Mannose Binding Lectin Polymorphisms as a Disease-Modulating Factor in Women with Systemic Lupus Erythematosus from Canary Islands, Spain

M. ISABEL GARCÍA-LAORDEN, IÑIGO RÚA-FIGUEROA, PALOMA PÉREZ-ACIEGO, J. CARLOS RODRÍGUEZ-PÉREZ, M. JESÚS CITORES, FAYNA ÁLAMO, CELIA ERAUSQUIN, and CARLOS RODRÍGUEZ-GALLEGO

ABSTRACT. ***Objective.*** To determine whether mannose binding lectin (MBL) polymorphisms are associated with clinical characteristics and with susceptibility to systemic lupus erythematosus (SLE) in women from the Canary Islands, Spain.

Methods. MBL alleles and genotypes were determined by polymerase chain reaction in 89 female patients and 188 female controls.

Results. No differences in the allelic or genotypic frequencies were observed between patients and controls. Anti-U1RNP autoantibodies were less frequent in association with mutated alleles (p = 0.037), and in association with MBL deficient genotypes, although this association was not statistically significant. The patients with low or nonproducer genotypes exhibited a decreased frequency of anti-Sm antibodies (p = 0.059). A nonsignificant trend toward lower prevalence of anti-Sm and anticardiolipin antibodies in association with both mutated alleles and low or nonproducer genotypes was also observed. The prevalence of more than one autoantibody was lower in association with mutated alelles (p = 0.022) and with low or nonproducer genotypes (p = 0.052). Homozygous or heterozygous patients with mutated alleles were significantly older at disease onset and at SLE diagnosis (p = 0.005, p = 0.014, respectively). An increase in the mean age at disease onset and at SLE diagnosis was observed with regard to the number of nonproducer alleles present (p = 0.021, p = 0.038, respectively).

Conclusion. MBL deficiency is not a risk factor for SLE in women from the Canary Islands, but it is associated with lower prevalence of autoantibodies and with later age at disease onset and at SLE diagnosis. (J Rheumatol 2003;30:740–6)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS AUTOANTIBODIES
MANNOSE BINDING LECTIN COMPLEMENT POLYMORPHISM

Systemic lupus erythematosus is a prototypical autoimmune disease involving the formation of multiple autoantibodies and the recurrent inflammation of various organ systems. Development of SLE is related to genetic as well as environmental factors[1]. Evidence of a hereditary predisposition to SLE comes from family studies that describe a high concordance among homozygotic twins and a high prevalence of disease among relatives of patients with SLE[2]. Deficiency in any one of the early complement components (C1q, C2, and C4) represents a higher risk of predisposition to SLE[3,4]. Many hypotheses have been developed to explain the relationship between complement defect and autoimmune disease[4]: impaired clearance of immune complexes, humoral immunity disorders, or impaired clearance of apoptotic bodies.

Mannose binding lectin (MBL) is a calcium dependent serum lectin that is secreted by the liver as an acute-phase protein[5] whose gene is located on the long arm of chromosome 10[6]. The protein is a multimeric molecule of up to 6 functional subunits, each consisting of 3 polypeptide chains, a structure analogous to C1q[6]. The MBL plays an important role in the innate immune defence system, opsonizing mannose- and N-acetylglucosamine-rich microorganisms[7] and activating macrophages through the C1q receptor[8]. MBL activates the third pathway of the complement system, the lectin pathway, through 2 associated serine proteases (MASP1 and MASP2)[6,7]. Deficient and low MBL concentrations in serum are mainly due to the presence of 3 point mutations encoding nonproducer structural alleles *B*, *C*, and *D* (codons 54, 57, and 52, respectively, termed *0* alleles in contrast to the normal allele, *A*)[9-11]. Additionally, 3 polymorphic sites in the promot-

From the Department of Immunology, Department of Rheumatology and Research Unit, Hospital de Gran Canaria Dr. Negrín, Las Palmas de Gran Canaria, Spain, and Fundación LAIR, Madrid, Spain.

Supported in part by Fondo de Investigaciones Sanitarias (Ministerio de Sanidad) grant FIS 98/1513.

M.I. García-Laorden, BSc, Department of Immunology; I. Rúa-Figueroa, MD, Department of Rheumatology; J.C. Rodríguez-Pérez, PhD; F. Álamo, BS, Research Unit; C. Erausquin, PhD, Department of Rheumatology; C. Rodríguez-Gallego, PhD, Department of Immunology, Hospital de Gran Canaria Dr. Negrín; P. Pérez-Aciego, PhD; M.J. Citores, PhD, Fundación LAIR.

Address reprint requests to Dr. C. Rodríguez-Gallego, Department of Immunology, Hospital de Gran Canaria Dr. Negrín, Barranco de la Ballena s/n 35020, Las Palmas de Gran Canaria, Spain.
E-mail: jrodgal@gobiernodecanarias.org

Submitted May 1, 2002; revision accepted September 18, 2002.

er and 5' untranslated regions of the *MBL* gene (*H*/*L*, *X*/*Y*, and *P*/*Q* at positions –550, –221, and +4, respectively) have an important effect on MBL serum concentrations[12,13]. As a consequence of strong linkage disequilibrium, 7 alleles have been observed, *HYPA*, *LYQA*, *LYPA*, *LXPA*, *HYPD*, *LYQC*, and *LYPB*, combining in 28 different genotypes. *HYPA* and *LYQA* alleles are associated with high levels of MBL, the allele *LYPA* with middle to low levels, and allele *LXPA* with low MBL production[12,13].

Allelic and genotypic frequencies have been defined in different population groups[6,13]. The frequency of nonproducer alleles and individuals presenting MBL deficiencies in the Canary Islands population is higher than that reported in other European and Asian populations[14]. Studies suggest that MBL deficiency is associated with a high risk of infection both in children and in adults[7,9,15,16], and controversy has arisen concerning its role in the etiopathogenesis of SLE and rheumatoid arthritis[17-27].

To examine the proposed role of MBL deficiency in SLE, we investigated the association of *MBL* alleles and genotypes with susceptibility to SLE or to clinical or serological characteristics in a group of women with SLE in the Canary Islands.

MATERIALS AND METHODS

Patients and controls. The patient population consisted of 89 unrelated white Spanish women who were followed at the Hospital de Gran Canaria Dr. Negrín, Canary Islands. All patients fulfilled 4 or more of the revised criteria proposed by the American College of Rheumatology 1982 for the classification of SLE[28]. The median age at onset of SLE was 30 years (range 12–68). The clinical characteristics analyzed were the following: the SLE classification criteria (malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, serositis, renal disorder, neurologic disorder, hematologic disorder, immunologic disorder, and antinuclear antibody), presence of some autoantibodies (anticardiolipin, anti-dsDNA, anti-Sm, anti-U1RNP, anti-La and/or anti–Ro), the mean age at disease onset and at SLE diagnosis, hypocomplementemia, thrombosis, miscarriage, cytopenias, ictus, Raynaud's phenomenon, avascular osteonecrosis, Systemic Lupus Activity Measure (SLAM), and SLE Disease Activity Index (SLEDAI). Demographic and clinical data were obtained retrospectively from medical records, and the data collection criteria were uniform for all patients. Unrelated white Spanish women (n = 188) who attended outpatient clinics to be evaluated for bone marrow donation were selected as the control group. Genomic DNA was isolated from whole blood according to standard phenol-chloroform procedure[29].

MBL typing. Genetic analyses were carried out using modifications of the procedures of Madsen, *et al*[12,13] and Sullivan, *et al*[17] (for details see reference 14). Sequences of oligonucleotides are shown in Table 1. The *B* and *C* alleles were detected by polymerase chain reaction with restriction fragment length polymorphism (PCR-RFLP) using the MBP3 and MBP17 primers. The *D* and *P*/*Q* alleles were detected by site directed mutagenesis (SDM)-PCR-RFLP using the MBP2 and MBP4 and the PQD and PQI primers, respectively. Genotyping of the promoter variants *H*, *L*, *X*, and *Y*, and *L* and *H* relative to *X* and *Y*, was performed by PCR using sequence-specific primers (SSP) using the forward primers MBP13, MBP5, MBP7, and MBP8 for alleles *H*, *L*, *X*, and *Y*, respectively, and the reverse primer MBP2, and the MBP5, MBP13, and reverse primers MBP14 and MBP15 for *L* and *H* relative to *X* and *Y*. The cis/trans-location of the promoter variants relative to the structural variants was determined by nested PCR (*B* and *C* variants) or SDM-PCR (*D* allele) on the PCR-SSP products, followed by an RFLP analysis with the relevant enzymes. *0*/*0* homozygous genotype was confirmed by direct sequencing.

PCR were performed in a volume of 50 µl that contained 250 to 500 ng of genomic DNA, 1.25 U (structural alleles) or 2 U (promoter variants) of AmpliTaq DNA polymerase (Perkin-Elmer, Norwalk, CT, USA), 0.18 to 0.5 µM of specific primers, 0.2 mM of deoxynucleotide triphosphate, and 2.4 mM (PCR-SSP) or 1.5 mM (PCR and SDM-PCR) of $MgCl_2$. All PCR are initiated by a 2 min step of denaturation at 94°C and completed by a 5 min step of extension at 72°C. The temperature cycles were as follows: PCR 30 cycles of 30 s at 94°C, 60 s at 62°C, and 120 s at 72°C; SDM-PCR 35 cycles of 30 s at 94°C, 30 s at 58°C (for *D* allele) or 55.5°C (for *P*/*Q* variants), and 60 s at 72°C; PCR-SSP 30 cycles of 30 s at 94°C, 60 s at 65°C, and 60 s at 72°C; PCR-SSP for the cis/trans location of the promoter variants 35 cycles of 30 s at 95°C, 30 s at 60°C, and 30 s at 72°C. All these amplifications were visualized in 2% agarose gel electrophoresis.

Table 1. Oligonucleotides used in genotyping of the mannose binding lectin.

Primer	Sequence
MBP3	5′-CCCAGATTGTAGGACAGAG-3′
MBP17	5′-AGTTGTTGTTCTCCTGTCCAG-3′
MBP2	5′-CAGGCAGTTTCCTCTGGAAGG-3′
MBP4	5′-CATCAACGGCTTCCCAGGCAAAGACGCG-3′
PQD	5′-ACCCAGATTGTAGGACAGAGGGCAAGC-3′
PQI	5′-TTGCAGAGACAGAACAGC-3′
MBP5	5′-TTACCCAGGCAAGCCTGTC-3′
MBP13	5′-GCTTACCCAGGCAAGCCTGTG-3′
MBP7	5′-CATTTGTTCTCACTGCCACC-3′
MBP8	5′-CATTTGTTCTCACTGCCACG-3′
MBP14	5′-GGAAGACTATAAACATGCTTTCG-3′
MBP15	5′-GGAAGACTATAAACATGCTTTCC-3′

For the RFLP analysis of *B* and *C* alleles, the PCR product was digested with the restriction enzymes BanI (New England BioLabs, Beverly, MA, USA) and MboII (New England BioLabs), respectively. The restriction fragments were all separated by 2% agarose gel electrophoresis. For the RFLP analysis of *D* allele, the SDM-PCR product was digested with the restriction enzymes MluI (New England BioLabs) and HhaI (New England BioLabs). For the RFLP analysis of *P* and *Q* alleles, the SDM-PCR product was digested with the restriction enzyme HindIII (New England BioLabs). For *D* and *P*/*Q* analysis, the restriction fragments were separated by 6% polyacrylamide gel electrophoresis.

Statistical analysis. To test the null hypothesis for the distribution of clinical categorical variables between patients and controls, as well as the allelic and genotypic frequencies of the *MBL* gene for the 2 groups, Fisher's exact test was used. The risk of SLE associated with MBL genotypes as genetic factor and the strength of the association of the genotypes with the frequency of the clinical characteristics was estimated by calculation of odds ratios with 95% confidence interval. The Hardy-Weinberg equilibrium for the genotypic frequencies was tested by chi-square analysis. Deviations from normal distributions were evaluated by the Kolmogorov-Smirnof z statistic. Comparison of mean differences for age at disease onset and age at SLE diagnosis, related to MBL genotypes, was performed by Student t test for 2 independent samples or by ANOVA when we compared more than 2 categories. All tests were 2 tailed. Statistical significance was taken as p value < 0.05. Statistical analysis was performed using the SPSS statistical package (version 10.0 for Windows).

RESULTS

The frequencies of the 7 MBL alleles were not significantly different between patients and controls (Table 2). The total frequency of nonproducer alleles (*0* alleles) was also similar between the 2 groups [49 alleles in patients (27.5%) vs 101 alleles in controls (26.9%); p = 0.92] (Table 2). The overall

Table 2. Frequencies of mannose binding lectin alleles in patients with SLE and controls. *0* is the designation for the mutated alleles (*HYPD*, *LYQC*, and *LYPB*).

Alleles	Patients, n = 89	Controls, n = 188
HYPA	0.236	0.253
LYQA	0.264	0.223
LYPA	0.067	0.074
LXPA	0.157	0.181
HYPD	0.056	0.072
LYQC	0.034	0.037
LYPB	0.185	0.16
0	0.275	0.269

distribution of the 28 genotypes did not differ between patients and controls, and the genotypic frequencies were in Hardy-Weinberg equilibrium in both groups (data not shown). Table 3 shows the frequencies of the genotypes grouped on the basis of the presence of structural variants: 45 patients (50.6%) carried genotypes *A*/*A*, 39 (43.8%) had *A*/*0* genotypes, and 5 (5.6%) had *0*/*0* genotypes. This overall distribution was also similar to that observed in the control group: 53.2% for homozygous *A*/*A*, 39.9% for *A*/*0* heterozygous, and 6.9% for *0*/*0* homozygous (chi-square 0.462, 2 df, p = 0.794; *A*/*0* comparison, p = 0.60 by Fisher's exact test; *0*/*0* comparison, p = 0.80). When we evaluated the presence of *LXPA* alleles (which are associated with low serum MBL levels) in the different genotypes, we observed that the frequency of *LXPA* homozygosity did not differ from patients [3 (3.4%)] to controls [6 (3.2%)]. In addition, the frequency of *LXPA*/*0* genotypes was not significantly different in patients versus controls: 8 (7.9%) versus 26 (13.8%), respectively (p = 0.17). Moreover, the frequencies of low or nonproducer genotypes (*LXPA*/*0* + *0*/*0*) did not differ significantly between patients and controls (13.5% vs 20.7%; p = 0.184) (Table 3).

The frequencies of the main clinical characteristics in the patient group are shown in Table 4. Some clinical data were unknown for some patients. Allelic and genotypic frequencies were examined according to clinical characteristics. No differences in the allelic or genotypic frequencies were observed when SLE classification criteria and other clinical characteristics (miscarriage, all types of cytopenias, ictus, Raynaud's phenomenon, and avascular osteonecrosis) were analyzed. However, an association with the *LYQC* allele was observed with regard to thrombosis (13.6% in patients with thrombosis vs 1.9% in patients without; p = 0.026, OR 8.05, 95% CI 0.99-63.19).

Table 3. Frequencies of mannose binding lectin genotypes in patients with SLE and controls. *A* is the designation for wild-type and *0* the mutated alleles *HYPD*, *LYQC*, and *LYPB*. *YA*/*0* is the designation for the *HYPA*/*0*, *LYQA*/*0*, and *LYPA*/*0* genotypes. Values are number (%).

Genotype	Patients, n = 89	Controls, n = 188
A/*A*	45 (50.6)	100 (53.2)
A/*0*	39 (43.8)	75 (39.9)
0/*0*	5 (5.6)	13 (6.9)
0/*0* + *LXPA*/*0*	12 (13.5)	39 (20.7)
A/*A* + *YA*/*0*	77 (86.5)	149 (79.3)

Table 4. Characteristics of patients with SLE. Values are number (%), except for age, which is the mean current age in years.

Characteristic	
Mean age, yrs	40.55
Classification criteria	
Malar rash	49 (55.1)
Discoid rash	11 (12.4)
Photosensitivity	47 (52.8)
Oral ulcers	34 (38.2)
Arthritis	75 (84.3)
Serositis	25 (28.1)
Renal disorder	39 (43.8)
Neurologic disorder	12 (13.5)
Hematologic disorder	69 (77.5)
Immunologic disorder	80 (89.9)
ANA	89 (100)
Hypocomplementemia	71 (80.7)*
Thrombosis	11 (12.4)
Autoantibodies	
Anticardiolipin	42 (48.8)*
Anti-dsDNA	78 (87.6)
Anti-Sm	22 (25.3)*
Anti-U1RNP	33 (37.5)*
Anti-La/Ro	38 (43.2)*

* N = 89 except for anticardiolipin (86), anti-Sm (87), and hypocomplementemia, anti-U1RNP and anti-La/Ro (88).

When the distribution of the autoantibodies among the different genetically defined subgroups was analyzed (Table 5), it was observed that the frequencies of anticardiolipin, anti-Sm, and anti-U1RNP autoantibodies were lower in those subgroups of alleles or genotypes with associated deficient MBL production. Notably, *0* alleles were significantly associated with a lower prevalence of anti-U1RNP autoantibodies (24.5%) compared with *A* alleles (42.5%) (p = 0.037, OR 0.44, 95% CI 0.19-0.96). A similar, although nonsignificant, trend toward lower prevalence of anti-U1RNP autoantibodies was observed when patients with *A*/*0* or *0*/*0* genotypes were compared with patients with *A*/*A* genotypes (27.3% vs 47.7%, respectively; p = 0.077). In addition, the group of patients with *LXPA*/*0* or *0*/*0* genotypes exhibited a decreased frequency of anti-Sm antibodies (0%) compared with patients without these genotypes (29%), although this difference did not achieve statistical significance (p = 0.059).

We also analyzed the number of different autoantibodies present (anticardiolipin, anti-dsDNA, anti-Sm, anti-U1RNP, and anti-La/Ro) with regard to the MBL genotypes (ANA were excluded because of their prevalence of 100% in our patients) (Table 5). The prevalence of more than one autoantibody versus none or one autoantibody (mostly anti-dsDNA)

Table 5. Prevalence of autoantibodies in patients with SLE by mannose binding lectin alleles and genotypes. Values are number (%).

	A	*0*	*A/A*	*A/0 + 0/0*	*A/A + YA/0*	*LXPA/0 + 0/0*
Anticardiolipin, n = 86	64 (52.0)	20 (40.8)	24 (57.1)	18 (40.9)	38 (51.4)	4 (33.3)
Anti-Sm, n = 87	34 (27.0)	10 (20.8)	12 (27.3)	10 (23.3)	22 (29.0)[a]	0 (0)[a]
Anti-U1RNP, n = 88	54 (42.5)[b]	12 (24.5)[b]	21 (47.7)[c]	12 (27.3)[c]	31 (40.8)	2 (16.7)
Anticardiolipin, Sm, U1RNP,[d] n = 84	92 (76.7)[e]	28 (58.3)[e]	34 (82.9)[f]	26 (60.5)[f]	55 (75.3)[g]	5 (45.5)[g]
Anti-dsDNA, n = 89	115 (89.2)	41 (83.7)	41 (91.1)	37 (84.1)	68 (88.3)	10 (83.3)
Anti-La/Ro, n = 88	54 (42.5)	22 (44.9)	19 (43.2)	19 (43.2)	33 (43.4)	5 (41.7)
More than 1 autoantibody[h], n = 84	100 (83.3)[j]	32 (66.7)[j]	36 (87.8)[k]	30 (69.8)[k]	60 (82.2)[m]	6 (54.5)[m]

[a] p = 0.059. [b] p = 0.037, OR 0.44, 95% CI 0.19–0.96. [c] p = 0.077. [d] Analysis of the presence of anticardiolipin and/or anti-Sm and/or anti-U1RNP vs none of these autoantibodies. [e] p = 0.023, OR 0.43, 95% CI 0.21–0.87. [f] p = 0.030, OR 0.31, 95% CI 0.1–0.96. [g] p = 0.069. [h]Analysis of more than one of the 5 autoantibodies vs none or one autoantibody. [j] p = 0.022, OR 0.4, 95% CI 0.17–0.93. [k] p = 0.062. [m] p = 0.052. All 2 tailed Fisher's exact test.

was 66.7% associated to *0* alleles compared with 83.3% associated to *A* alleles (p = 0.022, OR 0.4, 95% CI 0.17-0.93). In addition, the prevalence of more than one autoantibody was also decreased in patients with *A/0* or *0/0* genotypes: 30 (69.8%) versus 30 (87.8%) in patients with *A/A* genotypes (p = 0.062) and in patients with *LXPA/0* or *0/0* genotypes, 6 (54.5%) versus 60 (82.2%) in patients with the other genotypes (p = 0.052). On the other hand, we evaluated the frequency of only those autoantibodies in which some differences were observed (anticardiolipin, anti-Sm, and anti-U1RNP). *0* alleles were associated with a lower frequency of these autoantibodies compared with *A* alleles, 58.3% versus 76.7% (p = 0.023, OR 0.43, 95% CI 0.21-0.87), and these differences were also statistically significant when we compared *A/0* or *0/0* genotypes versus *A/A* genotypes: 26 (60.5%) versus 34 (82.9%) (p = 0.030, OR 0.31, 95% CI 0.1-0.96). In addition, we observed a nonsignificant decrease in the frequency for patients with any of these autoantibodies among *LXPA/0* or *0/0* genotypes: 5 (45.5%) versus 55 (75.3%) in patients without these genotypes (p = 0.069).

When the relationships of MBL deficient genotypes with both age at disease onset and age at SLE diagnosis were analyzed, we observed that the mean age at disease onset was 7.7 years lower in patients with *A/A* genotypes than in those with *A/0* or *0/0* genotypes (24.0 ± 9.05 vs 31.7 ± 11.9 yrs, respectively; p = 0.005 by Student t test). Similarly, the mean age at SLE diagnosis was 7.7 years lower in patients with *A/A* than in those with *A/0* or *0/0* genotypes (25.75 ± 10.8 vs 33.4 ± 12.4 yrs, respectively; p = 0.014, Student t test). Further, an increase in the mean age at disease onset was observed with regard to the number of nonproducer alleles present: mean age 24.0 ± 9.05 years for patients with *A/A* genotypes, 31.5 ± 12.3 years for patients with *A/0* genotypes, and 33.7 ± 5.9 years for patients with *0/0* genotypes (Figure 1). These differences were statistically significant (p = 0.021, F statistic by ANOVA). Similar results were observed when we analyzed the mean age at SLE diagnosis. The mean age at SLE diagnosis was 25.75 ± 10.8 years in patients with *A/A* genotypes, 33.0 ± 12.7 in patients with *A/0* genotypes, and 39.4 ± 0.1 in patients with *0/0* genotypes. These differences were also statistically significant (p = 0.038, F statistic by ANOVA) (Figure 1).

DISCUSSION

We observed a high similarity in the allelic and genotypic frequencies between patients with SLE and controls in women from the Canary Islands. We also documented a lower prevalence of certain autoantibodies in patients with defective protein genotypes as well as an association between the *MBL* defect and higher ages at disease onset and at SLE diagnosis.

It has been suggested that a deficiency in MBL is associated with a greater susceptibility to SLE. The first studies carried out to ascertain the relationship between MBL and SLE revealed a significant increase in the frequency of *B* and *C* structural alleles in Afro-American patients[17]. Conversely, no significant increases in either the frequency of allele *B* or the frequency of homozygous *BB* were detected in English patients[18]. However, the direct primer employed in these and other studies included the polymorphic site *P/Q*, which, as noted, could lead to an overestimation of allele *B* and of the homozygous *BB*[30]. Increases in the frequency of the structural mutations of the gene in South African[19], Chinese[20], and Danish[21] patients were observed in subsequent studies. While the statistical analyses of these studies did not prove to be conclusively significant, it has been suggested that deficiency in MBL could be a minor risk factor for SLE. As for the promoter variants of the *MBL* gene, in Chinese populations a significant increase in low producer allele *LX* was observed[22], and in Afro-American SLE patients a significant increase in the frequency of those who were homozygous for this allele was observed[17]. These results were not confirmed by the study that was subsequently carried out in the Danish population[21].

On the other hand, a recent study[23] has reported similarities between the frequency of the structural and promoter variants found in SLE patients compared with those for the control group in a Japanese population. The investigators concluded that in their population it is unlikely that MBL deficiency plays a major role in the pathogenesis of SLE. MBL deficiency could therefore be of more significance in some populations than in others.

Considering the analysis of structural variants and variants in the promoter region, our results show a high degree of similarity in the allelic and genotypic frequencies between patient and control groups. It can therefore be concluded that for our

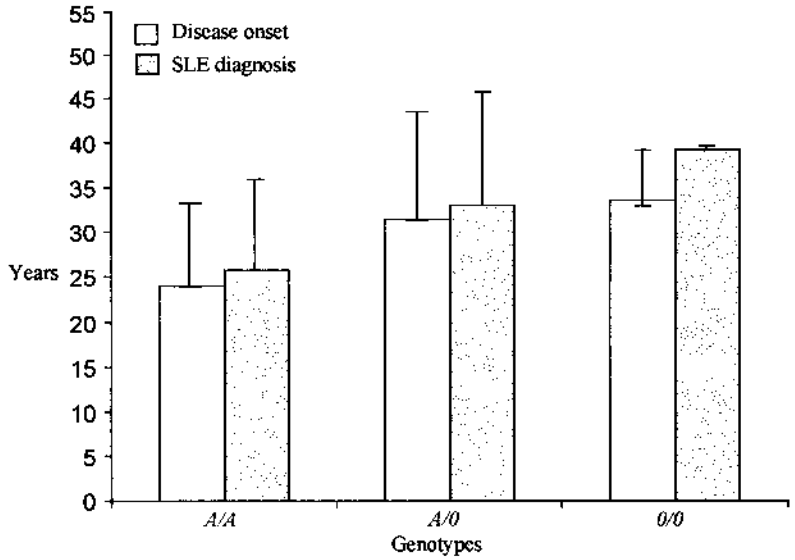

Figure 1. Ages at disease onset and at SLE diagnosis with respect to the mannose binding lectin genotypes. Values are the mean age ± SD (p = 0.021 for mean age at disease onset and p = 0.038 for mean age at SLE diagnosis, F statistic by ANOVA).

population the data would not support the hypothesis that MBL deficiency represents a risk factor for SLE. Studies in a Spanish SLE population from Barcelona have shown an increase in the number of patients possessing the *B* allele[24,25] and a decreased frequency of the *HYPA* allele[25]. The differences in the frequencies observed between the 2 studies as well as the differences in allelic and genotypic frequencies within our population[14] might account for the observed discrepancies with regard to MBL deficiency and SLE susceptibility.

In support of our data, genome screening studies for linkage to SLE to date have not found an association within the region of the *MBL* gene[31,32]. Thus, given that SLE has a strongly skewed sex distribution, it was necessary to carry out a study limited to female patients only. Such a criterion has not been considered previously. Indeed, Garred, *et al*[21] found an overestimation of males in the group of mutated homozygous subjects in their SLE patients, and suggested that MBL deficiency may increase the risk of acquiring SLE among men. However, if analysis had been carried out only on their female patients, MBL deficiency would not represent SLE susceptibility either.

Our results show a lower prevalence of determined autoantibodies associated with MBL deficiency, although in previous studies[17,18] mutated *MBL* alleles were not found to exert an influence on the prevalence of autoantibodies. We found that the prevalence of anti-U1RNP was lower, associated with defective alleles, and a lower (although not statistically significant) prevalence was also observed in MBL deficient genotypes. In addition, a trend to lower prevalence of anti-Sm and anticardiolipin antibodies in association with MBL deficiency was observed. However, these differences were not statistically significant, which might be attributed to the low number of individuals in each subgroup. On evaluating the joint analysis of the studied autoantibodies, we also observed a protective role for defective MBL-producing alleles and genotypes when we compared the prevalence of no or one autoantibody (mostly anti-dsDNA) versus more than one autoantibody, as well as for the subsequent joint analysis of anticardiolipin, anti-Sm, and anti-U1RNP autoantibodies. Our results clearly suggest that MBL deficiency may play a protective role in autoantibody production in SLE.

The prevalence of some autoantibodies is also lower in patients with other complement deficiencies. Indeed, it has been established that the complement system plays an important role in the normal humoral immune response to T-dependent antigens[3,4]. Our observations seem to suggest that MBL deficiency is associated with a low prevalence of some autoantibodies, but not with increased susceptibility to SLE. It is tempting to speculate that MBL may play a role in humoral antibody response but does not exert an influence on the maintenance of tolerance.

When the relationships between MBL genotypes and age at disease onset and age at SLE diagnosis were evaluated, an increase in the mean age at disease onset (p = 0.021) was found in patients with defective protein genotypes. Similar results were observed when mean age at SLE diagnosis was analyzed (p = 0.038). Thus, patients with MBL deficiency underwent disease onset and had SLE diagnosis at later ages, a finding not observed in a previous study[21]. It has been observed in some but not all studies that a delay in the onset of disease is associated with prolonged survival[33-36]. These data, together with the fact that the presence of certain autoantibodies has been associated with determined clinical charac-

teristics that may define the course of the disease, would suggest that MBL deficiency may be a factor that contributes to a lower degree of severity of the disease. The ability of MBL genotypes to influence more than one aspect of a disease is apparent in other inflammatory conditions[27,37,38], possibly as a consequence of a reduced capacity for complement activation and thereby lesser host damage[6]. However, evaluating the association between *MBL* alleles and genotypes and SLE clinical characteristics, no direct relationship was encountered, except in the case of a significant increase in the frequency of allele *LYQC* in patients with thrombosis ($p = 0.026$). On the other hand, it has been reported that renal involvement was more frequent in *0/0* homozygous patients[21] and in patients carrying *B* alleles[24]. However, in our study and in others[17,18] no association with renal involvement was observed.

Additionally, we evaluated the SLAM and SLEDAI indexes, and we found no association with MBL deficiency (data not shown). Since SLAM and SLEDAI are only disease activity indices and they did not correlate directly with disease severity, it would be interesting to analyze the data from the Systemic Lupus International Collaborating Clinics damage index to clarify the relationship between SLE severity and MBL deficiency. Although our data may suggest a possible protective role for MBL in the severity of SLE, it is not supported by the clinical findings.

In summary, statistical differences in allelic or genotypic frequencies of structural or promoter variants of the *MBL* gene among patients with SLE and controls were not encountered. These results suggest that, at least in our female population group, MBL deficiency did not represent a risk factor for SLE. However, the lower prevalence of autoantibodies observed in patients with defective protein genotypes, and delay in the age at disease onset and at SLE diagnosis in such patients, suggests that MBL deficiency is a disease-modulating factor. In view of the disparity in the results published to date, additional large studies are needed to clarify the relationship between MBL polymorphisms and SLE.

ACKNOWLEDGMENT

We thank the medical staff of the Rheumatology and Internal Medicine Departments from de Hospital de Gran Canaria Dr. Negrín for their collaboration, Ana Bilbao and Aránzazu Anabitarte for participating in sample collection, and Miguel Rúa-Figueroa for the manuscript revision.

REFERENCES

1. Reveille JD. The molecular genetics of systemic lupus erythematosus. Curr Opin Rheumatol 1991;3:722-30.
2. Deapen D, Escalante A, Weinrib L, et al. A revised estimate of twin concordance in systemic lupus erythematosus. Arthritis Rheum 1992;35:311-8.
3. Walport MJ. Complement deficiency and disease. Br J Rheumatol 1993;32:269-73.
4. Pickering MC, Botto M, Taylor PR, Lachmann PJ, Walport MJ. Systemic lupus erythematosus, complement deficiency, and apoptosis. Adv Immunol 2000;76:227-324.
5. Thiel S, Holmskov U, Hviid L, Laursen SB, Jensenius JC. The concentration of the C-type lectin, mannan-binding protein, in human plasma increases during an acute phase response. Clin Exp Immunol 1992;90:31-5.
6. Turner MW. Mannose-binding lectin: the pluripotent molecule of the innate immune system. Immunol Today 1996;87:455-64.
7. Jack DL, Klein NJ, Turner MW. Mannose-binding lectin: targeting the microbial world for complement attack and opsonophagocytosis. Immunol Rev 2001;180:86-99.
8. Tenner AJ, Robinson SL, Ezekowitz RAB. Mannose binding protein enhances mononuclear phagocytic function via a receptor that contains the 126,000 Mr component of the C1q receptor. Immunity 1995;3:485-93.
9. Sumiya M, Super M, Tabona P, et al. Molecular basis of opsonin defect in immunodeficient children. Lancet 1991;337:1569-70.
10. Madsen HO, Garred P, Kurtzhals JAL, et al. A new frequent allele is the missing link in the structural polymorphism of the human mannan-binding protein. Immunogenetics 1994;40:37-44.
11. Lipscombe RJ, Sumiya M, Hill AVS, et al. High frequencies in African and non African populations of independent mutations in the mannose binding protein. Hum Mol Genet 1992;9:709-15.
12. Madsen HO, Garred P, Thiel S, et al. Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. J Immunol 1995;155:3013-20.
13. Madsen HO, Satz ML, Hogh B, Svejgaard A, Garred P. Different molecular events result in low protein levels of mannan-binding lectin in populations from Southeast Africa and South America. J Immunol 1998;161:3169-75.
14. García-Laorden MI, Manzanedo A, Figuerola A, Sanchez-Garcia F, Rodriguez-Gallego C. Mannose-binding lectin polymorphisms in a Canary Islands (Spain) population. Genes Immun 2001;2:292-4.
15. Summerfield JA, Sumiya M, Levin M, Turner MW. Association of mutations in mannose binding protein gene with childhood infection in consecutive hospital series. BMJ 1997;314:1229-32.
16. Summerfield JA, Ryder S, Sumiya M, et al. Mannose binding protein gene mutations associated with unusual and severe infections in adults. Lancet 1995;345:886-9.
17. Sullivan KE, Wooten C, Goldman D, Petri M. Mannose-binding protein genetic polymorphisms in black patients with systemic lupus erythematosus. Arthritis Rheum 1996;39:2046-51.
18. Davies EJ, Snowden N, Hillarby MC, et al. Mannose-binding protein gene polymorphism in systemic lupus erythematosus. Arthritis Rheum 1995;38:110-4.
19. Davies EJ, Tikly M, Wordsworth BP, Ollier WER. Mannose-binding protein gene polymorphism in South African systemic lupus erythematosus. Br J Rheumatol 1998;37:465-6.
20. Lau YL, Lau CS, Chan SY, Karlberg J, Turner MW. Mannose-binding protein in Chinese patients with systemic lupus erythematosus. Arthritis Rheum 1996;39:706-8.
21. Garred P, Madsen HO, Halberg P, et al. Mannose-binding lectin polymorphisms and susceptibility to infection in systemic lupus erythematosus. Arthritis Rheum 1999;42:2145-52.
22. Ip WK, Chan SY, Lau CS, Lau YL. Association of systemic lupus erythematosus with promoter polymorphisms of the mannose-binding lectin gene. Arthritis Rheum 1998;41:1663-8.
23. Horiuchi T, Tsukamoto H, Morita C, et al. Mannose binding lectin gene mutation is not a risk factor for systemic lupus erythematosus and rheumatoid arthritis in Japanese. Genes Immun 2000;1:464-6.
24. Davies EJ, Teh LS, Ordi-Ros J, et al. A dysfunctional allele of the mannose binding protein gene associates with systemic lupus erythematosus in a Spanish population. J Rheumatol 1997;24:485-8.
25. Villareal J, Crosdale D, Ollier W, et al. Mannose binding lectin and FcγRIIa (CD32) polymorphism in Spanish systemic lupus erythematosus patients. Rheumatology 2001;40:1009-12.
26. Stanworth SJ, Donn RP, Hassall A, Dawes P, Ollier W, Snowden N. Absence of an association between mannose-binding lectin polymorphism and rheumatoid arthritis. Br J Rheumatol 1998; 37:186-8.

27. Garred P, Madsen HO, Marquart H, et al. Two edged role of mannose binding lectin in rheumatoid arthritis: a cross sectional study. J Rheumatol 2000;27:26-34.
28. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.
29. Blin N, Stafford DW. A general method for isolation of high molecular weight DNA from eukaryotes. Nucl Acids Res 1976;3:2303-8.
30. Madsen HO, Garred P, Kurtzhals JA, et al. A new frequent allele is the missing link in the structural polymorphism of the human mannan-binding protein. Immunogenetics 1994;40:37-44.
31. Gaffney PM, Kearns GM, Shark KB, et al. A genome-wide search for susceptibility genes in human systemic lupus erythematosus sib-pairs families. Proc Natl Acad Sci USA 1998;25:14875-9.
32. Shai R, Quismorio FP, Li L, et al. Genome-wide screen for systemic lupus erythematosus susceptibility genes in multiplex families. Hum Mol Genet 1999;8:639-44.
33. Ginzler EM, Diamond HS, Weiner M, et al. A multicenter study of outcome in systemic lupus erythematosus. I. Entry variables as predictors of prognosis. Arthritis Rheum 1982;25:601-11.
34. Wallace DJ, Podell T, Klinenberg JR, Forouzesh S, Dubois EL. Systemic lupus erythematosus — survival patterns. Experience with 609 patients. JAMA 1981;245:934-8.
35. Ward MM, Pyun E, Studensky S. Long-term survival in systemic lupus erythematosus. Patient characteristics associated with poorer outcomes. Arthritis Rheum 1995;38:274-83.
36. Abu-Shakra M, Urowitz MB, Gladman DD, Gough J. Mortality studies in systemic lupus erythematosus. Results from a single center. II. Predictor variables for mortality. J Rheumatol 1995;22:1265-70.
37. Rector A, Lemey P, Laffut W, et al. Mannan-binding lectin gene polymorphisms in ulcerative colitis and Crohn's disease. Genes Immun 2001;2:323-8.
38. Hibberd ML, Sumiya M, Summerfield JA, Booy R, Levin M. Association of variants of the gene for mannose-binding lectin with susceptibility to meningococcal disease. Lancet 1999;353:1049-53.