Mannose-Binding Lectin Concentrations, MBL2 Polymorphisms, and Susceptibility to Respiratory Tract Infections in Young Men

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Background. Mannose-binding lectin (MBL) is an important component of innate immunity, and its deficiency is associated with susceptibility to recurrent infections.

Methods. This exploratory study investigated the association of serum MBL concentrations and MBL2 gene polymorphisms with respiratory tract infections in young men. We genotyped 6 single-nucleotide polymorphisms (SNPs) in the promoter region (alleles H/L, X/Y, and P/Q) and exon 1 (variant alleles B, C, and D and wild-type allele A) of the MBL2 gene by real-time polymerase chain reaction and measured serum MBL concentrations in 111 Finnish military recruits with asthma and 362 without.

Results. An MBL level below the median concentration was a significant risk factor for infections (asthma status–adjusted odds ratio [OR], 2.5 [95% confidence interval {CI}, 1.4 – 4.5]). Among the 6 SNPs, there was a significant association between the promoter Y/Y genotype and infections (OR, 2.3 [95% CI, 1.2– 4.4]) and a borderline significant association between exon 1 variant alleles and infections (OR, 1.7 [95% CI, 0.9 –3.1]), after adjustment for asthma status.

Conclusion. These preliminary results suggest, for the first time, an association between MBL level and respiratory tract infections in young men and a possible association between infections and MBL2 polymorphisms.

Mannose-binding lectin (MBL) is an important serum protein of the innate immune response. MBL protein selectively recognizes the patterns of carbohydrates of infectious agents or infected cells and then opsonizes antigens and activates the lectin pathway of the complement system [1]. Complement activation results in opsonization of microorganisms and induction of inflammatory reactions.

There are 2 human MBL genes, but MBL1 is a pseudogene and only MBL2 (on 10q21.1) encodes a protein product. Previous studies have indicated that the 6 known single-nucleotide polymorphisms (SNPs) of MBL2 have a major effect on MBL protein structure and serum concentration. Three of these SNPs are located in exon 1 at codons 52 (CGT→TGT) [2], 54 (GGC→GAC) [3], and 57 (GGA→GAA) [4]. The wild-type alleles of these polymorphisms are designated as A, and the other alleles are known as D, B, and C, respectively. There are also 2 important SNPs in the 5′ regulatory region at positions −550 (alleles H and L) and −221 (alleles X and Y) and 1 in the 5′ untranslated region at position +4 (alleles P and Q) (table 1) [2, 5].

MBL insufficiency caused by polymorphisms in the MBL2 gene has been reported to lead to a defect in opsonization [6], which has been associated with susceptibility to recurrent infections, especially among children [6–8], but the association has also been reported in adults [9, 10]. There have been discussions on whether MBL deficiency increases susceptibility to infectious agents generally or whether it is a significant risk factor only for those who also have some other defect in im-
munity [1, 11]. Asthma is a chronic inflammatory disease of the airways that results from an abnormal immune response to antigens. We wanted to study the association of MBL2 genotypes and serum MBL concentrations with respiratory tract infections in young men with asthma and those without.

METHODS

Study participants and specimens. The study population included 111 military recruits with asthma and 362 without asthma from the January 2005 intake group (n = 1861) for compulsory military service in the Kajaani Garrison, Kainuu Brigade, in northern Finland. All of the men with medically diagnosed asthma in this intake group were invited to participate. Randomly chosen men were invited after the men with asthma had been excluded from the intake group. Twenty-five percent of all of the men declined to participate, and 3% were discharged because of health problems. All of the men who agreed to participate in the study signed an informed consent form. Serum samples were obtained at the beginning and at the end of their service, and paired serum samples were obtained for each infectious episode. More than half (60.0%) of the participants served 6 months (55% of those with asthma and 62% of those without), 7.0% served 9 months, 25.4% served 12 months, and 7.6% dropped out. The ages of the participants ranged from 17.4 to 26.3 years (median, 19.6 years). The study participants were Finns, and 100% were of white European ancestry. The study protocol was approved by the Medical Ethics Committee of the Kainuu Central Hospital. This study population was a part of a larger series [12]. The sample size was not determined for the purposes of genetic analysis; therefore, this is an exploratory study.

Blood samples were collected, and leukocytes and serum were separated and stored at −70°C or −20°C, respectively, for later analysis. The leukocytes were homogenized with 400 μL of homogenization buffer (50 mmol/L Tris-HCl, 10 mmol/L NaCl, 50 mmol/L EDTA, and 1% sodium dodecyl sulfate [pH 8.0]) and 20 μL of proteinase K (Sigma-Aldrich) 3 times at 4 m/s for 20 s. DNA was isolated from the entire homogenate with a MagNA Pure LightCycler instrument (Roche Diagnostics) using a Large Volume Kit (Roche Diagnostics) by first following the DNALVBlood30_500 protocol and later the DNACVcells protocol. DNA was eluted with 200 μL of elution buffer. DNA concentrations were measured in the samples with a LightCycler instrument using a Quant-it PicoGreen dsDNA Assay Kit (Invitrogen–Molecular Probes). λ-DNA (100 μg/mL) was used as a standard at concentrations of 2, 0.2, 0.02, and 0.002 μg/mL. The DNA was stored at −20°C.

Genotyping by real-time polymerase chain reaction (PCR). Genotyping with the LightCycler Instrument (Roche Diagnostics) was performed as described elsewhere [13], with slight modifications in the X/Y probe design and the PCR conditions for the promoter region. The X allele–specific X/Y detection probe 5'-TCTCAGTGCACCAGAAACAT-3'-FLU was used, and 0.2 μmol/L each of the detection and anchor probes were used in X/Y and P/Q genotyping. To verify the exon 1 genotypes, samples representing different genotypes were sequenced by Macrogen. In addition, known controls were analyzed together with the samples in each genotyping assay.

Serum MBL concentrations. The MBL concentrations of the serum samples obtained at the beginning of service were measured using a human MBL (lectin assay) ELISA kit (Hycult Biotechnology) according to the manufacturer’s instructions. Absorption at 450 nm was measured with a Multiscan Ascent plate reader (Thermo Electron Corporation). A standard curve was created, and the concentrations were defined using Ascent software (version 2.6; Thermo Electron Corporation).

Data on respiratory tract infections. Infections (bacterial or viral) were determined by means of a combination of respiratory tract symptoms, physical findings, and, if necessary, laboratory and radiological examination findings. The conscripts with acute or acutely aggravated respiratory tract symptoms were checked by a nurse and examined by a physician, if necessary, in the military primary care clinic in the Kainuu Brigade. The episode was included in our database if a respiratory tract infection was diagnosed by the physician. Mild episodes not referred to a physician were not included in our analyses. Consultations within 2 weeks of each other were considered to constitute a single episode. Symptoms, clinical findings, and drug prescriptions were recorded. The statistical analysis of infectious episodes was done in the group of men in service for 180 days (n = 283; 61 with asthma and 222 without), because the men with 270- or 362-day service were partly trained in other units in Finland, and therefore not all of their infectious episodes may have been included in our analysis. This study group included only the men in 180-day service who completed the follow-up.

Statistical analyses.XA, YA, and YO haplotypes were re-constructed with the statistical PHASE program described by Stephens et al. [14]. The current version (version 2.1; code by Stephens; 2004) was used, with some extensions to the original method. Statistical analyses were done using SPSS software (ver-

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### Table 1. Locations and alleles of the 6 MBL2 variants.

<table>
<thead>
<tr>
<th>dbSNP no.</th>
<th>SNP location</th>
<th>Alleles</th>
<th>Allele names</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs5030737</td>
<td>Exon 1 codon 52</td>
<td>C/T</td>
<td>A/Q</td>
</tr>
<tr>
<td>rs1800450</td>
<td>Exon 1 codon 54</td>
<td>G/A</td>
<td>A/B</td>
</tr>
<tr>
<td>rs7096206</td>
<td>Promoter −221</td>
<td>C/G</td>
<td>X/Y</td>
</tr>
<tr>
<td>rs1103125</td>
<td>Promoter −550</td>
<td>G/C</td>
<td>H/L</td>
</tr>
<tr>
<td>rs7095891</td>
<td>5'UTR +4</td>
<td>C/T</td>
<td>P/Q</td>
</tr>
</tbody>
</table>

**NOTE.** SNP, single-nucleotide polymorphism; UTR, untranslated region.
levels; and homozygous variant genotypes B/C, B/B, B/D, and the L/L with low (586 ng/mL) MBL levels (P values were corrected using the Benjamini-Hochberg procedure (P_corrected), which considered the number of comparisons, of which 5 were in associations for MBL level and 7 were in associations for infection [15]. Odds ratios (ORs), adjusted for asthma status, with 95% confidence intervals [CIs]) were estimated by logistic regression analysis.

RESULTS

MBL2 genotype and MBL concentration. The serum MBL concentrations of the whole study group were available from 110 subjects with asthma and 362 without. The median MBL level was 1087.5 ng/mL (interquartile range, 454.8–2068.5 ng/mL; range, 0–7342.0 ng/mL), and 0.6% of the men had an MBL level of 0 ng/mL. There was no difference between the median MBL levels of those with asthma and those without (P = .950; P_corrected = .950).

The MBL2 genotype and MBL concentration distributions are shown in table 2 and in figure 1, respectively (H/L data were available from 469, X/Y data from 470, P/Q data from 471, and exon 1 genotype data from 472 participants). There was no difference between the genotype distributions of subjects with asthma and those without (table 2). Allele frequencies in the combined study population were in Hardy-Weinberg equilibrium. The H/H genotype was associated with high (median MBL level, 1594 ng/mL), the H/L with intermediate (1121 ng/mL), and the L/L with low (586 ng/mL) MBL levels (P < .001; P_corrected < .001). All of the X/X (median MBL level, 707 ng/mL), X/Y (976 ng/mL), and Y/Y (1131 ng/mL) genotypes were associated with intermediate MBL levels (P = .157; P_corrected = .188). The P/P genotype was associated with intermediate (median MBL level, 928 ng/mL), the P/Q with high (1267 ng/mL), and the Q/Q with very high (1716 ng/mL) concentrations (P = .004; P_corrected = .006). The wild-type MBL2 exon 1 genotype A/A was associated with high MBL levels; heterozygous variant allele genotypes A/D, A/B, and A/C with low levels; and homozygous variant genotypes B/C, B/B, B/D, and D/D with almost undetectable MBL levels (P < .001; P_corrected < .001). To further analyze this phenomenon, the structural genotypes were categorized as A/A, A/O, and O/O genotype groups, where O stands for any of the exon 1 variant alleles B, C, or D. A significant difference was seen between these genotype groups (median MBL concentrations, 1646, 329, and 2 ng/mL, respectively; P < .001; P_corrected < .001) (figure 1).

Table 2. Frequencies of the MBL2 genotypes in the total population, in those with asthma, and in those without asthma.

<table>
<thead>
<tr>
<th>MBL2 genotype</th>
<th>Total population (n = 473)</th>
<th>With asthma (n = 111)</th>
<th>Without asthma (n = 362)</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>H and L alleles</td>
<td>71.5</td>
<td>67.6</td>
<td>72.8</td>
<td>.501</td>
</tr>
<tr>
<td>X and Y alleles</td>
<td>24.6</td>
<td>28.8</td>
<td>23.3</td>
<td>.001</td>
</tr>
<tr>
<td>P and Q alleles</td>
<td>3.8</td>
<td>3.6</td>
<td>3.9</td>
<td>.778</td>
</tr>
<tr>
<td>A–D alleles</td>
<td>64.8</td>
<td>64.9</td>
<td>64.8</td>
<td>.977</td>
</tr>
<tr>
<td>A/O</td>
<td>30.3</td>
<td>30.6</td>
<td>30.2</td>
<td>.50</td>
</tr>
<tr>
<td>A/A</td>
<td>64.8</td>
<td>64.9</td>
<td>64.8</td>
<td>.977</td>
</tr>
<tr>
<td>A/O</td>
<td>30.3</td>
<td>30.6</td>
<td>30.2</td>
<td>.50</td>
</tr>
<tr>
<td>A/A</td>
<td>64.8</td>
<td>64.9</td>
<td>64.8</td>
<td>.977</td>
</tr>
</tbody>
</table>

<sup>a</sup> Unless otherwise indicated, P values were determined by the Pearson χ<sup>2</sup> test for the comparison between those with asthma and those without.

<sup>b</sup> Fisher’s exact test.

<sup>c</sup> O stands for any of the exon 1 variant alleles B, C, or D.

MBL and respiratory tract infections. We studied the association of respiratory tract infections with asthmatic status, MBL levels, and MBL2 genotypes among men in 180-day service. Two hundred eighty-three men in 180-day service experienced a total of 269 episodes, 87 in those with asthma and 182 in those without. The conscripts with at least 2 infectious episodes were compared with those with 0–1 infections, because the number of cases with 3–5 infectious episodes was low. A comparison of subjects with asthma and those without revealed significantly more subjects with asthma than control subjects having ≥2 episodes of infection during their 180-day service (38% vs. 19%, respectively; P = .001; P_corrected = .007).

MBL levels were categorized into 2 groups, using a median MBL concentration of 1087.5 ng/mL as a cutoff. Significantly more conscripts with MBL concentrations under the median MBL level had at least 2 infectious episodes during their service (30% vs. 16%, respectively; P = .003; P_corrected = .011) com-
pared with those with MBL levels over the median. In addition, in a binary logistic regression analysis, an MBL level below the median proved to be a significant risk factor for infections: the OR was 2.4 (95% CI, 1.3–4.2) without adjustment and 2.5 (95% CI, 1.4–4.5) with adjustment for asthma status (those with asthma vs. those without).

Because there was no difference between the genotype distributions of subjects with asthma and those without, the associations between infections and genotypes could be considered in both sample types together. The association between infections and exon 1 genotypes was analyzed between the high-MBL-producing wild-type genotype (A/A) and the low-MBL-producing combined heterozygous and homozygous variant allele genotype groups (A/O + O/O) (table 3). Twenty-eight percent of those who had the low-producing A/O or O/O genotype and only 21% of those with the high-producing A/A genotype had ≥2 infections ($P = .190; P_{corrected} = .266$). In further analysis, the conscripts with the A/O or O/O genotype had a borderline significant risk of infections after adjustment for asthma status (OR, 1.7 [95% CI, 0.9–3.1]) (table 3). Analyzing the dependence between the MBL concentration groups, <1087.5 and >1087.5 ng/mL, and the MBL2 structural geno-

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**Figure 1.** Mannose-binding lectin (MBL) concentrations for the different MBL2 genotypes and the exon 1 genotype groups. A refers to the wild-type allele, and O refers to any of the exon 1 variant alleles B, C, or D. The statistical differences between the genotypes were analyzed by the Kruskal-Wallis test. The nos. of genotypes are shown.
type groups, A/A and A/O + O/O, we found that as many as 94% of those with the A/O or O/O genotype and 26% of those with the A/A genotype had MBL levels below the median (P < .001; data not shown).

Twenty-eight percent of the conscripts with the Y/Y genotype and 15% of those with the X/X or X/Y genotype had ≥2 infections during their service (P = .013; P_corrected = .025) (table 3). Furthermore, the risk was up to 2.3-fold when asthma status was adjusted for. To study the combined effect of the high-risk SNPs, the Y/Y genotype, and the O allele, we analyzed the infection episodes in the genotype groups YA/YO + YO/YO and XA/XA + XA/YA. The YA/YA and XA/YO groups were omitted from this analysis. The difference was significant (30% vs. 14%, respectively; P = .014; P_corrected = .025), and the risk was up to 3.4-fold with adjustment for asthma status (table 3). The interaction terms between MBL2 genotypes or MBL levels and asthma status were not statistically significant.

**DISCUSSION**

We investigated the associations between MBL levels, MBL2 gene polymorphisms, and respiratory tract infections in young men and demonstrated that a below-median MBL level is a significant risk factor for infections. In the earlier studies byAITONIEMI ET AL. [11] and THORARINSDOTTIR ET AL. [16], MBL serum levels did not differ between children with repeated respiratory tract infections and healthy individuals. However, Thorarinsdottir et al. found that sustained low levels of MBL were associated with recurrent otitis media.

Among the MBL2 structural genotype groups, there were more men with the A/O or O/O genotype than the A/A genotype who had at least 2 infectious episodes during their service, although this difference was not significant. However, there was a borderline significant infection risk of 1.7-fold for men with the A/O or O/O genotype after adjustment for asthma status. Furthermore, we demonstrated a clear association between the groups with high and low MBL concentrations (below or above the median) and the MBL2 structural genotype groups A/A and A/O + O/O, suggesting that the low-producing MBL2 exon 1 genotypes should also affect susceptibility to respiratory tract infections. It seems that there may be an association between exon 1 variant alleles and respiratory tract infections, but, evidently due to the small sample size in the different groups, a significant association was not found.

The analysis of the association between the promoter variant and infection episodes showed that the Y/Y genotype was a significant risk factor for infections. Furthermore, the Y/Y genotype and the exon 1 variant alleles seemed to have a possible combined effect on respiratory tract infections. There is proba-
bly some interaction between the Y allele and the exon 1 variant alleles, but this requires further study.

As far as we know, there have been 2 studies on the association between MBL2 polymorphisms and respiratory tract infections among adults, with contradictory results [17, 18]. Gomi et al. [17] found the structural variant allele B to be a risk factor for recurrent respiratory tract infections among adult patients in a hospital-based study. On the other hand, Dahl et al. [18] found no association between MBL2 polymorphisms and respiratory tract infections among adults in a large population-based study. There have been several studies of MBL2 variants and respiratory tract infection susceptibility among children, and most of them identified the A/O and O/O genotypes as risk factors for infections [7, 8, 19].

Earlier studies have shown that the promoter polymorphism X/Y and 3 exon 1 polymorphisms (variant alleles D, B, and C) of the MBL2 gene have an effect on MBL protein levels. The X allele has been described to have a potent decreasing effect on MBL levels [5, 20]. In the present study, the results suggested that the Y/Y genotype would be associated with higher MBL levels than the X/Y and X/X genotypes, but the difference was not significant. The structural variant alleles seemed to have larger effect on MBL levels. The wild-type genotype A/A correlated with very high serum MBL levels, the heterozygous variant genotype A/O correlated with intermediate levels, and subjects with the homozygous variant genotype O/O had almost undetectable MBL levels. This kind of distribution has been reported previously [5, 20, 21].

In Finland, all men aged 18–19 years are called up for compulsory military service. Ninety-eight percent of them attend a call-up examination to establish their fitness for military service [22], and 80%–85% of all men, including >80% of men with asthma, complete their service [23]. Thus, we can consider that military conscripts represent well the young Finnish male population. Respiratory tract infections are a major cause of sick days during military service. Military trainees are at a high risk of respiratory epidemics because of their crowded living conditions, stressful working environment, frequent travel, and exposure to novel strains of respiratory pathogens [24]. Training during the winter months, when most viral epidemics occur, further predisposes military recruits to respiratory tract infections. The living conditions in military service could be compared with kindergartens, where infections spread easily. The high infection load present during military service may help us easily pick out the persons most susceptible to infection and analyze more easily the potential association between MBL genotypes and infections. The present study design also allowed a relatively high number of infectious episodes to be included, and therefore this study population provided us with a good opportunity to investigate genetic susceptibility to respiratory tract infections. On the other hand, this study population is quite small, and therefore prospective studies in other populations are needed.

This study included military recruits with and without asthma from the January intake group, because wintertime is favorable to respiratory epidemics. We showed that conscripts with asthma were more prone to respiratory tract infections than were those without asthma during 180-day military service. This was also shown by Juvonen et al. [12], who studied the risk factors for acute respiratory tract illness among military recruits in 180-day service in the Kajaani Garrison between July 2004 and July 2005 (our study population is part of this series). It is well known that people with asthma are more susceptible to respiratory infections than are people without asthma and that respiratory tract infections are important inducers of asthma exacerbations [25, 26]. MBL deficiency has been shown to predispose especially immunocompromised patients [27–29] and those with some immunodeficiency to infections [11, 30]. Yang et al. [29] showed that the low-MBL-producing MBL2 codon 54 B allele was associated with infectious exacerbations of chronic obstructive pulmonary disease. Attoniemi et al. [11] found MBL deficiency to be a potentially significant risk factor for infections only in association with another form of humoral immunodeficiency. To our knowledge, there are no studies of the association between MBL deficiency and susceptibility to infection in those with asthma. The immune response of the respiratory tract is weaker in persons with asthma, because continuous inflammation makes the respiratory epithelium more vulnerable to bacterial and viral infections [31] and might thus be considered a kind of immunocompromised condition. In our study group, we found that the effect of MBL deficiency is not dependent on asthma status; on the other hand, the persons with the most serious asthma cases were already eliminated on arrival into military service, and the number of subjects with asthma was also quite low (61/283).

Previous studies of the association between MBL2 variants and respiratory tract infections have included patients or immunodeficient subjects with severe respiratory tract infections requiring hospitalization or antibiotic treatment. Our study included Finnish military recruits, who represent a healthy and young Finnish population. They experienced mainly moderately severe respiratory tract infections requiring consultation by a physician. Thus, our findings suggest that low MBL levels and MBL2 polymorphisms predispose not only to serious infections, especially in immunodeficient patients, but also to common respiratory infections in otherwise healthy people.

We studied for the first time the association between MBL levels and MBL2 polymorphisms with susceptibility to respiratory tract infections among young men with or without asthma who were otherwise healthy. We demonstrated, partly contrary to previous studies, that an MBL level below the median concentration was a significant risk factor for respiratory tract infections. In addition, we found that exon 1 variant alleles may affect susceptibility to respiratory infections in young men. Our findings support the role played by MBL in susceptibility to respiratory tract infections.
infections. However, a statistically significant association in a single study does not guarantee a genetic association; on the other hand, a lack of formal statistical significance does not exclude a possible association. Therefore, the present study, like genetic association studies in general, needs replication [32].

Acknowledgments

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References