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

Hans O. Madsen, M. Leonardo Satz, 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 Chiriguans (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 Chiriguans (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) 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 inter racial 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 Chiriguans Indians (n = 43) from Northern Argentina and the Mapuche Indians (n = 25) from Southern...
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 MgCl₂, 0.2 mM of deoxynucleotide triphosphate, 30 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.001% (v/v) gelatin, and 0.5 to 2 U of Taq DNA polymerase (Life Technologies, Gaithersburg, MD) or AmpliTag DNA polymerase (Perkin-Elmer, Norwalk, CT), including TaqStart Ab (Clonetech, 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 BanI and MboII restriction enzyme digestions of the 685-base pair (bp) product that had been amplified by the MBL exon 1 PCR primers (upstream primer: 5'-AGTTCAGCAGGTTGAAGGACCAG-3'; downstream primer: 5'-AGTTTGGTTCTCTCTGTCCAG-3'), followed by a 2% agarose gel electrophoresis. The PCR product contained one nonpolymorphic BanI 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 MboII 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'-CATCAAC GGCATTCCAGGCAAAGACGCG-3'; downstream primer: 5'-AGGAT CCAGCGCGCCCTCCTGGAGGAAAG-3'). The MboII 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 HhaI 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 (BanI and MboII for the B and C variants and MluI and HhaI 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'-AAGCCTGTTGAAAACACC-3'.
Comparison of LYPA

This analysis did not reveal any "hybrids" between these LYQA (LYPA MBL) haplotypes in the promoter region when compared with the known deletions in the promoter region. An isolated comparison of the influence of the selected individuals that were homozygous for LYPA (LYQA) genes from South America.

Discussion

Previous population studies have shown that sub-Saharan 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 HYPA and the defective haplotype LYPB. The presence of the LYQA 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

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 LYQA (LYPA 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 intermediate 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 µg/L, 1472 µg/L, and 2896 µg/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 individuals that were otherwise heterozygous for the structural mutation C (median serum concentrations: 330 and 180 µg/L, respectively; Mann-Whitney, p = 0.16, Table III).

Discussion

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

<table>
<thead>
<tr>
<th>Population</th>
<th>HYPA</th>
<th>LYQA</th>
<th>LYPA</th>
<th>LXPA</th>
<th>LYPB</th>
<th>LYQC</th>
<th>HYPD</th>
<th>Frequency of Homozygous-Defective Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOZ</td>
<td>0.06</td>
<td>0.27</td>
<td>0.30</td>
<td>0.13</td>
<td>0</td>
<td>0.24</td>
<td>0</td>
<td>0.06</td>
</tr>
<tr>
<td>CHI</td>
<td>0.54</td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
<td>0.42</td>
<td>0</td>
<td>0</td>
<td>0.14</td>
</tr>
<tr>
<td>MAP</td>
<td>0.38</td>
<td>0</td>
<td>0.08</td>
<td>0.04</td>
<td>0.46</td>
<td>0.04</td>
<td>0</td>
<td>0.16</td>
</tr>
<tr>
<td>CAU†</td>
<td>0.31</td>
<td>0.19</td>
<td>0.04</td>
<td>0.26</td>
<td>0.11</td>
<td>0.03</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>KEN‡</td>
<td>0.08</td>
<td>0.25</td>
<td>0.13</td>
<td>0.24</td>
<td>0.02</td>
<td>0.24</td>
<td>0.04</td>
<td>0.13</td>
</tr>
<tr>
<td>ESK§</td>
<td>0.81</td>
<td>0</td>
<td>0.04</td>
<td>0.03</td>
<td>0.12</td>
<td>0</td>
<td>0</td>
<td>0.03</td>
</tr>
</tbody>
</table>

† 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).
‡ The expanded population of Danish Caucasian.
§ For previously analyzed populations see Reference 25.

Dominating haplotypes are indicated in bold.

Table II.  Median MBL concentrations of the different genotypes

<table>
<thead>
<tr>
<th>Structural Genotype</th>
<th>Mozambique Population</th>
<th>Chiriguano</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (µg/L)</td>
<td>Range (µg/L)</td>
</tr>
<tr>
<td>AA</td>
<td>1248</td>
<td>70–4352</td>
</tr>
<tr>
<td>AO</td>
<td>217</td>
<td>0–2960</td>
</tr>
<tr>
<td>OO</td>
<td>0</td>
<td>0–42</td>
</tr>
</tbody>
</table>

A = functional genotype, O = defective genotype.
In other populations (e.g., the Eskimos). This could indicate that type were significantly lower in the South American Indians than the Africans are dominated by lower-producing haplotypes (Table I). However, the MBL levels of the Indians are highly dominated by the high-producing haplotype, HYPA, while the Africans are positioned in the large "mutational gap" of the evolutionary tree.

The observation is somehow surprising, because the South American differentiation in the levels expressed by the different haplotypes. This phenomenon seems to have occurred in sub-Saharan Africa through the later on by a secondary wave of migration through North to South America from Siberia to Eastern Canada and Greenland that was followed through migration theories that postulate a primary migration of the Eskimos and the South American populations were probably not contemporary, they all appear to descend from populations carrying the HYPA and LYPB 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 LYPB 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 sub-Saharan 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, HYPA, while the Africans are dominated by lower-producing haplotypes (Table I). However, the MBL levels of the HYPA haplotype were significantly lower in the South American Indians than in other populations (e.g., the Eskimos). This could indicate that 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 HYPA 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 (LYPB, LYQC, and HYPD), and five were functional haplotypes with different expression levels (i.e., a low-producing LXPA haplotype, two LYA haplotypes consisting of a high-producing LYQA haplotype and an intermediate-producing LYPA haplotype, and finally a high-producing HYPA haplotype).

A difference in MBL expression of the LYPA and LYQA haplotypes was found when the MBL levels of these haplotypes were compared in the Mozambique population. This finding means that the known ancestral gene seems to have passed through steps that have given rise to both higher- (HYPA and LYQA) as well as lower- (LYPB and LXPA) 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 LYQA and LYQC 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 LYQA and HYPA haplotypes and not from the HYPA or the LYPA 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 LYPA and LYQA 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. 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)).

FIGURE 2. Comparison of MBL serum levels in the different functional homozygous genotypes: LYPA/LYPA (n = 15), LYPA/LYQA (n = 28), and LYQA/LYQA (n = 9) of the Mozambique population. The median protein levels are indicated by horizontal bars. See Results for further explanation.

FIGURE 3. Comparison of MBL serum levels in the different functional homozygous genotypes: LYPA/LYPA (n = 15), LYPA/LYQA (n = 28), and LYQA/LYQA (n = 9) of the Mozambique population. The median protein levels are indicated by horizontal bars. See Results for further explanation.
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 cellular bacteria and parasites has recently been provided (17–19).

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 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.

Acknowledgments

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References


![FIGURE 4](image-url). 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.


