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Posttransplant lymphoproliferative disorder in pediatric renal transplantation

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Abstract Of 84 renal transplants performed in our center since 1986, six recipients (7.1%) developed posttransplant lymphoproliferative disorder (PTLD). All received quadruple immunosuppression with Minnesota anti-lymphoblastic globulin or anti-thymocyte globulin, methylprednisolone, cyclosporine, and azathioprine or mycophenolate mofetil. Five were seronegative for Epstein-Barr virus (EBV) when they received their renal transplant. All patients received prophylactic acyclovir treatment postrenal transplant and none developed a cytomegalovirus (CMV) infection. All patients were positive for EBV by serology and polymerase chain reaction at the time of diagnosis of PTLD. Clinical features at presentation included fever (6/6), adenopathy (4/6), hypertrophied adenoids (4/6), liver involvement (2/6), and allograft involvement (2/6), 2–78 months (4/6<6 months) postrenal transplant. Histopathology of PTLD tissue revealed T cell rich/ Hodgkin disease-like B cell PTLD in one patient, polymorphic PTLD in four, and monomorphic (large B cell lymphoma) PTLD in one. Immunophenotyping of the PTLD biopsy specimen revealed predominant T cells in three, mixed B and T cells in two patients, and B cell in one. No aneuploid populations were identified by flow cytometric DNA ploidy assay. DNA from the PTLD tissue revealed weak to moderate IgH gene rearrangement in four of six patients but no T cell receptor β-chain or c-*myc* gene rearrangement on Southern blot analysis. The child with monomorphic (large B cell lymphoma) PTLD was clonal with λ light chain restriction on immunophenotyping. Treatment consisted of reduced immunosuppression and ganciclovir/ acyclovir in all patients. CMV hyperimmune globulin was used as an adjunctive therapy in two patients. Chemotherapy was needed in only one patient. A single rejection episode occurred in two children following reduction in immunosuppression, which reversed following intravenous methylprednisolone therapy. PTLD resolved in all patients and at present all patients are alive with functional grafts 2–54 months post diagnosis. Our experience suggests that reduced immunosuppression and anti-viral treatment is adequate in most cases of PTLD, but chemotherapy and hyperimmune globulin therapy may be beneficial in cases resistant to first-line therapy. Since all but one of our patients were EBV seronegative at the time of transplant, vigilance is especially important for early detection of PTLD in this group of the pediatric renal transplant population.

Key words Posttransplant lymphoproliferative disorder · Renal transplantation · Epstein-Barr virus

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Introduction

Posttransplant lymphoproliferative disorder (PTLD) is an abnormal proliferation of lymphocytes seen in an immunocompromised host following transplantation. The histopathological spectrum ranges from infectious mononucleosis-like to monomorphic proliferation of lymphocytes similar to non-Hodgkin lymphoma. Epstein-Barr virus (EBV) plays a central role in the pathogenesis of PTLD, which occurs as a result of a complex interplay of multiple factors, including impaired T cell surveillance, immunosuppressive drugs, cytomegalovirus (CMV) infection, B cell proliferating interleukins (IL) such as IL-4, IL-6, and IL-10, chronic antigenic stimulation by the allograft, and the activation of cellular oncogenes [1–5]. The EBV infection associated with PTLD can be either

primary or reactivation of the latent virus. The risk for PTLD is 24 times higher for a seronegative recipient compared with a seropositive recipient [6]. The incidence of PTLD has been reported to be 1% in adult renal transplant recipients, and 1.8%, 2.2%, and 4.6% for recipients of heart, liver, and heart-lung transplants, respectively [7]. The frequency of PTLD is higher in children than in adults, which may be a result of the more frequent EBV seronegative status of children (49%) compared with adult (8%) recipients at the time of transplantation [8]. The prevention and treatment strategies for PTLD remain ill defined. In this report, we describe our experience with six pediatric patients who developed PTLD following renal transplantation. The successful outcome of these patients provides insight into potential approaches to treatment.

Patients and methods

We reviewed the medical records of 84 children who underwent renal transplantation at our institution between December 1986 and July 1998. Six children (7.1%) developed PTLD. All patients were tested for EBV infection by EBV polymerase chain reaction (PCR) and EBV serology. EBV PCR was performed by Thermal Cycler (Perkin Elmer Cetus, Norwalk, Conn., USA) using EBV-specific primers for the early antigen gene of EBV (upstream: CAC CAC CTT GTT TTG ACG GG, downstream: GTC AAC CAA CAA GGA CAC AT) (Oligos ETC, Wilsonville, Ore., USA). Negative and positive control DNA samples were run with test DNA samples on every EBV PCR test. The EBV PCR results were initially reported semi-quantitatively and only since November 1997 were they quantified by the number of gene copies; hence all results in this report are graded as positive or negative. Serology was performed for anti-EBV IgM antibodies (Ab), EBV anti-viral capsid antigen (anti-VCA) IgG Ab, EBV anti-EBV nuclear antigen (anti-EBNA) IgG Ab, and EBV anti-early antigen (anti-EA) IgG Ab by indirect fluorescent antibody assay (Gull, Salt Lake City, Utah, USA).

The PTLD tissue specimens (tonsils, adenoids, lymph nodes, and/or liver) obtained from the patients were fixed in formalin for histopathological study. Unfixed fresh tissue was used for immunophenotyping and DNA ploidy assay. Portions of fresh tissue were snap frozen in liquid nitrogen for molecular genetics. The histopathological classification was based on *Posttransplant lymphoproliferative disorders: summary of Society for Hematopathology Workshop* [9]. Flow cytometric immunophenotyping was performed on PTLD tissue for pan T cells (CD7), E-rosette receptor (CD2), mature T cells (CD3), T helper cells (CD4), T suppressor cells (CD8), thymocyte (CD1 A), pan B cells (CD19), CALLA (CD10), monocyte (CD14), and lambda and kappa light chains. Immunohistochemical stains for CD 45 RO (memory T cells), CD 20, EBV latent membrane protein (EBV-LMP), leukocyte common antigen CD45, CD 30, and CD 3 (Dako) were performed on formalin fixed paraffin sections following 25 min incubation at 100°C in citrate buffer. Slides were then stained with specific antibodies by an indirect technique using labelled streptavidin, according to the manufacturer's (Dako) directions. Cell suspension was prepared from the lymphoid tissue, lyzed, digested with RNAase, and stained with propidium iodide for ploidy assay.

We also evaluated PTLD DNA specimens for rearrangement of the c-*myc* gene for evidence of malignant transformation, and immunoglobulin heavy chain (IgH) and T cell receptor β-chain (TCR-β) genes for clonality, using Southern blot hybridization. DNA was prepared from peripheral blood and solid tissue using proteinase K digestion and phenol: chloroform extraction using an Applied Biosystems (Foster City, Calif., USA) Genepure 341. Southern blots were prepared as previously described [10]. For detection of c-*myc* rearrangements we used separate digestions with the restriction enzymes *Eco*RI, *Hind*III, and *Bam*HI, and for probe we used E3-*myc*, a 1,400-base pair *Cla*I-*Eco*RI fragment from the third exon of human c-*myc* [11]. To detect IgH rearrangement we used separate digestions with the restriction enzymes *Eco*RI and *Hind*III, and simultaneous double digestion with *Bam*HI and *Hind*III. For IgH probe we used a 1020-bp DNA fragment from the J region (Dako, Carpenteria, Calif, USA). To detect rearrangements of the TCR-β gene we used the same set of restriction enzymes as for c-*myc* analysis, and the probe TCRBC (Dako), which is a 855-bp DNA fragment from the constant region. The DNA was labelled using [α^{32}P]dCTP (New England Nuclear, Boston, Mass., USA) and the Decaprime II random primed DNA labelling kit (Ambion, Austin, Tex., USA). Hybridization and washing were performed as previously described for Northern blots [12]. Data were collected by digital autoradiography with a PhosphorImager (Molecular Dynamics, Sunnyvale, Calif., USA) and analyzed using Imagequant software.

Results

The clinical features of our patients at the time of transplantation are summarized in Table 1. Immunosuppression consisted of Minnesota anti-lymphoblastic globulin (ALG) or antithymocyte globulin (ATGAM), methylprednisolone, cyclosporine and azathioprine (5/6), or mycophenolate mofetil (1/6). All children received antiviral prophylaxis with intravenous ganciclovir (5 mg/kg per day), with modification for abnormal renal function while receiving either ALG or ATGAM therapy as part of induction therapy. Bactrim and nystatin were prescribed for pneumocystis and fungal prophylaxis, and daily oral acyclovir (30–40 mg/kg per day) was given following hospital discharge.

None of the children developed CMV disease or sepsis prior to the diagnosis of PTLD. Patient 3 had an acute cellular rejection 1 month prior to developing PTLD, which was treated with methylprednisolone 10 mg/kg for 3 days followed by OKT3 2.5 mg for 10 days. Patient 2

Table 1 Clinical features of patients at the time of transplantation (*M* male, *LRD* live related donor, *CAD* cadaveric donor, *EBV* Epstein-Barr virus serology, *CMV* cytomegalovirus serology, *R* recipient, *D* donor, *N*A not available)

Patient no.	Sex	Diagnosis	Age (years)	D	EBV(R)	EBV(D)	CMV(R)	CMV(D)
1	M	Posterior urethral valves	1.75	LRD	+	NA	+	–
2	M	Renal dysplasia	2.5	CAD	–	NA	+	–
3	M	Posterior urethral valves	5.0	CAD	–	+	+	+
4	M	Renal dysplasia	4.5	CAD	–	+	–	+
5	M	Alport syndrome	18	CAD	–	+	+	–
6	M	Fibromuscular dysplasia	2.25	LRD	–	+	+	+

Table 2 Clinical features at the time of diagnosis of posttransplant lymphoproliferative disorder (PTLD) (*CSF* cerebrospinal fluid)

Patient no.	1	2	3	4	5	6
Time since transplantation (months)	78	70	2	2	3	3
Fever ≥ 40°C	+	+	+	+	+	+
Tonsil/ adenoid enlargement	–	+	+	+	–	+
Adenopathy						
Mesenteric adenopathy	+	+	–	–	+	–
Mediastinal adenopathy	+	+	–	–	–	–
Superficial neck adenopathy	+	–	+	–	–	–
Abnormal CSF	+[a]	–	+[b]	–	–	–
Liver involvement	–	–	–	–	+	+
Spleen involvement	+	–	–	–	+	–
Allograft involvement	–	–	–	–	+	+

[a] CSF EBV polymerase chain reaction positive
[b] CSF lymphocytosis

Table 3 Immunosuppression at the time of diagnosis of PTLD

Patient no.	1	2	3	4	5	6
Prednisone (mg/kg per day)	0.17	0.15	1.1	0.55	0.33	0.33
Azathioprine (mg/kg per day)	1.6	1.6	2.1	2.4	–	2.5
Mycophenolate (mg/m^2 per day)	–	–	–	–	1.400	–
Cyclosporine (mg/kg per day)	8.7	9.8	24	13.6	10	25
Cyclosporine trough level[a] (ng/ml)	150	205	309	303	313	281

[a] Cyclosporine monoclonal antibody (TDX) assay

had three episodes of acute cellular rejection that were treated with high-dose steroids, the last rejection having occurred 2.5 years prior to the diagnosis of PTLD. The remaining four children had no prior episodes of acute rejection.

Presentation of PTLD

The clinical features of the six children at the time of presentation of PTLD are summarized in Table 2. The initial anti-viral prophylaxis (e.g., acyclovir) was discontinued 6 months posttransplantation in patients 1 and 2, while the rest were on anti-viral prophylaxis at the time of diagnosis of PTLD. Children who presented >6 months from transplantation (patients 1 and 2) were on maintenance doses of prednisone (0.15–0.17 mg/kg per day), cyclosporine (8.7–9.8 mg/kg per day), and azathioprine (1.6 mg/kg per day) or mycophenolate mofetil (1400 mg/m^2 per day). In contrast, children who presented <6 months following transplantation were on a tapering schedule of the triple immunosuppression at the time of PTLD diagnosis (Table 3).

All patients had positive peripheral blood EBV PCR at the time of PTLD diagnosis. In five patients, serological tests were suggestive of primary EBV infection (positive EBV anti-IgM Ab and elevated EBV anti-VCA Ab) in a previously seronegative patient, while patient 1 had reactivation of EBV (non-reactive EBV anti-IgM Ab and elevated EBV anti-VCA Ab).

The morphological, immunophenotypic, and molecular genetic findings are summarized in Table 4. No evidence of rearrangement of the TCR-β gene or the c-*myc* gene was found in any specimens. However, rearrangement of the IgH gene was frequent, being found in some specimens from four of the six patients. In two of the patients, the rearranged bands were faint, representing 5% or less of the total signal. Patient 6 was clonal and morphologically, immunophenotypically, and molecularly typical of monomorphic large B cell lymphoma with strong IgH chain rearrangement and λ light chain restriction. Patient 2 had identical rearrangements in left and right tonsils with an intensity in the rearranged bands consistent with 10%–15% of the cells being in a clonal population of B lymphocytes. The identical rearrangement in two different sites is regarded as molecular evidence of metastasis. On propidium iodide DNA ploidy assay no aneuploid populations were identified.

Management of PTLD

PTLD was treated by decreasing immunosuppression and initiating intravenous anti-viral therapy. Azathioprine or mycophenolate mofetil was discontinued in all six children. Cyclosporine was discontinued at the time of diagnosis in four children, decreased (8.7 to 4.3 mg/kg per day) and then discontinued 6 months after diagnosis in patient 1 and decreased (24 to 10 mg/kg per day) at diagnosis in Patient 3. Prednisone (0.15–0.85 mg/kg/day) was the sole immunosuppressive therapy in five children, while patient 3 received prednisone (0.48 mg/kg per day) and cyclosporine (10 mg/kg per day). Initial anti-viral treatment was intravenous ganciclovir (5 mg/kg per dose twice a day) modified for abnormal renal function for 8–20 weeks and then changed to oral acyclovir or ganciclovir. Oral acyclovir was discontinued in patients 3 and 6 after 10 and 12 months respectively, while patients 1, 2, 4, and 5 continue to receive anti-viral treatment for 15, 14, 16, and 2 months, respectively.

The progress of the PTLD in each patient was followed by monitoring clinical status (fever, palpable ad-

Table 4 Histopathology, immunophenotype, and molecular studies in children with PTLD (*LMP* latent membrane protein, *ND* not determined, *TCR* T cell receptor, *R* gene rearranged, *G* germ line)

Patient no.	Biopsy tissue	Histopathology	Immunophenotype study Flow cytometry		Immunohistochemistry	EBV-LMP	Molecular study Molecular rearrangements		
			Predominant population	Immunoglobulin light chain restriction			IgH	TCR	c-*myc*
1	Lymph node	T cell-rich/ Hodgkin disease-like B cell PTLD	T cell >90%	No	Scattered large B cell	+	R	G	G
2	Tonsil	Polymorphic PTLD	ND	ND	Mixed T and B cells	+	R	G	G
3	Tonsil	Polymorphic PTLD with focal monomorphism	T cell >90%	No	Few scattered large B cell	ND	G	G	G
4	Tonsil	Polymorphic PTLD with focal monomorphism	T cell >85%	No	Few scattered large B cell	+	R	G	G
5	Liver	Polymorphic PTLD with focal monomorphism	T cell 58%, B cell 26%	No	Few scattered large B cell	+	G	G	G
6	Liver	Monomorphic B cell PTLD	B cell 80%	λ	B cell	+	R	G	G

enopathy, organomegaly), allograft function, serial EBV PCR, and sequential computed tomographic (CT) scans (adenopathy and extranodal tissue involvement). Response to treatment was clinically determined to be present when there was an absence of fever, adenopathy, and organomegaly, with resolution of changes seen on initial CT scans and a negative EBV PCR. Patient 1 responded poorly to decreased immunosuppression and anti-viral therapy, as he continued to have high-grade fever with no significant change in adenopathy. He received chemotherapy consisting of six cycles given 3 weeks apart of cyclophosphamide 600 mg/m^2, intrathecal methotrexate 5 mg, and prednisone 2 mg/kg per day for 5 days. He received CMV hyperimmune globulin 100 mg/kg per week as adjunctive therapy. Patients 4 and 5 developed biopsy-proven acute cellular rejection following a decrease in immunosuppression. The rejection episodes were completely reversed in both cases with intravenous methylprednisolone 10 mg/kg per day for 3 days.

Outcome of PTLD

Sequential CT scans showed resolution of adenopathy, lesions in liver, spleen, and allograft in all six cases. Fever lasted for 3–6 weeks in five children, while patient 1 had persistent fever for more than 8 weeks until he responded to chemotherapy. The immunosuppressive agents were reintroduced after a satisfactory clinical response and a negative EBV PCR was achieved. Immunosuppression was reinstituted 3 months after diagnosis in patients 2, 3, 4, and 6, and 13 months after diagnosis in patient 1. Patient 5 is currently on prednisone only and is just 2 months post diagnosis of PTLD. In each case, either azathioprine or mycophenolate mofetil was reintroduced initially, followed by the addition of cyclosporine at a fraction of the prior dose while monitoring the clinical status and EBV PCR. The immunosuppression, serum creatinine, and time since diagnosis of PTLD are summarized in Table 5. All children are currently alive with a functioning renal allograft.

Discussion

Of 84 children, 6 (7.1%) developed PTLD following renal transplantation. The Cincinnati Transplant Tumor Registry found PTLD to be the most-common neoplasm in pediatric patients following transplantation, accounting for 52% of all lesions [13]. EBV has been implicated to be a major determinant in the pathogenesis of PTLD [1–5]. The supportive evidence of EBV in PTLD comes from demonstration of EBV DNA [14] and *EBER-1* gene expression in PTLD tissue [15], increased oropharyngeal EBV shedding [16], and an increase in EBV-infected peripheral B cells [17] in patients with PTLD. In all of our patients, PTLD was associated with an EBV infection that was primary in five patients and a reactivation in one, and EBV-LMP antigen could be demonstrated in

Table 5 Follow-up data on children with PTLD

Patient no.	1	2	3	4	5	6
Immunosuppression						
Prednisone (mg/kg per day)	0.22	0.15	0.15	0.30	0.30	0.30
Mycophenolate mofetil (mg/m^2 per day)	1000	725	900	650	650	0
Azathioprine (mg/kg per day)	0	0	0	0	0	0.60
Cyclosporine (mg/kg per day)	2	3.5	10	6	0	13
Serum creatinine (mg/dl)	0.8	1.0	1.2	0.7	1.1	0.8
Follow-up since PTLD diagnosis (months)	15	14	55	16	2	49

five of five PTLD tissue specimens by immunohistochemistry. An additional eight patients in our transplant population whose EBV status was monitored and who were seronegative at transplantation and received a seropositive donor kidney did not develop PTLD.

The abnormal lymphoproliferation observed in PTLD following EBV infection is felt to be related to the level and type of immunosuppression. In an immunocompetent host, resolution of EBV infection is characterized by elimination of most infected cells by HLA-restricted EBV-specific cytotoxic T cells. Subsequently, EBV is present in latent form as an episomal or circularized DNA in the host cell for the remainder of the host's life [4]. Latent infection is characterized by limited expression of only 10 (e.g., EBV-LMP) of the more than 80 genes encoded by the virus [4]. In a transplant recipient, the T cell function is compromised from heavy immunosuppression, particularly cyclosporine, FK506, methylprednisolone pulse therapy, and OKT3 [1–5]. Elevated IL-4 and IL-10 in PTLD increase B cell proliferation [18–20]. Finally, a PTLD-associated EBV infection upregulates EBV LMP and bcl-2 protein expression, which inhibits apoptosis giving the infected cell a survival advantage [21, 22]. Thus, therapeutic immunosuppression together with cellular oncogenes and elevated ILs lead to an uncontrolled lymphoproliferation that results in PTLD.

EBV-seronegative status, CMV disease, usage of cyclosporine, FK506, anti-lymphocytic antibody, and OKT3, the number of methylprednisolone pulses, acute rejection episodes, and younger age have been suggested to be risk factors associated with the development of PTLD [8, 23]. Quadruple immunosuppression and primary EBV infection were the risk factors identified in our patients. None of the children developed CMV disease. Only patient 3 had an acute cellular rejection that was treated with methylprednisolone and OKT3 near the time PTLD was diagnosed. A high index of suspicion is necessary for diagnosing PTLD, because its clinical presentation can range from an infectious mononucleosis-like presentation to sepsis and multiorgan failure. Our patients with PTLD presented with high-grade temperatures (>40°C), adenopathy, tonsil/adenoid enlargement, abnormal cerebrospinal fluid (CSF) (lymphocytosis and CSF EBV PCR positive), liver, spleen, and allograft involvement. None of our patients developed central nervous system lymphoma, gastrointestinal involvement (perforation or obstruction) or pulmonary lesions. Miller et al. [24] observed that with the introduction of cyclosporine, a primary site of PTLD involvement has changed from the central nervous system, which was the predominant site of disease prior to the use of cyclosporine, to thoracic and abdominal presentations. They also observed that PTLD isolated to the renal allograft is strongly associated with the use of OKT3.

Whereas the serological findings in our patients were supportive of EBV infection, one has to be cautious interpreting such data, as false-negative serology is frequently noted in immunocompromised individuals [4]. The EBV PCR assay may be a better tool in immunocompromised patients for, in addition to confirming the presence of EBV viremia, it can be used to assist in monitoring the clinical course of the disease. Unfortunately, we were unable to correlate the number of gene copies by EBV PCR with the clinical course in all of our patients because of the semi-quantitative nature of our early test results. We also evaluated our patients by analyzing the c-*myc* gene for evidence of rearrangement. Rearrangement of the c-*myc* gene is an alteration found frequently in Burkitt lymphoma [25, 26] and non-Burkitt lymphomas from human immunodeficiency virus-infected individuals [27]. The association of c-*myc* rearrangement with lymphoma in EBV-infected or immunocompromised individuals has also been found following organ transplantation [28]. We found no evidence for c-*myc* rearrangement in any of our patients. We did not look for any other mutational changes in oncogenes or tumor suppressors in these patients.

Once PTLD was diagnosed, our patients were treated with a reduction in immunosuppression designed to allow for sufficient recovery of the host immune response to control the lymphoproliferation, hopefully without causing rejection of the allograft. They also received anti-viral treatment with intravenous ganciclovir followed by oral acyclovir or oral ganciclovir to suppress the actively replicating EBV cell population. Five of the six children responded completely to a decrease in immunosuppression and anti-viral treatment alone, including patient 6 with monomorphic (large B cell lymphoma) PTLD. In fact, our experience reveals that neither evidence of clonality (patient 6) nor metastatic spread (patient 2) necessarily indicates the presence of an aggressive malignancy justifying the use of chemotherapy designed for the treatment of lymphoma. Nalesnik et al. [7] also observed that both polyclonal and a subpopulation of clonal PTLDs are capable of regression following reduced immunosuppression, indicating that it is an appropriate first line of treatment. The use of reduced immu-

nosuppression and anti-viral therapy in all of our patients makes it impossible to judge the individual contributions of the two components of therapy. Whereas most reports addressing treatment of PTLD include the use of intravenous ganciclovir, acyclovir would likely be as effective with less likelihood of complications such as bone marrow suppression [7, 29, 30].

In contrast to our other five patients, patient 1 had a uncommon form of PTLD, T cell-rich/ Hodgkin disease-like B cell PTLD, that did not respond to the initial course of chemotherapy. Whereas his clinical presentation and molecular findings were similar to the other patients, he was uncharacteristically EBV positive at the time of transplantation and had late-onset PTLD. Persistent fever and a rising number of gene copies by EBV PCR prompted the use of a combination of chemotherapy and CMV hyperimmune globulin to which he responded. Like others, we prescribed the use of CMV hyperimmune globulin as a means of providing passive immunity against EBV without an accompanying assessment of our patients' anti-EBV antibody level [29]. CMV hyperimmune globulin has also been reported to be beneficial adjunctive therapy for PTLD in pediatric liver transplant recipients, presumably because the preparation is known to contain a significant titer of antibodies to EBV [30]. Although indications for the use of CMV hyperimmune globulin in PTLD remain poorly defined, like Cao et al. [29], we believe its use should be considered in all patients after the diagnosis of PTLD and especially in those patients who do not fully respond to decreased immunosuppression and anti-viral therapy. Other therapeutic options available are chemotherapy (CHOP and ProMACE CytaBOM), α-interferon, surgical resection, and radiotherapy. Anti-B cell monoclonal antibodies (specific for CD21 and CD24) [31] and lymphokine activated killer cells [32] have been used in difficult situations with some success.

A single episode of allograft rejection occurred in two children (patients 4 and 5) on reduced immunosuppression that responded to steroids and did not recur following the reinstitution of a portion of the patient's baseline immunosuppression. Purighalla et al. [33] observed that the risk of rejection following reduced immunosuppression in PTLD is 50% and post-PTLD rejection left untreated led to graft loss. Hence, a stringent watch must be maintained for occurrence of acute rejection at the time of reduced immunosuppression. The pathologist evaluating an allograft biopsy in this setting must also recognize the potential for PTLD involvement of the renal tissue [34, 35].

In summary, early detection and reduction of immunosuppression appear to be key components of the management of PTLD. Intravenous ganciclovir followed by oral acyclovir prophylaxis given during and for ≥3 months after anti-lymphocytic globulin may also decrease the incidence of PTLD in EBV-seronegative individuals [36, 37]. However, since all six of our patients had received or were on anti-viral prophylaxis when they developed PTLD, one has to be constantly vigilant for PTLD, especially in the high-risk setting of an EBV-seronegative recipient receiving a positive donor kidney. Based on our experience, we recommend following this high-risk group of patients with EBV PCR every 1–2 months in the 1st year following transplantation and to be alert to the development of symptoms of EBV infection, such as high fever and adenopathy. In our small series, this approach to diagnosis and subsequent prompt management appears to be associated with an excellent patient and graft outcome.

Addendum

Since submitting our manuscript, patient 1 had a relapse of PTLD with recurrence of fever, progressive adenopathy, and an elevated number of EBV PCR gene copies. He is currently receiving chemotherapy with prednisone, vincristine, cyclophosphamide, and methotrexate. Mycophenolate mofetil and cyclosporine have been discontinued and his serum creatinine is 0.7 mg/dl.

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