Polymorphism in the LMP2 gene influences susceptibility to extraspinal disease in HLA-B27 positive individuals with ankylosing spondylitis

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Abstract

Objective—To investigate the potential influence of the HLA-linked LMP2 gene on disease susceptibility in HLA-B27 individuals with ankylosing spondylitis (AS).

Methods—A polymorphic CfoI restriction enzyme site in the coding region of the LMP2 gene was evaluated in genomic DNA samples from 193 white and 49 Chinese B27 individuals with well documented AS, 97 of whom had had acute anterior uveitis (AAU) and 97 peripheral arthritis; 42 samples from normal, white, B27 positive blood donors in whom AS was excluded were also evaluated.

Results—Analysis of B27 white AS individuals with AAU, peripheral arthritis, or both, revealed significant differences in genotypic distribution of this bi-allelic locus compared with B27 AS patients without extraspinal manifestations (p < 0.005) or B27 controls (p < 0.01). Furthermore, homozygosity for one LMP2 gene allele was significantly more prevalent in AS patients with AAU (71.3%) (p < 0.01) or peripheral arthritis (68.3%) (p < 0.02) than in B27 controls (45.2%). A similar genotypic distribution was noted in B27 Chinese AS individuals with extraspinal manifestations compared with those with axial disease alone.

Conclusions—These findings support the involvement of the HLA linked LMP2 gene in the expression of disease in B27 individuals and represent a novel finding in rheumatic disease.


The strong association between ankylosing spondylitis (AS) and HLA-B27 is now generally accepted, although it is also clear that only a minority of all B27 positive individuals (2%) develop the disease. The prevalence may be as high as 20% in B27 positive first degree relatives of probands with AS, suggesting a significant role for additional genetic or other factors in the development of AS in B27 positive individuals.

A number of genes mapping to the class II region of the human major histocompatibility complex (MHC) appear to be involved in antigen processing for presentation via the HLA class I pathway. Two such genes, LMP2 and LMP7, encode proteins demonstrating homology to subunits of a large multicatalytic cytosolic proteinase complex, the proteasome. The proteasome is thought to be involved in the cellular degradation of proteins and generation of oligopeptides before transport into the lumen of the endoplasmic reticulum where binding of peptides to HLA class I molecules normally occurs. Indirect evidence suggests that the LMP2 and LMP7 genes modify the proteolytic action of the proteasome complex by influencing both the efficiency and spectrum of peptides generated which are suitable for binding to HLA class I molecules.

It is possible that polymorphism in the LMP2 or LMP7 genes may influence the expression of disease in HLA-B27 individuals by influencing the spectrum of peptides available, firstly for binding to HLA-B27 and then for presentation to auto-reactive cytotoxic T cells. This is consistent with a preliminary observation from our laboratory which described an association between LMP2 gene polymorphism and the development of acute anterior uveitis (AAU) in 125 white AS patients. This study describes an extension of our analysis of LMP2 gene polymorphism in two ethnically diverse and clinically well characterised populations of B27 positive individuals with AS.

Patients and methods

PATIENTS AND CONTROLS

The patient population included 193 HLA-B27 positive, unrelated white individuals with AS as defined by the modified New York criteria. Four had associated Reiter’s syndrome, 13 had psoriasis, and 14 had inflammatory bowel disease. Patients included 152 males and 41 females of average age 41.5 years (range 21–77) and average age at onset of disease of 24.7 years (range 12–58). One hundred and twenty five of these patients have been described previously.

Peripheral synovitis was defined as inflammation, documented by a rheumatologist, occurring in joints outside the axial skeleton excluding adjacent joints (shoulders and hips), and was noted in 60 (31.1%) patients of average age 43.6 years (range 23–67) (15 females and 45 males). Average age at onset of AS for patients developing peripheral arthritis was 22.1 years (range 12–54).

Eighty seven (45.1%) of the AS patients (67 males and 20 females) had had at least one
attack of AAU. The average age of these individuals was 42.9 years (range 21–67) and average age at onset of AS was 22.5 years (range 12–54). The greater prevalence of AS patients with AAU in this population compared with other series reflects a selection bias for inclusion of AS patients with AAU in view of our interest in this area.

A second population of AS patients included 49 unrelated, HLA-B27 positive Chinese individuals from Taiwan. This population included 44 males and five females of average age 37.4 years (range 16–61) and average age at onset of disease 18.6 years (range 12–28). Peripheral arthritis was noted in 37 (75.5%) and AAU in 10 (21.3%). The higher prevalence of peripheral arthritis has been noted previously in this ethnic group.

The control population included 42 normal, unrelated, ethnically matched, white blood donors typed serologically as HLA-B27 at the Canadian Red Cross and has been described previously. An additional random control group included 163 normal, unrelated white individuals.

DNA EXTRACTION
Anticoagulated blood was obtained using sodium EDTA tubes and genomic DNA was extracted using a modified salt precipitation method.

LMP2 GENE POLYMORPHISM
LMP2 CfoI amplified fragment length polymorphism (AFLP) genotypes were assigned after polymerase chain reaction (PCR) amplification of the LMP2 gene and digestion with CfoI restriction enzyme as described previously, with the following modifications. Oligonucleotides spanning the restriction site (primer A: 5′ GAATTCGAGCCTTCA T3; primer B: 5′ GTGAACCGATGGTTT GAC 3′) were constructed to generate a 140 base pair (bp) fragment after 30 cycles at 94°C for 30 seconds and 60°C for 30 seconds. The presence of a CfoI site yielded fragments of 98 and 42 base pairs (allele B) and absence of the site a 140 bp fragment (allele A) after CfoI digestion.

HLA TYPING
HLA-B27 typing was performed at the Canadian Red Cross using standard microcytotoxicity assays and typing serum. Molecular typing for HLA-B27 was also performed as described previously when serological typing was not available (for example for samples drawn over the weekend).

STATISTICS
Comparisons of patient and control groups were performed using the Pearson χ² test. Stated p values are uncorrected. Odds ratios (OR) and corresponding 95% confidence intervals (CI) were estimated for the variables of interest.

Results
Comparison of B27 positive AS patients with B27 positive controls revealed an increased prevalence of the BB genotype and decreased prevalence of the AA allele in AS patients, although these differences were not statistically significant (p > 0.05) (table). Genotypic and allelic distribution in B27 positive AS patients was also similar to that observed in a random population of unrelated, white individuals. Significant differences in genotypic distribution became apparent when AS patients with extraspinal disease were compared with AS patients with axial disease alone (p < 0.005; χ² = 12.74, 2 df) or with normal B27 positive controls (p > 0.01; χ² = 11.22, 2 df).

An increased prevalence of the BB genotype and decreased frequency of the AA allele was evident also in patients who had had AAU or peripheral arthritis, or both, compared with either AS patients with axial disease alone, or B27 controls. Among the AS patients with AAU, 71.3% were homozygous for the B allele, compared with 49.2% of B27 positive controls (OR = 3.0; p = 0.004) (table) and 46.25% of AS patients with axial disease alone (OR = 2.9; 95% CI = 1.5 to 5.4; p = 0.001). Thirty-five of the 87 patients with AAU also had peripheral arthritis (40.2%); the prevalence of the BB genotype was 69.2% in the 52 patients with AAU alone (p = 0.02 v B27 controls).

Analysis of AS patients who had developed peripheral arthritis showed that 68.3% were homozygous for the B allele compared with 45.2% of B27 positive controls (OR = 2.6; p = 0.02) (table), and 46.25% of AS patients with axial disease alone (OR = 2.5; 95% CI = 1.3 to 5.0; p = 0.009). The prevalence of the BB genotype was 60% in the 25 patients with peripheral arthritis alone (p > 0.05 v B27 controls). AS patients were grouped into those without peripheral arthritis, some of whom had had AAU, or those without AAU, some of whom had peripheral arthritis (table). Neither group had any significant difference in genotypic distribution or prevalence of the BB

### LMP2 Gene CfoI AFLP Genotypic distribution in normal subjects and patients with AS, with and without extraspinal disease

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Genotype (No (%)</th>
<th>Odds ratio†</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AB</td>
<td>BB</td>
</tr>
<tr>
<td>Random controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 163)</td>
<td>9 (5.5)</td>
<td>65 (39.9)</td>
<td>89 (54.6)</td>
</tr>
<tr>
<td>B27 controls</td>
<td>2 (4.8)</td>
<td>21 (50.0)</td>
<td>19 (45.2)</td>
</tr>
<tr>
<td>Total AS patients</td>
<td>7 (3.6)</td>
<td>71 (36.8)</td>
<td>115 (59.6)</td>
</tr>
<tr>
<td>(n = 113)</td>
<td>0 (0.0)</td>
<td>35 (31.0)</td>
<td>78 (69.0)</td>
</tr>
<tr>
<td>AS/extraspinal disease +ve</td>
<td>0 (0.0)</td>
<td>5 (45.0)</td>
<td>27 (50.0)</td>
</tr>
<tr>
<td>(n = 80)</td>
<td>0 (0.0)</td>
<td>25 (31.3)</td>
<td>62 (68.7)</td>
</tr>
<tr>
<td>AS/extraspinal disease -ve</td>
<td>7 (8.7)</td>
<td>36 (45.0)</td>
<td>37 (46.25)</td>
</tr>
<tr>
<td>(n = 87)</td>
<td>0 (0.0)</td>
<td>25 (28.7)</td>
<td>62 (71.3)</td>
</tr>
<tr>
<td>AS/AAU +ve</td>
<td>7 (6.6)</td>
<td>35 (43.4)</td>
<td>53 (50.0)</td>
</tr>
<tr>
<td>(n = 106)</td>
<td>0 (0.0)</td>
<td>15 (59.0)</td>
<td>69 (41.0)</td>
</tr>
<tr>
<td>AS/AAU -ve</td>
<td>7 (5.3)</td>
<td>52 (39.1)</td>
<td>74 (55.6)</td>
</tr>
<tr>
<td>(n = 133)</td>
<td>0 (0.0)</td>
<td>15 (59.0)</td>
<td>69 (41.0)</td>
</tr>
</tbody>
</table>

†Calculated for BB v AA + AB combined with 95% confidence intervals; disease category is compared with B27 positive controls.
*Calculated using χ² (1 df).
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The prevalence of the BB genotype in the 35 patients who had both AAU and peripheral arthritis was 74.3% (p = 0.01 v B27 controls).

The 31 AS patients with psoriasis, Reiter's disease, or inflammatory bowel disease included 14 with AAU and 16 with peripheral arthritis. An increased prevalence of the BB genotype was also observed in these individuals with extraspinal disease (68.2%) compared with those without extraspinal disease (44.4%).

Although genomic DNA was not available from B27 positive Taiwanese normal individuals, a within group comparison of the 49 Taiwanese AS patients revealed a similar pattern of genotypic distribution, namely, an increased prevalence of the BB genotype in AS individuals with extraspinal disease (59.5%) compared with AS individuals who had axial involvement alone (41.7%). However, the differences between these small subgroups of patients were not statistically significant (p > 0.05).

Discussion

Our data support an association between polymorphism in the LMP2 gene and the development of extraspinal disease in HLA-B27 positive AS patients from two ethnically diverse populations. This work not only confirms and extends our previous preliminary observation documenting an association between polymorphism in the LMP2 gene and development of AAU in patients with AS, but also suggests that the association extends to those individuals who develop peripheral arthritis.

It is not clear from our data whether the primary LMP2 gene association centres on susceptibility to AAU, peripheral arthritis, or both complications, as more than 50% (35/60) of our patients who developed peripheral arthritis had at least one episode of AAU. This accounts for the finding that the subgroup of AS patients without AAU or that subgroup without peripheral arthritis did not differ significantly from B27 controls or AS patients with axial disease alone. Furthermore, the rather small number of AS patients who had developed peripheral arthritis as the sole extraspinal manifestation (25 patients) precludes valid conclusions as to the primary association.

The involvement of an additional HLA-linked gene in the pathogenesis of AS is consistent with the findings from a family study in which it was shown that peripheral and axial disease segregated according to the different B27 haplotypes in two families with separate B27 haplotypes. A previous population analysis has shown an association between HLA-DR7 and the development of peripheral arthritis in patients with AS, raising the possibility that the association with LMP2 gene polymorphism may reflect linkage disequilibrium with flanking HLA genes. HLA-DR class II DNA typing using PCR and sequence specific primers of an unrelated, random, white population has not revealed evidence of significant linkage disequilibrium between the LMP2 gene AFLP and HLA-DR7 in our laboratory (unpublished observations), suggesting that our observation does not reflect a primary association with HLA-DR7.

A different analysis showed no association between LMP2 CfoI AFLP genotypes and a history of AAU in AS. Possible considerations accounting for the discrepancy with our findings include ascertainment differences and primary association with other clinical parameters such as disease severity.

Our finding of an increased prevalence of homozygosity for the allele associated with extraspinal disease also supports the involvement of the non-B27 haplotype in the development of extraspinal disease in patients with AS. Although a population based study has implicated HLA-Bw60 as an additional risk factor in predisposition to AS, LMP2 genotypic distribution in 11 HLA-Bw60 positive individuals with AS in our study group was similar to that observed in remaining B27 positive AS individuals with AS (unpublished data). However, our data do not preclude the potential involvement of additional HLA and non-HLA linked genes predisposing to both AS and extraspinal disease.

The apparent requirement for homozygosity for allele B may reflect a dosage effect for generating a cryptic B27 binding uveitic self peptide which does not elicit an autoreactive T cell response under normal circumstances, but may do so when induced by a cross reactive exogenous antigen. Allele A may generate a higher avidity competitor peptide which induces tolerance in autoreactive T cells. Previous work has shown that T cells which escape tolerance induction are specific for cryptic determinants on an antigen.

A recent report described reduced MHC class I cell surface expression and inefficient presentation of the endogenous antigen HY in mice with a targeted deletion of the gene encoding LMP7. Preliminary data from our laboratory suggest no association between polymorphism in the LMP7 gene and disease in HLA-B27 positive individuals (unpublished data).

In conclusion, our population analysis of B27 positive individuals with AS from two different ethnic populations supports the hypothesis that inherited differences in antigen processing influence the pathogenesis of disease.

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References


