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Primary tacrolimus (FK506) therapy and the long-term risk of post-transplant lymphoproliferative disease in pediatric liver transplant recipients

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Abstract: While the overall incidence of post-transplant lymphoproliferative disease (PTLD) in pediatric liver transplant recipients has been reported to be 4–11%, the long-term risk of PTLD associated with primary tacrolimus therapy is unknown. Therefore, in order to determine the incidence and long-term risk of PTLD, the present study examined 131 pediatric recipients who underwent liver transplantation (LTx) between October 1989 and December 1991 and received primary tacrolimus therapy. This cohort of children was evaluated over an extended time-period (until December 31 1996) with a mean follow-up of 6.3 yr. Actuarial Kaplan–Meier analysis was utilized to determine the risk of PTLD over time. The overall incidence of PTLD was 13% (17/131) with an average age of 4.3±0.75 yr at diagnosis. Pretransplant Epstein–Barr virus (EBV) serologies were negative in 82%, positive in 12%, and not available in 6% of the patients. The median time to diagnosis of PTLD post-Tx was 11.9 months (mean 16.4±3.9, range 1.7–63.0 months). Mean tacrolimus dose and plasma trough level (as evaluated by enzyme-linked immunosorbent assay [ELISA]) at the time of diagnosis was 0.32±0.06 mg/kg/day and 1.3±0.3 ng/mL, respectively. The cumulative long-term risk of PTLD was found to increase over time: 3% at 6 months, 8% at 1 yr, 12% at 2 yr, 14% at 3 yr, and 15% at 4 and 5 yr. Mortality from PTLD was 12% (two of 17 patients). Primary tacrolimus use in pediatric LTx has a long-term risk of PTLD approaching 15%, with the majority of episodes (78%) occurring in the first 2 yr, suggesting that intense EBV surveillance should occur early post-transplantation.

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The introduction and widespread clinical use of CsA (Sandimmune®; Sandoz, Basel, Switzerland) in the early 1980s resulted in OLTx becoming a viable treatment modality for end-stage liver disease, with present-day survival rates being 88% at 1 yr post-Tx (1). More recently, in 1989, tacrolimus (FK506; Prograf®; Fujisawa USA, Deerfield, IL, USA), a new macrolide immunosuppressant, was introduced into clinical trials at the University of Pittsburgh. Preliminary data from 1990 showed a decreased incidence and severity of rejection episodes (2). Further results from a US multi-center trial confirmed the Pittsburgh data (1). Tacrolimus has been shown to be 10 to 100 times more potent than CsA (3, 4);

Abbreviations: CMV, cytomegalovirus; CsA, cyclosporin A; CT, computerized tomography; CTL, cytotoxic T lymphocyte; EBER-1, EBV-encoded small RNA; EBV, Epstein–Barr virus; ELISA, enzyme-linked immunosorbent assay; GI, gastrointestinal; HIV, human immunodeficiency virus; OLTx, orthotopic liver transplantation; POD, post-operative day; PTLD, post-transplant lymphoproliferative disease; Tx, transplantation.

however, the comparative potential for adverse side-effects (including infections) is not clearly understood.

EBV infections and EBV-associated PTLD are critical complications following OLTx in children. Early data from the Children's Hospital of Pittsburgh noted that an increasing percentage of pediatric OLTx recipients developed PTLD after receiving CsA-based immunosuppression (5). It identified an incidence of PTLD approaching 20% by 7 yr after LTx. The introduction of tacrolimus, with its increased potency, raises the concern that EBV disease and PTLD may be more frequent in children who receive this therapy. This concern was enhanced by a recent report of a very high incidence of PTLD in pediatric OLTx recipients receiving tacrolimus immunosuppression (6). However, the vast majority of cases of PTLD identified in this study occurred in children who received tacrolimus as rescue therapy after initial exposure to CsA and failure to respond to conventional management of rejection (including OKT3). Therefore, these patients do not serve as an accurate predictor of the incidence of PTLD after pediatric OLTx under tacrolimus-based immunosuppression.

While a number of studies have attempted to identify risk factors for the development of PTLD following pediatric OLTx (6–8), little data is available that has specifically investigated the long-term incidence of this complication in a large cohort of children transplanted under tacrolimus-based immunosuppression. The availability of a large cohort of children who received tacrolimus as primary immunosuppression between 1989 and 1991 allows us the unique opportunity of determining (in patients receiving tacrolimus therapy) the true incidence and long-term risk over time of developing PTLD.

Patients and methods

Between October 1989 and December 1991, 131 children underwent primary OLTx at the Children's Hospital of Pittsburgh.

Immunosuppressive regimen

All children received primary tacrolimus induction therapy (children receiving tacrolimus as rescue therapy were excluded), starting in the operating room after graft reperfusion, at a dose of 0.075 mg/kg/12 h via continuous intravenous infusion. Patients were converted to oral tacrolimus when tolerated. Tacrolimus dosages were initially adjusted to maintain 12-h plasma trough levels of 0.5–2.0 ng/mL, as measured by double-antibody ELISA (9). The most recent tacrolimus dose and level preceding the onset of diagnosis of PTLD was recorded.

Initial corticosteroid therapy consisted of a 10 mg/kg bolus of intravenous methylprednisolone in the operating room followed by a corticosteroid taper of 5 mg/kg at POD 1, decreasing to 1 mg/kg at POD 5 and a reduction of 20% per week thereafter. Maintenance prednisone therapy was individualized with attempts to wean completely off steroids as soon as possible. No patients received anti-lymphocyte/thymocyte preparation or azathioprine as induction therapy. Biopsy-proven steroid-resistent rejection was treated with OKT3 (Orthoclone®; Ortho Biotech, Inc., Raritan, NJ, USA) at a dose of 2.5 mg/day for 7–14 days, depending on the response. Antiviral prophylaxis for CMV consisted of oral acyclovir at a dose of 800 mg/m^2 q.i.d. $\times 3$ months in all patients.

Diagnosis of PTLD

A diagnosis of EBV infection and PTLD was suspected in patients with unexplained fever, tonsillitis, lymphadenopathy, upper airway obstruction, rash or GI disturbances. The diagnostic evaluation for EBV/PTLD included EBV serologic studies and a CT scan of the chest and abdomen. Additionally, colonoscopy and/or upper endoscopy was performed in patients with symptoms of GI disease. Biopsy and histologic evaluation of enlarged lymph nodes and other affected tissue was performed in all patients for a definitive diagnosis. All patients met the histologic diagnosis of PTLD, which ranged from lymphoid hyperplasia to malignant lymphoma, according to the criteria of Nalesnik et al. (10). The presence of EBV within PTLD lesions was confirmed by performing EBER-1 *in situ* hybridization on biopsied tissue (11).

Management of PTLD

Initial treatment of PTLD consisted of reduction or total withdrawal of tacrolimus and prednisone in combination with either intravenous acyclovir (1500 mg/m^2/day) or ganciclovir (10 mg/kg/day). Interferon therapy (α or γ) was used in patients who did not respond to the initial treatment. Patients with progressively non-responsive disease despite reduction/withdrawal of immunosuppression, or patients with lymphoma (non-Hodgkin's, Burkitt's), received chemotherapy and/or radiation therapy, depending on the site of involvement. Surgical intervention (excluding

tissue biopsies for diagnosis) was supportive and reserved for complications of PTLD.

Response to therapy

Patients were evaluated for response of PTLD to therapy, as previously defined by Nalesnik:

- 'regression': reduction and disappearance of tumor by decreasing or withdrawal of immunosuppression plus treatment with antiviral agents;
- 'resolution': disappearance of tumor with the use of additional treatment modalities (radiation, chemotherapy, or surgical); and;
- 'no response': progression of disease despite all therapeutic interventions (10).

Statistics

Data are expressed as mean±SEM. Patients were followed-up until 12 December 1996 (mean follow-up: 6.3 yr). The long-term risk of PTLD was calculated using inverse Kaplan–Meier survival statistics, with development of PTLD (rather than patient death) considered as an end-point.

Results

Seventeen of 131 patients developed PTLD, giving an overall incidence of 13%. The mean age at OLTx was 2.9±0.7 yr (median 1.5, range 0.2–10.9 yr). Pretransplant EBV serologies were negative in 82%, positive in 12%, and not available in 6% of patients. The median time to diagnosis of PTLD post-Tx was 11.9 months (mean 16.4±3.9, range 1.7–63.0 months). Tacrolimus dosages at the time of diagnosis varied from 0.025 to 1.09 mg/kg/day (mean dose 0.32±0.06 mg/kg/day) while the mean 12-h plasma trough level was 1.3±0.3 ng/mL (range <0.5–11.6 ng/mL). Steroid-resistant rejection developed in two of 17 children (12%) and was successfully treated with OKT3 therapy. Nine of 17 (53%) patients were totally weaned off prednisone and were receiving tacrolimus monotherapy at the time of diagnosis of PTLD.

The clinical and pathologic characteristics of PTLD in these 17 patients are depicted in Table 1.

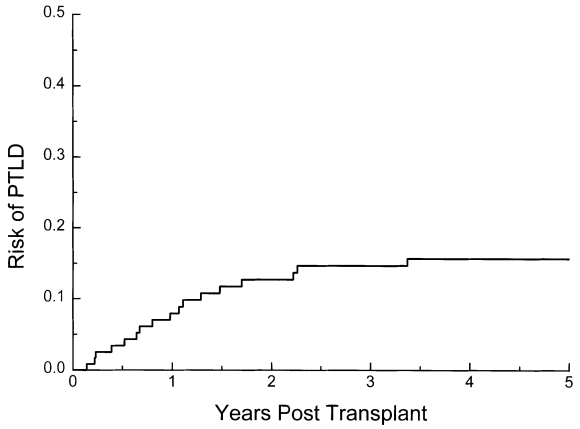

Fig. 1. Cumulative long-term risk of developing post-transplant liver disease (PTLD) in pediatric liver transplant recipients receiving primary therapy with tacrolimus. Kaplan–Meier statistics were used, with end-points representing onset of PTLD rather than patient deaths.

Table 1. Clinical and pathologic characteristics of post-transplant lymphoproliferative disease (PTLD)

Age at PTLD (yr)	Signs and symptoms	PTLD presentation site	PTLD pathologic type	Primary treatment*	Additional treatment	Response of tumor to therapy†
7.5	Fever, malaise	Middle ear, colon	Polymorphic	D/C TAC	None	Regression
1.1	Fever, adenopathy	LN	Polymorphic	D/C TAC	None	Regression
1.3	Fever, N & V, adenopathy	LN, stomach	Polymorphic	D/C TAC	None	Regression
3.3	Fever, N & V	Small bowel	Lymphoma	D/C TAC	Small bowel resection	Resolution
5.3	Fever, adenopathy	LN, larynx	LH	D/C TAC	None	Regression
6.3	Adenopathy	LN, Burkitts	Monomorphic	D/C TAC	Chemo-tx	Resolution
7.8	Fever, adenopathy	LN	Polymorphic	D/C TAC	None	Regression
2.5	Fever, dyspnea	LN	Polymorphic	D/C TAC	None	Regression
11.0	Fever, increased LFTs, adenopathy	LN, GI, liver	Polymorphic	D/C TAC	None	No response
2.5	Fever, adenopathy	LN	Polymorphic	D/C TAC	None	Regression
2.1	Fever, adenopathy	LN, GI	Polymorphic	D/C TAC	None	Regression
1.5	Fever, tonsillitis URI	LN, GI	Monomorphic	D/C TAC	None	Regression
2.5	Fever, adenopathy	LN, liver, spleen	Polymorphic	D/C TAC	None	No response
2.5	Fever, adenopathy	LN, lung	Polymorphic	D/C TAC	Radiation	Resolution
7.1	Adenopathy	LN	Polymorphic	Reduced TAC	None	Regression
1.3	Fever, adenopathy	LN	LH	D/C TAC	None	Regression
8.2	Fever, adenopathy increased LFTs, hemolytic anemia	LN, spleen	Polymorphic	Reduced TAC	Splenectomy	Resolution

*Primary treatment included reduction or discontinuation of TAC and either intravenous acyclovir or ganciclovir.
†See the Patients and methods section.
Chemo-tx, chemotherapy; D/C, discontinue; GI, gastrointestinal; LFTs, liver function tests; LH, lymphoid hyperplasia; LN, lymph node; TAC, tacrolimus; URI, upper respiratory infection.

The mean age at diagnosis of PTLD was 4.3±0.75 yr (median 2.5, range 1.3–11.6 yr). Fever and lymphadenopathy, the most common sign and symptom, occurred in 88% and 71% of patients, respectively. Sites of involvement included: lymph nodes, 15 patients; GI tract, six patients; spleen, two patients; liver, two patients; middle ear, one patient; larynx, one patient; and lung, one patient; with 10 patients (59%) having multiple sites. The majority of patients were successfully treated with reduction or withdrawal of immunosuppression (11/17, 65%), while four patients (23%) required surgery (small bowel resection, one patient; and splenectomy, one patient), chemotherapy (one patient) or radiation therapy (one patient) for resolution of disease. Overall mortality was 24% (4/17). Two children died as a direct result of disseminated PTLD after being treated unsuccessfully with discontinuation of immunosuppression and intravenous acyclovir. The remaining two children recovered from PTLD but died of chronic rejection and recurrent giant cell hepatitis.

The long-term risk of developing PTLD associated with the use of primary tacrolimus-based immunosuppression is shown in Fig. 1. The cumulative PTLD risk increases over time and is 3% at 6 months, 8% at 1 yr, 12% at 2 yr, 14% at 3 yr, and 15% at 4 and 5 yr.

Discussion

Serologic evidence of prior EBV infection is found in up to 80% of the normal adult population (12). While EBV infection is generally self-limited, an increased risk of tumor development has been observed in EBV-infected immunocompromised individuals (HIV-infected patients and transplant recipients) (13). The usual mode of transmission of EBV in immunocompetent children is through oral epithelial cells, which then undergo lysis with viral replication and subsequent infection and immortalizaton of B cells (14). During acute infection, proliferation of these transformed B cells is kept in check by cellular immune responses, including natural killer cells and $CD4^+$ and $CD8^+$ CTLs, which recognize various EBV nuclear antigens and membrane proteins (15, 16). These gene products are then down-regulated during latency, thereby allowing EBV-infected B cells to escape normal immune surveillance mechanisms (17). During this convalescence phase, EBV-infected B cells may be re-activated and express additional viral nuclear antigens and membrane proteins (18). As with acute infection, host CTLs are able to recognize these gene products and control EBV and B-cell proliferation.

PTLD occurs when EBV-infected B cells undergo transformation and proliferation secondary to a host's inability to mount an effective immune response. Recipients of solid organ transplants in general, and pediatric OLTx recipients in particular, are at increased risk of developing EBV infections and PTLD compared to individuals in the normal population, as a result of the iatrogenic manipulation of the host's immune system with anti-T-cell agents, particularly CsA, tacrolimus, and poly- and monoclonal antibodies (6–8, 10). In addition, $\approx 50\%$ of children are seronegative for EBV prior to Tx; two-thirds of these seronegative children experience primary EBV infection following Tx and thereby are at a greater risk of developing PTLD (19).

The present report found the overall rate of development of PTLD in pediatric OLTx recipients under primary tacrolimus immunosuppression to be 13%. When analyzed by actuarial analysis for development of PTLD over time, the rate rose from 8% at 1 yr to 15% at 5 yr, with the greatest increment in risk for PTLD occurring during the first 2 yr following Tx. This rate compares favorably with data generated in a similar analysis of the initial clinical experience with CsA-based immunosuppression in pediatric OLTx recipients transplanted between 1981 and 1984 at our center (5). In that study, Malatak and colleagues found that the cumulative risk of acquiring PTLD was nearly 20% at 7 yr post-OLTx. Thus, the rate of development of PTLD in patients receiving therapy with tacrolimus appears to be similar to or less than the risk in patients receiving CsA, at a similar level of experience with the two drugs. While the risk of PTLD did not differ, patients on tacrolimus appeared to fare better than those receiving the CsA-based immunosuppression. Overall mortality from PTLD appeared to be substantially greater in the CsA era (42%) compared to the tacrolimus era (12%). One reason may be the more advanced nature of the disease in the CsA patients. Five of 12 CsA patients had a 'lymphomatous presentation' with only one child surviving and having a disease-free course. In contrast, most patients in the tacrolimus group presented at an earlier stage of the disease with only one patient developing lymphoma. This patient was treated with discontinuation of tacrolimus, underwent a small bowel resection for a perforation secondary to PTLD, and ultimately survived. Earlier detection in the tacrolimus patients is probably because

of an enhanced awareness of EBV/PTLD, better diagnostic tools (e.g. EBER-1 stain), and a higher vigilance on the part of the transplant team. Although this might contribute to an increased incidence of PTLD in tacrolimus patients, similar rates of PTLD were identified in both cohorts. In addition, two of six surviving patients from the CsA group required re-Tx for chronic rejection after resolution of PTLD compared to only one of 15 patients surviving PTLD in the tacrolimus group. While it is difficult to draw conclusions from data from two different eras of immunosuppression, it does appear that pediatric OLTx recipients receiving tacrolimus as primary therapy are not at an increased risk of developing PTLD when compared to CsA patients.

The rate of PTLD seen in this study is lower than that reported by Cox et al., who described an increased incidence of PTLD in children receiving tacrolimus after LTx (6). Cox described a significant increase in PTLD in children less than 5 yr of age receiving tacrolimus compared to those receiving CsA (19% vs. 3%). However, the overwhelming majority of children receiving tacrolimus had already failed primary CsA therapy and hence were 'rescue' patients who probably received more cumulative immunosuppression. The failure to differentiate between patients receiving primary tacrolimus and 'rescue' therapy does not allow for a fair comparison between the two immunosuppressive agents. The data in the present analysis is a realistic representation of the risk of PTLD in children treated with tacrolimus, as all patients received primary therapy only and were followed-up for a mean of longer than 6 yr post-Tx. With tacrolimus now commercially available to all liver transplant centers, its use as rescue therapy may decrease in the future as more centers adopt it as primary therapy.

When a new immunosuppressant is used in Tx, the most efficacious dosing regimen, as well as the best way to monitor drug levels, evolves over time. At the time of this study, the initial tacrolimus dose was at 0.075 mg/kg/12 h intravenously, a dose which has subsequently been reduced to 0.05 mg/kg/12 h. This reduction in tacrolimus dosage may contribute to a lowering of the risk of PTLD in the future. Tacrolimus levels, initially measured by ELISA in plasma, are now performed by the IMx analyzer method on samples of whole blood, a technique generally felt to be more accurate (20). The present study shows tacrolimus levels to be in the desired range in 12 patients at the time of diagnosis of PTLD, with only three patients having levels slightly higher than recommended at the time of diagnosis, inferring that tacrolimus levels, while a useful guide for dosing, are not in and of themselves predictive of PTLD. More stringent guidelines for tacrolimus dosing and levels at various time intervals post-Tx have recently been published and should serve to help further balance its immunosuppressive properties with side-effects (21).

The present report is limited by a number of factors. Most importantly, this is a retrospective study. As such, there was no opportunity to have a head-to-head comparison between the children on tacrolimus and those on CsA-based immunosuppressive regimens. However, as only a limited number of pediatric liver transplant recipients were ever enrolled in randomized trials comparing tacrolimus and CsA (1), prospective data comparing rates of development of PTLD under both regimens are not available. Accordingly, we and others (6, 9) have used historical experience to attempt to compare the relative risk of developing PTLD under these different regimens. By comparing long-term follow-up data from the same center using the same sets of definitions, the current study provides a reasonable comparison of the true incidence of PTLD under both drugs.

In conclusion, the long-term risk of the development of PTLD in children receiving primary tacrolimus therapy after LTx does not differ from an early historical group of children receiving CsA therapy at the same institution. At 5-yr post-Tx, the risk of developing PTLD with primary tacrolimus therapy approaches 15%, with the majority of episodes occurring in the first 2 yr post-Tx, suggesting the need for a keen awareness of this complication early after transplant on the part of physicians caring for these patients. The group of children studied in this analysis represent the earliest cohort of liver transplant recipients receiving primary tacrolimus therapy followed-up long-term for PTLD. As refinements have been made in the dosage and monitoring of tacrolimus drug levels in recent years, it is probable that the prevalance of PTLD, as well as the long-term risk of developing PTLD, will decrease in pediatric liver transplant recipients in the future.

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