The contribution of \textit{HLA-DQB1} coding and \textit{QBP} promoter alleles to anti-Ro alone autoantibody response in systemic lupus erythematosus

D. Logar, B. Vidan-Jeras\textsuperscript{1}, V. Dolžan\textsuperscript{2}, B. Božič and T. Kveder

\textit{Department of Rheumatology, University Medical Centre, \textsuperscript{1}Tissue Typing Centre, Blood Transfusion Centre and \textsuperscript{2}Institute of Biochemistry, Faculty of Medicine, Ljubljana, Slovenia}

Abstract

\textbf{Objective.} To analyse the influence of \textit{HLA-DR}, \textit{DQ} and corresponding \textit{DQA1} and \textit{DQB1} promoter alleles (\textit{QAP} and \textit{QBP}) on the anti-Ro alone autoantibody response in systemic lupus erythematosus (SLE).

\textbf{Methods.} Sixty-five unrelated anti-La antibody-negative SLE patients, 37 of them with and 28 without anti-Ro antibodies, were included. Anti-Ro antibodies were determined by both counter-immunoelectrophoresis and enzyme-linked immunosorbent assay. Seventy-four healthy individuals were selected as controls. The patients and controls were analysed for \textit{HLA-DRB1}, \textit{QAP}, \textit{DQA1}, \textit{QBP} and \textit{DQB1} alleles by DNA typing. The allelic frequencies of anti-Ro alone-positive and anti-Ro-negative SLE patients and healthy controls were compared using the $\chi^2$ test or Fisher’s exact test as appropriate.

\textbf{Results.} The \textit{DQB1*0202} allele showed a significant positive correlation with anti-Ro alone antibodies [odds ratio (OR) = 16.949, $P = 0.0015$, corrected $P = 0.018$], while the \textit{QBP5.11} allele and the combination of \textit{DQB1*0301} and its promoter \textit{QBP3.1} were under-represented in anti-Ro-alone-positive SLE patients ($P = 0.01$, corrected $P = 0.048$ and corrected $P = 0.048$ respectively).

\textbf{Conclusions.} The above-mentioned alleles may contribute to the presence or absence of anti-Ro alone autoantibodies in SLE patients.

\textbf{Key words:} Systemic lupus erythematosus, Autoantibodies, HLA-class II polymorphism, Promoter polymorphism.

Systemic lupus erythematosus (SLE) is a complex autoimmune disorder characterized by spontaneous B-cell activation and overproduction of autoantibodies, abnormal T-cell regulation and dysregulation of the complement cascade, resulting in insufficient clearance of immune complexes [1]. The results of genetic studies indicate that susceptibility to SLE is polygenic [2–4]. Besides other genetic factors, the disease is associated with certain class-II major histocompatibility complex (MHC)-encoded molecules, particularly with HLA-DR3 and weakly with DR2 [5–8]. \textit{HLA} alleles and haplotypes associated with SLE vary among racial and ethnic groups [9, 10]. Among Caucasians, associations with the extended haplotypes A1-B8-DRB1*0301-DQA1*0501-DQB1*0201 and DRB1*1501-DQA1*0102-DQB1*0602 have been recorded [9, 11] and thoroughly reviewed [12].

Autoantibodies to Ro antigens are particularly important in the pathogenesis of skin changes in patients with SLE and, associated with anti-La autoantibodies, in the congenital heart block which appears sometimes in infants of anti-Ro-positive mothers [13, 14]. The induction and regulation of the immune response against the various Ro polypeptides are not fully understood. It is known that the genetic background leading to the anti-Ro alone autoantibody response is different from that leading to concomitant anti-Ro-La autoantibodies [15]. Anti-Ro-La antibodies were associated with \textit{DR3} and \textit{DQ1/DQ2} heterozygosity in a white population [16–18]. The combination of the allele \textit{DQBI*0201} with one of the alleles DQA1*0101, DQA1*0102 or DQA1*0103 was also associated with anti-Ro antibodies [19].

\textbf{Correspondence to:} D. Logar, Department of Rheumatology, University Medical Centre, Vodnikova 62, 1000 Ljubljana, Slovenia.

Disease association studies frequently implicate HLA-DQ molecules, which are more closely involved in repertoire selection, rather than DR molecules, which are more closely involved in peripheral presentation, in the predisposition to autoimmune diseases [20]. HLA class-II antigens can all present immunogenic peptides to human CD4+ T cells, but HLA-DR restriction overwhelmingly predominates. HLA-DQ-restricted T-cell clones are rare, reflecting the low level of expression of the dimer on antigen-presenting cells [21]. However, the linkage disequilibrium between particular HLA-DR and DQ alleles complicates the interpretation of which locus is of primary importance in the induction of the autoimmune response [20, 22].

In addition to the structural differences among HLA antigens due to the polymorphism of their coding regions, the process of antigen presentation may be influenced by polymorphism in their promoter regions [23, 24], which may influence the rate of transcription and consequently the density of the class-II heterodimeric proteins [25]. The different levels of expression may, in addition to the structural differences in HLA-class II antigens, affect antigen presentation and may lead to autoimmunity [26]. While the role of the polymorphism of DQA promoters in susceptibility to SLE has already been investigated [27], the influence of DQA (QAP) and DQB (QBP) promoters on the secretion of anti-Ro antibodies has not yet been studied.

The purpose of our study was to investigate if the presence or absence of anti-Ro alone autoantibodies in a cohort of 65 Slovenian patients with SLE could be attributed to differences in the antigen-presenting capacity of HLA-DRB, DQA1 and DQB1 molecules coded by polymorphic genes and/or to differences in the expression level of DQ molecules due to the polymorphism of QAP and QBP promoters.

Patients and methods

Patients
A cohort of 65 unrelated anti-La-negative SLE patients (all were female; mean age 36.2 ± 8.3 yr) followed up at the Department of Rheumatology, University Medical Centre, Ljubljana, were included in the study. All patients met at least four of the American College of Rheumatology classification criteria for SLE [28]. The majority of them had moderate disease activity at the time of entry into the study [mean European Consensus Lupus Activity Measurements (ECLAM) score 3.5] [29] and were receiving low-dose corticosteroid therapy (mean dose of methylprednisolone 6.4 mg). The rheumatologists taking part in the study reviewed all available medical records of the selected patients (mean retrospective follow-up period 93 months). On the basis of the data from their medical records, the patients were classified into two groups: anti-Ro-alone-positive and anti-Ro-negative. Only patients who were consistently either positive or negative for anti-Ro antibodies [detected by counter-immunoelectrophoresis (CIE) on at least three occasions] were taken into consideration. The presence or absence of anti-Ro alone antibodies was additionally confirmed by two independent detection techniques: CIE and enzyme-linked immunosorbent assay (ELISA) before HLA typing.

The control group consisted of 74 healthy Slovenians who were chosen randomly from the group who were typed as part of the 12th International Histocompatibility Workshop (12th IHW) Anthropology Component for HLA-DRB, DQB1 and DQA1 alleles [30, 31], and were additionally typed for QBP and QAP alleles as part of the 12th IHW DQA1 and DQB1 promoter polymorphism study [32, 33].

Detection of autoantibodies
Antinuclear antibodies (ANA) were detected by an immunofluorescence test on Hep-2 cells (Immuno-concepts, Sacramento, CA, USA) and antibodies to double-stranded DNA by the Farr assay [34]. The presence of autoantibodies against Ro and La antigens and against Sm, U1RNP, PL4, SL and proliferating cell nuclear antigen was ascertained by CIE, following the proposed guidelines of the European laboratory consensus group [35]. Antibodies to Ro and La antigens were confirmed subsequently in all patients’ sera with a commercially available ELISA (Relisa SS-A(Ro)/SS-B(La); Immunoconcepts) according to the manufacturer’s instructions. Each serum sample was tested in duplicate. A serum sample for which the calculated optical density (OD) value at 450 nm was higher than 30 ENA units (OD at 450 nm value multiplied by the conversion factor for Ro and La) was considered positive. Patients’ sera for which calculated values were less than 20 ENA units were considered negative and values between 20 and 30 units were considered borderline positive and were re-examined. The ELISA and CIE techniques were chosen in order to detect the whole populations of antibodies against the native proteins.

Typing of HLA coding and promoter regions
Genomic DNA was isolated from peripheral blood by a modified salting-out method [36]. All patients were typed for HLA-DRB1, DQA1 and DQB1 alleles. Polymerase chain reaction-based sequence-specific oligonucleotide hybridization (PCR-SSO), using the 12th IHW primers and probes, was used for the generic typing of DRB1 and DQB1 alleles. Second exons of DRB1 and DQB1 genes were amplified and hybridized with 23 oligonucleotide probes specific for DRB1 alleles and 23 oligonucleotide probes specific for DQB1 alleles [37, 38]. The subtyping of DRB1 and DQB1 alleles and DQA1 typing were performed by amplification of particular alleles with sequence-specific primers (PCR-SSP) [39, 40] selected from updated Dynal kits (Dynal, Oslo, Norway). Promoter regions of DQA1 and DQB1 genes were analysed using reagents and protocols provided during the 12th IHW [32, 33]. Briefly, the 10 alleles of the proximal promoter region QAP were...
resolved by amplification of the 315-base pair (bp) fragment and hybridization with a set of 20 SSO probes. Three additional SSOs were used to resolve variability in the Y box [32]. The 13 alleles of the DQBI proximal promoter region (QBP) were resolved by the amplification of a 590-bp fragment and hybridization with a set of 20 SSO probes. Sequence-specific PCR was used to discriminate 6.2 and 6.3 alleles [33]. All amplification products were dot-blotted on a Hybond N+ membrane (Amersham, Amersham, Buckinghamshire, UK). SSOs used for the detection of the alleles of the coding and promoter regions were labelled with digoxigenin-11-ddUTP and hybridizations were performed according to the method of Nevinny-Stickel et al. [41].

Statistical analysis

For the comparison of HLA phenotype frequencies, the proximal promoter region within the borders of the PCR-amplified region was first considered as a separate locus and the polymorphic variants were considered as individual alleles. The promoter–functional allele haplotype assignment was based on the previously observed QAP-DQAI and QBP-DQBI haplotypes in Caucasians [31, 33]. The DRBI-DQAI-DQBI haplotypes were deduced according to the previously observed linkage between DRBI and DQBI [42] and between DQAI and DQBI [43].

Phenotype frequencies (f) were expressed as percentages of controls or patients in a particular group that typed positively for one of the alleles. The frequencies of individual DRBI, DQBI and DQAI alleles in anti-Ro-alone-positive SLE patients, anti-Ro-negative patients and control subjects were compared using the $\chi^2$ test. The one-tailed Fisher’s exact test was used when appropriate [44]. The level of significance was set at 0.05. An extra correction factor ($P_c$) was used because the patients’ group had been divided into anti-Ro-alone-positive and anti-Ro-alone-negative groups by multiplying the number of alleles at each locus as follows: by $13 \times 2$ for DRBI, by $11 \times 2$ for DQAI, by $6 \times 2$ for DQBI, by $8 \times 2$ for QAP and by $8 \times 2$ for QBP. Odds ratios (OR) were calculated using the cross-product formula.

Results

Clinical and serological features of SLE patients

The SLE patients fell into two groups according to their anti-Ro antibody profiles. The anti-Ro-alone-positive group consisted of 37 patients and the anti-Ro-negative group consisted of 28 patients. Repeated detection of serum anti-Ro autoantibodies confirmed stable antibody profiles against native Ro proteins in all patients included in the study, and no evidence of the spreading of the immune response to anti-La antibody was observed. There was no significant difference between the two groups of patients in age (anti-Ro-alone-positive 35.3 ± 11.2 yr, anti-Ro-negative 37.2 ± 12.3 yr), disease duration (anti-Ro-alone-positive 93.2 ± 22.3 months, anti-Ro-negative 85.4 ± 17.5 months) or ECLAM score at presentation (anti-Ro-alone-positive 3.6, range 1–9; anti-Ro-negative 2.9, range 2–7). Comparison of clinical and serological features did not reveal any statistically significant differences between the anti-Ro-alone-positive and anti-Ro-negative patients.

HLA class-II phenotype and deduced haplotype frequencies in anti-Ro-alone-positive patients

As shown in Table 1, the frequencies of individual DRBI*03, QBP2.1, DQBI*0201 and DQBI*0202 alleles were significantly higher in the group of anti-Ro-alone-positive patients compared with the control group, although the significance of DQBI*0201 was lost when the P values were corrected for the number of comparisons made. On the basis of haplotypes observed previously [30, 42], we deduced that all these alleles, with the exception of DQBI*0202, were transmitted on the DRBI*03-QBP2.1-DQBI*0201 haplotype. The QBP2.1 promoter allele can be seen to be linked to either DQBI*0201 or DQBI*0202 [33], DQBI*0202, which has been reported to be closely linked to DRBI*07 [30] and only weakly linked to DRBI*03, represented the highest risk factor (OR = 16.95) for the formation of anti-Ro alone antibodies.

The frequency of the DRBI*15 allele was also increased in the anti-Ro-positive group of patients when compared with the controls. However, the significance was lost after correction of the P value.

On the contrary, the frequencies of DRBI*04, DRBI*11, QBP3.1 and DQBI*0301 were all significantly decreased in the anti-Ro-alone-positive patients when compared with the controls. After correction of the P values, the significance was lost for all except DQBI*0301 and its promoter QBP3.1. On the basis of the well-known linkage disequilibrium between DRBI*04 and DQBI*0301 as well as between DRBI*11 and DQBI*0301 [32] and the linkage between DQBI*0301 and QBP3.1 [33], we deduced that the two haplotypes DRBI*04-QBP3.1-DQBI*0301 and DRBI*11-QBP3.1-DQBI*0301 were under-represented in Slovenian anti-Ro-alone-positive SLE patients.

Decreased frequencies of QBP5.11 and DQBI*0502 were also observed in anti-Ro-positive patients when compared with controls ($P = 0.01$ and $P = 0.004$ respectively; Fisher’s exact test).

HLA class-II phenotype and deduced haplotype frequencies in anti-Ro-negative patients

The frequencies of DRBI*03, QAP4.1 and DRBI*15, DQBI*0602 were significantly increased among anti-Ro-negative patients compared with controls. However, none of the differences remained statistically significant after correction of the P values, as shown in Table 1. QAP4.1 was found to be linked with DQA1*0501 [32]. Because of the strong linkage between DQA1*05 and
DQB1*0502 or DQB1*0503 promoter allele can be linked with DQB1*0501, but rather to the DQB1*0502 or the QBP-5.11 promoter allele, which was present in only one anti-Ro-alone-positive patient and six anti-Ro-negative patients, was never found to be linked to DQB1*0501. On the other hand, QBP5.12 was always linked with DQB1*0501. While the association of both SLE itself and the anti-Ro-La antibody response with the two haplotypes HLA-DRB1*03-DQA1*0501-DQB1*0201 and HLA-DRB1*15-DQA1*0102-DQB1*0602 or their particular components is well established [8, 9, 11, 15, 18, 47], data on the association of HLA antigens with the production of anti-Ro alone antibodies are conflicting.

In this study we investigated the possible influence of particular HLA-DRB1, DQA1 and DQB1 coding alleles and QAP and QBP promoter alleles on the anti-Ro alone antibody response in SLE patients. All anti-Ro-alone-positive SLE patients had a stable anti-Ro alone antibody profile. In none of them did we observe spreading to the anti-La autoantibody response, which is in agreement with a study published previously [46]. While the association of both SLE itself and the anti-Ro/La antibody response with the two haplotypes HLA-DRB1*03-DQA1*0501-DQB1*0201 and HLA-DRB1*15-DQA1*0102-DQB1*0602 or their particular components is well established [8, 9, 11, 15, 18, 47], data on the association of HLA antigens with the production of anti-Ro alone antibodies are conflicting. Some studies showed that DR3 was not associated with the anti-Ro alone antibody response [3, 19]. On the other hand, Buyon et al. [48] found an association of the extended haplotype DRB1*0301-DQA1*0501-DQB1*0201 with combined anti-52-kDa Ro antibodies and low titres of anti-60-kDa Ro antibodies. Conversely, our data showed increased frequencies of

**Table 1. Phenotype frequencies (f) of HLA class-II alleles that differed significantly between anti-Ro alone antibody-positive SLE patients (Ro⁺) and healthy controls (C)**

<table>
<thead>
<tr>
<th>HLA allele</th>
<th>f/C (n=74)</th>
<th>f/Ro⁺ (n=37)</th>
<th>f/Ro⁻ (n=28)</th>
<th>Ro⁺ vs C</th>
<th>Ro⁻ vs C</th>
<th>Ro⁺ vs Ro⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>P⁺</td>
<td>OR</td>
<td>P</td>
<td>P⁺</td>
<td>OR</td>
</tr>
<tr>
<td>DRB1*03</td>
<td>21</td>
<td>52</td>
<td>50</td>
<td>0.001</td>
<td>0.026</td>
<td>4.273</td>
</tr>
<tr>
<td>QAP4.1</td>
<td>44</td>
<td>59</td>
<td>70</td>
<td>n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DQB1*0501</td>
<td>32</td>
<td>52</td>
<td>50</td>
<td>0.040</td>
<td>n.s.</td>
<td>2.19</td>
</tr>
<tr>
<td>QBP2.1</td>
<td>33</td>
<td>67</td>
<td>52</td>
<td>0.0008</td>
<td>0.013</td>
<td>4.081</td>
</tr>
<tr>
<td>DRB1*07</td>
<td>15</td>
<td>24</td>
<td>7</td>
<td>n.s.</td>
<td></td>
<td>n.s.</td>
</tr>
<tr>
<td>DQB1*0202</td>
<td>1</td>
<td>19</td>
<td>3</td>
<td>0.0015*</td>
<td>0.018</td>
<td>16.95</td>
</tr>
<tr>
<td>DRB1*11</td>
<td>25</td>
<td>8</td>
<td>25</td>
<td>0.031</td>
<td>n.s.</td>
<td>0.276</td>
</tr>
<tr>
<td>DRB1*04</td>
<td>20</td>
<td>5</td>
<td>21</td>
<td>0.045</td>
<td>n.s.</td>
<td>0.224</td>
</tr>
<tr>
<td>QBP3.1</td>
<td>35</td>
<td>10</td>
<td>22</td>
<td>0.003*</td>
<td>0.048</td>
<td>0.223</td>
</tr>
<tr>
<td>DQB1*0301</td>
<td>35</td>
<td>10</td>
<td>22</td>
<td>0.003*</td>
<td>0.048</td>
<td>0.223</td>
</tr>
<tr>
<td>DRB1*15</td>
<td>20</td>
<td>40</td>
<td>39</td>
<td>0.05</td>
<td>n.s.</td>
<td>2.50</td>
</tr>
<tr>
<td>QBP3.0602</td>
<td>17</td>
<td>27</td>
<td>39</td>
<td>n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1*01</td>
<td>17</td>
<td>27</td>
<td>7</td>
<td>n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QAP1.1</td>
<td>18</td>
<td>27</td>
<td>7</td>
<td>n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DQA1*0105</td>
<td>17</td>
<td>24</td>
<td>3</td>
<td>n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QBP5.12</td>
<td>27</td>
<td>35</td>
<td>7</td>
<td>n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QBP3.0501</td>
<td>19</td>
<td>30</td>
<td>7</td>
<td>n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QBP5.11</td>
<td>20</td>
<td>2</td>
<td>22</td>
<td>0.010*</td>
<td>n.s.</td>
<td>0.21</td>
</tr>
<tr>
<td>QBP5.0502</td>
<td>19</td>
<td>5</td>
<td>18</td>
<td>0.004*</td>
<td>n.s.</td>
<td>0.25</td>
</tr>
</tbody>
</table>

*Fisher’s exact test.

Deduced haplotypes: aDRB1*03-QAP4.1-QBP2.1-DQB1*0201; bDRB1*07-QBP2.1-DQB1*0202; cDRB1*11-QBP3.1-DQB1*0301; dDRB1*04-QBP3.1-DQB1*0301; eDRB1*15-QBP3.1-DQB1*0602; fDRB1*01-QAP1.1-DQA1*0101-QBP5.12-DQB1*0501; gQBP5.11-DQB1*0502.

DRB1*03 that has been observed in the Slovenian population [45], we assumed that the alleles QAP4.1 and DRB1*03 were transmitted on the same haplotype. The DRB1*15-DQB1*0602 haplotype could also be deduced from the previously published data [30].

Comparison of HLA class-II phenotype and deduced haplotype frequencies between anti-Ro-alone-positive and anti-Ro-negative patients

Comparison of individual HLA-class II allele frequencies between anti-Ro-alone-positive or negative SLE patients revealed that the frequencies of HLA-DQB1*0301 and its promoter QBP3.1 and the frequency of QBP5.11 were all decreased in the group of anti-Ro-alone-positive patients. On the contrary, the alleles DRB1*0101, DQB1*0501, DQA1*0101, QBP-5.12 and QAP-1.1 were decreased in the anti-Ro-negative group. After correction for multiple testing, the differences were no longer statistically significant, probably because of the relatively small numbers of patients in the two groups. In spite of this, we found that each of the DRB1*01, QAP1.1, DQA1*0101, QBP5.12 and DQB1*0501 alleles conferred a high OR for the anti-Ro alone antibody response in the SLE patients studied (Table 1). On the basis of the well-known linkage disequilibria between DRB1*0101, QBP5.12, DQB1*0501, QAP1.1 and DQA1*0101 [30, 32, 42] we considered that the whole haplotype was under-represented in anti-Ro-negative patients.

The QBP5.12 promoter allele has been reported to be always linked with DQB1*0501, while the QBP5.11 promoter allele can be linked with DQB1*0501, DQB1*0502 or DQB1*0503 [32]. In agreement with this, our typing data showed that QBP5.12 was always linked with DQB1*0501. On the other hand, the QBP-5.11 promoter allele, which was present in only one anti-Ro-alone-positive patient and six anti-Ro-negative patients, was never found to be linked to DQB1*0501, but rather to the DQB1*0502 or the DQB1*0503 allele.

Discussion

In this study we investigated the possible influence of particular HLA-DRB1, DQA1 and DQB1 coding alleles and QAP and QBP promoter alleles on the anti-Ro alone antibody response in SLE patients. All anti-Ro-alone-positive SLE patients had a stable anti-Ro alone antibody profile. In none of them did we observe spreading to the anti-La autoantibody response, which is in agreement with a study published previously [46].

While the association of both SLE itself and the anti-Ro/La antibody response with the two haplotypes HLA-DRB1*03-DQA1*0501-DQB1*0201 and HLA-DRB1*15-DQA1*0102-DQB1*0602 or their particular components is well established [8, 9, 11, 15, 18, 47], data on the association of HLA antigens with the production of anti-Ro alone antibodies are conflicting. Some studies showed that DR3 was not associated with the anti-Ro alone antibody response [3, 19]. On the other hand, Buyon et al. [48] found an association of the extended haplotype DRB1*0301-DQA1*0501-DQB1*0201 with combined anti-52-kDa Ro antibodies and low titres of anti-60-kDa Ro antibodies. Conversely, our data showed increased frequencies of...
individual DRB1*15 and DRB1*03 alleles not only in anti-Ro-alone-positive SLE patients, but also in the group of patients without these autoantibodies, compared with controls. Furthermore, the frequencies of the other components of the DRB1*03 and DRB1*15 linked haplotypes also did not differ significantly between the anti-Ro-alone-positive and anti-Ro-negative patients, suggesting that these alleles were not all preferentially associated with the production of the whole population of anti-Ro alone antibodies. The QBP2.1 promoter, which was found to be over-represented in the anti-Ro-alone-positive patients compared with controls, is a component of the extended haplotype DRB1*03-QAP4.1-DQA1*0501-QBP2.1-DQB1*0201 as well as of the two other common haplotypes, namely DRB1*07-QBP2.1-DQB1*0202 and DRB1*07-QBP2.1-DQB1*0201. In our view, the role of this promoter in directing the anti-Ro alone antibody response is therefore not crucial.

DQB1*0202 was the most significantly increased allele in the anti-Ro-alone-positive patients (19%) compared with controls. Furthermore, it was found in only one of the 28 anti-Ro-negative patients and its frequency did not differ significantly between the anti-Ro-negative patients and controls (3 and 1% respectively). Although the frequencies of this allele differed substantially between the two subgroups of SLE patients (19 vs 3%), significance was not reached, probably because of the relatively small numbers of patients in the two groups. Interestingly, DQB1*0202 was always found in association with DRB1*07 in control subjects as well as in both groups of SLE patients. However, the frequency of DRB1*07 was higher than that of DQB1*0202 within each of the three groups studied, due to the linkage of DRB1*07 with some other DQB1 alleles (data not shown). The frequencies of DRB1*07 itself did not differ significantly between the three study groups. Therefore, we believe that, within the DRB1*07-DQB1*0202 haplotype, the DQB1*0202 allele might play a more important role in directing the anti-Ro alone antibody response.

Recently, Gladman et al. [8] found a significant negative association of SLE with the DR1, DR6, DR7, DQ1 and DQ3 antigens in a large cohort of 217 SLE patients. The present study also demonstrated that DRB1*01 and DQB1*0501 (DQ1) were under-represented in anti-Ro-negative patients. Among the DRB1*01, QBP5.12, DQB1*0501, QAP1.1 and DQA1*0101 alleles, which are very probably transmitted on the same haplotype, QBP5.12 represented the highest risk for the anti-Ro alone antibody response. On the other hand, QBP5.11 seemed to be a protective factor.

A strong combined anti-Ro/La antibody response was ascribed to the DQα1 allelic coding for glutamine at position 34 of the DQα polypeptide chains and the DQB1 alleles coding for leucine at position 26 of the DQβ polypeptide chains of the DQ molecule [49]. In our study, the frequency of DQB1*0202 coding for leucine at position 26 was increased in the anti-Ro alone antibody-positive patients, while the frequency of DQB1*0301 coding for tyrosine at position 26 was decreased in the same group of patients. This indicates that the structural difference between DQB1*0202 and DQB1*0301 may influence the effectiveness of Ro antigen presentation and the anti-Ro alone antibody response. Stephens et al. [15] reported decreased frequencies of DR4 and DQB1*0301 in anti-Ro-alone-positive patients, which is in agreement with our finding. DQB1*0301 and its promoter allele QBP3.1 had similar decreased frequencies in anti-Ro-negative patients and healthy controls and were always increased when compared with anti-Ro-alone-positive patients.

There is little published data concerning the influence of promoter polymorphism of class-II genes on the development of various rheumatic diseases, such as rheumatoid arthritis [50] and early-onset pauciarticular juvenile chronic arthritis [51]. In SLE, Yao et al. [27] found the DRB1*02-QAP1.2-DQA1*0102-DQB1*0602 susceptibility haplotype in the SLE patients they studied. The results of our study indicate that the structural variability of the QBP promoter region may provide an explanation for the assumed protective effect of QBP5.11 and QBP3.1 on the anti-Ro antibody response. It was shown that the structural variability of the QBP3.1 promoter accounts for the high level of expression of DQB1*0301 molecules [33]. A higher level of expression of DQB1*0301 may thereby influence the composition of the α–β heterodimer. The protective role of the DQB1*0301 molecule may be ascribed to its inability to participate in the effective presentation of Ro antigens to potentially autoreactive helper T cells. However, the lack of anti-Ro antibodies could also be ascribed to the highly expressed DQB1*0301 molecule on the T-cell repertoire during thymic selection, thus preventing the onset of anti-Ro alone humoral autoreactivity.

In summary, our results suggest a primary association of the DQB1 molecules with the anti-Ro alone antibody response. While the DQB1*0202 allele seems to promote the anti-Ro alone antibody response, DQB1*0301 may have an opposite effect. The structural variability of the QBP3.1 and QBP5.11 promoter alleles may also contribute to the absence of the anti-Ro alone antibody response.

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