Resolution of renal allograft-associated post-transplant lymphoproliferative disorder with the introduction of sirolimus

Jeffrey S. Zaltzman1, Ramesh Prasad1, Kathy Chun2 and Serge Jothy3

1Division of Nephrology and 3Department of Laboratory Medicine and Pathobiology, St. Michael’s Hospital and
2Department of Pathology, University Health Network University of Toronto, Ontario, Canada

Keywords: post-transplant lymphoproliferative disorder; sirolimus

Introduction

Lymphoid neoplasia has remained a serious complication following solid organ transplantation. Recent data suggest an 11.8-fold higher risk of lymphoma compared with the general population over a 10 year period [1]. Although the risk is greatest within the first year post-transplant, the incidence remains high throughout the entire post-transplant period. Risk factors include unrestrained proliferation of Epstein–Barr virus (EBV) and the overall immunosuppression burden [2,3]. Post-transplant lymphoproliferative disorder (PTLD) may also occur within the allograft and may be mistaken for acute rejection. The mTOR inhibitor sirolimus is currently under investigation as an antitumour agent, and its use as an immunosuppressive agent in solid organ transplants has been associated with a lower risk of post-transplant malignancies, including PTLD [4,5].

Case

A 32-year-old patient with end-stage renal disease secondary to vesicoureteric reflux received a one haplotype-matched kidney from his sister. He had been on thrice weekly haemodialysis for 2.5 years. Both donor and recipient were cytomegalovirus IgG negative, but EBV IgG positive. At the time of transplant, a donor kidney biopsy was performed as per standard practice at our institution. The biopsy revealed normal renal histology. The recipient had a panel-reactive antibody of 0% and was a one-haplotype match with his donor. At the time of transplant, he was enrolled into a prospective three-arm clinical trial comparing steroid avoidance, rapid steroid tapering and standard steroid dosing. He had been randomized to an immunosuppressive regimen of basiliximab 20 mg on day 0 and day 4 post-transplant, solumedrol 1 mg/kg pre-transplant and 1 mg/kg bid for 48 h post-transplant, followed by a rapid oral prednisone taper with discontinuation of prednisone by day 7, EC-MPS 720 mg bid and cyclosporin microemulsion, dosed to keep 2 h post-dose (C2) levels between 1200 and 1600 ng/ml. There were no immediate or early surgical complications and the allograft functioned well with an immediate decline in serum creatinine. However, by day 5 post-transplant, the creatinine had stabilized at 200–240 μmol/l with a C2 cyclosporin level of 1430 ng/ml. At that time, the white blood cell count was 6.2 × 10⁹/l and the lymphocytes were normal at 2.2 × 10⁹/l. The patient remained asymptomatic and afebrile. Renal ultrasound and Dopplers were normal, and there was no response to reduction in cyclosporin dose. A renal biopsy was performed on day 7 post-transplant (Figure 1). There was no tubulitis, vasculitis or glomerulitis. Despite intensive screening of all biopsy sections, no evidence for either cellular- or antibody-mediated rejection could be found. However, there was a dense intense infiltrate of mononuclear cells occupying approximately one-quarter of the renal cortex. The cells were monotonous and medium size, fairly atypical with a moderate degree of mitotic activity. Although some T cells were present, the predominant cells were B lymphocytes (CD20⁺) demonstrating both κ and λ light chain positivity, but negative for EBV virus by in situ hybridization.

To identify whether the infiltrating lymphocytes were of donor or recipient origin, in situ hybridization was performed based on the identification of markers associated with the X and Y chromosome. A 4 μm
paraffin section on a positively charged slide was pre-treated using the Vysis (Downers Grove, IL) Paraffin Pretreatment Kit as described by the manufacturer. Fluorescence in situ hybridization was carried out according to established methods. Briefly, the slide was dehydrated by sequential immersion in 70, 90 and 100% ethanol for 2 min each and then air-dried. The CEP X/Y DNA Probe Kit (Vysis) was used to determine the sex complement of the cells. The X centromere was labelled with SpectrumOrange and the Y centromere was labelled with SpectrumGreen. Female (donor) cells would have two orange (X chromosome) signals and male (recipient) cells would have one orange (X chromosome) and one green (Y chromosome) signal. Probe and target were co-denatured and then allowed to hybridize overnight at 37°C. The slide was washed in 0.4x SSC/0.3% NP-40 at 72°C for 2 min, then in 2x SSC/0.1% NP-40 at room temperature for 1 min. The nuclei were counterstained with a 1:2 mixture of 4',6-diamidino-2-phenylindole II to mounting media. The slide was evaluated on an epi-fluorescent microscope using the PowerGene imaging system with MacProbe software 4.4 (Applied Imaging, Santa Clara, CA). As shown in Figure 2, the renal tubular cells were female in origin; however, the B-lymphocyte cell infiltrate was clearly from the male recipient.

The final diagnosis was consistent with a variant of PTLD; however, the absence of EBV positivity and the very early presentation post-transplant were atypical features. The patient was given oral valganciclovir 450 mg bid. The EC-MPS was discontinued and prednisone 15 mg per day was reinstituted, not because of concerns regarding the diagnosis, but as prophylaxis against possible acute rejection given the discontinuation of the EC-MPS and planned reduction in the cyclosporin. Over the next 4 weeks there was neither an improvement nor a deterioration in renal function despite a lowering of cyclosporin dosage, with resultant C2 levels ranging between 600 and 800 ng/ml. A second allograft biopsy was done 4 weeks later, which showed pathology similar to the previous biopsy. Again there was no evidence of tubulitis, or antibody-mediated rejection, with the predominant feature consisting of a dense lymphocytic infiltrate compatible with PTLD.

At this point, tacrolimus was substituted for cyclosporin (trough levels 5–7 ng/ml) and sirolimus 3 mg daily was begun, with [SRL] maintained at 5–8 ng/ml. Prednisone was continued at 5 mg/day. Within 2 weeks,
the serum creatinine had improved from 236 to 170 µmol/l. A third post-transplant biopsy was performed 4 months later, which revealed complete resolution of the previously seen lymphocytic infiltrates.

Discussion

This case is unique in several respects. First, the development of an atypical lymphocytic infiltration occurred within 1 week post-transplant. The time course for this was quite rapid, and unexpected. Secondly, the patient was not at particularly high risk for the development of PTLD, being EBV positive, and he had not received aggressive immunosuppressive therapy. Thus, the diagnosis of PTLD was questioned. The cells were predominantly CD20+, although there were up to 40% of T-cells present in some areas as well. This combination of B and T cells is often observed in early cases of PTLD [6]. The B cells were polyclonal, as shown by the dual expression of κ and λ light chains. What is clear, however, is that these cells were not present at the time of transplantation, as the donor biopsy was normal. Because in situ hybridization showed both X and Y chromosome signals, we conclude that the infiltrating lymphocytes were of recipient origin. In addition to ongoing allograft dysfunction, the infiltrate persisted on the second biopsy despite reduction of immunosuppression. One certainty, however, was the absence of acute cellular or antibody-mediated rejection seen on any of the three biopsies. However, the most remarkable finding was the complete resolution of the lymphocytic infiltrate and improvement of renal function with the addition of sirolimus. The improvement in creatinine could not be attributed to the withdrawal of cyclosporin alone, since this was substituted for another calcineurin inhibitor, tacrolimus. Prior to this, the dosage of cyclosporin A (CsA) had been continuously reduced, with no improvement in renal allograft function. The antiviral agent valgancyclovir did not appear to have any beneficial effects, as it had been used for 4 weeks with no change in renal function. Given the absence of EBV positivity, this was not unexpected.

The diagnosis of PTLD is not always readily established. Descriptions of monomorphic vs polymorphic PTLD have been used extensively in the classification of these lesions. What makes the diagnosis likely in this case relates to both the pattern of involvement and the histological features. PTLD involves the allograft 18% of the time, which in fact may be an underestimation [3]. Most lymphomas are B cell in origin. Involvement of the renal parenchyma often results in a peritubular infiltrate with expansion of the interstitium; however, typical features of rejection are usually absent. The cells are atypical, demonstrating varying stages of maturation. Although often present, EBV positivity is not required in establishing the diagnosis.

Using the above criteria, it seems reasonable to classify our patient as having PTLD.

The dramatic improvement in both renal function and associated histology with the addition of sirolimus was a welcome but not totally unexpected finding. TOR is an essential regulator of protein synthesis through its action on ribosomal biogenesis. Pathways upstream from TOR and the TOR pathway are frequently unregulated in human cancers [7]. Koehl et al. have demonstrated anti-cancer effects of sirolimus in one tumour–transplant model whereby BALB/c mice received syngeneic colon adenocarcinoma prior to C3H heart transplant. This group also reported a second mouse model of melanoma and transplant. In both models, the allograft was protected from rejection, but tumour growth was inhibited with sirolimus compared with cyclosporin, resulting in improved animal survival [8]. Campistol and colleagues demonstrated successful treatment of post-transplant Kaposi’s sarcoma in two patients converted to sirolimus [9].

CCI-779, an ester of sirolimus with comparable potency but longer half-life, is currently being investigated as an anti-cancer agent [10]. In addition, sirolimus has shown benefit in the resolution of
haematological malignancies in animal models [11]. Majewski and colleagues demonstrated significant growth inhibition of human PTLD-derived cells with RAD (everolimus) both in vitro and in a mouse xenotransplant model for severe combined immunodeficiency. They were able to show up to a 60-fold reduction in tumour volume [12]. In a review of five multi-centre studies, the 2 year incidence of malignancy in sirolimus-treated renal transplant recipients without CsA, or with early CsA withdrawal was lower than in those recipients maintained on CsA therapy [4]. Finally, Kahan and his group reported a very low incidence of PTLD of 0.4% over 10 years in his sirolimus-treated renal transplant recipients [13].

In summary, this case demonstrated a very rapid development of PTLD, which did not respond to reduction in immunosuppressive therapy and antiviral therapy, but did resolve with the administration of sirolimus. The mTOR inhibitors may be of benefit in the prevention and treatment in PTLD in recipients of solid organ allografts.

Conflict of interest statement. None declared.

References

11. Brown VI, Fang J, Alcorn K et al. Rapamycin is active against B-precursor leukemia in vitro and in vivo, an effect that is modulated by IL-7-mediated signaling. Proc Natl Acad Sci USA 2330; 100: 15113–15118

Received for publication: 21.12.04
Accepted in revised form: 12.4.05