Analysis of the MHC class II encoded components of the HLA class I antigen processing pathway in ankylosing spondylitis

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Abstract

***Objectives*—The evaluation of the role of polymorphism within the class II encoded antigen processing genes, LMP2 and TAP, in susceptibility to ankylosing spondylitis (AS).**

***Methods*—Eighty five patients with ankylosing spondylitis, 35 B27 positive healthy controls, and 55 unrelated healthy controls were studied. TAP1 and TAP2 alleles were assigned by ARMS PCR, and LMP2 alleles were assigned by restriction enzyme digestion of a PCR product.**

***Results*—The TAP1C allele was increased in the AS group (6%) compared with random controls (1%), p = 0·03 and TAP2E was increased in AS (3·5%) compared with random controls (0%), p = 0·05. However, the frequencies of these alleles were also increased in B27 matched controls. There were no differences in LMP2 allele or genotype frequencies between AS and either of the control groups. Partitioning of patients according to presence or absence of uveitis did not reveal any significant associations.**

***Conclusions*—Increases of the minor TAP alleles, 1C and 2E, in AS reflect linkage disequilibrium between these alleles and HLA-B27. Polymorphism of the class I antigen processing pathway does not contribute significantly to AS susceptibility nor to the development of anterior uveitis associated with AS.**

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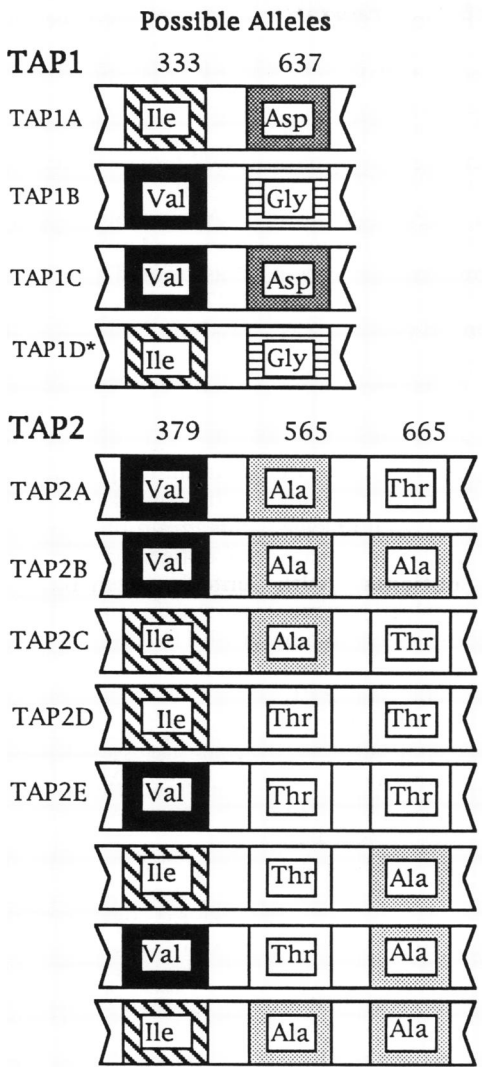
In the 20 years since the association between HLA B27 and ankylosing spondylitis (AS) was first described a great deal has been learned about the molecular biology of HLA Class I molecules and their role in the presentation of peptide antigens to the immune system.[1 2] The structure of HLA B27 has been solved and it has been shown that peptides presented by this molecule are typically nine amino acids in length with a highly conserved binding motif, including the presence of arginine at position 2 and another basic residue at position 9.[3 4] However, the precise nature of the association between HLA B27 and AS remains unclear and any explanation should take account of the fact that only certain individuals with the B27 antigen develop AS and a small proportion of patients (5%) are B27 negative.

It has been suggested that differences in the peptides presented could account for the differences in susceptibility to AS between different B27-positive individuals.[5] It is interesting therefore that some B27-positive individuals fail to present viral epitopes that are B27 restricted in other individuals.[6] One possible explanation for this could lie in the well described polymorphisms of a group of genes encoded within the class II region of the major histocompatibility complex. These encode components of the pathway by which complex antigens are broken down into peptide fragments and transported to the endoplasmic reticulum where they can associate with nascent class I molecules such as B27, subsequently to be presented on the cell surface to T lymphocytes.[7 8] Two of these genes, known as transporters, associated with antigen processing (TAP 1 and TAP 2) encode a member of the superfamily of ABC (ATP binding cassette) transporters. In the rat these have been shown to exhibit considerable polymorphism which influences the range of peptides presented by Class I HLA molecules.[9] Two other genes in this region LMP2 and LMP7 (large multi-functional protease) are believed to be involved in the cytosolic processing of antigen in the class I pathway of antigen presentation.[8] Since polymorphisms in these genes could influence both the generation of peptides and their transport to the endoplasmic reticulum before presentation by B27 we have investigated their frequencies in patients with AS, a disease with the strongest known HLA class I association.

Patients and methods

PATIENTS AND CONTROLS

Eighty five patients with typical AS, defined by the New York criteria, who were the index cases from multi-case families were included in this study. Eighty two (96%) were HLA B27 positive and 9 of these (11%) had the HLA-B27/B60 genotype that has previously been shown to be strongly associated with AS.[10] For comparison we examined a group of 55 unrelated healthy whites, and 35 B27 positive controls from the North West Regional Tissue Typing Laboratory. DNA was prepared from peripheral blood and stored at 4°C before use.



	Allele Frequencies		
	AS Patients (n = 85)	B27 Controls (n = 35)	Random Controls (n = 55)
TAP1A	82.9%	74.2%	90.9%
TAP1B	11.2%	15.7%	8.2%
TAP1C	5.9%	10.0%	0.9%
TAP1D*		Not Observed	
TAP2A	58.8%	52.9%	55.5%
TAP2B	23.5%	22.9%	33.6%
TAP2C	4.7%	8.6%	5.5%
TAP2D	9.4%	11.4%	5.5%
TAP2E	3.5%	4.3%	0.0%
		Not Observed	
		Not Observed	
		Not Observed	

The possible combinations of the amino acid dimorphisms at the TAP1 and TAP2 loci is demonstrated. Certain combinations have not been observed in this or previous studies. These include the hypothetical TAP1 D allele which is alluded to in the text. Individual allele frequencies in each of the study groups are indicated.*

DEFINITION OF ALLELES OF THE TAP LOCI

Three alleles at the TAP 1 locus and five alleles at the TAP 2 locus which have previously been well characterised were defined in this study from genomic DNA amplified by PCR using the amplification refractory mutation system as previously described.[7 11] Because these alleles are defined by different combinations of single amino acid substitutions it is not always possible to assign alleles unambiguously. For example, at TAP1 the precise allele is defined by amino acid dimorphisms at positions 333 and 637 (figure). Individuals typed as heterozygotes with val/ile at position 333 and gly/asp at position 637 could theoretically have the combination of alleles B/A or C/D*, that is, val(333)/gly(637), ile(333)/asp(637) or val(333)/asp(637), ile(333)/gly(637). In practice, however, this difficulty could be resolved because of the observation that certain combinations are either rare or have yet to be observed.[7 11] Thus in the example above the potential combination of val(333)/asp(637) which would be found in the theoretical allele D* (see figure) has never been observed, allowing the genotype to be assigned as A/B with a high degree of confidence.

DEFINITION OF ALLELES AT THE LMP2 LOCUS

Alleles at the LMP2 locus were characterised using a dimorphic *Cfo*1 restriction site described by Monaco and McDevitt.[12] *Cfo*1 digests were performed on a 2255 base pair fragment of the third exon amplified by PCR using the two primers: 5'-GG-CAGTGGAGTTTGACGG-3' and 5'-GG-CTGTCGAGTCAGCATTC-3'.[13] Cleavage of the DNA when this site was present yielded fragments 1167 and 1088 base pairs in length, as predicted by analysis of genomic DNA sequence.[14] DNA fragments generated in all these experiments were visualised by agarose gel electrophoresis under ultraviolet light after staining with ethidium bromide.

The statistical significance of differences in the individual allele and genotype frequencies between the groups was assessed by the chi-square or Fisher's exact tests as appropriate. Results were also analysed after partitioning the patients for the presence or absence of anterior uveitis since it has previously been suggested that inflammatory eye disease is associated with the presence of the minor LMP2 allele.[13]

Results

The allele frequencies at TAP1 and TAP2 are illustrated in the figure, assuming that the previously rarely or never observed alleles were also infrequent in the populations that we have studied. The TAP1C allele was increased (Fisher's exact $p = 0{\cdot}03$) in the AS group (10/170) compared with random controls (1/110) but the frequency of TAP1C was also increased in the B27 positive controls ($p = 0{\cdot}01$). There was no significant difference between AS patients and B27 positive controls suggesting that the increase in AS patients was probably secondary to linkage disequilibrium.

At TAP2 no significant differences were apparent between the patients and either of the control groups except for an excess of TAP2E in both the B27 control ($p = 0{\cdot}05$) and AS groups ($p = 0{\cdot}05$) compared with random controls. These results appear to favour linkage disequilibrium with B27 rather than a disease specific effect. There were no differences in the LMP2 allele frequencies between AS patients and either of the control groups. The minor allele frequency was 40/170 (24%) in patients and 30/110 (27%) in random controls. TAP1, TAP2 and LMP2 assumed genotype frequencies were similar between all three groups (data not shown). Furthermore, no differences were revealed at TAP1, TAP2 or LMP2 after partitioning the patients according to whether they had experienced acute anterior uveitis (37%).

Discussion

These studies indicate that there is probably linkage disequilibrium between certain alleles at the TAP loci and HLA B27 in both the B27 positive controls and the patients with AS. Thus TAP1C and TAP2E, both of which are rare in the general population, are increased in B27 positive individuals. However, this was a weak effect and not of primary importance in susceptibility to AS. Formal confirmation of linkage between the TAP and HLA-B loci would require family studies which were beyond the scope of this investigation. In our analysis we made certain assumptions in assigning alleles based on the previously observed rarity of certain alleles.[7] Unequivocal assignation of alleles was possible in about 85% of subjects in each of the study groups. However, to avoid the possibility that we had inadvertently underestimated the frequency of certain alleles we also analysed our results after reassigning the combinations of amino acid dimorphisms to give rare genotypes in the AS patients (data not shown), but this did not materially affect our conclusions. Similarly, assumed genotypes and individual dimorphisms at each of the five polymorphic sites were compared and showed no differences.

The previous observation of an excess of HLA B27/B60 genotypes in patients with AS[10] raises the possibility that a small sub-group of patients may have particular genetic features. We therefore took the opportunity to analyse our small sub-set of HLA B27/B60 positive AS patients but they did not reveal preferential associations with alleles at the TAP1, TAP2 or LMP2 loci when compared with the AS group overall.

A previous study has reported that a decreased prevalence of the minor LMP2 allele is associated with both AS, and uveitis in HLA-B27 positive individuals.[13] The results of our study, however, do not support the involvement of either LMP2 or TAP genes in phenotypic expression of disease. This is in agreement with uveitis in AS being a random event rather than a predominantly genetically determined phenomenon.[15]

In conclusion, although polymorphism of this group of genes involved in the processing of antigens before presentation by the HLA class I antigen B27 is an attractive hypothesis to explain why only a proportion of individuals with HLA B27 develop AS, our results do not support this.

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