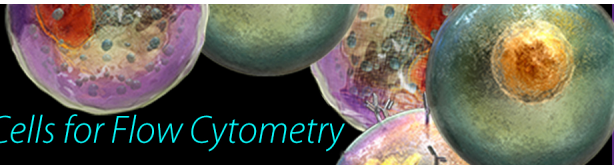


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Different Molecular Events Result in Low Protein Levels of Mannan-Binding Lectin in Populations from Southeast Africa and South America¹

Hans O. Madsen,^{3*} M. Leonardo Satz,^{2‡} Birthe Hogh,[†] Arne Svejgaard,^{*} and Peter Garred^{*}

Previous studies have shown that three point mutations in exon 1 and a particular promoter haplotype of the mannan-binding lectin (*MBL*) gene lead to a dramatic decrease in the serum concentration of MBL. In this study, *MBL* genotypes and serum concentrations were determined in unrelated individuals in a population from Mozambique ($n = 154$) and in two native Indian tribes from Argentina (i.e., the Chiriguano ($n = 43$) and the Mapuches ($n = 25$)). In both populations, the *MBL* concentrations were low compared with those found in Eskimo, Asian, and European populations. In Africans, the low serum concentrations were due to a high allele frequency (0.24) of the codon 57 (*C*) variant, which resulted in a high frequency of individuals with *MBL* deficiency (0.06), and were also due to the effect of a relatively high frequency (0.13) of low-producing promoter haplotypes. The low concentrations in the South American populations were primarily due to an extremely high allele frequency of the codon 54 (*B*) variant in both the Chiriguano (0.42) and the Mapuches (0.46), resulting in high frequencies of individuals with *MBL* deficiency (0.14 and 0.16, respectively). In the search for additional genetic variants, we found five new promoter mutations that might help to elucidate the evolution of the *MBL* gene. Taken together, the results of this study show that different molecular mechanisms are the basis for low *MBL* levels on the two continents. *The Journal of Immunology*, 1998, 161: 3169–3175.

Human mannan-binding lectin (*MBL*)⁴ is a liver-produced, C-type serum lectin that seems to play an important role in innate immunity (1). Upon binding to certain carbohydrate moieties on various pathogens, *MBL* may mediate phagocytosis by newly discovered receptors on phagocytes (2) and use *MBL* serine proteases-1 and -2 to activate the *MBL* pathway of complement (3–5).

MBL deficiency and low levels of serum *MBL* are the basis for a common opsonic deficiency (6) and are associated with recurrent infections in infancy (7, 8) and adult life (9), with an increased susceptibility to sexually transmitted HIV-1 infection (10), and with autoimmune diseases such as systemic lupus erythematosus (11–13) and rheumatoid arthritis (14). In contrast, it has been suggested that high serum concentrations of *MBL* may be disadvantageous by facilitating the entrance or increasing the pathogenicity of certain pathogens, especially intracellular microorganisms (15–19).

MBL is a multichain molecule of up to six subunits; each subunit consists of three identical 32-kDa polypeptide chains that contain a cysteine-rich region, a collagenous region, a “neck” region,

and a carbohydrate-binding domain (20). *MBL* deficiency and low levels of serum *MBL* are strongly associated with the presence of variant *MBL* alleles that encode three different structural variants, *B*, *C*, and *D* (codons 54, 57, and 52, respectively) (7, 21, 22), of the *MBL* polypeptide. The normal allele is known as *A*. Each variant is the result of a single point mutation that disrupts the collagen-like structure of the *MBL* polypeptide. This leads to a reduction of functional *MBL* to ~10% in individuals that are heterozygous for defective alleles compared with the functional *MBL* found in individuals with two functional alleles (7). The variant alleles are quite frequent in normal, healthy populations of African, Caucasian, Asian, and Eskimo origin, in that they are present in 20 to 50% of such individuals (15, 21–25).

Other variants (*H*, *L*, *X*, and *Y*) that are found upstream of the gene also have a dramatic effect on serum *MBL* levels, and different frequencies of both types of variants account for the large interracial differences in *MBL* serum levels (25). Initially, three promoter haplotypes were identified and subjected to investigation (*HY*, *LY*, and *LX*) (25). However, further division of the haplotypes is possible when a polymorphism (*P/Q*) located in the 5'-untranslated portion of the gene (position +4) is taken into account (22).

In this study, we have analyzed populations from two different continents on the southern hemisphere, namely black Africans from Mozambique and two South American populations of native Indians from different parts of Argentina, to elucidate whether selective forces may have influenced the genetics of *MBL* and thereby the *MBL* serum levels. Moreover, we looked for additional polymorphisms in the *MBL* gene.

Materials and Methods

Subjects and samples

Blood samples were randomly selected from a nonselected black African population of children ($n = 154$) from a suburban area of Maputo in Mozambique in Southeast Africa that were participating in a prospective malaria study (26) and also from two different Indian tribes from different parts of Argentina in South America (i.e., the Chiriguano Indians ($n = 43$) from Northern Argentina and the Mapuche Indians ($n = 25$) from Southern

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⁴ Abbreviations used in this paper: *MBL*, mannan-binding lectin; SSP, sequence-specific priming; SDM, site-directed mutagenesis; SSO, sequence-specific oligonucleotide.

Argentina) (27). All individuals were unrelated. In addition, a previously analyzed population of healthy and unrelated Danes comprising 42 laboratory staff members and 81 blood donors (22, 25) was expanded to include 60 laboratory staff members and 190 blood donors (i.e., a total of 250 individuals). Plasma was stored at -80°C , and genomic DNA was isolated from whole blood, granulocytes, or mononuclear cells according to a standard procedure (28).

Genomic PCR

PCRs were performed in 10 to 50 μl volumes that contained 100 to 500 ng of genomic DNA, 0.1 to 0.25 mM of specific primers in the presence of 1.5 mM MgCl_2 , 0.2 mM of deoxynucleotide triphosphate, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.001% (w/v) gelatin, and 0.5 to 2 U of *Taq* DNA polymerase (Life Technologies, Gaithersburg, MD) or AmpliTaq DNA polymerase (Perkin-Elmer, Norwalk, CT), including TaqStart Ab (Clontech, Palo Alto, CA) to prevent unspecific amplification. DNA was amplified by general PCR, by PCR using sequence-specific priming (SSP), and by site-directed mutagenesis (SDM)-PCR (22).

All PCRs were initiated by a 2-min denaturation step at 94°C and completed by a 5-min extension step at 72°C . The temperature cycles for the different types of PCRs were as follows: general PCR: 35 cycles of 30 s at 94°C , 60 s at 58°C , and 120 s at 72°C ; SDM-PCR: 35 cycles of 30 s at 94°C , 30 s at 55°C , and 60 s at 72°C ; PCR-SSP: 10 cycles of 10 s at 94°C and 60 s at 65°C followed by 20 cycles of 10 s at 94°C , 50 s at 61°C , and 30 s at 72°C .

Polymerase chain reaction-restriction fragment length polymorphism

The *B* and *C* alleles were detected by *Ban*I and *Mbo*II restriction enzyme digestions of the 685-base pair (bp) product that had been amplified by the MBL exon 1 PCR primers (upstream primer: 5'-AGTCGACCCAGATTGTAGGACAGAG-3'; downstream primer: 5'-AGTTGTGTCTCTCTGCCAG-3'), followed by a 2% agarose gel electrophoresis. The PCR product contained one nonpolymorphic *Ban*I site, which served as internal control of the enzyme digestion and gave rise to two fragments of 116 bp and 569 bp. The latter fragment was cleaved into two fragments of 308 bp and 261 bp in the *A*, *C*, and *D* genotypes; it remains uncleaved in the *B* genotype. The PCR product also contained two nonpolymorphic *Mbo*II sites, giving rise to three fragments of 83 bp, 94 bp, and 508 bp. The latter fragment was cleaved into two fragments of 280 bp and 228 bp in the *C* genotype but remained uncleaved in the *A*, *B*, and *D* genotypes. The *D* allele was detected by RFLP that was performed on SDM-PCR products; these products were produced under nonstringent primer annealing conditions (55°C) using a mutated 5'-primer (upstream primer: 5'-CATCAACGGCTTCCCAGGCCAAAGACGCG-3'; downstream primer: 5'-AGGATCCAGGCAGTTTCTCTGGAAGG-3'). The *Mlu*I restriction enzyme cleaved the 125-bp PCR product that was specific for the *D* allele into two fragments of 100 bp and 25 bp, while the *Hha*I restriction enzyme cleaves the *A*, *B*, and *C* alleles. SDM-PCR restriction fragments were separated by 4% MetaPhor (FMC, Rockland, ME) agarose gel electrophoresis.

MBL haplotyping

The *cis/trans*-location of the promoter variants *L*, *X*, and *Y* relative to the structural variants *B*, *C*, and *D* was determined by a nested PCR, initiated by PCR-SSP, and succeeded by a general PCR using MBL exon 1 primers (*B* and *C* variants) or by an SDM-PCR using allele *D* primers (*D* variant) (25). An RFLP analysis of the PCR products was subsequently performed using the relevant restriction enzymes (*Ban*I and *Mbo*II for the *B* and *C* variants and *Mlu*I and *Hha*I for the *D* variant). For the *P* and *Q* variants, an RFLP analysis was performed directly on the PCR-SSP product when determining the *cis/trans*-location to the *B* and *C* alleles. In an analogy, the *cis/trans*-location of the promoter variant *L* relative to the *X* and *Y* variants is determined by PCR-SSP using the MBL *cis*-LX and MBL *cis*-LY primer pairs; these pairs combine a downstream specificity for the *L* allele with upstream specificities for the *X* and *Y* alleles, respectively. The sequences of the PCR primers are listed in Reference 25.

Sequence-specific oligonucleotide (SSO) hybridization

The genotyping of the *H*, *L*, *X*, *Y*, *P*, and *Q* alleles was performed by a dot-blot hybridization of SSO probes to the PCR product essentially as described previously (25). The SSO probe that is specific for the *H* allele was previously mistyped (25), and the correct sequence is as follows: 5'-AAGCCTGTGTAACACC-3'.

Direct sequence analysis

A DNA sequence analysis was performed by conventional manual sequencing according to the dideoxy-termination method (29) using Sequenase version 2.0 DNA polymerase (United States Biochemical, Cleveland, OH) and [^{35}S]dideoxyATP (Amersham, Bucks, U.K.). The sequencing was performed directly on a biotinylated ssPCR product that had been isolated by the binding of the biotin to superparamagnetic, streptavidin-coupled M-280 Dynabeads (Dyna, Oslo, Norway) and followed by a magnetic separation (30). The sequencing was performed on a mixture of at least four independent PCR products in each case to avoid the detection of mutations that were artificially produced by PCR. Some PCR primers and sequencing primers are listed in Reference 25; the rest were deduced from the genomic sequence of the MBL gene (20, 31).

Assay for MBL

MBL serum concentrations were measured in a double enzyme immunoassay that was based on an anti-MBL mAb (clone HYB-131) against a repeating epitope in the MBL molecule (State Serum Institute, Copenhagen, Denmark) essentially as described previously (15, 32).

Statistical analysis

Mann-Whitney and Kruskal-Wallis tests for unpaired group comparisons were used to compare the *LYPA/LYQA* haplotypes in the African population. All analyses were two-tailed.

Results

Population studies

MBL genotyping was performed on samples from a Mozambique population and from the Chiriguano and Mapuche tribes by combining various PCR-based methods (e.g., PCR-RFLP, PCR-SSP, and PCR-SSO hybridization (25)). Haplotyping was performed on unselected subpopulations and did not reveal any deviation from the previously determined haplotype patterns (25). Therefore, the major part of the haplotyping was deduced on the bases of the strong linkage disequilibrium found between the different genotypes. The frequencies of the haplotypes were compared with those of other previously typed populations (25), including an expanded ($n = 250$) population of healthy, unrelated Danes (Table I). All four populations obeyed the Hardy-Weinberg expectations with respect to the distribution of MBL haplotypes (data not shown). Of particular interest was the finding that the allele frequency of the *B* allele was as high as 0.42 and 0.46 in the two South American Indian populations, 0.11 in the Caucasian population, and absent from the Mozambique population. By contrast, the *C* allele was frequent in Mozambique (0.24) and of low frequency in the Caucasians and the South American Indians (0.03 and 0.01, respectively). The variant *D* allele was only observed in the Caucasian population (0.06). The frequencies of individuals that were homozygous for defective alleles were quite different: High frequencies were seen in the African and South American populations (0.06–0.16) compared with the low frequencies in the Caucasian and Eskimo populations (0.03). In addition, the different populations were dominated by different functional haplotypes.

MBL serum concentrations were measured in unselected subpopulations of the Mozambique population and the Chiriguano; we found comparable low levels of serum MBL in both populations. The median concentrations and ranges of serum MBL of different structural genotypes are shown in Table II.

Sequence analysis of MBL gene variants

The previously identified MBL haplotypes (25) were based on the sequencing of the promoter region and exon 1 of the MBL gene and on other PCR-based methods defining the *cis/trans*-localization of the variant sites. To achieve a more complete description of the known MBL haplotypes, we chose to sequence the promoter region and all of the protein-coding portion of the MBL gene. This

Table I. *MBL haplotype frequencies and frequencies of homozygous-defective individuals in different populations^a*

Population	HYP A	LYQA	LYPA	LXP A	LYP B	LYQ C	HYP D	Frequency of Homozygous-defective Individuals ^b
MOZ	0.06	0.27^c	0.30	0.13	0	0.24	0	0.06
CHI	0.54	0.01	0.02	0.01	0.42	0	0	0.14
MAP	0.38	0	0.08	0.04	0.46	0.04	0	0.16
CAU ^c	0.31	0.19	0.04	0.26	0.11	0.03	0.06	0.03
KEN ^d	0.08	0.25	0.13	0.24	0.02	0.24	0.04	0.13
ESK ^d	0.81	0	0.04	0.03	0.12	0	0	0.03

^a MOZ = Mozambique population ($n = 154$), CHI = Chiriguano population ($n = 43$), MAP = Mapuche population ($n = 25$), KEN = population from Kenya ($n = 61$), CAU = Danish Caucasian population ($n = 250$), and ESK = Eskimo population from Eastern Greenland ($n = 72$).

^b Includes the following combinations of defective genotypes: *B/B*, *C/C*, *D/D*, *B/C*, *B/D*, and *C/D*.

^c The expanded population of Danish Caucasian.

^d For previously analyzed populations see Reference 25.

^e Dominating haplotypes are indicated in bold.

analysis was performed on DNA from individuals that were homozygous for each of the known haplotypes as judged by PCR-based typing methods. The different *MBL* haplotypes are shown in Figure 1. Most interestingly, the sequencing of *MBL* genes from individuals that were homozygous for the *LYQ* haplotypes (i.e., *LYQA* and *LYQC*) revealed five additional base substitutions/deletions in the promoter region when compared with the known *MBL* haplotypes (Figs. 1 and 2). To search for possible haplotypes that are intermediary to the *LYPA* and *LYQA* haplotypes, we sequenced the regions including the new mutations in randomly selected individuals that were homozygous for *LYPA* ($n = 7$) and *LYQA* ($n = 12$) and were from ethnically different backgrounds. This analysis did not reveal any "hybrids" between these haplotypes.

Comparison of *LYPA* and *LYQA* haplotypes

An isolated comparison of the influence of the *P* variant vs the *Q* variant on serum MBL levels is not meaningful, because the *P* variant is part of both high- and low-expressing haplotypes. The only means of studying this influence is by comparing the serum MBL levels in individuals with the functional haplotypes *LYPA* and *LYQA*. This comparison was not possible previously because of the skewed frequency of the *LYPA* and *LYQA* haplotypes in the populations investigated. However, a high frequency of both haplotypes in the Mozambique population allowed this comparison. Serum samples were available in 136 of the 154 individuals analyzed for *MBL* genotypes, and MBL concentrations were measured in these cases. A total of 15 and 9 individuals were homozygous for *LYPA* and *LYQA*, respectively, and 28 were heterozygous for *LYPA/LYQA*. The median MBL serum concentrations in *LYPA* homozygous individuals, *LYPA/LYQA* heterozygous individuals, and *LYQA* homozygous individuals were 1072 $\mu\text{g/L}$, 1472 $\mu\text{g/L}$, and 2896 $\mu\text{g/L}$, respectively (Kruskal-Wallis, $p = 0.009$) (Fig. 3 and Table III). Although not significant, a similar trend was observed when we tested the functional promoter haplotype *LYQA* ($n = 21$) against the likewise functional haplotype *LYPA* ($n = 12$) in indi-

viduals that were otherwise heterozygous for the structural mutation *C* (median serum concentrations: 330 and 180 $\mu\text{g/L}$, respectively; Mann-Whitney, $p = 0.16$, Table III).

Discussion

Previous population studies have shown that subSaharan Africans have high frequencies of *MBL*-defective variant *MBL* alleles, particularly the *C* type. Moreover, the promoter type *HYP* that is associated with high serum MBL levels has only been observed with low frequencies in African populations. Thus, to test the hypothesis of selective advantages of low MBL levels in populations living under a high parasitic burden, we analyzed native populations both from the southern part of Africa and from South America.

As would be expected based on the previous population studies performed on African populations (21, 25), the Mozambique population had a high frequency of defective *MBL* haplotypes (0.24) and a high frequency of individuals that were homozygous for defective *MBL* alleles (0.06). The Mozambique population was very homogenous for the *MBL* genotypes/haplotypes when compared with a previously typed population from Kenya and was dominated by the functional haplotypes *LYQA* and *LYPA*. The only defective allele found was the typical African *MBL* variant *C* allele (codon 57 variant) that is located on the *LYQC* haplotype.

The two native Indian tribes from Argentina were quite similar with respect to *MBL* haplotype distribution; they were dominated by the functional haplotype *HYP A* and the defective haplotype *LYP B*. The presence of the *LYQ C* haplotype in 2 of 25 individuals of the Mapuche tribe may indicate some ethnic admixture with settlers of Old World origin, and this possibility is in agreement with the 6% Old World origin admixture that was estimated previously in this population by the use of blood genetic markers (27).

The haplotypes found in these original South American populations are essentially the same as those found in Eskimos from

Table II. *Median MBL concentrations of the different genotypes^a*

Structural Genotype	Mozambique Population			Chiriguano		
	Median ($\mu\text{g/L}$)	Range ($\mu\text{g/L}$)	Number (n)	Median ($\mu\text{g/L}$)	Range ($\mu\text{g/L}$)	Number (n)
AA	1248	70–4352	80	1088	512–1616	7
AO	217	0–2960	48	153	44–528	12
OO	0	0–42	8	0		1

^a A = functional genotype, O = defective genotype.

-600
 LYFA ccaacgtagtaagaaatttcagagaaaatgcttaccaggcaagcctgtctaaaacaccaaggggaagcaaactccagtttaattctgggctgggttgg
 LYFB -----
 HYFA -----g-----
 HYPD -----g-----
 LXFA -----
 LYQA -----
 LYQC -----

-500
 LYFA gactaaggttgaggttgatctgaggttgagaccttctcttggatcaccagcttcagctcagggcctgccaatgagtaaataatgtagttaacaggtcct
 LYFB -----
 HYFA -----
 HYPD -----
 LXFA -----
 LYQA -----c-----
 LYQC -----c-----

-400
 LYFA ggaggggaatcagctgccagatacaaaatgggatcaggtggcagatggacccgaagaggacatggagagaagaggaagctcctacagacacctggg
 LYFB -----
 HYFA -----
 HYPD -----
 LXFA -----
 LYQA -----g-----g-----
 LYQC -----g-----g-----

-300
 LYFA ttccactcattctcattccctaagctaacaggcataagccagctggcaatgcacggctccatttgttctcactgccacggaagcatgtttatagtctt
 LYFB -----
 HYFA -----
 HYPD -----
 LXFA -----c-----
 LYQA -----
 LYQC -----

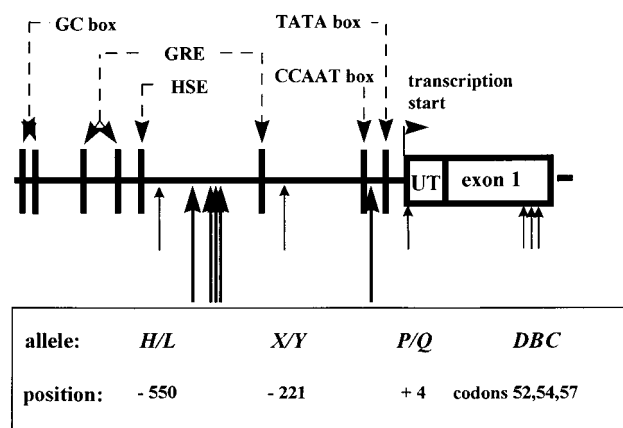
-200
 LYFA ccagcagcaacgccaggtgtctagggcacagatgaacccctccttaggatccccactgctcatcatagtgctaccttgttaaagtactagtcacgcagt
 LYFB -----
 HYFA -----
 HYPD -----
 LXFA -----
 LYQA -----
 LYQC -----

-100
 LYFA gtcacaaggaatgtttacttttccaaatcccagctagaggccagggatgggtcatctatttctatatagcctgcaccagattgtagggacagagggcat
 LYFB -----
 HYFA -----
 HYPD -----
 LXFA -----
 LYQA -----t-----
 LYQC -----t-----

→transcription →translation
 1
 LYFA gctcggtaaatatgtgttcattaactgagattaaccttccctgagttttctcacaccaaggtgaggaccATGTCCCTGTTTCCATCACTCCCTCTCCTTC
 LYFB -----
 HYFA -----
 HYPD -----
 LXFA -----
 LYQA -----t-----
 LYQC -----t-----

101
 LYFA TCCTGAGTATGGTGGCAGCGTCTTACTCAGAAACTGTGACCTGTGAGGATGCCCAAAGACCTGCCCTGCAGTGATTGCCTGTAGCTCTCCAGGCATCAA
 LYFB -----
 HYFA -----
 HYPD -----
 LXFA -----
 LYQA -----
 LYQC -----

201 256
 LYFA CGGCTTCCCAGGCAAAGATGGGCGTGA TGGCACCAAGGGAGAAAAGGGGAACCAG
 LYFB -----A-----
 HYFA -----
 HYPD -----T-----
 LXFA -----
 LYQA -----
 LYQC -----A-----



↑ points at the hitherto known polymorphic sites in the MBP gene, which generate 7 MBP haplotypes: *HYP A*, *LYP A*, *LYQ A*, *LXP A*, *LYP B*, *LYQ C* and *HYP D*.

↑ points at 5 new mutations at positions -427, -349, -336, del(-329 to -324) and -70, which are found in the *LYQ A* and *LYQ C* haplotypes.

▬ indicate elements involved in the regulation of gene expression: GC -box, Glucocorticoid Response Element (GRE) and Heat Shock Element (HSE) and in basal gene expression: CCAAT-box and TATA-box

FIGURE 2. Organization of the promoter region and exon 1 of the human MBL gene. The arrows point to the position of the variant sites that are responsible for the genotypes *B*, *C*, *D*, *P*, *Q*, *H*, *L*, *X*, and *Y*. The dotted arrows point to the promoter elements that are known to participate in basal transcription (TATA box, CCAAT box, GC box) and induced transcription (glucocorticoid receptor element (GRE) and heat-shock element (HSE)).

Eastern Greenland (Table I and Ref. 25) and those found in populations of Asian origin (our unpublished observations). This observation is in agreement with the well-established population migration theories that postulate a primary migration of the Eskimos from Siberia to Eastern Canada and Greenland that was followed later on by a secondary wave of migration through North to South America. Accordingly, although the migrations of Eskimos and the South American populations were probably not contemporary, they all appear to descend from populations carrying the *HYP A* and *LYP B* haplotypes. Although a “bottle-neck effect” can never be excluded as the cause of the different haplotype distributions in the Eskimos and the South American Indians, the high frequencies of the defective haplotype *LYP B* that are found in both of these populations could very well be due to a selection pressure favoring low serum MBL in this region, especially since the same phenomenon seems to have occurred in subSaharan Africa through the *C* allele.

When serum MBL levels were compared in subpopulations of the Chiriguano and Mozambique populations, we found no difference in the levels expressed by the different haplotypes. This observation is somehow surprising, because the South American Indians are highly dominated by the high-producing haplotype, *HYP A*, while the Africans are dominated by lower-producing haplotypes (Table I). However, the MBL levels of the *HYP A* haplotype were significantly lower in the South American Indians than in other populations (e.g., the Eskimos). This could indicate that

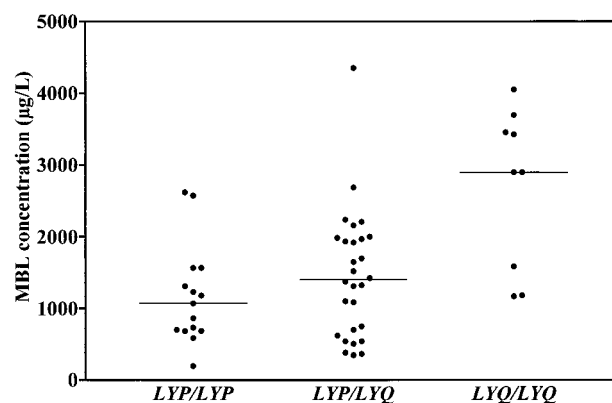


FIGURE 3. Comparison of MBL serum levels in the different functional homozygous genotypes: *LYP A/LYP A* ($n = 15$), *LYP A/LYQ A* ($n = 28$), and *LYQ A/LYQ A* ($n = 9$) of the Mozambique population. The median protein levels are indicated by horizontal bars. See Results for further explanation.

the MBL serum levels of the South American Indians may have been lowered by consumption of the protein. Alternatively, it cannot be excluded that other down-regulating variants exist outside of the region of the Indian *HYP A* haplotype that we have analyzed.

In addition to the evidence of a selective advantage of low serum MBL, this study revealed the existence of at least seven MBL haplotypes: Three were defective haplotypes (*LYP B*, *LYQ C*, and *HYP D*), and five were functional haplotypes with different expression levels (i.e., a low-producing *LXP A* haplotype, two *LYA* haplotypes consisting of a high-producing *LYQ A* haplotype and an intermediate-producing *LYP A* haplotype, and finally a high-producing *HYP A* haplotype).

A difference in MBL expression of the *LYP A* and *LYQ A* haplotypes was found when the MBL levels of these haplotypes were compared in the Mozambique population. This finding means that each of the identified promoter haplotypes has different influences on the MBL level. The evolution of the MBL gene seems to have passed through steps that have given rise to both higher- (*HYP A* and *LYQ A*) as well as lower- (*LYP A* and *LXP A*) expressing variants in addition to the structural variants with the massive down-regulating effect on the MBL serum levels.

Previously, we have proposed a model for the evolution of the MBL gene based on the MBL haplotypes (25). With the finding of five additional mutations in the *LYQ A* and *LYQ C* haplotypes this model must now be slightly modified (Fig. 4). We still propose that a high-producing haplotype may be the original haplotype, but based on the findings of the new mutations, we find that the known haplotypes more likely have evolved from an ancestral haplotype positioned in the large “mutational gap” of the evolutionary tree between the *LYQ A* and *LYP A* haplotypes and not from the *HYP A* or the *LYP A* haplotype as indicated previously (25). Perhaps the original haplotype has been lost from present populations, because our analysis did not indicate the existence of any haplotypes that were intermediate to the *LYP A* and *LYQ A* haplotypes. Thus, the ancestral gene may originate somewhere in between the present day haplotypes.

It is now recognized that a lack of MBL is associated with an increased risk of infections (7–9). However, the findings in the

FIGURE 1. Aligned sequences of the seven different MBL haplotypes. Only 600 bases upstream of the gene and exon 1 are shown. The rest of the protein coding portion of the gene (20, 31) was identical in the different haplotypes. Symbols: “—” indicates identity, “.” indicates deletion, and a letter indicates a base substitution compared with the sequence of the *LYP A* haplotype. The complete sequences reported herein have been submitted to the EMBL Nucleotide Sequence Database and assigned accession numbers Y16576–Y16582.

Table III. Genotypes and MBL concentrations in the Mozambique population (n = 154)^a

Structural Genotype	Complete Genotype	Individuals with Genotype (n = 154)	Individuals with MBL (n = 136)	Median MBL Concentrations (μg/L)	Range (μg/L)
A/A	LYQA/LYQA	11	9	2896	1168–4048
	LYQA/LYPA	28	28	1400	352–4352
	LYPA/LYPA	16	15	1072	198–2624
	LYQA/LXPA	7	7	1248	560–3616
	LYPA/LXPA	11	9	992	528–2832
	LXPA/LXPA	2	1		
	HYPALYQA	4	4		
	HYPALYPA	7	5		
	HYPALXPA	3	2		
A/O	LYQA/LYQC	24	21	330	35–2960
	LYPA/LYQC	13	12	180	0–1776
	LXPA/LYQC	14	11	0	0–157
	HYPALYQC	4	4		
O/O	LYQC/LYQC	10	8	0	0–42

^a Only groups of a sufficient size (n > 5) with regard to both genotype and MBL serum level were considered.

African and South American populations also indicate a selection pressure against high-producing MBL haplotypes, thereby giving rise to a balanced genetic system. Complement-dependent opsonization has been shown to be involved in the uptake of certain viruses, bacteria, and parasites (33). Experimental and clinical evidence that MBL may indeed facilitate infection with some intracellular bacteria and parasites has recently been provided (17–19). Thus, the presence of high frequencies of variant MBL alleles that are responsible for low MBL levels may confer relative protection against some infections or, alternatively, may result in a reduction of the deleterious effects of complement-mediated inflammation (21, 32). However, these two hypotheses are not mutually exclusive.

Consequently, we still propose in our revised model that a high-producing haplotype may be the original haplotype, and that this haplotype subsequently evolved into lower-producing haplotypes by the introduction of both structural and regulatory mutations.

In conclusion, this study has revealed that the MBL genetic system is even more complex than suggested previously. In addition, the high frequencies of two different variant alleles that are associated with low MBL concentrations on different continents support the assumption that such high frequencies may have evolved in response to the deleterious effects of certain types of infections.

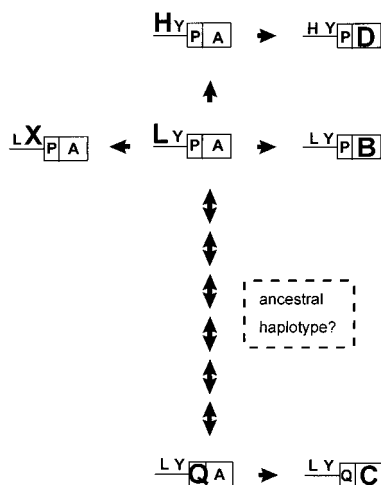


FIGURE 4. Evolution of the MBL gene. The haplotypes are indicated using the same symbols shown in Figure 2. The number of mutational steps is indicated by arrows.

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