Mannose binding lectin and FcγRIIa (CD32) polymorphism in Spanish systemic lupus erythematosus patients

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Abstract

Objective. Mannose binding lectin (MBL) and FcγRIIa (CD32) polymorphisms have both been implicated as candidate susceptibility genes in systemic lupus erythematosus (SLE). The aim of this study was to evaluate the relationship of these polymorphisms with SLE.

Methods. We studied a cohort of 125 SLE patients from Barcelona, Spain and 138 geographically matched controls. Sequence-specific primer–polymerase chain reaction (SSP–PCR) amplification was used to determine CD32 and MBL structural polymorphisms. MBL haplotypes were established using sequence-specific oligonucleotide probing techniques.

Results. Patients carried the MBL codon 54 mutant allele more frequently than controls [odds ratio (OR) 2.2; 95% confidence interval (CI) 1.2–4.0; P = 0.007] and the haplotype HY W52 W54 W57 was found to be significantly lower in cases compared with controls (OR 0.6; 95% CI 0.4–0.9; P = 0.016).

Conclusion. The MBL gene codon 54 mutant allele appears to be a risk factor for SLE, whilst haplotypes encoding for high levels of MBL are protective against the disease. Differences between controls and patients were not significant when considering the FcγRIIa polymorphisms; similar results were observed for renal affectation.

Key words: MBL, FcγRIIa, Polymorphism, SLE.

Systemic lupus erythematosus (SLE) is a complex autoimmune disease influenced by both genetic and environmental factors [1, 2]. Homozygosity for a number of complement and immunity-related genes has been implicated in the susceptibility to SLE. These include deficiencies of complement components such as C1q, C2, C4, [3–5], or mannose binding lectin (MBL) [6–9] and the abnormal distribution of FcγRIIa allotypes [10–14].

MBL is an acute-phase serum protein [15] of the innate immune system, which participates in complement activation and the opsonization of antigens. The protein coding region of the gene consists of four exons, and there are five known polymorphic sites within the MBL gene that are thought to affect the amount of protein in serum and have been associated with SLE. Two are situated within the promoter region of the MBL gene [H or L (G to C) at –550 and Y or X (G to C) at –221] [16]. The remaining three polymorphic sites are situated within exon 1 of the MBL gene at codon 52 (Arg to Cys) [17], codon 54 (Gly to Asp) [18] and codon 57 (Gly to Glu) [16]. A number of MBL alleles exist in linkage together. The codon 52 mutation is only found together with the promoter haplotype HY, whereas codon 54 and 57 mutant alleles are found to exist with LY [17].

Human immunoglobulin Fc receptors (FcR) on leucocytes exhibit considerable structural and functional diversity. Within the groups of receptors for IgG there
are three distinct subgroups; FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16), each containing several different members. The ‘A’ isofrom of FcγRII is the only receptor on human phagocytic cells that is capable of significant interaction with IgG2 [19]. Two isofroms of FcγRIIa are FcγRIIa R and FcγRIIa H; these differ by one amino acid at position 131 (Arg to His) which results from a single base substitution. The variant containing histidine at this position (FcγRIIa-H131) binds human IgG2, and IgG3, more strongly than the ‘low-affinity’ isofrom containing arginine (FcγRIIa-R131) [19]. Fcγ receptors play an essential role in the clearance of immune complexes. Impaired clearance and removal of immune complexes by the mononuclear phagocyte system can result in the deposition of immune complexes in organs and tissues. In SLE patients, the FcγRIIa-R131 allotype of CD32 receptor has been associated with renal disease; it was observed that there is an over-representation of FcγRIIa-R131 homozygosities in SLE patients with renal involvement, compared with patients homozygous for the FcγRIIa-H131 allele [13, 14].

We hypothesized that within our population of Spanish SLE patients there would be an over-representation of MBL mutant alleles and FcγRIIa alleles compared with controls. There would also be an increase in FcγRIIa allele frequency in patients with renal involvement compared with those without.

Materials and methods

One hundred and twenty-five SLE patients were recruited from the Vall d’Hebron Hospital in Barcelona, Spain. These were compared with an ethnically matched random healthy control population recruited from the same geographical region (n = 137). All patients satisfied the 1982 American College of Rheumatology revised criteria for SLE [20]. The clinical manifestations studied were renal involvement (n = 48), articular affection (n = 102), cutaneous lesions (n = 90), neurological disease (n = 20), and serositis (n = 45).

**MBL typing**

DNA was extracted from ethylene diamine tetraacetic acid (EDTA) blood samples using the DNAce MaxiBlood Purification System (Bioline). MBL codon 52, 54 and 57 wild and mutant alleles were detected using sequence-specific primer–polymerase chain reactions (SSP–PCR) as previously described by Crosdale et al. [21]. In total, 1042 bp of the promoter and the majority of exon 1 of the MBL gene were amplified. A 464 bp segment of the gene for human growth hormone was amplified as an internal control for each PCR reaction. SSP–PCR reactions were performed on each sample, both utilizing a common primer set amplified 256 bases of the MBL gene and promoter polymorphisms are summarised in Table 2. The allele frequencies of the structurally encoding wild alleles as determined by SSP–PCR and being positive for X, Y, L and H polymorphisms. In such a situation, it was impossible to determine the cis/trans orientation of the promoter alleles. This was resolved by performing further SSP–PCR reactions with H/L forward primers (5’ to 3’) and X/Y reverse primers as described by Crosdale et al. [21].

A number of samples were cloned and sequenced (MBL gene exon 1 and promoter region) to provide control material for this study.

**FcγRIIa typing**

Each sample was typed for the presence of FcγRIIa-H and FcγRIIa-R alleles using the method and primers described by Smyth et al. [23]. Two PCR reactions were performed on each sample, both utilizing a common anti-sense primer located downstream of the H/R polymorphism and one of the two allele-specific primers. A second amplification was also made between the anti-sense primer and a common sense primer situated upstream of the H/R polymorphism as an internal control. The control primers amplified 256 bases of the FcγRIIa gene, whilst the H/R-specific reaction amplified 224 bases.

**Statistical analysis**

χ² analysis (using Yates’ correction, where applicable) was performed to determine the significance of a frequency difference between the two groups (significance level of 5%). Odds ratios (OR) were calculated for significant associations and expressed with 95% confidence intervals (CI). An OR was considered to be significant if the 95% CI did not include 1.0.

**Results**

The allele frequencies of the structurally encoding MBL gene and promoter polymorphisms are summarized in Table 1. There was a significant increase in the frequency of −550 L allele in the patient group compared with the control population (OR 1.5; 95% CI 1.0–2.1; P = 0.039) and also in the number of patients possessing the codon 54 mutant allele compared with controls (OR 2.2; 95% CI 1.2–4.0; P = 0.007). The codon 57 mutant allele frequency was also increased in the patient group, but this result did not reach statistical significance.

MBL haplotype frequencies for both the SLE and control populations are shown in Table 2. The frequency of the HY W52 W54 W57 haplotype
TABLE 1. Allele (promoter variants) and phenotype (structurally encoding) frequencies (%) of MBL polymorphisms in Spanish SLE patients and controls

<table>
<thead>
<tr>
<th>Allele</th>
<th>Patients (n = 125)</th>
<th>Controls (n = 138)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−550 H/L</td>
<td>H</td>
<td>30.4</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>69.6</td>
</tr>
<tr>
<td>−221 X/Y</td>
<td>X</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td>Y</td>
<td>85.4</td>
</tr>
<tr>
<td>Codon 52</td>
<td>W</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>10.8</td>
</tr>
<tr>
<td>Codon 54</td>
<td>W</td>
<td>97.5</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>2.5</td>
</tr>
<tr>
<td>Codon 57</td>
<td>W</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>5</td>
</tr>
</tbody>
</table>

W, wild type; M, mutant or dysfunctional allele.

OR 1.5; 95% CI 1.0–2.1; P = 0.039.

OR 2.2; 95% CI 1.2–4.0; P = 0.007.

TABLE 2. MBL haplotype frequencies (%) in SLE patients and controls

<table>
<thead>
<tr>
<th>MBL haplotype</th>
<th>Patients (n = 125)</th>
<th>Controls (n = 138)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HY W52 W54 W57</td>
<td>23.3</td>
<td>32.9</td>
</tr>
<tr>
<td>LY W52 W54 W57</td>
<td>40.4</td>
<td>32.2</td>
</tr>
<tr>
<td>LX W52 W54 W57</td>
<td>14.2</td>
<td>18.5</td>
</tr>
<tr>
<td>HY M52 W54 W57</td>
<td>5.4</td>
<td>6.2</td>
</tr>
<tr>
<td>LY W52 M54 W57</td>
<td>14.2</td>
<td>9.1</td>
</tr>
<tr>
<td>LY W52 W54 M57</td>
<td>2.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

OR 0.6; 95% CI 0.4–0.9; P = 0.016.

was significantly decreased in the patient population (23.3%) compared with the control group (32.9%) (OR 0.6; 95% CI 0.4–0.9; P = 0.016). Haplotypes containing structurally encoding mutant alleles were generally of increased frequency in the SLE population, compared with controls.

With regard to FcγRIIa, no differences were found between patients (H = 41.3%, R = 58.7%) and controls (H = 42.3%, R = 57.7%). An increased genotype frequency of R/R homozygotes was seen in SLE patients with renal involvement (32.7%) compared with those without renal involvement (22.1%). There was also an increase in the R allele frequency in those patients with renal involvement (63.3%) compared with those without (55.8%). However, both of these findings failed to reach statistical significance. Similar results were observed when considering cutaneous affection, neurological disorders, arthritis or serositis.

Possession of both FcγRIIa-R131 and any structurally encoding MBL mutant allele was increased within the SLE population (35.3%) compared with controls (25.0%). Possession of MBL codon 54 mutant alleles together with FcγRIIa-R131 alleles also showed an overall increase from 14.0% in the control group to 23.2% in the patients. Both of these differences, however, failed to reach statistical significance.

Discussion

Previous studies have shown MBL gene mutations at codons 54 and 57 as being additive risk factors for susceptibility to SLE in different populations [6–9]. In this study we found an increased phenotypic frequency of codon 54 and 57 mutant alleles in SLE patients compared with controls. Nevertheless, the increase observed for codon 57 mutant alleles was not sufficiently high within this sample size to reach significance, which may reflect the rarity of the codon 57 mutant allele in populations of Spanish descent.

The MBL haplotype distribution within our control population was consistent with those of previous studies [22]. Codon 52 mutant alleles were found to be in linkage disequilibrium with HY promoter polymorphisms and both codon 54 and 57 mutant alleles carried only LY promoter alleles on their haplotypes. We hypothesized that MBL haplotypes encoding for low-level production of the protein would be more prevalent within an SLE population compared with an ethnically matched control population. Our results suggest that this is indeed the case.

There was an increase in the frequency of the intermediate-level MBL-producing haplotype LY W52 W54 W57 within the SLE population compared with controls and a significant increase in the high-level MBL-producing HY W52 W54 W57 haplotype frequency within the control group. This finding provides evidence that MBL haplotypes encoding for high serum levels of the protein are protective against the development of SLE. The protective nature of the high serum level-producing MBL haplotypes may become more apparent during an acute-phase response when baseline levels of MBL can increase up to 4-fold [24].

A significantly increased number of codon 54 mutant alleles was observed when comparing SLE patients with renal disease (18/90 alleles, 20.0%) with those without (15/150 alleles, 10.0%) (OR 2.3; 95% CI 1.1–4.7; P = 0.029). The codon 54 mutant allele therefore appears to be acting as a susceptibility factor for the development of renal disease in patients with SLE. Heterozygotes for the MBL codon 54 mutation have approximately one-eighth of the serum protein concentration they would have if encoded by wild alleles [18]. This would account for a reduction in immune complex clearance and complement activation in these individuals, which could ultimately lead to increased susceptibility to renal disease in SLE patients. FcγRIIa-R131 homozygosity has previously been associated with renal involvement in SLE patients [13]. In this study, we found no differences between R/R homozygosity in SLE patients compared with controls. This result, together with the R allele frequencies that are approximately equal between populations, suggests that there are no differences in IgG3 and IgG1 immune complex clearance between patients and controls and that the FcγRIIa-R131 allele is not a susceptibility factor to the development of SLE. However, a 10% increase in FcγRIIa-R131 homozygosity was observed...
in patients with renal disease. This finding, although not significant, suggests decreased levels of IgG2 and IgG3 immune complex clearance in patients with renal disease. A lack of immune complex clearance could result in their accumulation within the blood, especially within densely packed capillary areas such as the kidneys, possibly resulting in the development or onset of renal disease.

We analysed our data to look for any differences in the frequencies of both FcγRIIa and MBL structural mutant alleles between the SLE population and controls. Increases were seen in the frequencies of both codon 54 mutant and FcγRIIa-R131 alleles in the SLE population. These results approached statistical significance and therefore suggest that within our SLE population there is a trend towards impaired immune complex clearance when compared with the control group. This impaired immune complex clearance may, together with a number of other additive factors such as early complement component deficiencies, contribute to disease susceptibility.

References