High anti-A titres may not preclude ABO-incompatible renal transplantation: an autoantibody could be the culprit

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Abstract
ABO-incompatible live donor renal transplantation is a growing field. To avoid hyperacute rejection, pre-operative ABO antibody titres should be <8. There are a number of therapeutic measures used to reduce these titres if they are high. This case report describes a patient initially found to have an extremely high anti-A IgG titre (512). The high titre results were concomitant with a positive atypical antibody screen, which showed no specificity on identification. A strategy to assess true titre levels and remove sub-clinical autoantibodies was devised, leading to successful transplantation.

Keywords: ABO blood group system; autoantibodies; kidney transplantation

Case report
Mrs SP, a 62-year-old woman, was due to receive a renal transplant from her daughter. This was an ABO-incompatible transplant (A₁ donor to O recipient) with a 1–1–1 mismatch. She had renal failure due to renovascular disease and had not yet commenced dialysis. The flow cytometry cross-match performed a month prior to transplant had been negative.

She was given a single dose of rituximab at 375 mg/m² 1 month prior to transplantation. Seven days prior to transplantation, she was started on tacrolimus 0.1 mg/kg bd and MMF 500 mg qds.

On Days 3, 2 and 1 before transplantation, she underwent immunoadsorption using carbohydrate anti-A columns (Glycorex).

The initial anti-A antibody titres performed at Guy’s and St Thomas’ laboratories were measured as 512 using an indirect antiglobulin test (IAT) with gel column agglutination. It was immediately identified that this result was unreliable as the atypical antibody screen showed a positive result, indicating an autoantibody. An increased reaction on the IAT was found at room temperature (compared with 37 °C), indicating that the autoantibody was IgM. The laboratory was unable to correctly measure the true anti-A titre due to the masking effect of the stronger autoantibody. The case was transferred to the NHSBT RCI reference laboratories. The autoantibody was removed by adsorption. To ensure complete removal of the IgM autoantibody, the sample was then treated with dithiothreitol (DTT).

As shown in Figure 1, the first assay performed using this method showed a corrected titre of 64. This reduced...
to 8 after the first immunoabsorption and 1 after the second. On the day of transplantation, titres were again 8, and post-operatively, they have remained between 2 and 4. The patient’s graft is functioning well.

Discussion

This patient had falsely elevated levels of anti-A due to the presence of a cross-reacting IgM autoantibody. All samples requiring anti-A and/or B titres are also routinely tested for atypical antibodies against a three-cell screen by an IAT. The value of this routine atypical antibody screen is demonstrated by the fact that it allowed accurate antibody titre measurement and a successful transplant.

Autoantibodies are classically IgM and of no clinical significance. However, they do present problems for blood transfusion laboratories as red cell serological techniques are often affected. In these cases, the autoantibodies can (depending on strength) be adsorbed to produce ‘clean’ plasma for retesting. This autoadsorption was performed three times in succession on a small aliquot of serum: the autoantibody is gradually removed until the activity is non-detectable. This process will not remove anti-A as the patient’s own red cells do not carry the A antigen. Anti-A can then be measured without interference from the autoantibody. At this stage, a direct agglutination test was performed to assess the IgM anti-A antibody levels. No IgM antibody could be detected, but to ensure that any residual undetected IgM did not interfere with the true anti-A assay, the plasma was also treated with DTT, which cleaves disulphide bonds in the pentameric IgM basic antibody structure, resulting in monomeric forms with reduced ability to elicit agglutination.

The RCI laboratories perform IAT under direct vision. In this case, agglutination was seen only after the addition of anti-human globulin, not before, so confirming the absence of IgM anti-A antibody and the presence of IgG anti-A antibody. It was only once this had been confirmed that DTT was used. If IAT is not performed under direct vision (GSTS Pathology use DiaMed gel card technology for all blood group serology), the use of DTT could lead to an IgM antibody being missed, and is not recommended.

Desensitization protocols do not differentiate between IgG and IgM antibodies (and there would be no purpose in doing so). Our approach to pre-operative ABO-incompatible titre assays is to measure the total immunoglobulin load using gel cards for IAT and make therapeutic decisions based on that total level, rather than measuring IgG and IgM levels separately. In this case, there were no IgM antibodies, so clinical decisions were based purely on the IgG level.

As Jordan [3] suggests, centres undertaking ABO-incompatible transplantation should be fully equipped for all issues arising from the incompatibility. In this case, access to appropriate expertise enabled a previously unencountered autoantibody to be identified and dealt with; therefore allowing the transplant to continue with a successful outcome.

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Osseous metaplasia in a kidney allograft

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Abstract
Osseous metaplasia is defined by the presence of heterotopic normal bone tissue in a soft tissue. The bone matrix is associated with osteoblasts, osteoclasts, adipocytes and haematopoietic stem cells. Osseous metaplasia pathophysiology is not well known, but many factors have been incriminated including chronic inflammation and chronic ischaemia. We describe the second case of osseous metaplasia in a kidney allograft. Numerous factors might favour its development including factors linked to transplantation failure environment.

Keywords: bone; kidney; metaplasia; transplantation

Case report
A 21-year-old woman received a first renal transplant in 1995. The cause of end-stage renal disease was interstitial nephritis due to a vesico-ureteral reflux in her unique congenital kidney.

The transplant was from a 19-year-old cadaveric male donor dead from trauma. Cold ischaemia time was 19 h, and the number of HLA mismatches between recipient and donor was four. Donor and recipient were seropositive for cytomegalovirus and Epstein–Barr virus. Immunosuppressive regimen included cyclosporine, azathioprine and corticosteroids after an initial induction treatment with anti-thymocyte globulin. The patient required haemodialysis during the first week. Then, renal function improved, and at 1 year, mean serum creatinine was 150 μmol/L (MDRD 40 mL/min/1.73 m2).

Transplantation was complicated by several urinary tract infections including more than five graft pyelonephritis with classical urinary tract germs. Infections were favoured by a permanent vesico-ureteral reflux in the transplant. Despite macroplastic injection and ureteral surgical re-implantation in 1999, reflux was not corrected, and pyelonephritis episodes continued. She had no hypertension and no acute rejection. The native kidney reflux did not favour these infections since our patient was anuric at