

ELISA TESTING ON CYTOTOXICITY NEGATIVE BUT FLOW CYTOMETRY POSITIVE RENAL PATIENTS' SERA

WELSH BLOOD SERVICE
GWASANAETH GWAED CYMRU

WELSH TRANSPLANTATION AND
IMMUNOGENETICS LABORATORY



R WALTERS AND C DARKE

ELISA ID testing of ELISA positive sera

Sera from the 28 ELISA positive patients were tested with the appropriate Quik-ID® Class I or II ELISA kits in an attempt to determine their specificity. All ELISA testing was done according to the manufacturers' instructions.

Although some variation was seen in the reactivity of different sera, overall the 28 patients were assigned HLA-class I and/or II antibody specificities by ELISA ID testing. Of the 65 individual component specificities identified 47.7% were directed towards HLA-B specificities, 36.9% against DR, 10.8% against HLA-A, 3.1% against DQ and 1.5% were directed towards HLA-C.

Of the 28 patients 11 (39.3%) had ELISA-reactive antibodies that corresponded to the mismatched specificities (HLA-A, B, C, DR, DR51, DR52) of a previous graft (2 patients with class I and II specificities, 4 with class I only (1 was anti-HLA-C), and 5 with class II antibodies only) - Table 2.

Table 2. Antibodies identified by ELISA corresponding to a previous graft mismatch

No. of patients	HLA specificities	
1	A2	Note that of the 17 patients who had class II antibodies 7 were directed towards a previous mismatched specificity and 4 of these were anti-DR13 (Fisher's p=0.06).
1	B44	
1	B7, B40	
1	B7, DR4	
1	B61, DR52	
1	Cw5	
4	DR13	
1	DR51	

HLA antibody screening of renal patients

We have a proven strategy for HLA antibody 'screening' of sera from prospective renal transplant patients. This includes: Complement dependent cytotoxicity (CDC) testing, against Dynabeads® prepared B-cells from 36 random 5-locus PCR-SSP HLA typed blood donors and IgG flow cytometry (FC) testing against two pools of locally produced EBV-transformed B-cell lines. The pools, each of cells from 11 donors, are selected to cover the majority of HLA-A, B, C, DR and DQ specificities.

Antibodies detected by FC but not by CDC

Between June 1997 and January 2002 we tested 4,260 sera from 707 different patients by CDC and FC.

470 of these sera, from 136 patients (19.2% of the total number of patients tested), were CDC negative (after dithiothreitol treatment) but were positive when tested by our FC method.

ELISA screening of CDC negative / FC positive sera

A total of 501 sera from the 136 patients were tested by solid phase ELISA (all GTI™) for antibodies directed toward HLA class I (QuikScreen®) and HLA class II antigens (B-Screen). All ELISA testing was done according to the manufacturer's instructions.

Table 1 shows that 48.5% of patients were negative by both class I and class II ELISA techniques while the remaining 51.5% of patients were positive with either class I and/or class II ELISA screening kits.

Table 1. ELISA test results on 136 patients who were CDC negative but FC positive on antibody screening

Class I positive Class II positive	Class I negative Class II positive	Class I positive Class II negative	Class I negative Class II negative
20 (14.7%)	25 (18.4%)	25 (18.4%)	66 (48.5%)

Considerable variation was seen in the ELISA reactivity of patients' sera (largely taken at 3-monthly intervals). However, sera from 28 patients (40% of ELISA positive patients), who had consistent ELISA screening reactions over a minimum of three sera, were further examined for the specificity of their HLA-class I and/or II antibodies. These were 8 patients who were ELISA screen class I+ II+, 12 patients who were class I+ II-, and 8 patients who were ELISA screen class I- II+.

Summary

- 19.2% (136/707) of renal patients were negative by CDC screening for IgG antibodies but positive by FC testing.
- About 50% (70/136 - 51.5%) of these patients were positive by HLA-class I and/or class II ELISA antibody screening kits.
- 40% (28/70) of these "CDC negative/FC and ELISA positive" patients gave consistent ELISA screening tests - while 60% give very variable reactivity.
- Of the 28 patients with consistent ELISA reactivity 11 appeared to have ELISA reactive antibodies directed towards previous graft mismatches.

Comments

The finding that some specificities identified by ELISA ID tests corresponded to mismatches in previous grafts goes some way to authenticate the antibody specificities generally identified by ELISA ID tests on this group of "CDC negative/flow cytometry positive/ELISA positive" patients. In addition, the identification of HLA antibodies by the ELISA technique supports our long held and cautious policy of treating all previous HLA-A, B and DR graft mismatches as 'unacceptable antigens' when considering subsequent transplants.