

SEQUENCE, GENETICS AND SEROLOGY OF A NEW HLA ALLELE - HLA-B*2723

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However, two donors were both A26 and DR12 positive and both possessed B*2723.

One B*2723 donor was B44 positive and the other was A32 positive. In both cases this had confounded detection of the B*2723 product by reactivity with particular Bw4 antisera.

PCR-SSP HLA-A, B typed donors

A search was undertaken of 20,666 PCR-SSP typed donors for subjects that appeared homozygous for HLA-B alleles that could confound the detection of B*2723 by the PCR-SSP Bw4.2 mixture.

Sixteen donors were found (B*37=1, B*27=15) that also possessed A*26 and/or DRB1*12. One possessed both A*26 and DRB1*12. None of these 16 donors had B*2723.

Frequency of HLA-B*2723

The two examples of B*2723 were found in a total of 29,851 blood donors residing in Wales. Thus, this allele has an estimated phenotype frequency of <0.01% and a gene frequency of <0.00004 in this predominantly Northern European Caucasoid population.

Clearly other examples of B*2723 could exist that are not associated with A*26 or DRB1*12.

Contribution of B*2723 to unravelling HLA-B27 and B7 'CREG' epitopes

Inspection of HLA class I amino acid sequences, and consideration of the lack of serological reactivity of 'HLA-B2723' suggested that an important epitope for HLA-B27 involves: 63E, 67C, 69A, 70K, 71A.

Similarly, 71A, 72Q, 73T, 74D, 75R, 76E, present on B7, and most B27, B42, B54, B55 and B56 specificities, probably represents an important HLA-B7 'cross-reactive group' epitope.

Summary

- ! HLA-B*2723 is a new allele that probably occurred following a gene conversion-like event involving B*27 and B*35
- ! The HLA haplotype possessing B*2723 has been identified
- ! The B*2723 product is difficult to detect serologically
- ! HLA-B*2723 has an estimated gene frequency of <0.00004 in blood donors resident in Wales

Note: An EBV-transformed B-cells line is available from a B*2723 donor.

Identification

Routine serological and PCR-SSP HLA typing of the family of a potential bone marrow transplant recipient (GI) uncovered a novel HLA-B allele. From serological HLA-B typing the patient appeared to be HLA-B7 (Bw6) only, but reacted weakly with 3 out of 8 Bw4 antisera/mabs. This suggested the presence of a second HLA-B specificity. In addition, HLA-A,B,C typing by PCR-SSP revealed an unexpected amplification with a primer mixture for Bw4.2, a motif possessed by, for example, B*27, B*37 and B*47. This likely new allele, temporarily called HLA-B*"GI" was investigated further.

Sequencing

Nucleotide sequencing of exons 2 and 3 showed that B*"GI" differed from the common B*27052 allele by 9 nucleotides. These all occur in B*35 alleles and encode 7 amino acid substitutions at positions 63 (E to N), 67 (C to F), 69 (A to T), 70 (K to N), 71 (A to T), 74 (D to Y) and 77 (D to S).

This suggests that HLA-B*"GI" occurred following a gene conversion-like event involving B*27 as recipient and probably B*35 as donor.

HLA-B*"GI" was later designated as **HLA-B*2723** by the WHO Committee for Factors of the HLA System.

A new PCR-SSP mixture was designed to specifically detect B*2723.

Family studies

Tests on GI's immediate family unequivocally defined the HLA-B*2723 bearing haplotype as:

A*26; B*2723; Cw*0202; DRB1*12; DRB3*02; DQB1*0301.

More examples of B*2723 in the Welsh Bone Marrow Donor Registry?

Serologically HLA-A, B typed donors

The B*2723 product had failed to react with 10 B27 antisera. Therefore, we reasoned that more examples of this allele might be present in the 9,185 Welsh Bone Marrow Donor Registry donors who had been HLA-A, B typed by serology alone.

Using HLA-A26 and DR12 as 'markers' for B*2723. The Registry was searched for serologically typed donors with a single HLA-B specificity and who were A26 and/or DR12 positive.

A total of 47 donors were identified who were A26 or DR12 positive. These were typed by PCR-SSP. None possessed B*2723.