transplanted into nude mice were killed by the intratumoural or subcutaneous administration of a recombinant vaccinia virus carrying the human MBL gene by a complement-independent mechanism [120]. The authors of this work suggest the mechanism is analogous to the antibody-dependent cell-mediated cytotoxicity of the adaptive immune system.

5. Substitution therapy

The first attempts at substitution therapy used whole plasma [1,121]. This corrected the opsonic defect and appeared to benefit the patients clinically. Many years later, Valdimarsson et al. [122] treated two patients with MBL affinity-purified from the plasma fraction, Cohn fraction III. No adverse effects were noted, no antibody response to MBL was detected, and a biological half-life of the product was estimated at 5–7 days. A normal concentration of circulating MBL was achieved by this means, as was normalisation of complement activation ability (C4 deposition) and opsonic activity towards *Saccharomyces cerevisae*. Significant clinical improvement apparently resulted in a 2-year-old girl with a history of frequent infections since birth. It could be concluded, therefore, that the isolated plasma protein product was safe and that there were grounds for optimism regarding efficacy.

It is clearly time to move on to randomised clinical trials, but several considerations need to be addressed. Would a plasma product be better than a recombinant protein? If so, would purified MBL be better than a modified Cohn fraction III; the latter contains several factors contributing to innate immunity [123], and the mixture may be more effective than a single component. What would be the most suitable target disease for a clinical trial ? RA and CF are obvious candidates, but hepatitis C infection, recurrent miscarriage and severe recurrent childhood infections are other possible indications for therapy. The benefits in RF and CF may only become evident over the medium to long term; this consideration makes those disorders less attractive for clinical trials. Also, several treatments have been shown to be effective in RA over the short-term, so MBL therapy would only be an important addition to the therapeutic options (for the minority of patients who are MBL

insufficient) if it could be shown to be cost-effective in the long term.

Plasma-derived MBL has the advantage that it is natural and therefore has the natural distribution of oligomers. Recombinant MBL, on the other hand, could in principle be produced in much greater amounts and could be free from the theoretical risks associated with any blood product. It has been claimed that recombinant MBL produced in human embryonic kidney cells is structurally and functionally similar to natural MBL [124]. An apparently similar product was obtained in vivo by hepatic synthesis after tail-vein injection of mice with an MBL expression construct in the form of naked plasmid DNA [125].

It seems likely that recombinant proteins could be produced with different molecular characteristics, particularly with regard to oligomerization. It has already been mentioned that non-complement activating recombinant MBL can neutralise influenza A virus and protect against experimental colon cancer. As it is believed that complement activity in MBL may be harmful in some settings (see next section), there may be some clinical contexts in which the MBL form associated with ''insufficiency'' is preferred. Certainly, a great many randomised trials comparing different MBL products with each other as well as with placebos are required before the value (if any) of MBL as a prophylactic or therapeutic agent can be fully evaluated.

6. Concluding comments

The common occurrence of mutant alleles of the MBL gene in various populations (Table 1) could be regarded as evidence of some selective advantage for the heterozygous state. One possibility is that relative inability to activate complement might reduce damage to the host associated with the release of pro-inflammatory mediators. This hypothesis has been examined most carefully in relation to RA, and, as discussed in Section 4.2, can be considered to have been refuted. More recently, high MBL levels (and the haplotypes HYPA and LYPA) have been associated with primary biliary cirrhosis [126]; this could be explained by excessive complement activation and excessive inflammation.

An alternative view is that high MBL levels promote uptake of intracellular pathogens that utilise C3 receptors to enter host cells. This view is supported by the recent finding that serum MBL levels directly correlated with the probability of developing visceral leishmaniasis [127]. The best evidence for this hypothesis, however, comes from independent studies of mycobacterial infections [57,128–131]. Those studies, despite involving different species of bacteria, are consistent in finding circumstantial evidence for a protective influence of low MBL concentrations.

While it seems likely, if not fully proven, that high MBL levels may be harmful in certain contexts, there is much more evidence to support the view that MBL insufficiency, either alone or in combination with other relative deficiencies, may contribute to susceptibility to, and progression of, a wide spectrum of infectious diseases. There is likely to be considerable redundancy within the innate immune system, with the ficolins [13], P35 and Hakata antigen, the prime candidates to take the place of MBL. It is not so surprising that there are some inconsistencies in the literature; the bulk of the evidence suggests that MBL plays some role in host defence. It remains to be determined how important a role MBL plays, in which clinical contexts its replacement would be most effective, and which molecular forms are most suited to specific clinical situations. It is to be hoped that some or all of these outstanding questions will be addressed by replacement therapy in the not too distant future.

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