Differential contribution of HLA-DR, DQ, and TAP2 alleles to systemic lupus erythematosus susceptibility in Spanish patients: role of TAP2*01 alleles in Ro autoantibody production

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Abstract

Objective—To study the influence MHC class II and TAP2 alleles exert on systemic lupus erythematosus (SLE) susceptibility and on the clinical and serological manifestations of the disease, in a cohort of Spanish patients.

Methods—HLA-DR serological typing and HLA-DQA, DQB, and TAP2 DNA sequence specific oligotyping, were carried out in 85 unrelated Spanish SLE patients and 186 healthy controls. Autoantibodies detection was carried out by indirect immunofluorescence and counter immunoelectrophoresis.

Results—Total SLE group: the frequency of HLA-DR3 and HLA-DQA1*0501 is significantly increased in this group (p<0.005, δ=0.34 and p<0.005, δ= 0.45, respectively) although the highest δ value (δ=0.87) is obtained when the TAP2*01 alleles are considered. No DQB allele shows significant deviation from the control group. Renal damage: it mainly occurs in HLA-DR3 patients (p<0.0005 and δ=0.72). HLA-DQA1*0501 (p<0.05, δ=0.57) and DQB1*0201 (p=NS, δ=0.56) are weaker susceptibility factors. Ro+ (but not La) group: this autoantibody response is associated with TAP2*01 alleles in homozygosity (p<0.05, δ=0.81). Ro/La+ group: it has a different genetic background as HLA-DQA1*0501 (δ=1) and HLA-DQB1*0201 (δ=1) are the main susceptibility factors.

Conclusions—A differential association between HLA-DR, DQA1, and DQB1 alleles and SLE or its clinical and serological manifestations are found. Furthermore, the associations are different to the ones reported in other ethnic groups. Finally, TAP2*01 group of alleles are associated with the highest susceptibility to SLE (higher than HLA-DR3) and may influence Ro (but not La) autoantibodies production, whereas HLA-DQA1*0501 and DQB1*0201 mediates concomitant Ro and La production.

Systemic lupus erythematosus (SLE) is an autoimmune disorder with a large spectrum of clinical manifestations and a variety of immunological features. The disease is autoimmune in nature, and its precise aetiology remains poorly understood. Several studies have associated the polymorphic genes of the major histocompatibility complex (MHC) with susceptibility to SLE. Recent data suggest that the HLA system may exert its influence in the outcome and expression of the disease, and several HLA-DR and DQ markers or residues, or both, have been implicated in the appearance of specific autoantibodies. No single HLA locus has been involved in SLE susceptibility and more than one HLA locus seem to be involved. To dissect the specific HLA susceptibility locus, it is necessary to study unusual haplotypes in which SLE associated alleles occur separated from their neighbours in individual families or in certain populations. The Spanish population displays differential associations of the disease with the HLA antigens, because the susceptibility allele DR3 is associated with the A30-B18-BfF1-C2C-C4A*3-C4B*Q0 haplotype, instead of the A1-B8-BfS-C2C-C4A*Q0-C4B*1 haplotype found in the North European populations. This fact makes the Spanish population suitable to carry out these studies.

TAP1 and TAP2 genes (TAP stands for transporter for antigen processing) are placed between HLA-DP and DQ and encode for proteins that deliver cytosolic peptides across the endoplasmic reticulum membranes (where TAP molecules are placed) to nascent intraluminal HLA class I molecules. Peptide binding is necessary for the conformational correctness and export to plasma membrane of HLA molecules. TAP2 genes, located close to DR-DQ, may confer SLE susceptibility, as TAP polymorphic proteins may be involved in the processing and presentation of putative autoantigens by HLA-class I proteins. TAP2*01 include a group of alleles with a stop codon at position 687. Because in the Spanish population DR3 susceptibility genes are placed in HLA chromosomes different from North European and other white populations, it is therefore a good and complementary model to pinpoint possible SLE susceptibility genes placed close to the DR/DQ region. Thus, the purpose of this work was to study: (1) the association of SLE to HLA-DR, DQA, and DQB alleles in the Spanish population and (2) whether TAP2 alleles are SLE (or any of its clinical and serological
manifestations) susceptibility factors and whether any of them increases the risk conferred by HLA class II alleles, as TAP2 has been found increased in other autoimmune disease (diabetes) in Spaniards.4

**Methods**

**PATIENTS**

Eighty five unrelated SLE patients, diagnosed according to the American College of Rheumatology Criteria1 were studied. Among the patients, 25 with clinical evidence of renal disease had undergone kidney biopsy and classified according to the World Health Organisation criteria, slightly modified, as suffering from diffuse proliferative glomerulonephritis (DPGN). Patients lacking clinical evidence of renal disease were not biopsied. In a previous unpublished study of 20 SLE patients with no clinical evidence of renal disease, renal biopsy revealed only one with DPGN. Thus, patients with no evidence of renal disease were included in the non-DPGN group.

One hundred and eighty six unrelated adult healthy subjects of Spanish origin were used as controls. None had symptoms of SLE or other rheumatic disease and no ANA or ENA autoantibody was found in any of the healthy subjects tested.

**HLA-DR AND DQ PHENOTYPING**

HLA-DR antigens (HLA-DR1 through HLA-DR14, polymorphism encoded by the HLA-DRB1 gene) were determined by serology as previously described.3 HLA-DQA1 and HLA-DQB1 alleles recognised in the 11th Histocompatibility Workshop were detected by oligotyping as described elsewhere.11

The degree of HLA-DR polymorphism achieved by serology is adequate for the purpose of this work. However, given the limited HLA-DQ polymorphism obtained by serology, oligotyping techniques were used to study it; a decision also based on the fact that it has been recently suggested that there is a relevant role for HLA-DQ, rather than HLA-DR, in SLE susceptibility.12

**SEQUENCE SPECIFIC OLIOTYPING (SSO) ANALYSIS OF TAP2 VARIANTS.**

Amplification products were obtained with the specific primers 5'-GGGGATCGCACAGT GCTGTTG and 5'-CTGGAATTCAGGA ACAGCTAT that contain the region between positions 1954 and 2204 of the TAP2 gene. The presence of TAP2*01, which comprises a group of alleles with a stop codon at residue 687 (Thr-665, Stop-687) and thus have a short cytoplasmic domain, and TAP2*0201 (Ala-665, Gin-687) were tested by oligonucleotide typing using the oligoprobes 5'-AGGCT GCAGACAGTTCCAG and 5'-AGGCTGC AGGCAGTTCAG to detect the variation in position 665 and 5'-ATTCCCGCCTGGT GCAGC and 5'-ATTCCCGCCTGGT GCAGC to detect the variation in position 687, as previously described.7

**DETECTION OF AUTOANTIBODIES**

Indirect immunofluorescence testing for anti-nuclear antibodies (ANA) was done in all serum samples using a human epithelioid cell line as substrate. Anti-ds-DNA autoantibodies were tested by indirect immunofluorescence using *Crithidia lucilliae* as substrate. The presence of precipitating autoantibodies to SSA/Ro, SSB/La, Sm, and RNP was ascertained by double immunodiffusion and counter immunoelectrophoresis in agarose by using prototype reference serum with known anti-Ro, anti-La, anti-Sm, and anti-RNP activity.13

**STATISTICS**

The distribution of the frequencies of each HLA-DR, DQA, DQB, and TAP2 allele in SLE patients (in the total SLE group and in the different subgroups) and control subjects was compared calculating χ² test (with Yates’s correction) or Fisher test when appropriate, δ (aetiological fraction value), relative risk (RR), and their corresponding level of significance corrected by the number of alleles tested when appropriate (p), as previously described.14 All these calculations were made using two Fortran IV programs.15

Aetiological fraction value (δ) indicates the extent to which a given allele contributes to the appearance of the disease at the population level (compared with other factors that conspire to produce the disease).16 δ Value has advantages over RR values when the association is caused by linkage disequilibrium between a genetic marker and the true “diseased” genetic marker, both markers being very close at the genomic level. As the true susceptibility marker is unknown, this type of analysis will assign higher δ values to the genetic markers placed closer to the true susceptibility markers or loci, irrespective of any difference in the frequency of the two antigen alleles. The δ value may be used to quantify the strength of association of the HLA markers as an almost absolute measure of the strongest linkage disequilibrium. It is calculated as δ=d−p1−p, where d denotes the frequency of a given allele in the disease population, and p in the control healthy population. Its value ranges between 0 and 1.0 denoting no contribution at all to the appearance of the disease, and 1 absolute contribution.

The mode of inheritance (recessive or dominant) of TAP2*01 group of alleles or TAP2*0201, susceptibility or resistance alleles, respectively, was tested by the Thomson and Bodmer method.17

**Results**

**SEROLOGICAL HLA-DR FREQUENCIES**

Table 1 shows the relevant associations between HLA class II antigens and SLE or its clinical or serological manifestations. These results confirm and complete previous data obtained by our group.18

**HLA-DR3 antigen**

It shows a significant increase in the SLE group (52% vs 27% in controls, p<0.005) and in the DPGN+ (79%, p<0.0005), Ro+ (65%,
Table 1 Relevant serological HLA class II alleles associations in SLE patients and its clinical and serological manifestations (%)

<table>
<thead>
<tr>
<th>SLE (n=81)</th>
<th>DPGN+ (n=24)</th>
<th>DPGN~ (n=57)</th>
<th>Ro (n=20)</th>
<th>Ro/La (n=8)</th>
<th>Ro alone (n=12)</th>
<th>Control (n=186)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR1</td>
<td>10</td>
<td>8</td>
<td>5§</td>
<td>10</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>DR3</td>
<td>52†</td>
<td>79‡</td>
<td>43†</td>
<td>65*</td>
<td>88*</td>
<td>50</td>
</tr>
<tr>
<td>DR4</td>
<td>15</td>
<td>8</td>
<td>14</td>
<td>5</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>DR7</td>
<td>19*</td>
<td>17</td>
<td>22§</td>
<td>25</td>
<td>25</td>
<td>38</td>
</tr>
<tr>
<td>DR11</td>
<td>25</td>
<td>17</td>
<td>35‡</td>
<td>30</td>
<td>13</td>
<td>42</td>
</tr>
<tr>
<td>DR12</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>DR13</td>
<td>35*</td>
<td>33</td>
<td>43‡</td>
<td>35</td>
<td>50‡</td>
<td>25</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, †p<0.005, ‡p<0.0005. p Values are corrected by the number of alleles tested. □Denotes significances lost after correction.

Table 2 Relevant HLA-DQA1 and DQB1 antigens associations in SLE patients and its clinical and serological manifestations (%)

<table>
<thead>
<tr>
<th>SLE (n=81)</th>
<th>DPGN+ (n=25)</th>
<th>DPGN~ (n=37)</th>
<th>Ro (n=19)</th>
<th>Ro/La (n=7)</th>
<th>Ro alone (n=12)</th>
<th>Control (n=176)</th>
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</thead>
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<tr>
<td>DQA1*0101</td>
<td>19</td>
<td>24</td>
<td>13‡</td>
<td>21</td>
<td>14</td>
<td>25</td>
</tr>
<tr>
<td>DQA1*0201</td>
<td>23‡</td>
<td>24</td>
<td>27</td>
<td>37</td>
<td>14</td>
<td>50</td>
</tr>
<tr>
<td>DQB1*0201</td>
<td>69†</td>
<td>76*</td>
<td>70*</td>
<td>74</td>
<td>100*</td>
<td>58</td>
</tr>
<tr>
<td>DQB1*0501</td>
<td>13†</td>
<td>12</td>
<td>8*</td>
<td>18</td>
<td>0‡</td>
<td>33</td>
</tr>
<tr>
<td>DQB1*0604</td>
<td>11</td>
<td>16†</td>
<td>8</td>
<td>14</td>
<td>22</td>
<td>8</td>
</tr>
<tr>
<td>DQB1*0201</td>
<td>62</td>
<td>60‡</td>
<td>62</td>
<td>86‡</td>
<td>100‡</td>
<td>75</td>
</tr>
<tr>
<td>DQB1*0301</td>
<td>32</td>
<td>16</td>
<td>46‡</td>
<td>29</td>
<td>22</td>
<td>33</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, †p<0.005, ‡p<0.0005. p Values are corrected by the number of alleles tested. □Denotes significances lost after correction.

Table 3 Relevant TAP2 antigens associations in SLE patients and its clinical and serological manifestations (%)

<table>
<thead>
<tr>
<th>SLE (n=85)</th>
<th>DPGN+ (n=25)</th>
<th>DPGN~ (n=40)</th>
<th>Ro (n=21)</th>
<th>Ro/La (n=9)</th>
<th>Ro alone (n=12)</th>
<th>Control (n=155)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAP2*01</td>
<td>99*</td>
<td>96</td>
<td>98</td>
<td>100</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>0201</td>
<td>28</td>
<td>30</td>
<td>14‡</td>
<td>22</td>
<td>22</td>
<td>8</td>
</tr>
<tr>
<td>01/01</td>
<td>68</td>
<td>72</td>
<td>70</td>
<td>86*</td>
<td>78</td>
<td>92*</td>
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<tr>
<td>01/0201</td>
<td>31</td>
<td>24</td>
<td>28</td>
<td>14*</td>
<td>22</td>
<td>8</td>
</tr>
<tr>
<td>0201/0201</td>
<td>1*</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01.

HLA-DQA1*0501 allele

It is found increased though not significantly (p>0.05) when compared with the control group, although HLA-DR3 shows a very high frequency in the total SLE group and several subgroups (DPGN+, Ro+, and Ro/La+). This can be explained by the high proportion of HLA-DR7 (in linkage with HLA-DQB1*0201) found in healthy individuals (38%, see table 1).

HLA-DQB1*0201 allele

This allele, present in HLA-DR3 haplotypes, is found increased though not significantly (p>0.05), when compared with the control group, although HLA-DR3 shows a very high frequency in the total SLE group and several subgroups (DPGN+, Ro+, and Ro/La+). This can be explained by the high proportion of HLA-DR7 (in linkage with HLA-DQB1*0201) found in healthy individuals (38%, see table 1).
Differential contribution of HLA-DR, DQ, and TAP2 alleles to SLE based on the

It is uncertain whether TAP2*0201 protection only reflects TAP2*01 susceptibility, because the mathematical processing of our data has taken both “alleles” as true alleles of a diallelic system.

Inheritance of TAP2 alleles in SLE patients

Table 4 shows the δ values obtained with different HLA markers, in the SLE total group and its clinical and serological subgroups.

SLE group

The highest susceptibility to SLE is present in TAP2*01 positive subjects because it displays the highest δ value (δ=0.87). If non-DR3 subjects were considered (controls n=114, patients n=39), the susceptible TAP2*01 alleles (controls 89%, patients 97%, p>0.05, results not shown) still remain as such, although significance is not reached, probably because of the low number of patients tested. These results suggest that TAP2*01 allele may exert an additional and independent susceptibility to suffer from SLE, in HLA-DR3 people.

HLA-DQA1*0501 is, according to its δ value (δ=0.45), the second SLE susceptibility allele. This allele is present in HLA-DR3 and in some DR3 bearing haplotypes and both alleles are found significantly increased in SLE Spanish patients (see table 1); this therefore explains the δ value obtained. In fact HLA-DR3 and DR13 show a lower δ value (δ=0.34 and δ=0.21, respectively).

Renal damage (DPGN+)

It is associated to HLA-DR3 (79% v 27% in controls p<0.0005 and δ=0.72, see tables 1 and 4), whereas HLA-DQA and DQB factors do not play such an important part in this group of patients. This finding confirms our previously published data.

If DPGN+ SLE patients are compared with DPGN- SLE patients, a HLA-DR3 increase and a DQB1*0301 decrease is observed, though significance is lost upon correction in both instances (79% v 43%, p< 0.065 and...
The appearance of Ro autoantibodies, whether alone or concomitantly with La, is associated to different genetic markers in Spanish patients.

Ninety two per cent of the patients presenting Ro (without La) autoantibodies are TAP2*01/01 homozygous v 56% of controls (p<0.05, δ=0.81, see tables 3 and 4). HLA-DR11 is increased in the patients group when compared with the control group, although significance is lost when corrected by the number of comparisons made (p>0.05, δ=0.3). When both genetic markers are considered together (DR11 and TAP2*01 homozygosity) a δ=1 is obtained (p value after correction p>0.05, results not shown). If non-DR11 subjects were considered (controls n=72, Ro+ alone patients n=6) susceptibility associated to TAP2*01/01 homozygosity still remains as such, although significance is not reached (55% v 86%, p>0.05, δ=0.68, results not shown) probably because of the low number of patients tested. These results suggest that TAP2*01 mediated susceptibility acts independently of the DR locus. Finally, the inheritance of the TAP2 alleles in this group of patients shows a clear recessive model (recessive: expected frequency TAP2*01/01 92%, TAP2*01/0201 8%, TAP2*0201/0201 0%, p>0.05; dominant: TAP2*01/01 30%, TAP2*01/0201 67%, TAP2*0201/0201 3%, p<0.00005). This model of inheritance differs from the one found in the total SLE group (see results).

However, when the simultaneous production of Ro and La autoantibodies is considered, different genetic susceptibility markers are found. As table 2 shows, HLA-DQA1*0501 is present in 100% of patients v 44% of controls (p<0.05) and HLA-DQB1*0201 in 100% patients v 54% controls (p>0.05). Both alleles display the highest δ value (δ=1, table 4) and meet the postulated hypothesis that associates susceptibility to produce Ro autoantibodies to the presence of a residue of glutamine at position 34 of the DQα chain and leucine at position 26 of the DQβ chain. The DQA1*0501/DQB1*0201 heterodimer, either in cis or trans, has been implicated in the susceptibility to other autoimmune diseases, such as dermatitis herpetiformis in Spaniards and coeliac disease in white patients. Interestingly, all but one of our Ro/La+ patients are HLA-DR3, bearing this DQ heterodimer in cis. The remaining one is HLA-DR7/DR11 thus bearing it in trans.

TAP2 mediated SLE susceptibility has already been studied in other ethnic groups. TAP2C frequency (variant included in the TAP2*01 group of alleles) was increased in patients with circulating anti-Ro (but not anti-La) antibodies, although this significance was lost upon correction. However, Davies et al failed to analyse whether homozygosity would increase the susceptibility risk. On the other hand, the concomitant production of Ro and La autoantibodies is mainly mediated by HLA-DQA1*0501 and DQB1*0201 alleles and no TAP2 allele appears implicated (see table 4). Thus, the genetic background leading to the production of Ro autoantibodies alone is different from that leading to concomitant Ro/La autoantibodies. A similar conclusion was already reached by Reichlin and coworkers. In his work, SLE patients with Ro autoantibody production alone or with concomitant Ro and La production were HLA phenotyped, and it was concluded that genetic factors (HLA) were responsible for the serological differences between these two groups, and it was suggested that there was an independent control of antibodies to Ro on the one hand and joint regulation in the production of antibodies to Ro and La on the other.

The finding of an association between the TAP2*01 allele and Ro (but not La) autoantibodies, may be explained by the fact that this is the only autoantigen of those tested in this study that shows a clear cytoplasmic distribution. Thus, there is a hypothetical possibility (among others) that the shorter group of alleles would have a functional difference (enhancing) in transporting a putative Ro autoimmunogenic peptide and ease its presentation to CD8 aggressive T lymphocytes. When present in homozygosity, the patients would be more prone to producing Ro autoantibodies.

The implication of TAP genes has been studied in rheumatic diseases other than SLE. These studies disclosed little evidence for TAP alleles in disease susceptibility, although a minor effect on disease phenotype has been proposed. However, TAP alleles showed linkage disequilibrium with HLA-DR4 in the populations in which these studies were carried out, and it was concluded that the associations found were secondary to the linkage disequilibrium. This is not the case in our population, and thus the conclusions reached in this study are firmly substantiated.

Further challenges to the study of TAP alleles and disease susceptibility come from the work by Obs et al. They concluded that TAP polymorphism had no influence on peptide selection and that its contribution to disease progression and autoimmunity was not very likely. It can, however, be argued that: (1) as already suggested by the authors, subtle differences among TAP alleles were noted in the transport of a given peptide and as the reasons for these minor differences are unclear, it cannot be concluded that they can have functional consequences (that is, autoimmunity), (2) the fact that no differences were found in the peptides assayed in the experiment does not necessarily parallel physiological conditions after antigenic challenge with pathogens, where a wide array of peptides may be generated and variations at positions other than the shown in that experiment may have a dramatic effect in peptide transport, (3) the methodology used to detect peptide transport may be far less efficient than T cell activation and thus, differences in the transport, roughly detected in the experimental model used may be readily detected by T lymphocytes and have functional
consequences, and (4) because TAP2 molecules interact with HLA class I molecules acting as chaperones, in the delivery process of foreign peptides to HLA molecules, differences either in the TAP or HLA class I alleles, may also affect the travel of foreign peptides to the cell surface.

If comparisons between SLE patients with or without Ro autoantibodies (concomitantly or not with La autoantibodies) are carried out, several deviations are found although none reach significance upon correction.

The fact that comparisons between SLE patients with clinical manifestations or autoantibodies and SLE patients without them, do not reach significance upon correction (whereas it is maintained when comparisons are carried out with the healthy group) can be explained by the low number of patients present in some of the groups.

Our results show that the appearance of autoantibodies in Spanish SLE patients, is associated to different HLA alleles to those found in other populations. It should not be considered remarkable because it must be noted that the frequency with which these autoantibodies appear depends on the ethnic group studied. The pattern of production of autoantibodies in our population is closer to that of Mexican patients than to, for instance, black American patients. This differential production pattern has been stated in international collaborative studies. Thus, the appearance of autoantibodies is a genetic trait that differs depending on the population studied. This would explain the contradictory results found in the literature concerning the linkage between HLA alleles and autoantibodies in SLE patients.

From this study, it can be deduced that there is a differential HLA association to SLE or to its clinical and serological manifestations in Spanish SLE patients, supporting the notion that the HLA genes mediate susceptibility to the manifestations of the disease, rather than to SLE itself.

In summary, our results show that: (1) there is a differential association of HLA markers to SLE and its clinical or serological manifestations, as different genes underlie their appearance; (2) these associations differ to what has been reported in other ethnic groups; and (3) TAP2*01 allele is associated to SLE susceptibility and, more prominently, in the production of Ro (without La) autoantibodies.

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3 Reveille JD, MacLeod MJ, Whitington K, Arnett FC. Specific aminoacid residues in the second hypervariable region of HLA-DQA1 and DQB1 chain genes promote the Ro (SS-A)/La (SS-B) autoantibody responses. J Immunol 1991;146:3871–6.