SHORT REPORT

Laboratory investigations following an unexpectedly positive crossmatch result in a patient awaiting renal transplantation

J Cole, A Wortley, J Stoves, B Clark

J Clin Pathol 2002;**55**:627–628

In the preparation of patients for renal transplantation tests of human leucocyte antigen (HLA) sensitisation are performed to detect "unacceptable" HLA antigens that, if present on donor cells, would be expected to result in a positive crossmatch. Individuals bearing such specificities may then be excluded from consideration as donors. Unexpected positive crossmatch results are sometimes obtained when a serum specificity has not been detected on screening. Failure to identify a donor relevant HLA antibody in a recipient at the time of crossmatch may result in hyperacute rejection of the graft. This report describes laboratory investigations performed after a positive crossmatch result in a live donor situation. The pattern of crossmatch results indicated that reactivity resulted from HLA class I antibody. Previously performed serum screening using a standard complement dependent cytotoxicity technique had failed to identify donor relevant antibody specificities in the recipient. Retrospective flow cytometric screening of the same serum samples identified an HLA-A24 specificity of donor relevance. The lower sensitivity of methods used for routine serum screening compared with those used for crossmatching accounts for the findings in this case. The laboratory has amended its serum screening protocol to include flow cytometric analysis.

Longitudinal serum screening of patients awaiting renal transplantation is performed to define human leucocyte antigens (HLA) against which a patient has produced antibodies in response to a sensitising event. Comprehensively defining a patient's HLA antibody profile enables recognised antigens to be designated as "unacceptable" for transplantation, in that their presence on a donor organ would result in antibody mediated hyperacute rejection. As a safeguard against donor relevant HLA antibodies being overlooked on serum screening, the crossmatch test between donor cells and recipient serum is performed immediately before transplantation. A crossmatch negative result is required for the transplant to proceed.

"Our laboratory has now amended its serum screening protocol to include flow cytometric analysis"

Here, we present findings obtained in the investigation of a patient scheduled for transplantation from a living donor. The local crossmatch policy in this situation requires negative T and B cell results in both the complement dependent cytotoxicity (CDC) and flow cytometric assays, implying the complete absence of donor relevant HLA class I and II antibodies from the patients serum. The results of previously performed serum screening by the conventional methodology (CDC) predicted a

negative crossmatch but, contrary to expectation, a crossmatch positive result was obtained. Further analysis by novel techniques demonstrated the presence of donor relevant antibodies in the patient's serum.

CASE REPORT

We report the case of a 32 year old man with end stage renal failure secondary to chronic pyelonephritis who was awaiting retransplantation from a living, unrelated donor following first (cadaveric) graft loss as a result of chronic rejection. The first donor was mismatched for HLA A3, B17, and B27. Other relevant events in the patient's history included a two unit transfusion of non-lymphocyte depleted packed red blood cells.

LABORATORY FINDINGS

The crossmatch against the living donor was performed in a 221 mismatch situation with potential for recipient reactivity to mismatched antigens HLA A24, A26, B7, B38, and DR14. Prior screening of the recipient had shown him to be sensitised, with a 52% panel reactive antibody including HLA A3, A11, B17, and B27 plus 11% undefined reactivity. A review of the patient's history showed that no sensitising events had occurred in the period between screening and crossmatching.

At crossmatch, a profile of allo T cell negative/B cell positive, auto T cell negative/B cell negative serum reactivity was obtained by standard CDC testing,¹ whereas flow cytometric analysis² returned an allo T cell positive/B cell positive result. Because these results were consistent with recipient sensitisation to donor class I antigen we performed FlowPRA specificTM (one-Lambda)³ analysis identifying reactivity against HLA A24, one of the antigens for which the potential donor was mismatched. On the basis of these findings the donor and recipient were informed of their incompatibility and their investigation for live unrelated transplant was concluded.

Our choice of the FlowPRA method to define serum specificity in this situation permitted the unequivocal identification of donor relevant HLA antibody, increasing confidence in the interpretation of crossmatch results. Evaluations based on panels of HLA typed cells can be confounded by the unrecognised presence of non-HLA antibody in the sample. Such antibodies may also cause positive crossmatches and may therefore inappropriately deny the patient the opportunity of receiving a transplant.

On reanalysing previous results of serum screening it was found that of five HLA A24 bearing cells in the panel two had given positive results. Importantly, one of these was a chronic

Abbreviations: CDC, complement dependent cytotoxicity; HLA, human leucocyte antigen

Take home messages

- The methods for the routine screening of serum should offer an equivalent or better degree of sensitivity than the methods used at final crossmatch
- Standard complement dependent cytotoxicity techniques should be complemented by more sensitive techniques, such as flow cytometric analysis

lymphoblastic leukaemia cell but reactivity had been attributed to the HLA A11 specificity that was also carried by the cell. The reaction against the second cell had not been attributed to a particular HLA specificity.

In a further investigation of the profile of crossmatch results we have sequenced the promoter region of the donor HLA A*24 allele to establish whether our findings were related to low level expression of the antigen.⁴ This has confirmed that the donor possessed the normal expression level variant HLA A*2402101. Moreover, the cell surface antigen was detectable by conventional serology.

DISCUSSION

The relative insensitivity of serum screening by CDC in comparison with methods used at final crossmatch appears to account for the unexpected positive crossmatch result obtained in our case. This experience underlines the need to

use routine serum screening methods offering an equivalent or better degree of sensitivity than those used at final crossmatch. It also demonstrates the value of methods providing high confidence serum specificity analysis in decision making relating to a positive crossmatch.

Our laboratory has now amended its serum screening protocol to include flow cytometric analysis.

Authors' affiliations

J Cole, A Wortley, B Clark, Transplant and Cellular Immunology, St James's University Hospital, Leeds LS9 7TF, UK

J Stoves, Department of Renal Medicine, St James's University Hospital

Correspondence to: Dr B Clark, Transplant and Cellular Immunology, St James's University Hospital, Leeds LS9 7TF, UK; Brendan.Clarke@leedsth.nhs.uk

Accepted for publication 2 April 2002

REFERENCES

- 1 **Zachary AA**, Klingman L, Thorne N, *et al*. Variations of the lymphocytotoxicity test *Transplantation* 1995;**60**:498–503.
- 2 **Talbot D**. Flow cytometry crossmatching in human organ transplantation. *Transpl Immunol* 1994;**69**:719–23.
- 3 **Rebibou JM**, Chabod J, Bittencourt MC, *et al*. Flow-PRA evaluation for antibody screening in patients awaiting kidney transplantation. *Transpl Immunol* 2000;**8**:125–8.
- 4 **Laforet M**, Froelich N, Parissiadis A, *et al*. An intronic mutation responsible for a low level expression of an HLA-A*24 allele. *Tissue Antigens* 1997;**50**:340–6.

Browsing made easy

Collections

With a single click Collections allows you to find all articles that have been published in your chosen subject. Select from over 200 clinical and non-clinical topic collections and/or cross search other specialist journals, the BMJ and Cochrane Reviews

www.jclinpath.com