

Flow Cytometry-Based HLA-B57/B58 Screening Prior to Abacavir Prescription



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Introduction

Abacavir, used to treat HIV/AIDS patients, may induce potentially life-threatening hypersensitivity reactions that are strongly associated with the possession of B*57:01 - found in up to some 8% of patients.

Using flow cytometry we have evaluated the HLA-B17 (B57/B58) reactivity of two monoclonal antibodies to screen for B17 with the proposal of testing positive samples for B*57:01 by a PCR-based method.

Materials and Methods

Monoclonal antibodies

There were used essentially according to the manufacturers' protocols and were:

- **0196HA (One Lambda)** an IgM anti-B17 used with goat anti-mouse IgM-FITC (F9259, Sigma-Aldrich)
 - **BIH0243 (One Lambda)** an IgM biotinylated anti-B57, B58 used with ExtrAvidin-FITC (E2761, Sigma-Aldrich)
- and an anti-CD3-PC5 (A07749, Beckman Coulter).

Flow cytometry

Fluorescence was measured on a FC500 MCL flow cytometer (Beckman Coulter). A FITC median channel fluorescence intensity (MCFI) of >4.0 was considered positive.

Reference samples

85 reference samples (HLA typed by CDC and PCR-SSP/SBT) were tested: 55 were B17 negative and included all examples of HLA-A, B, C specificities except A203, A210, A2403, B17, B3901, B3902, B4005, B5102 and B5103.

Specificities were each covered by 1-22 donors (mean 3.9 donors). HLA-C*12 and C*14 to C*18 were also covered (1-7 donors, mean 3.0).

Thirty were HLA-B17 positive and included B*57:01 (n=19 donors), B*57:02 (n = 1), B*57:03 (n = 3), B*57:04 (n = 1), B*57:08 (n = 1), B*58 (n = 5).

Random donor samples

The antibodies were also blind tested with 142 random donors being HLA typed by PCR-SSP prior to registration on the Welsh Bone Marrow Donor Registry's haemopoietic stem cell panel.

These random samples were between 1 and 7 days old.

Results

Reference samples

- Neither antibody reacted with B17 negative cells.
- 0196HA was positive with all B*57 samples except B*57:08 and reacted with 4/5 B58 subjects.
- BIH0243 was positive with all B*57 and B*58 samples.

Random samples

Both antibodies detected all 11 B*57 subjects and the 2 B*58 donors. There were no other positive tests.

The MCFI of negative tests, for both monoclonal antibodies, ranged from 0.39 to 2.49, mean 0.69, SD ± 0.35 and for positive tests ranged from 4.02 to 31.20, mean 12.42, \pm SD 6.12.

Comment

These data clearly indicate that using both 0196HA and BIH0243 monoclonal antibodies together will accurately detect HLA-B57 and B58 specificities for subsequent B*57:01 testing by a PCR-based method.