

Case Report

Acute vascular rejection mediated by HLA antibodies in a cadaveric kidney recipient: discrepancies between FlowPRA™, ELISA and CDC vs luminex screening

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Introduction

Sensitization is defined as the presence of preformed alloantibodies (usually anti-HLA class I antibodies, but sometimes also anti-HLA class II or non-HLA antibodies) in the serum of a prospective transplant recipient. Sensitization is measured by testing the patient's serum for cytotoxicity against a panel of lymphocytes of various HLA types. The donor's vascular endothelium, particularly of the microcirculation, is the major target of alloantibodies. The antibodies' ability to recruit effector systems—among which are complement, leucocytes, including neutrophils, natural killer cells and macrophages—results in the destruction of target organs [1]. Thus, a positive T-cell cross-match is an absolute contraindication to transplantation, because of the risk of hyperacute rejection and immediate graft dysfunction and loss. Indeed, alloantibodies may cause not only hyperacute rejection, the most severe type of humoral graft injury, but they also may contribute to acute or chronic rejections [2,3].

We present an interesting case entailing the pre-sensitization of a dialysis patient, and compare different methods of sensitization testing. If preformed alloantibodies are not detected, this may respectively influence the clinical outcome of transplantation.

Case

A 45-year-old-woman with end stage renal disease (polycystic kidney disease) received a kidney graft in our hospital. Four years before admission for transplantation, the patient's creatinine concentration had began rising from 3.4 mg/dl baseline; and 3.5 years before admission, advanced renal failure occurred and haemodialysis was begun. She did not have a history of chest pain, dyspnea, cough, fever, vomiting, or use of tobacco, alcohol, or illicit drugs. The tests for hepatitis B surface antigen and antibodies to hepatitis C were negative, as was a serologic test for VIH, although antibodies to cytomegalovirus were positive.

She did not appear to be sensitized to HLA antigens. She had a panel-reactive antibody (PRA) level approaching 0%, determined by a standard complement-dependent cytotoxicity (CDC) technique, and no history of transfusion or transplants (she had been included in the transplantation waiting list 3 years earlier). Before transplantation, a cross-match of the patient's serum and the donor's T and B cells by standard CDC assay was negative. This cross-match test (with extended times for greater sensitivity) was performed as previously published [4,5].

On the evening of her admission, a left cadaveric kidney was transplanted into the left iliac fossa without incident—except that an endarterectomy of the left common iliac artery was necessary before the anastomosis. Subsequently, the graft became pink, and a left popliteal pulse was palpable. The flow of urine was sluggish until furosemide, mannitol and fluid were infused.

During the post-transplantation inpatient days, prednisone, dopamine, albumin, mycophenolate mofetil, ranitidine and tacrolimus were given.

On the second hospital day, two units of packed red cells were transfused. Renal ultrasonography was apparently normal on that day. Though the initial

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oliguria persisted (despite administration of furosemide and mannitol) there was no hydronephrosis. A Doppler evaluation of the renal artery showed a slightly elevated resistive index (RI) (0.78); no perinephric fluid collection was identified.

On the third hospital day, radionuclide scanning showed that the perfusion of the transplanted kidney was severely reduced, with a parallel decrease in renal function—manifested as impaired tracer uptake and clearance. A Doppler study showed that the RI was further elevated (1.0), indicating poor diastolic flow in the transplanted kidney.

Surgical exploration on the next day revealed a well-perfused allograft that appeared healthy but felt soft. The examination of frozen sections of a wedge biopsy obtained at that time revealed features strongly suggestive of acute humoral rejection [6,7]. Humoral or antibody-mediated rejection is characterized by the aggregation of neutrophils in the peritubular capillaries and glomeruli, fibrin thrombi in the capillaries, and vasculitis, with occasional fibrinoid necrosis of vessel walls. Moderate focal interstitial oedema and clusters of tubules lined by flattened epithelium in our patient indicated tubular injury, without thrombosis. On the following day, treatment with plasmapheresis and three boluses of methylprednisolone (500 mg each) was begun, and surgical exploration revealed a globular graft that maintained arterial and venous blood flow.

On the seventh day, two units of packed red cells were transfused, due to a decreased haematocrit; and echography indicated a perirenal haematoma. The patient had abdominal pain. A third surgical procedure revealed a violet graft with a thrombosed vein and perirenal bruising; therefore, the grafted kidney was removed.

Post-transplant cross-matching by the CDC assay using serum drawn at the time of the first biopsy was strongly positive (as would be expected in a case of acute humoral rejection) as were other antibody screening tests—CDC (PRA = 53.2%), ELISA (GTI Diagnostic, Waukesha, WI) (PRA = 61.2%) and FlowPRA™ (OneLambda, Inc., CA) (PRA = 68.3%). FlowPRA™ microparticle evaluations were performed according to the manufacturer's recommended procedure, as previously published [5]. The ELISA and FlowPRA™ tests also detect antibodies that are not cytotoxic in a standard CDC (i.e. non-complement-fixing antibodies). The CDC assay also showed that the antibodies detected were anti-immunoglobulin G (IgG) antibodies. Indeed, we detected a high titre of an anti-A2 antibody in this screening (the donor's typing had been positive for A2). Then we performed ELISA and FlowPRA™ screening in the pre-transplant sera, and thereby confirmed the negative result (0%) of the pre-transplantation CDC screening. Figure 1 compares pre- and post-transplantation FlowPRA™ determinations. In view of these conflicting results, and because it was available in our laboratory, we applied the new luminex technology (Labscreen, OneLambda), and found in the pre-transplant sera low titres of

anti-A2 and anti-B7 antibodies, which had not been detected with the CDC, ELISA and FlowPRA™ procedures.

We then proceeded to family typing of the patient's sons (the patient had three sons and two abortions, her last pregnancy was 17 years ago). HLA-A and -B class I antigens were determined using the standard microlymphocytotoxicity technique [4,8]. HLA-DRB1 and -DQB1 genotyping was performed by the PCR-SSP method (OneLambda), as previously published by us [9].

HLA class I and II typings of the patient, donor, husband and sons are summarized in Table 1. Two of the sons presented A2, B7, DR15 and DQ6 mismatches with the mother, the other son presented A2, DR4 and DQ8 mismatches. Indeed, cross-matchings between the patient's pre-transplant serum and the sons' and husband's T and B cells by the standard CDC assay were negative, while those done with the patient's post-transplant serum were positive.

We therefore surmised that the sensitization of the patient was caused by her previous exposure to alloantigens, such as multiple pregnancies and deliveries, with exposure to paternal HLA antigens expressed by fetal cells. These alloantibodies are directed against class I and II molecules, generally in the form of high-affinity IgG. The patient probably had low titres of pre-transplant anti-donor antibodies that were not detected prospectively by the lymphocytotoxicity and flow cytometry assays. These antibodies were detected by luminex technology after the patient lost her graft; but they might have been detected before transplantation.

Discussion

For more than 30 years, the CDC assay has been the standard test for detecting preformed anti-HLA

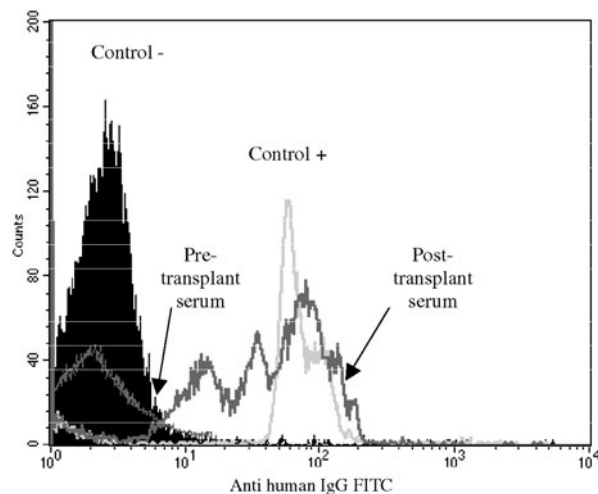


Fig. 1. FlowPRA™ screening of pre- and post-transplant sera from our patient. The histograms show the presence of IgG antibodies in the post-transplant serum and its absence from the pre-transplant serum.

Table 1. HLA class I and II typing of the patient, organ donor, husband and sons

	HLA			
	Locus A	Locus B	Locus DRB1	Locus DQB1
Patient	A23, A32	B8, B44	DRB1*07, *13	DQB1*02, *04
Organ donor	A2, A29	B8, B44	DRB1*03, *07	DQB1*02, *02
Husband	A2, A2	B7, B44	DRB1*04, *15	DQB1*03, *06
First son	A2, A23	B7, B8	DRB1*13, *15	DQB1*06, *06
Second son	A2, A23	B7, B8	DRB1*13, *15	DQB1*06, *06
Third son	A2, A23	B8, B44	DRB1*04, *13	DQB1*04, *03

antibodies in the sera of potential kidney transplant recipients. However, this assay has been criticized for being unable to detect non-complement binding, low-affinity, or low-titre antibodies. Nevertheless, the clinical relevance in kidney transplantation of additional antibodies detected by the more sensitive ELISA or flow cytometry techniques is a matter of debate [2,3,10]. In this respect, there are a number of reports that compare the different methods of HLA antibodies screening [11], although reports are limited in number where the clinical relevance and applicability of these comparisons are clearly discussed. Our present study shows that there are clear differences between these methods, and that those differences could be of major clinical importance, as in our patient. Only the luminex technology allowed the identification of antibodies, which were strongly deleterious to allograft outcome.

Although antibody-mediated rejection is associated with increased graft loss [1], in general, recovery of renal function can be achieved with early aggressive treatment. The main purpose of all available therapeutic modalities is to remove alloantibodies or control their production. The preferred treatment of early antibody-mediated rejection is the removal of alloantibodies by plasmapheresis or immunoadsorption, with or without the additional aid of immunosuppressive agents; however, this type of treatment was not sufficient to avoid the graft loss suffered by our patient.

Other surprising aspects of the present case could be the fact that the patient had circulating antibodies resulting from a sensitizing event that occurred 17 years ago, and that these antibodies in low titre were able to produce an immune response leading to graft failure in spite of an active immunosuppressive regimen. In patients with pre-existing and long-standing alloantibody responses, however, immunosuppressive agents may have little effect, possibly due to the presence of long-lived plasma cells [1]. The longevity of antibody responses is maintained by such plasma cells or by the persistence of antigen. Long-term antibody responses, however, are maintained by non-dividing, long-lived plasma cells that reside mainly in the bone marrow and produce high-affinity antibody. The competition for limited survival niches regulates the

survival of long-lived plasma cells. Once they have found their survival niches, they do not require the presence of antigen to persist [12]. In our patient, either the presence of low-titre antibodies in the recipient or the activation of memory B cells could have mediated these processes, for IgG antibodies were present in her. In this regard, exposure to antigen following transplantation can stimulate memory cells, resulting in an anamnestic response and rapid production of anti-HLA antibodies. At the time of rejection, the strong production of INF-g in the graft ensures high MHC expression, allowing IgG alloantibodies to effectively activate complement [1].

This case report also underlines the importance of identifying patients who are already sensitized by using several screening methods, and of cross-matching with remote (historic) sera. At least some antibody-mediated rejection could be prevented by the use of very sensitive screening and cross-matching methods. Indeed, the luminex technique has recently been noted to be more sensitive than other procedures for antibody detection [13].

Conclusion

The different methods used to detect HLA sensitization could lead to discrepant results. At present, the luminex technology in our opinion seems to be the most sensitive for antibody detection, and should be considered for use in transplantation or to determine modulation of immunosuppressive regimens.

Conflict of interest statement. None declared.

References

1. Vongwiwatana A, Tasanarong A, Hidalgo LG, Halloran P. The role of B cells and alloantibody in the host response to human organ allografts. *Immunol Rev* 2003; 196: 197–218
2. Susal C, Opelz G. Kidney graft failure and presensitization against HLA class I and class II antigens. *Transplantation* 2002; 73: 1269–1273
3. Terasaki PI, Ozawa M. Predicting kidney graft failure by HLA antibodies: a prospective trial. *Am J Transplant* 2004; 4: 438–443
4. Ontañón J, Muro M, García-Alonso AM *et al.* Effect of partial HLA-class I match on acute rejection in viral preinfected human liver allograft recipients. *Transplantation* 1998; 65: 1047–1053
5. Muro M, Sánchez-Bueno F, Marín L *et al.* Relationship between CDC crossmatch in liver recipients and antibodies screening by flow cytometry. *Transplant Proc* 2003; 35: 1894–1895
6. Böhmig GA, Exner M, Habicht A *et al.* Capillary C4d deposition in kidney allografts: a specific marker of alloantibody-dependent graft injury. *J Am Soc Nephrol* 2002; 13: 1091–1099
7. Sayegh MH, Colvin RB. Case 8–2003—a 35-year-old man with early dysfunction of a second renal transplant. *N Eng J Med* 2003; 348: 1033–1044
8. Muro M, Marín L, Torío A *et al.* HLA polymorphism in the Murcia Population (Spain) in the Cradle of the Archaeologic Iberians. *Hum Immunol* 2001; 62: 910–921

9. Muro M, Álvarez-López MR, Torío A *et al.* HLA-DRB1 and -DQB1 polymorphism in liver recipients: relationship between HLA-DQB1*0302 allele frequency and acute rejection. *Hum Immunol* 1997; 56: 70–76
10. Fernández-Fresnedo G, Pastor JM, López-Hoyos M *et al.* Relationship of donor-specific class-I anti-HLA antibodies detected by ELISA after kidney transplantation on the development of acute rejection and graft survival. *Nephrol Dial Transplant* 2003; 18: 990–995
11. Dyer PA, Taylor C, Martin S. HLA and clinical solid organ transplantation: recent publications and novel approaches. In: Terasaki PI, Gjertson GW, eds. *HLA 1998*. Lenexa, Kansas, 1998; 13–45
12. Slifka MK, Antia R, Whitmire JK, Ahmed R. Humoral immunity due to long-lived plasma cells. *Immunity* 1998; 8: 363–372
13. Pretl K, Chesterton KA, Scholander JT, Leffell MS, Zachary AA. Accurate, rapid characterization of HLA-specific antibody using luminex technology. *Hum Immunol* 2003; 64: S108

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