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, ±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.