Ankylosing spondylitis without B27: no evidence for gene conversion

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SUMMARY Isoelectric focusing gel electrophoresis was used to look for variant HLA molecules in five patients with HLA-B27 negative ankylosing spondylitis (AS). The isoelectric points of the HLA-A and B antigens from these patients and HLA paired controls were identical. This implies that the HLA-A and B antigens from the patients with AS and the controls are similar. Gene conversion of a nucleotide sequence from a B27 positive gene is thus unlikely to be the explanation for the existence of AS in patients who are HLA-B27 negative by alloantisera typing.

The strength of the association of HLA-B27 with ankylosing spondylitis (AS) in populations throughout the world strongly suggests a direct role for B27 in the aetiology of AS.¹ Patients with AS who are HLA-B27 negative by routine alloantisera typing are likely to have another genetic factor of importance which is instrumental in the aetiology of their disease.

The alloantisera in routine use define HLA-B27 as a single antigen. Heterogeneity of this antigen was first demonstrated by Grumet and Fendly using two dimensional polyacrylamide gel electrophoresis to differentiate variants of B27 with different isoelectric points.² Breuning *et al* reported that cytotoxic T lymphocytes were able to detect subtypes of HLA-B27,³ and, subsequently, the same group showed that the HLA-B27 subtypes also had different isoelectric points, a result which they attributed to differences in the polypeptide backbone of the heavy chain.⁴ So far, the development of AS has not been restricted to a particular variant of B27.^{5 6}

Approximately half of all patients with AS who are B27 negative have or will develop psoriasis vulgaris or inflammatory bowel disease,⁷ implying that genes for these diseases may also predispose to AS. This leaves many cases of AS without B27, however, who are not associated with skin or bowel disease. An explanation not previously investigated is gene conversion.⁸ This is a mechanism put forward to explain the appearance within a

particular gene of short nucleotide sequences that seem to be derived from a gene at another locus. Our hypothesis is that a critical part of the B27 gene may be inserted into other alleles and be expressed. This may permit class I protein to function as B27 in, for instance, antigen presentation to T cells. If present, it may be possible to determine such gene conversion by one dimensional isoelectric focusing gel electrophoresis. To test this possibility immunoprecipitated class I HLA antigens from patients with AS without B27 and from matched HLA identical controls were focused side by side to establish whether they had the same isoelectric points.

Patients and methods

PATIENTS

Five patients with AS (four male, one female) without B27 and with no clinical evidence of psoriasis or inflammatory bowel disease were selected. The HLA antigens detected in the patients by routine alloantisera testing were matched in healthy control subjects. Table 1 records the individual tissue types.

Table 1 HLA typing results for patients with ankylosing spondylitis

Patient No	HLA-A locus	HLA-B locus
1	3	8, 7
2	2, 32	21, 5
3	2, 10	12, 8
4	1, 33	7, 8
5	2	15, 12

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Fig. 1 Isoelectric focusing gel patterns of [^{35}S]methionine labelled HLA-A and B antigens from three patients with ankylosing spondylitis and HLA paired controls.

METHODS

Lymphocytes were metabolically labelled by incubation with [^{35}S]methionine for 16 hours (100 ml/ 5×10^6 cells). Cells were washed twice in phosphate buffered saline, then lysed in 0.5 ml of 50 mM trometamol (TRIS), 5 mM MgCl_2 , 0.1 mM phenylmethanesulphonyl fluoride, 0.5% Nonidet P-40. Lysates were precleared before immunoprecipitation by incubation for one hour with 10 μg of rabbit antimouse immunoglobulin followed by 50 μl of 10% formalin fixed *Staphylococcus aureus* Cowan strain 1 (SAC). W6/32 ascites (5 μg) was added and incubation at 4°C was continued for a further hour, followed by addition of 50 μl of SAC.⁹ After a further hour the SAC was pelleted and washed three times with lysis buffer. SAC bound immunoprecipitates were treated with neuraminidase before elution, by incubation at 50°C for 30 minutes in 9 M urea, 2% Nonidet P-40, 2% ampholines, 5% 2-mercaptoethanol. For isoelectric focusing in slab gels the method of van der Poel *et al* was followed.¹⁰ The gels had a pH gradient of approximately 4–7, were focused for 16 hours, then dried and autoradiographed. Exact experimental details have been described previously.¹¹

Results

Figure 1 shows the isoelectric focusing gel patterns of [^{35}S]methionine labelled HLA-A and B antigens from three patients with AS and from control subjects.

The isoelectric points for the corresponding HLA antigens are identical. Similar results were obtained for the other two patients with AS. The HLA antigens matched with control subjects were A1, 3, 10, 32, 33 and B5, 7, 8, 12, 15, and 21.

Discussion

One dimensional isoelectric focusing gel electrophoresis is sufficiently sensitive to detect variant HLA molecules that differ in only three to four amino acids.⁴ As the isoelectric points of the HLA-A and B antigens in the patients and controls were identical there was no evidence that gene conversion represents an alternative mechanism for the development of spondylitis in the absence of B27. We, therefore, consider it unlikely that there is insertion of a section of B27 into one of the HLA class I genes in these patients.

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