Systemic lupus erythematosus and the extended major histocompatibility complex—evidence for several predisposing loci

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Objective. Systemic lupus erythematosus (SLE) is an autoimmune disease reported to be associated with several alleles in the HLA complex. The purpose of this study was to systematically examine the extended HLA complex (xMHC) in order to get an overview of the primary predisposing genetic factors.

Materials and methods. One hundred and sixty-four SLE patients and 254 healthy, unrelated controls were genotyped for HLA-DRB1, -B and -A alleles, as well as 13 microsatellites markers covering the xMHC. Moreover, we selected 335 additional controls matched with the patients for the HLA haplotypes showing the strongest associations, in order to look for additional predisposing loci.

Results. Two regions of the xMHC showed associations: the region covering DRB1 to B, and the extended class I region. Explicitly, DRB1*03 and B*08 displayed strong associations with SLE, which seem to be independent of each other. Furthermore, associations were seen with alleles at microsatellites D6S2225 and D6S2223, located about 3.6 Mb telomeric of HLA-B, and these were not secondary to the associations found with DRB1*03 and B*08.

Conclusion. Both the DRB1*03 and the B*08 alleles display disease association, either implicating involvement of both alleles or caused by another yet unidentified gene(s) in linkage disequilibrium. The associations found in the extended class I region could be markers for a ‘novel’ predisposing locus (loci) in SLE, adding to the risk conferred by DRB1*03 and B*08. Interestingly, this region has been shown to also be associated with other autoimmune diseases, hence the gene(s) might confer a general propensity for autoimmunity.

Key words: SLE, MHC, HLA, Microsatellites.

Systemic lupus erythematosus (SLE) is a chronic and severe inflammatory autoimmune disease. Although its aetiology remains unknown, accumulating evidence suggests that SLE is caused by the combination of genetic and environmental factors [1]. The genetic component is considered to be polygenic. A substantial genetic contribution to SLE derives from genes in the major histocompatibility complex (MHC), in humans also referred to as the human leucocyte antigen (HLA) complex, covering 3.6 Mb on the short arm of chromosome 6, encoding numerous immunological molecules including the peptide-presenting HLA class I and II molecules. The HLA complex has been expanded, particularly by extending the class I region, as novel genes of similar function have been discovered. The extended MHC (xMHC) spans 7.6 Mb and at least 28% of the expressed transcripts have potential roles in the immune system [2].

The most consistent SLE associations within the HLA complex are with HLA-DRB1*0301, -DRB1*1501, -DRB1*08, -B*08 and -A*01 [3] (reviewed in [4, 5]). Moreover, given variants of the tumour necrosis factor α (TNFα) [6] and complement C2 and C4 factors (review in [7]) encoded in the HLA class III region, as well as MHC class I chain-related genes (MICA) [8], have also been suggested to be risk factors. However, the literature concerning HLA associations with SLE is often in disagreement, with discrepancies seen in the allelic distribution for different ethnic groups [9–12], which could be due to confounding factors caused by the strong linkage disequilibrium (LD) in the xMHC. Thus, which MHC loci are involved in SLE still remains unclear.

There is growing evidence in several autoimmune and immune-mediated diseases that HLA complex genes, other than the classical HLA class I and II genes (e.g. HLA-A, -B, -C, -DR, -DQ, -DP), influence disease susceptibility. We have previously shown the presence of an as yet unidentified gene(s), located telomeric in the extended HLA class I region, that confers risk both for type 1 diabetes [13] and coeliac disease [14] on the DRB1*03-DQB1*0201 haplotype. In SLE, microsatellite scans have previously been confined to the classical HLA complex [3, 6, 15], but none has so far covered the entire xMHC.

The purpose of the present study was to systematically screen the chromosomal region covering the xMHC for genetic associations with SLE in order to map primary predisposing genes, in particular, on the so-called ‘autoimmune’ DRB1*03-B*08 haplotype [16]. To look for disease associations in genetic regions marked by a high degree of LD, such as in the HLA complex, it is necessary to ensure that associations seen are not secondary to each other. Therefore, we precisely matched cases and controls for specific HLA haplotypes to ensure that observed associations are reflecting a separate susceptibility locus.

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Materials and methods

Patients and controls

This study included 164 Norwegian patients with SLE. One hundred and twelve patients were recruited from Diakonhjemmet Hospital [mean age 45 (±25) yr; disease duration 14.5 (±12.5) yr], as described previously [17]. Fifty-two of the patients were children, adolescents and young adults identified with childhood onset disease [disease onset before the age of 16 yr; mean age 27.6 (±9.9) yr; disease duration 11.3 (±8.6) yr]. Forty-seven of these patients were recruited from Rikshospitalet University Hospital, Department of Rheumatology, identified through the hospital’s patient register. Five patients were included from other hospitals. All patients met the American College of Rheumatology criteria for SLE [18]. As controls we randomly selected 253 healthy, unrelated individuals from the Norwegian Bone Marrow Donor Registry (NBMDR). In addition, we selected 142 controls homozygous for the DRB1*03 allele, 96 controls homozygous for the B*08 allele and 193 controls carrying DRB1*15, for investigations of the extended class I region.

The study was approved by appropriate ethical review boards.

Genomic HLA typing

Genotyping for DRB1 alleles was performed using sequence-specific oligonucleotide (SSO) probes, as described previously [19]. Patients were typed for HLA-A and -B alleles using a reverse dot blot kit with SSO probes (Dynal ReliSSO kit, Norway). For the control material, HLA-A and -B data from serological class I typing was available from the NBMDR. In the first screen, we selected five evenly spaced microsatellite markers in the classical HLA complex: D6S291, DQCar, D6S273, MIB and D6S265 [20]. In the second screen we chose eight microsatellites located in the extended HLA class I region: D6S2222, D6S1001, D6S464, D6S2223, D6S2225, D6S2219, D6S1260 and D6S2239 [20, 21]. The location of the microsatellites in xMHC is illustrated in Fig. 1.

Statistical analyses

The allele frequencies among patients and controls were compared using a $\chi^2$ test or Fisher’s exact test when appropriate. Odds ratios (OR) were calculated according to Woolf’s formula, and 95% confidence intervals (CIs) were obtained using Cornfield’s approximation. $P$ values were corrected according to the Bonferroni method ($n=72$ for the first microsatellite screen, and $n=33$ in the second screen; only alleles with a frequencies higher than 5% were included in the analysis).

Haplotypes were constructed using the PHASE program (http://www.stats.ox.ac.uk/mathgen/software.html) [22, 23]. We also reanalysed our results using haplotype frequencies estimated by the expectation maximization (EM) algorithm [24] (Cocaphase program, http://www.hgmp.mrc.ac.uk) to compare the estimates obtained using the two different statistical methods. The frequencies of estimated DRB1 microsatellite haplotypes were similar for both methods (data not shown), thereby suggesting that the associations found were not caused by a bias created by the uncertainty of the estimations.

To test the influence of LD between alleles at two adjacent associated loci, we tested association with one locus in the presence or absence of associated allele at the second locus [25].

Results

SLE associations in the classical HLA complex

The distribution of the most strongly associated alleles at the classical HLA class I or II loci and at the five microsatellites

![Fig. 1. Schematic outline of the extended HLA complex indicating the position of the microsatellites genotyped for in the present study.]
spanning the HLA complex is presented in Table 1. We observed a significant increase in the frequencies of the DRB1*03, HLA-B*08 and HLA-A*01 alleles in the patients compared with the controls. The frequency of DRB1*15 was also elevated, but did not reach statistical significance after correction of the P value. Moreover, we observed a decrease in the frequency of the DRB1*04 allele in the patient population. Haplotype analysis including the HLA-A, -B and -DRB1 loci showed that susceptibility to SLE is associated with the DRB1*03-B*08-A*01 haplotype (12% in patients vs 6% in controls; OR = 2.3; 95% CI = 1.3–3.8; Punc = 0.002), which is known to be an extended haplotype.

Further, we investigated the associations on the DRB1*03-B*08-A*01 haplotype to evaluate which of the DRB1*03, HLA-B*08 or HLA-A*01 alleles are likely to be primarily responsible for the associations seen for this haplotype, using the test described by Sveigaard and Ryder [25]. In this analysis, HLA-A*01 did not show association with SLE on DRB1*03-positive (OR=0.96; 95% CI = 0.45–2.01) or DRB1*03-negative haplotypes (OR = 1.5; 95% CI = 0.86–2.49), suggesting that HLA-A*01 is not an independent risk factor but is ‘hitch-hiking’ on the DRB1*03-B*08 haplotype. On the contrary, DRB1*03 was associated with SLE on HLA-A*01-negative (OR = 2.8; 95% CI = 1.6–5.1; Punc = 0.001) and on HLA-A*01-positive haplotypes (OR = 1.9; Punc = 0.06). The independent contribution of DRB1*03 and B*08 to SLE susceptibility is shown in Table 2. The frequency of DRB1*03 was increased on HLA-B*08-negative (OR = 2.7; Punc = 0.003), but not on HLA-B*08-positive haplotypes. HLA-B*08 was significantly associated with SLE on DRB1*03-negative (OR = 2.8; Punc = 0.008), but not on DRB1*03-positive haplotypes.

Next, we analysed the frequencies of five microsatellite alleles, covering the classical HLA complex (a schematic map of investigated microsatellites is given in Fig. 1). The microsatellite markers D6S291 and D6S265 did not show any associations with the disease. Strong positive association was observed for allele 99 at DQCar, allele 140 at D6S273 and allele 350 at MIB (Table 1). We next inspected haplotypes carrying all the associated alleles. Haplotype estimation revealed that the associated alleles are part of the extended A*01–B*08–DRB1*03 haplotype: DQCar*99–DRB1*03–D6S273*140–MIB*350–B*08–A*01 (11% in patients vs 5% in controls; OR = 2.2; 95% CI = 1.3–3.8; Punc = 0.002). None of these microsatellites showed associations after controlling for the DRB1*03 effect (data not shown). Thus, the DRB1*03 allele tagged the strongly associated haplotype and when this effect is controlled for no other associations were seen within the classical HLA complex.

### Investigation of the extended class I region

Subsequently we focused on the extended class I region. It has previously been shown that this region harbours a novel, as yet unidentified, gene marked by microsatellite marker D6S2225 predisposing to type 1 diabetes [14] and coeliac disease [13] on the DRB1*0301 haplotype. Therefore, we included a dense set of microsatellites, surrounding D6S2223 (Fig. 1). As it is necessary to exclude associations arising due to LD with the above described associations, it is essential to compare cases and controls matched for specific HLA alleles. To get a sufficient number of genotype matched cases and controls is, however, in many instances a considerable problem. An alternative approach, that will provide more statistical power, is to study HLA haplotypes by comparing DRB1-matched case and control haplotypes. This does not imply that any observed effect is only *cis*-acting, but merely that any observed association is not secondary to HLA-DRB1. We focused on the DRB1*03 haplotype because DRB1*03 showed the strongest association with SLE, and these haplotypes occurred in a considerably high number. Notably, B*08 did not show any additional association in DRB1*03-positive individuals and should therefore not act as a confounding factor in this analysis (Table 2). We included an additional 142 controls who were homozygous for DRB1*03 to increase the number of DRB1*03 control haplotypes. Finally, we compared the distribution of microsatellite alleles between patients and controls only on this high-risk haplotype.

Interestingly, allele 146 at marker D6S2225 showed a significant negative association with SLE on DRB1*03 haplotypes (1% in patients vs 15% in controls; Punc = 0.0006; P = 0.02; Table 3a). In addition, allele 170 at marker D6S2223, about 24 kb centromeric of D6S2225, was increased in patients compared with controls (OR = 2.2; Punc = 0.03), and allele 178 at microsatellite D6S1001, located about 180 kb centromeric of D6S2225, showed a negative association with SLE (OR = 0.9; Punc = 0.003), suggesting the presence of an additional predisposing locus in this region. The other markers, D6S464, D6S1260, D6S2219 and D6S2222.

### Table 1. The frequencies of associated HLA-DRB1, -B, -A and microsatellite alleles in SLE patients and random controls from the first screen

<table>
<thead>
<tr>
<th>Locus, associated allele</th>
<th>SLE patients (n = 328 [%])</th>
<th>Controls (n = 508 [%])</th>
<th>OR (95% CI)</th>
<th>Punc</th>
<th>Pc</th>
</tr>
</thead>
<tbody>
<tr>
<td>DQCar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>99</td>
<td>96 (29)</td>
<td>75 (13)</td>
<td>2.7 (1.9–3.8)</td>
<td>&lt;10^-6</td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>DRB1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>73 (22)</td>
<td>84 (17)</td>
<td>1.4 (1.0–2.1)</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td>03</td>
<td>83 (25)</td>
<td>58 (11)</td>
<td>2.7 (1.8–3.9)</td>
<td>&lt;10^-6</td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>04</td>
<td>38 (12)</td>
<td>110 (22)</td>
<td>0.5 (0.3–0.7)</td>
<td>0.0002</td>
<td>0.01</td>
</tr>
<tr>
<td>D6S273</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>140</td>
<td>79 (24)</td>
<td>62 (11)</td>
<td>2.6 (1.8–3.8)</td>
<td>&lt;10^-6</td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>B</td>
<td>78 (24)</td>
<td>54 (10)</td>
<td>2.8 (1.8–3.9)</td>
<td>&lt;10^-6</td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>MIB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>350</td>
<td>102 (31)</td>
<td>96 (17)</td>
<td>2.2 (1.6–3.1)</td>
<td>&lt;10^-5</td>
<td>&lt;10^-5</td>
</tr>
<tr>
<td>A 01</td>
<td>80 (24)</td>
<td>74 (13)</td>
<td>2.1 (1.5–3.1)</td>
<td>&lt;10^-5</td>
<td>&lt;10^-5</td>
</tr>
</tbody>
</table>

n = number of alleles; OR = odds ratio; 95% CI = 95% confidence interval; Punc = uncorrected P value; Pc = corrected P value (corrected for 72 alleles tested, i.e. with a frequency higher than 5%).

### Table 2. The contribution of the HLA-DRB1*03 and HLA-B*08 alleles to disease risk by comparing SLE patients and random controls

<table>
<thead>
<tr>
<th>HLA haplotype</th>
<th>Patients (n = 328)</th>
<th>Controls (n = 508)</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B<em>08-DRB1</em>03</td>
<td>19</td>
<td>09</td>
<td>2.0 (1.3–3.0)</td>
<td>0.00036</td>
</tr>
<tr>
<td>B<em>08-DRB1</em>X</td>
<td>05</td>
<td>02</td>
<td>2.8 (1.2–6.8)</td>
<td>0.008</td>
</tr>
<tr>
<td>B<em>X-DRB1</em>03</td>
<td>06</td>
<td>03</td>
<td>2.9 (1.4–6.2)</td>
<td>0.002</td>
</tr>
<tr>
<td>B<em>X-DRB1</em>X</td>
<td>70</td>
<td>86</td>
<td>0.4 (0.3–0.6)</td>
<td>&lt;10^-6</td>
</tr>
<tr>
<td>B*08 haplotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1*03 on</td>
<td>80</td>
<td>80</td>
<td>1.0 (0.4–2.5)</td>
<td>NS</td>
</tr>
<tr>
<td>B*08 haplotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1*03 on</td>
<td>08</td>
<td>03</td>
<td>2.7 (1.3–5.6)</td>
<td>0.003</td>
</tr>
<tr>
<td>B*X haplotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B<em>08 on DRB1</em>X haplotypes</td>
<td>75</td>
<td>74</td>
<td>1.0 (0.5–2.4)</td>
<td>NS</td>
</tr>
<tr>
<td>B<em>08 on DRB1</em>X haplotypes</td>
<td>07</td>
<td>02</td>
<td>2.8 (1.2–6.5)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

OR = odds ratio; 95% CI = 95% confidence interval; n = number of individuals with a given haplotype; DRB1*03 = non-DRB1*03 allele; B*X = non-B*08 allele; NS, not significant.

*Carriage of the given allele on a particular haplotype in patients versus controls.
did not show any association. Only the association with marker D6S2225 reached statistical significance after a conservative Bonferroni correction for the number of comparisons performed (see Table 3a).

To further ensure that the observed association with D6S2225 was not secondary to HLA-B*08, we also investigated the distribution of D6S2225 alleles after controlling for HLA-B*08. Hence, we selected 92 additional controls being homozygous for B*08 and compared the frequencies of the microsatellite alleles on B*08 haplotypes in patients and controls. The results for the B*08 haplotype were comparable with those seen for the DRB1*03 haplotype (D6S2225*146: 5% in patients vs 15% in controls; OR = 0.3; 95% CI = 0.1–0.9; \( P_{unc} = 0.03 \)). Moreover, we also looked at the frequencies of microsatellite alleles on DRB1*03-B*08 haplotypes among cases and controls. Again, we observed a significantly decreased frequency of allele *146 at D6S2225 in SLE patients compared with controls (1% vs 14%; OR = 0.01; \( P = 0.004 \)). Allele 170 at D6S2225 was still increased on the DRB1*03-B*08 haplotype in SLE patients, but did not reach statistical significance (14% in patients vs 7% in controls).

Furthermore, we performed analysis of two-marker microsatellite types including all markers in the extended class I region. The analyses confirmed that the strongest association was around 1 Mb encompassing most of the class III and class II regions. Similarly, Graham et al. [3] analysing HLA risk haplotypes in families with SLE showed that the risk region conferred by DRB1*03–B*08 was not associated with SLE, neither was marker D6S265. A disease association. The HLA-DRB1*15 allele previously suggested to be associated with SLE [11, 12, 26] was in our data set only weakly increased in patients compared with controls. This is in agreement with a previous study of a separate cohort of SLE patients of Norwegian origin [27].

The associations with DRB1*03 and B*08 could be provided by risk conferred by both alleles. However, it cannot be excluded that these results reflect the existence of another as yet unidentified susceptibility locus or loci on this haplotype, in the DRB1-B region, but not extending to the A locus, since HLA-A*01 itself was not associated with SLE, neither was marker D6S265. Similarly, Graham et al. [3] analysing HLA risk haplotypes in families with SLE showed that the region confounded by DRB1*03 haplotypes could not be narrowed beyond a region of about 1 Mb encompassing most of the class III and class II regions. Interestingly, D6S273, a marker in LD with TNF, previously suggested as a risk factor for SLE [6], is carried on the extended DRB1*03–B*08 haplotype.

Moreover, genetic markers in the extended class I region could be markers for an additional predisposing locus (loci) adding to the risk conferred by DRB1*03–B*08. It may be argued that comparing the distribution of microsatellite alleles between patients and controls on only one risk DRB1 haplotype is inappropriate, because the distribution of the other haplotype would influence the allele frequency observed at the tested locus. In our material, the frequency of the second uninvestigated DRB1 haplotype (non-DRB1*03 and non-DRB1*15) did not differ among the SLE patients and the control groups. The associations with the microsatellite markers in the extended HLA class I region was observed on both the DRB1*03 and DRB1*15 haplotype, thereby strengthening the evidence for a novel and independent SLE-predisposing locus. The fact that different microsatellite alleles are associated on different haplotypes could suggest either that the responsible associated allele(s) shows different LD on different haplotypes or that there is more than one predisposing locus for SLE within the extended HLA class I region.

The finding that alleles at D6S2225 are associated with SLE on the DRB1*03 haplotype, and possibly on the DRB1*15 haplotype, supports our hypothesis that the extended class I region

### Table 3a. Distribution of alleles at associated microsatellites on DRB1*03 haplotypes among patients with SLE and controls

<table>
<thead>
<tr>
<th>Marker, associated allele</th>
<th>SLE patients (N = 83)</th>
<th>Controls (N = 284)</th>
<th>OR (95% CI)</th>
<th>( P_{unc} )</th>
<th>( P_c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>D6S1001 *178</td>
<td>1 (01)</td>
<td>34 (12)</td>
<td>0.09 (0.0–0.6)</td>
<td>0.003 NS</td>
<td></td>
</tr>
<tr>
<td>D6S2223 *170</td>
<td>14 (17)</td>
<td>24 (08)</td>
<td>2.2 (1.1–4.7)</td>
<td>0.03 NS</td>
<td></td>
</tr>
<tr>
<td>D6S2225 *172</td>
<td>56 (67)</td>
<td>225 (79)</td>
<td>0.8 (0.5–1.6)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>D6S2225 *146</td>
<td>1 (01)</td>
<td>43 (15)</td>
<td>0.07 (0.0–0.5)</td>
<td>0.0006 0.02</td>
<td></td>
</tr>
<tr>
<td>D6S2225 *148</td>
<td>43 (51)</td>
<td>117 (41)</td>
<td>1.5 (0.9–2.4)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

* \( n \% = \) number (% of alleles); \( N = \) number of DRB1*03 haplotypes tested; \( OR = \) odds ratio; \( 95\% CI = \) 95% confidence interval; \( P_{unc} = \) uncorrected \( P \) value; \( P_c = \) corrected \( P \) value (corrected for 33 alleles tested, i.e. with frequency >5%); NS = not significant.

### Table 3b. Distribution of alleles at associated microsatellites on DRB1*15 haplotypes among patients with SLE and controls

<table>
<thead>
<tr>
<th>Marker, associated allele</th>
<th>SLE patients (N = 73)</th>
<th>Controls (N = 102)</th>
<th>OR (95% CI)</th>
<th>( P_{unc} )</th>
<th>( P_c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>D6S2223 *172</td>
<td>49 (67)</td>
<td>60 (59)</td>
<td>1.4 (0.7–2.8)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>D6S2225 *150</td>
<td>4 (6)</td>
<td>17 (16)</td>
<td>0.3 (0.08–0.98)</td>
<td>0.03 NS</td>
<td></td>
</tr>
</tbody>
</table>

* \( n \% = \) number (% of alleles); \( N = \) number of DRB1*15 haplotypes tested, \( OR = \) odds ratio; \( 95\% CI = \) 95% confidence interval; \( P_{unc} = \) uncorrected \( P \) value; \( P_c = \) corrected \( P \) value (corrected for 33 alleles tested, i.e. with frequency >5%); NS = not significant.

### Discussion

We have systematically screened the xMHC in order to look for primary associations with SLE. Our results confirmed previous findings [3–5], that both the DRB1*03 and the B*08 alleles display a disease association. The HLA-DRB1*15 allele previously suggested to be associated with SLE [11, 12, 26] was in our data set only weakly increased in patients compared with controls. This is in agreement with a previous study of a separate cohort of SLE patients of Norwegian origin [27].

The associations with DRB1*03 and B*08 could be provided by risk conferred by both alleles. However, it cannot be excluded that these results reflect the existence of another as yet unidentified susceptibility locus or loci on this haplotype, in the DRB1-B region, but not extending to the A locus, since HLA-A*01 itself was not associated with SLE, neither was marker D6S265. Similarly, Graham et al. [3] analysing HLA risk haplotypes in families with SLE showed that the risk region confounded by DRB1*03 haplotypes could not be narrowed beyond a region of about 1 Mb encompassing most of the class III and class II regions. Interestingly, D6S273, a marker in LD with TNF, previously suggested as a risk factor for SLE [6], is carried on the extended DRB1*03–B*08 haplotype.

Moreover, genetic markers in the extended class I region could be markers for an additional predisposing locus (loci) adding to the risk conferred by DRB1*03–B*08. It may be argued that comparing the distribution of microsatellite alleles between patients and controls on only one risk DRB1 haplotype is inappropriate, because the distribution of the other haplotype would influence the allele frequency observed at the tested locus. In our material, the frequency of the second uninvestigated DRB1 haplotype (non-DRB1*03 and non-DRB1*15) did not differ among the SLE patients and the control groups. The associations with the microsatellite markers in the extended HLA class I region was observed on both the DRB1*03 and DRB1*15 haplotype, thereby strengthening the evidence for a novel and independent SLE-predisposing locus. The fact that different microsatellite alleles are associated on different haplotypes could suggest either that the responsible associated allele(s) shows different LD on different haplotypes or that there is more than one predisposing locus for SLE within the extended HLA class I region.

The finding that alleles at D6S2225 are associated with SLE on the DRB1*03 haplotype, and possibly on the DRB1*15 haplotype, supports our hypothesis that the extended class I region
may harbour an unidentified gene involved in the predisposition to autoimmune diseases [13, 14].

The overlap of the disease associations in this region for several diseases may point toward common risk genes involved in pathological mechanisms related to the autoimmune nature of these diseases. There are a number of genes identified in this region [2] and some of them are potential candidates for SLE, like the butyrophilin genes [28] and several transcription factors, as well as thymus-specific serine protease (PRSS16), which has been suggested to play a role in positive selection of T cells in thymus [29, 30]. However, the responsible gene remains to be identified.

There are also examples that non-HLA complex genes play a role in the development of more than one autoimmune disease, e.g. CTLA4 gene polymorphisms are associated with the risk for type 1 diabetes and Grave’s disease [31] and PTPN22 is associated with a number of autoimmune diseases [32, 33]. This leads to the hypothesis of common predisposing genes for autoimmunity.

In conclusion, our results show, that both DRB1*03 and B*08 are associated with SLE. In addition there seems to be an additional locus in the extended HLA class I region associated with SLE. However, more studies are required, both to entangle the association described by DRB1*03-B*08 and to identify the predisposing risk factor in the extended class I region.

### Key messages

- SLE is associated with a novel locus in the extended HLA class I, marked by the microsatellite D6S2225, adding to the risk conferred by the DRB1*03-B*08 haplotype.
- This locus may be a common risk factor involved in pathological mechanisms related to the autoimmune nature of several diseases.

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Clinical Vignette

Cerebral MRI is necessary in patients with rheumatoid arthritis and uveitis before undergoing therapy with TNF-α blocking agents

Optic neuritis (ON) is often the first symptom of further demyelinating events within the central nervous system (CNS), eventually developing into multiple sclerosis (MS). As for rheumatoid arthritis (RA), TNF-α was considered to be the key mediator of the inflammatory response in autoimmune CNS disorders, such as MS. Subsequently, TNF-α-blocking agents were suggested as potential therapies for MS and related disorders. However, large clinical trials in patients with MS revealed that TNF-α-blocking agents, such as lenercept, cause an increase in both relapse rates and MRI lesion load. A recent study from our group suggests that up to 30% of patients with uveitis carry clinically silent inflammatory lesions at the time of presentation, as visualized by MRI, suggestive of a chronic inflammatory disorder of the CNS.

The cases reported by Tauber et al. in Rheumatology (2005;44:405) now provide additional evidence for the failing dogma that TNF-α is a purely inflammatory cytokine. The cases suggest that, in contrast to the joints, TNF-α plays a pivotal role in mechanisms of myelin repair within the CNS. Subsequently, given the high comorbidity of uveitis with ON/MS, we suggest that patients with both RA and uveitis should undergo cranial MRI and should be examined by an experienced neurologist in order to avoid activation of clinically silent inflammatory disorders of the CNS by TNF-α-blocking agents.

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