Introduction

The FITC conjugated ‘HLA-B27’ monoclonal antibody FD705 (catalogue number B27F50X, One Lambda) is commonly used for routine HLA-B27/B2708 typing by flow cytometry.

We have previously shown that this antibody fails to react with the products of B*27:02 and B*27:08 (1). We have determined its reactivity against other B*27 allele products and identified its likely epitope. Accordingly this has enabled us to predict its reactivity against other ‘rare’ B*27 allele products.

Materials and Methods

**HLA-B*27 allele products**

To further determine the reactivity of FD705 against B27 specificities we tested cells from 52 subjects possessing 14 different B*27 alleles, viz. B*27:01 n=10, B*27:02 n=8, B*27:03 n=1, B*27:04 n=2, B*27:05 n=8, B*27:06 n=1, B*27:07 n=3, B*27:08 n=5, B*27:09 n=1, B*27:10 n=2, B*27:12 n=5, B*27:14 n=2, B*27:17 n=1 B*27:23 n=3.

**Flow cytometry testing**

Testing used our routine flow cytometry-based B27/B2708 typing protocol (1).

Results

**FD705 reactivity**

The FD705 monoclonal antibody gave:


- Negative findings against the products of B*27:02, B*27:08, B*27:12 and B*27:23.

- Variable reactions with B*27:01 products - some examples were positive while others were borderline positive or negative.

**FD705 epitope**

Inspection of HLA class I amino acid sequences suggested that the Bw4/Bw6-related motif 80T 81L 82L 83R was important for FD705 reactivity.

Thus, all the B*27 products that were clearly FD705 positive possessed this motif, while all those that were negative, with the exception of the B*27:23 specificity, lacked the motif.

This finding with B*27:23 suggests that other residues are involved in the FD705 epitope; a possible candidate being 70K since 70K is possessed by all FD705 positive B27 products, including B*27:01, but is absent from B*27:23.

Importantly, the B*27:01 specificity, solely, has the closely related 80T 81A 82L 83R motif.

The substitution of 81L>A (with different side chain polarity and charge) may be the cause of the unpredictable reactions of B*27:01 products with FD705 due to impaired antibody binding.

**Predicted reactivity of FD705**

On the basis of identifying the FD705 epitope as including 70K 80T 81L 82L 83R, we predict that of the 84 expressed B*27 proteins (B*27:01-B*27:89) FD705 will react with 61 (72.6%).

It will be negative with 22 (26.2%) and, as seen in this study, it will be ‘variable’ with the B*27:01 specificity.

Comment

It is clearly important to be aware of an assay's ability to detect the products of ‘rare’ HLA-B*27 alleles, especially when testing patients from diverse ethnic backgrounds.

Reference