Mannose-Binding Lectin Gene Polymorphism Is a Modulating Factor in Repeated Respiratory Infections*

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**Study objective:** To clarify how mannose-binding lectin (MBL) participates in the pulmonary defense system.

**Design:** Multicenter retrospective study.

**Participants:** Sixty-two patients with unexplained recurrent respiratory infections, 50 patients with nontuberculous mycobacterial infection, 23 patients with aspergillosis, and 49 patients with diffuse panbronchiolitis (DPB). For controls, 52 blood samples were provided by the Blood Donation Center of the Japanese Red Cross Society. For BAL fluid (BALF) evaluation, there were five patients with acute phase pneumonia and five healthy volunteers.

**Measurement and results:** We demonstrated that MBL protein could be directly measured in the BALF from the lungs of patients with pneumonia by means of enzyme-linked immunosorbent assay. Furthermore, we demonstrated that the prevalence of the codon 54 mutation of the MBL gene in 62 patients having repeated respiratory infections was significantly higher compared with healthy control subjects (54.8% vs 32.7%). The prevalence of the MBL mutant genotype among patients with DPB was higher (51.1%) than in the rest of the patients. In contrast, the prevalence of the MBL mutant genotype among patients with nontuberculous mycobacteria or Aspergillus chronic infection was not significantly different from that in control subjects (44.0% and 34.8%).

**Conclusions:** Our results suggest that MBL may play an important role in modulating the inflammatory response against repeated microbial infections.

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**Key words:** immunocompromised host; lectins; natural immunity

**Abbreviations:** BALF = BAL fluid; bp = base-pair; DPB = diffuse panbronchiolitis; ELISA = enzyme-linked immunosorbent assay; MBL = mannose-binding lectin; NTM = nontuberculous mycobacteria; PCR = polymerase chain reaction

Various infectious microbes continuously challenge the respiratory system. The upper airway and lungs have multiple complex defense mechanisms, which detect most infectious microbes and destroy them within minutes by first-line defense mechanisms that constitute the innate immune system.1,2 The innate immune system in the lungs includes antimicrobial peptides and the complement system, with pattern recognition receptors-mediated phagocytosis. Disruption in either system might contribute to increased susceptibility and repeated respiratory infections.

Mannose-binding lectin (MBL) is a well-characterized pattern recognition receptor, and is a major component of the innate immune system.3,4 MBL binds to mannose or N-acetyl-glucosamine sugar chains, present on Gram-positive or Gram-negative bacteria,3,5 yeast,6,7 and some viruses,6,9 and directly enhances the phagocytosis of these infectious microbes. The human MBL gene consists of four exons,10 and three genetic polymorphisms at codons 52, 54 and 57 in exon 1 of the MBL gene have been reported.3,11,12 The codon 54 variant is significantly common in Japan.13–15 These variants result in a low
serum concentration of MBL protein and an insufficient opsonic function of MBL. In this study, we present direct evidence that the prevalence of the genotype related to a low level of serum MBL is significantly increased in patients with diffuse panbronchiolitis (DPB) who have repeated pulmonary infections, and that MBL genetic variants may constitute risk factors for repeated pulmonary infections.

Materials and Methods

Study Population

In this study, we carried out genomic analyses of blood samples from 184 patients having unexplained recurrent respiratory infections (62 patients), nontuberculous mycobacterial infection (50 patients), aspergillosis (23 patients), and DPB (49 patients). These patients were treated at Tohoku University Hospital, Tenri Hospital, Toranomon Hospital, and National Tokyo Hospital, between 1995 and 2000. The patients with recurrent respiratory infections had at least one episode of pneumonia per year, and showed no apparent abnormalities in the components of the immune system, such as number of circulating WBCs, Ig (IgG, IgA, IgM), and complement system (C3, C4, CH50). The diagnosis of pulmonary nontuberculous mycobacterial infection was established according to the criteria of the American Thoracic Society.17 The diagnosis of aspergillosis was made when characteristic radiographic features were found in a patient with serum precipitins positive for Aspergillus species and/or when Aspergillus was isolated from respiratory specimens. The diagnosis of DPB was made according to the criteria established by Homma et al.,18 characterized by coarse crackles and rhonchi detected on physical examination together with chest radiographs or CT scan images of characteristic diffuse small nodular lesions and hyperinflation. Patients with underlying pulmonary diseases, a history of immunosuppressive therapy, or an apparent immunologic disorder were excluded. For controls, blood samples were provided by the Blood Donation Center of the Japanese Red Cross Society in Sendai. For BAL fluid (BALF) evaluation, five patients with acute-phase pneumonia (three patients with Pneumocystis carinii pneumonia and two patients with bacterial pneumonia), and five healthy volunteers were newly enrolled at Tohoku University Hospital. The local ethics committee approved this study, and informed consent was obtained from all participants in this study.

Detection of Codon 54 Alleles by Ban1 Restriction Endonuclease

Genomic DNA of all patients and healthy control subjects was extracted from whole-blood samples by means of a DNA extractor WB kit (Nippon Gene; Tokyo, Japan). We used newly designed primers (5'-CTTCCCTGAGTTTTCTCAC-3' and 5'-ATCAGTCTCCTCATATCCCC-3') for the polymerase chain reaction (PCR) to amplify the 298 base-pair (bp) fragment, which included codon 54 of exon 1 of the MBL gene, according to the sequence data of human MBL available from GenBank (accession No. AF209479). The products were then digested with Ban1 restriction endonuclease for 2 h at 37°C, and subjected to electrophoresis on 1.5% agarose gels. Ban1 recognizes the sequence G/GYRCC, located at codon 54 of exon 1 of the MBL gene. The codon 54 wild-type allele (GGC) codes Gly (G), and the codon 54 variant (GAC) codes Asp (A). When the PCR products were from carriers of 54G/G (wild-type), 54G/A (heterozygote), and 54A/A (homozygote) alleles, the Ban1 restriction fragments had to be visualized as two bands, three bands, and one band respectively (Fig 1, top, A). In addition, we confirmed the amino acid sequence of exon 1 and promoter region of the MBL gene by direct sequencing. The PCR products were sequenced by ABI PRISM 310 Genetic Analyzer (Perkin-Elmer; Foster City, CA). The sequencing primers used were the same as the PCR primer for the MBL exon 1 gene. The results of direct...

Figure 1. Ban1 restriction analysis and direct sequencing for the MBL codon 54 alleles. Top, A: Ban1 restriction pattern is shown for representative genotype with 54G/G, 54G/A, and 54A/A. The codon 54 wild-type allele (GGC) are denoted G, and the codon 54 variant (GGC to GAC) are denoted A. Briefly, there is a Ban1 site in the 298-bp fragment of the wild-type MBL gene (G/G), resulting in two bands (195 bp and 103 bp) for 54G/G, three bands (298 bp, 195 bp, and 103 bp) for 54G/A (heterozygote), and a single band (298 bp) for 54A/A (homozygote). Bottom, B: Analysis by sequencing is presented for each genotype, 54G/G, 54G/A, and 54A/A.
sequencing agreed with genotyping by restriction fragment length polymorphism (RELP) [Fig 1, bottom, B], and no other mutation was detected (data not shown).

**Measurement of MBL Concentration by Enzyme-Linked Immunosorbent Assay**

MBL in sera was measured by a sandwich enzyme-linked immunosorbent assay (ELISA), as previously described. BALF specimens were obtained by three injections of 30 mL of saline solution. After the mucin components were removed by nylon mesh collation, the samples were centrifuged, and the supernatants were stored at −20°C until use. MBL in the BALF samples was directly measured by ELISA.

**Statistical Analysis**

Statistical analysis was performed by χ² test, and differences were regarded as statistically significant at p < 0.05 (Stat View; SAS Institute; Cary, NC).

**RESULTS**

To examine whether MBL participates in pulmonary defense mechanisms, we measured the concentration of MBL protein in the BALF samples from five patients with pneumonia and five healthy volunteers by ELISA. MBL protein was obviously detected in the BALF samples from patients with pneumonia. The BALF MBL ranged from 0.011 to 0.078 mg/mL (Table 1). In contrast, no MBL protein was detected in the BALF samples from the five healthy adults, indicating that MBL protein is absent in the normal alveolar space. In addition, we assessed the MBL genotype in patients with repeated respiratory infections by Ban1 RELP analysis. As a result, 30 of the 62 patients (48.4%) were found to show the homozygous variant at codon 54 of the MBL gene (54G/A), and 4 patients (6.5%) showed a homozygous variant at codon 54 (54A/A) [Table 2]. The 54G/A and 54A/A genotypes were associated with a low serum concentration of MBL, and the frequency of the low MBL variants (54G/A and 54A/A) among these patients was significantly higher compared to the control subjects (54.8% and 32.0%, respectively; p < 0.05). No other mutations in codon 52 or 57 were found in these Japanese individuals. These results indicated that BALF MBL plays an important role in the pulmonary defense system, and that the variants of the MBL gene are related to predisposition to pulmonary infectious diseases.

To further clarify how MBL participates in the pulmonary defense system, we focused on the potential role of MBL in several kinds of pulmonary infections. We assessed the prevalence of MBL genotype in DPB and chronic pulmonary infections, such as those caused by nontuberculous mycobacteria (NTM) and Aspergillus. Table 3 shows that, in patients with DPB, the frequency of the low MBL variant (54G/A and 54A/A) in patients with DPB was statistically higher compared with controls (51.0% and 32.7%, respectively; p < 0.05) (Table 3). On the other hand, the frequency of the low MBL variants in the patients with chronic infections caused by NTM or Aspergillus was not significant (44.0% and 34.8%, respectively).

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**Table 1—MBL Concentration in BALF Samples**

<table>
<thead>
<tr>
<th>No.</th>
<th>MBL, ng/mL</th>
<th>Status of Lung for BAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>78.0</td>
<td>P carinii pneumonia</td>
</tr>
<tr>
<td>2</td>
<td>11.0</td>
<td>P carinii pneumonia</td>
</tr>
<tr>
<td>3</td>
<td>29.0</td>
<td>Bacterial pneumonia</td>
</tr>
<tr>
<td>4</td>
<td>19.0</td>
<td>Bacterial pneumonia</td>
</tr>
<tr>
<td>5</td>
<td>11.0</td>
<td>Bacterial pneumonia</td>
</tr>
<tr>
<td>6</td>
<td>ND</td>
<td>Normal volunteer</td>
</tr>
<tr>
<td>7</td>
<td>ND</td>
<td>Normal volunteer</td>
</tr>
<tr>
<td>8</td>
<td>ND</td>
<td>Normal volunteer</td>
</tr>
<tr>
<td>9</td>
<td>ND</td>
<td>Normal volunteer</td>
</tr>
<tr>
<td>10</td>
<td>ND</td>
<td>Normal volunteer</td>
</tr>
</tbody>
</table>

*BALF samples were not concentrated. ND = not determined.

**Table 2—Frequency of the MBL Genotype in Patients With Recurrent Pulmonary Infections and Healthy Control Subjects**

<table>
<thead>
<tr>
<th>MBL Genotype</th>
<th>Serum Level</th>
<th>Patients (n = 62)</th>
<th>Control Subjects (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>54 G/G</td>
<td>Normal</td>
<td>28 (45.1)</td>
<td>35 (67.3)</td>
</tr>
<tr>
<td>54 G/A</td>
<td>Low</td>
<td>30 (48.4)</td>
<td>12 (23.1)</td>
</tr>
<tr>
<td>54 A/A</td>
<td>Low</td>
<td>4 (6.5)</td>
<td>5 (9.6)</td>
</tr>
<tr>
<td>Low MBL variant</td>
<td>54 G or A/A</td>
<td>34 (54.9)†</td>
<td>17 (32.7)</td>
</tr>
</tbody>
</table>

*Data are presented as No. (%) unless otherwise indicated. †Significant, p = 0.003.

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**Table 3—Frequency of the MBL Genotype in Patients With NTM, Aspergillus, and DPB**

<table>
<thead>
<tr>
<th>MBL Genotype</th>
<th>DPB (n = 49)</th>
<th>NTM (n = 50)</th>
<th>Aspergillus (n = 23)</th>
<th>Control Subjects (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>54 G/G</td>
<td>24 (49.0)†</td>
<td>28 (56.0)†</td>
<td>15 (65.2)</td>
<td>35 (67.3)</td>
</tr>
<tr>
<td>54 G/A</td>
<td>22 (44.9)†</td>
<td>21 (42.0)†</td>
<td>7 (30.4)</td>
<td>12 (23.1)</td>
</tr>
<tr>
<td>54 A/A</td>
<td>3 (6.1) §</td>
<td>1 (2.0) §</td>
<td>1 (4.3)</td>
<td>5 (9.6)</td>
</tr>
<tr>
<td>Low MBL variant</td>
<td>25 (51.0)†</td>
<td>22 (44.0)†</td>
<td>8 (34.7) †</td>
<td>17 (32.7)</td>
</tr>
</tbody>
</table>

*Data are presented as No. (%) unless otherwise indicated. †Significant, p = 0.15 vs control subjects. §Significant, p = 0.08 vs control subjects.
**Discussion**

We specifically evaluated the role of MBL against respiratory tract infection. MBL is a calcium-dependent C-type lectin and belongs to the collectin family, which includes pulmonary surfactant proteins A and D. The pulmonary collectins, surfactant proteins A and D, have a remarkable role in the defense of the respiratory tract since they can bind to a wide variety of pathogens, and thus effectively opsonize or aggregate these microbes. In contrast, the role of MBL has not been fully examined. In this study, we first demonstrated that MBL can be directly measured in BALF from patients with acute-phase pneumonia, suggesting that MBL, produced by the liver as one of the acute phase proteins may leak from the systemic circulation at the site of inflammation in the lungs, and work there. A number of serum proteins collectively termed acute-phase proteins show a dramatic increase as a result of inflammation of the lungs, and these components, including MBL, may provide an optional host defense mechanism. Interestingly, in contrast to surfactant proteins, MBL activates the complement cascade via specific serine proteases, termed MBL-associated serine proteases 1 and 2. These series of interactions have been recognized as the third pathway of complement activation, termed the lectin pathway. This suggests that MBL may assume a critical role at sites of pulmonary infection.

In addition, we showed that the codon 54 variant of the MBL gene is one of the risk factors for repeated pulmonary infections in patients without any apparent immunologic disorders. The human MBL gene is located on chromosome 10 at q11.2-q21 and consists of four exons. The MBL levels in serum are genetically determined, and three major variants, located at codons 52, 54, and 57 of exon 1 of the MBL gene, are associated with a low serum concentration of MBL. The codon 54 variant is significantly common in Asia, and it is present in a higher compared to codons 52 and 57 in the Japanese population. Interestingly, no other variant in codon 52 or 57 was found in our study, similar to the results of previous studies. However, two substitutional variants on the promoter region of the MBL gene (H/L in codon −550 and X/Y in codon −221) have been reported, and a variant allele in codon −221 (X variant) has been shown to have a significant down regulating effect on the serum concentration of MBL. Our preliminary examination revealed that the frequency of the X variant in the Japanese population is relatively low (0.07) [data not shown], which was in agreement with previous reports. Thus, the serum MBL level in the Japanese population is mainly affected by polymorphism at codon 54 on exon 1 of the MBL gene.

Recently, Garred et al. reported that the presence of MBL variant alleles in patients with cystic fibrosis was associated with a poor prognosis, and that the predicted annual survival rate of those bearing the alleles was reduced by approximately 8 years, when compared with patients with normal MBL alleles. DPB is a chronic recurrent infection of the lower respiratory tract common among Japanese people and characterized by a persistent Pseudomonas aeruginosa infection in the late stage. There are remarkable similarities between DPB and cystic fibrosis, with severe repeated pulmonary infections following early destruction of the lung. We have found that the frequency of the variant allele of MBL gene in patients with DPB was higher than that in healthy control subjects. This result explains why MBL gene polymorphism is one of the risk factors for recurrent infections in patients with DPB and patients with cystic fibrosis. Cystic fibrosis transmembrane regulator gene (ΔF508) is strongly associated with a poor prognosis due to repeated infections by opportunistic bacteria. Although MBL is not the sole determinant of infection, a low MBL concentration likely represents a persistent disadvantage in the long term.

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