

Distribution of HLA-B27 subtypes in patients with ankylosing spondylitis: the disease is associated with a common determinant of the various B27 molecules

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SUMMARY HLA-B27 subtypes can be defined by cellular, serological, and biochemical techniques. The seven subtypes^{4,9} so far identified represent structural variants of B27 with limited variations in the amino acid sequence of the B27 molecule. The routinely typed B27 'antigen' remains a common (shared, public) determinant present on various B27 molecules. The distribution of the subtypes varies strongly among different ethnic groups and they occur in different linkage disequilibria.⁴ In the healthy Dutch population only two subtypes were found: B27W (B27·1, B27M2+) (90%) and B27K (B27·2, B27M2-) (10%). A similar distribution of B27 subtypes was observed in 91 unrelated Dutch patients with ankylosing spondylitis (AS)—namely, 92% B27W and 8% B27K. In Oriental populations the subtype distribution is quite different: B27W occurs in less than 50%, whereas more than 50% individuals are of the B27C and B27D subtypes. Preliminary data indicate that the distribution of subtypes in healthy and diseased Oriental individuals is similar. These results suggest that the B27 and disease (AS) association is not correlated with the structural variations of one of the B27 subtypes, but with a common B27 determinant shared by various B27 subtypes. Consequently, the disease is older than the B27 variants. Further studies on disease and subtype distribution in various ethnic groups might contribute to a better understanding of the origin of both.

Key words: cytotoxic T lymphocytes.

The association between HLA-B27 and ankylosing spondylitis (AS), the HLA and disease association with the highest relative risk so far discovered, has been observed in various racial groups.¹ HLA-B27 is found in about 96% of patients with AS, but occurs in only 8% of the normal healthy Caucasian population.

Since the discovery of HLA-B27 subtypes with monoclonal antibodies (Mab)² and cytotoxic T lymphocytes (CTL)^{3,4} we have studied the role of the B27 subtypes in the association with AS.

Our results indicate that the disease susceptibility is not correlated with any of the B27 subtypes, but rather with a common determinant present on the variant forms of B27. Further studies of patients

with different ethnic origins are required to elaborate this finding.

Patients and methods

PATIENTS

Patients with AS in this study all satisfied the New York criteria of definite diagnosis of AS (sacroiliitis grade 3–4).⁵ More than 90% of the patients presented with, besides sacroiliitis, abnormalities of the spinal column, i.e., clear overbridging syndesmoses at various levels up to a complete bamboo spine. They could all be classified as having the classical form of AS, with age of onset less than 20 years and severely affected sacroiliac joints and spinal column. None of the patients had ulcerative colitis, Crohn's disease, or psoriasis.

Of 91 unrelated Dutch Caucasian AS patients, 63 were male and 28 female. Preliminary results indicated that the B27K subtype occurred more

Accepted for publication 10 November 1986.

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Table 1 Generation of HLA-B27 subtype specific CTL using selected responder and stimulator pairs

Designation of CTL	Responder		Stimulator		Specificity of CTL	⁵¹ Cr release* (%)
	HLA-A	-B	HLA-A	-B		
W1	A2 .11	B27K, 35	A2 .11	B27W, 35	B27W	41
W2	A2 . 3	B27K, 35	A3 . 3	B27W, 35	B27W	47
W3	A2 . 3	B27K, 35	A2 . 3	B27W, 35	B27W	29
K1	A2 .11	B27W, 35	A2 .11	B27K, 35	B27K	54
K2	A2 .28	B27W, 7	A2 .28	B27K, 27K	B27K	59
K3	A2 . 3	B27W, 35	A2 . 3	B27K, 35	B27K	52
C1	A24.11	B27W, 35	A24.11	B27C, 35	B27C/D	31
C2	A1 . 2	B27W, 8	A1 . 2	B27C, 8	B27C/D	48
C3	A2 .11	B27W, 51	A11.11	B27C, 27D	B27C/D	66

*The specific lysis on stimulator target cells at an effector to target ratio of 10:1.

frequently in female AS patients, and the number of female B27⁺ classical AS patients with abnormalities of the spinal column was increased. Ten patients with AS were of Oriental (Indonesian and Chinese) origin. The controls were healthy blood donors.

The HLA-B27⁺ AS⁺ patients and B27⁺ AS⁻ controls were HLA-A, B, C, and DR phenotyped by routine HLA serological typing. The B27 subtypes were determined in a standard, cell mediated lympholysis (CML) assay with subtype specific alloimmune CTL, as described.^{3 4}

DETERMINATION OF B27 SUBTYPES BY CYTOTOXIC T CELLS (TYPING OF CTL)

Generation of HLA-B27 subtype specific CTL and the CML assay have been described in detail previously.^{3 4 6} CTL were generated using selected responder cell and stimulator cell combinations as listed in Table 1.

Effector cells and ⁵¹Cr labelled target cells were incubated for eight hours at 37°C. Supernatants were harvested and the ⁵¹Cr release was determined. The cytotoxicity was calculated by the formula:

$$\frac{\text{cpm exp.} - \text{cpm in medium}}{\text{cpm 1\% saponin} - \text{cpm in medium}} \times 100$$

Experiments were repeated at least twice at three effector to target ratios.

BIOCHEMICAL ANALYSIS

Procedures for the one dimensional isoelectric focusing (1D-IEF) of HLA class I antigens have been described elsewhere.⁷ In brief, cells were metabolically labelled with [³⁵S]methionine for six hours. Cell lysates were prepared with the detergent Triton X-114. After preclearing with normal rabbit serum and staphylococcal protein A, HLA-A, B, and C antigens were precipitated with the Mab W6/32 as described. Immunoprecipitates were

digested with neuraminidase. Gel electrophoresis was carried out by the horizontal slab gel technique. Detection of radioactive products was performed by fluorography.

Results

The subtypes of B27⁺ cells were identified with series of B27W, B27K, and B27C/D subtype specific CTL (see Table 1). According to the reactivity patterns of the typing CTL on B27⁺ cells, we could identify B27W, B27K, and B27C or B27D subtypes in positive individuals.⁴

In parallel, and mainly to distinguish the B27W from the B27D subtype, 1D-IEF gel electrophoresis was performed with cells from selected B27⁺ individuals. The cell lysates were immunoprecipitated with the Mab W6/32, which recognises a monomorphic determinant of the HLA-A, B, and C heavy chains complexed with β₂ microglobulin. Four

Table 2 Distribution of HLA-B27 subtypes in Dutch control population and patients with ankylosing spondylitis and in a limited number of Oriental individuals

	Total	W	K	C/D
	n	n (%)	n (%)	n (%)
<i>Dutch</i>				
Control	70	63 (90)	7 (10)	0
Ankylosing spondylitis*	91	85 (93)	7 (8)	0
Total	161	148 (92)	14 (9)	0
<i>Oriental</i>				
Control	11	5 (45)	0 (0)	6 (55)
Ankylosing spondylitis	10	4 (40)	1 (10)	5 (50)
Total	21	9 (43)	1 (5)	11 (52)

*Sixty three men and 28 women, one patient with AS was B27W, B27K.

distinct isoelectric focusing patterns of B27 heavy chains (W, K, C, D) were identified.^{4 7}

In this study we examined the B27 subtypes from 91 unrelated Dutch Caucasian B27⁺ AS patients, 10 Oriental B27⁺ AS patients, and 81 B27⁺ control individuals. The results (summarised in Table 2) show an equal distribution of the B27W and B27K subtypes in the Dutch AS patients and the controls. No apparent preference was observed for one of the B27 subtypes and the disease susceptibility in the limited number of Oriental individuals.

The B27W subtype is prevalent in Caucasians, with a frequency of about 90% B27W and 10% B27K. None of the Caucasian cells was B27C or B27D. In contrast, subtypes B27C and B27D were observed in more than 50% of the cells of Oriental origin.

Discussion

The results of the present study again emphasise that, although B27 remains one of the best defined HLA antigens as detected by highly selected conventional alloimmune antisera,⁸ a surprisingly high number of HLA-B27 subtypes exist.^{4 9} These subtypes, which can be identified by cellular,^{3 4 10} serological,^{2 11 12} and biochemical^{7 9} (1D-IEF) techniques, occur in various linkage disequilibria.⁴ The distribution of the subtypes in various ethnic groups is clearly different.^{4 9 13 14}

The complete amino acid sequence of four HLA-B27 subtypes has been described, and the positions in which the molecules differ have been established.¹⁵⁻¹⁹ Various HLA-B27 genes have been isolated and sequenced.²⁰⁻²² Thus B27 is not only the HLA antigen with the highest disease association, but also the most studied and best understood.

HLA-B27 does not exist as a genetic entity or as the product of one B27 allele. What is routinely typed and designated as HLA-B27, is a family of (at least seven) closely related B27 molecules (variants/mutants). The B27 subtypes are structural variants occurring along about 338 amino acids of a 45 kD molecular mass polypeptide chain. The variations are due to point mutations, events similar to gene conversions, or other chromosomal rearrangements, mainly in the first or second domain, or both, of the B27 molecule.^{12 18-21 23}

The conventional anti-B27 sera that are routinely used to type the antigen B27 in HLA laboratories recognise a common B27 determinant present on the various B27 molecules. Interestingly, it is this common (public) determinant and not one of the subtype specific (private) determinants of B27 which is associated with AS. This is at variance with the conclusion from preliminary studies by Grumet *et*

al.^{12 14} They suggested that B27 variants might be disease related, especially in Orientals.

Although a relatively high number of Dutch patients and controls were tested, the data are still preliminary, particularly for other populations. Further studies on the distribution of B27 subtypes and the association with AS in various ethnic groups are needed before general conclusions can be made. Moreover, the possible role of the B27 subtypes in other disease associations, including some reactive arthropathies, can now be studied. Such studies will contribute to a better understanding of disease associations and the evolutionary aspect of HLA (B27) polymorphism.

The combined data indicate that the variations of the B27 molecule are of rather recent origin.^{18 20 21} Since the association of AS and HLA-B27 has been confirmed in patients with various racial backgrounds¹ the disease (AS) appears to be older than the origin of the B27 variants.

We thank all patients who volunteered to participate in this study and the late Bert Huis for excellent technical assistance. The study was financially supported in part by the Netherlands League against Rheumatism and in part by the Dutch Kidney Foundation.

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