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THE EFFECT OF IMMUNOSUPPRESSION ON POSTTRANSPLANT LYMPHOPROLIFERATIVE DISEASE IN PEDIATRIC LIVER TRANSPLANT PATIENTS

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Background. **Posttransplant lymphoproliferative disease (PTLD) is a serious complication associated with the use of chronic immunosuppression for solid organ transplantation. This study represents a retrospective analysis of UCLA's experience with PTLD in all pediatric liver transplant recipients between 1984–1997. We assessed the clinical presentation, risk factors, incidence density, immunological characteristics, management, and outcome of patients who developed PTLD when receiving either primary cyclosporin A (CsA) or tacrolimus.**

Methods. **A total of 251 children received primary CsA therapy of which 70 required OKT3 for steroid resistant rejection and 29 required tacrolimus rescue for OKT3 resistance and/or chronic rejection. One hundred forty one children received tacrolimus as primary therapy. Sixty patients who survived for less than 6 months after transplantation were excluded from the study.**

Results. **The total incidence density (ID) rate of PTLD was 1.8±0.4 per 100 patient-years (30/392). The overall ID rate of PTLD in the CsA group was 0.93±0.2 per 100 patient-years (15/251). Within this group of primary CsA-treated patients, the ID rate of PTLD was 0.49±0.1 without OKT3 or tacrolimus, 0.67±0.2 with OKT3, and 6.42±1.1 with tacrolimus rescue. The overall PTLD ID rate in the primary tacrolimus-treated patients was 4.86±1.2 per 100 person-years (15/141). There was a 5-fold increase in the ID rate of PTLD in the primary tacrolimus group when compared to the comparable, primary CsA group ($P<0.001$). The mean time to PTLD was 5-fold longer (49.7±20.7 months) in the CsA group when compared to the CsA/tacrolimus rescue group (9.8±3 months, $P<0.05$) or the tacrolimus primary group (12.6±5.1 months, $P<0.05$). Five patients had monoclonal disease in the CsA group, but only one in the tacrolimus group ($P<0.05$). Clinical presentations with enlarged lymph nodes, fevers, malaise, anorexia, weight loss, hypoalbuminemia, and gastrointestinal blood loss were common. Mortality was 20%, three patients died in each group.**

Conclusion. **The use of primary tacrolimus therapy was associated with a significant 5-fold higher rate of PTLD when compared to those treated with primary cyclosporine. Early diagnosis, decrease and/or discontinuation of potent immunosuppressive agents may contribute to decrease morbidity and mortality of this entity.**

The recent development of several new immunosuppressive agents has provided transplant centers with additional options to treat solid organ transplant patients. Several of these more potent immunosuppressive drugs have shown promise in decreasing the likelihood of developing overall rejection, including acute steroid resistant and chronic allograft rejection (*1, 2*). However, the expected side effects of a more potent immunosuppressive regimen would be an increased risk for developing an assortment of viral infections. A common viral pathogen in the transplant recipients is Epstein-Barr virus (EBV), which usually causes B cell proliferation with a broad clinical spectrum, ranging from infectious mononucleosis-like syndrome to frank lymphoma (*3*).

EBV infection induces B cell growth transformation by expressing certain viral factors, such as EBNA-2 and LMP-14–7. In the immunosuppressed state, these viral growth factors cause a sustained proliferation of B lymphocytes, which frequently result in malignant transformation (*8*). The presence of chromosomal alterations may enhance the rate of proliferation of a subset of B lymphocytes, which may initially result in polyclonal, then oligo- and monoclonal B cell proliferation (*9, 10*).

The pediatric liver transplant population has been reported to be at a particular high risk for developing posttransplant lymphoproliferative disease (PTLD) (*11–14*). When compared with their adult counterparts, the risk factors associated with the development of PTLD are: young age, EBV negative status at time of transplantation when receiving an EBV positive graft, and primary EBV infection (*14*). Because EBV transmission approaches 60–80% in EBV sero-negative children in the early posttransplant period, this group of patients is at a particularly higher risk for developing PTLD (*14*).

The other previously described risk factors associated with PTLD are type, duration, and intensity of immunosuppresive agents (*11–13*). Several studies have examined the relative risk of developing PTLD in transplant recipients on different immunosuppressive medications. The reported incidence for PTLD varied from 2–27% depending on the potency of the drug, the type of the organ transplanted, and the recipients age (*9, 14–16, 23–25*). However, all of these studies failed to take into account the longer follow-up time of patients treated with CsA when compared with those treated with tacrolimus.

In this retrospective study, we reviewed our experience of PTLD since the inception of our pediatric OLT program 13 years ago and assessed the incidence density rate, clinical presentation, histological findings, management, and outcome of PTLD in this group of patients. During this period we used two different immunosuppressive protocols namely cyclosporine (CsA) and tacrolimus, and demonstrate in this study that the length of therapy should be controlled for when comparing groups with different follow-up times.

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MATERIAL AND METHODS

Patient population. The clinical course of 452 children who underwent liver transplantation in the period between 1984–1997 at the Dumont-UCLA liver transplant program was retrospectively reviewed. Sixty patients died less than 6 months after their transplantation for causes unrelated to PTLD and were excluded from the study.

Immunosuppressive regimens. During this period we used two different immunosuppressive protocols. Between 1984–1994 primary immunosuppression was CsA (Sandimmune, Sandoz, East Hanover, NJ) combined with low-dose prednisone. After 1989 azathioprine was routinely added. In 1994 our program changed to primary therapy with tacrolimus (Prograf, Fujisawa, Deerfield, IL). Acute allograft rejection was treated with high-dose intravenous methylprednisolone (SoluMedrol, Upjohn, Kalamazoo, MI) and steroid resistant rejection was treated with monoclonal antibody OKT3 (Orthoclone OKT3, Ortho Biotech, Raritan, NJ). In patients treated with CsA, intractable acute rejection or chronic rejection was managed by conversion to tacrolimus (*17*). In the early posttransplant period, whole blood levels of CsA were kept at a range of 300–350 ng/ml, although later levels of 150–250 ng/ml were targeted. The methodology for CsA levels changed over the decade. Most recently a whole blood monoclonal radioimmunoassay technique is used (CYCLO-Trac, Incstar, Stillwater, MN). Methylprednisolone was given intravenously at 20 mg/kg/day divided every 6 hr, tapered to 0.3 mg/kg/day over 7 days. Maintenance azathioprine was given at 0.1–0.2 mg/kg/day.

Tacrolimus levels targeted (whole blood micro particle enzyme immunoassay: Imx, Abbott Labs, Abbott Park, IL) changed over time: 12–15 ng/ml during the first month after transplantation, 10–12 ng/ml in the posttranplant months 1 through 3, and 5–10 ng/ml thereafter. In some patients levels <5 ng/ml after the sixth posttranplant month were well tolerated (*18*). OKT3 was used in a dose of 2.5 mg/day i.v. for 10–14 days for patients with body weight less than 20 kg, and 5 mg/day for patients who weighted more than 20 kg. If CD3 lymphocyte cell count was higher than 5%, the OKT3 dose was increased by 2.5 mg increments.

Diagnosis of EBV infection. Until 1995, the diagnosis of EBV infection was established based upon the presence of IgM anti-viral capsid antigen (VCA) titers. EBV reactivation was based upon a more than 4-fold increase in IgG anti-VCA titers, or the reappearance of IgM VCA. Since 1995 we have been using EBV-PCR in peripheral blood to detect early infection (*19*). Routine donor testing was not performed until 1996.

Diagnosis of PTLD. Patients who presented with unexplained prolonged fevers, progressive lymph node enlargement, failure to gain weight or weight loss, stridor and/or obstructive apnea, seizures, headaches, hypoalbuminemia with protein losing enteropathy, chronic diarrhea with or without heme positive stools and/or graft dysfunction, underwent evaluation for PTLD. CAT scan, MRI studies of chest, abdomen, and occasionally the brain were performed to evaluate for adenopathy and/or mass lesions. Whenever possible biopsy evidence of suspected PTLD lesions was obtained. Evaluation for PTLD included immunohistochemical tissue analysis.

PTLD management. After the diagnosis of PTLD was established, all immunosuppressive agents except for low dose prednisone were stopped. Antiviral treatment with i.v. acyclovir or ganciclovir was initiated. Patients were monitored closely for evidence of allograft rejection at which time treatment with CsA or tacrolimus was started at low doses. Chemotherapy, interferon and high dose IgG were used occasionally in patients with refractory disease.

Statistical methods. Incidence density was computed per 100 patient years and was compared using exact binomial procedures (*20*). Proportions were compared via χ^2 methods and means were compared via t tests. Time to PTLD and percent PTLD free as a function of time were estimated via the Kaplan-Meier survival methods.

RESULTS

Demographics. During the period between March 1984 to June 1997, 452 children underwent liver transplantation at the Dumont-UCLA transplantation program. The indications for OLT were biliary atresia 52%, hepatitis and fulminant liver failure 15%, metabolic liver disease 10%, cryptogenic cirrhosis 10%, tumors 3%, others 10%. A total of 392 patients survived more than 6 months posttransplantation. The overall median age at transplantation was 6.7 years (range 3 months to 18 years), with a mean follow-up time of 4.3±2.8 years.

A total of 30 patients developed PTLD, during the time interval investigated. All except one had histological evidence of PTLD, including EBV-DNA. There were 251 patients treated with primary CsA with a mean follow-up time of 6.4±2.3 years. We defined primary CsA-treated patients as those who were started on CsA after OLT, regardless of subsequent immunosuppressive regiments, this included patients treated with either OKT3 (70 patients), or those rescued with tacrolimus (29 patients). In the primary tacrolimus-treated group, there were 141 patients with a mean follow-up time of 2.2±0.7 years. Primary tacrolimus-treated patients were defined as those who were started on tacrolimus post-OLT, regardless of subsequent immunosuppression. These data demonstrate that the posttransplant follow-up time is nearly 3-fold longer for the primary CsA group when compared to the tacrolimus. Because of this disparate length of follow-up time between the two groups, all subsequent analysis controlled for this variable by estimating incidence density.

Incidence density of PTLD by treatment group. The incidence density of PTLD for all patients in this study was 1.8±0.4 per 100 patient-years (30/392), with a prevalence of 7.6%. Fifteen patients developed PTLD in the primary CsA group, with an incidence density rate of 0.93±0.2 per 100 patient-year (Fig. 1). Fifteen patients developed PTLD in the primary tacrolimus group with an incidence density of 4.86±1.2 per 100 patient-years. The lowest incidence density

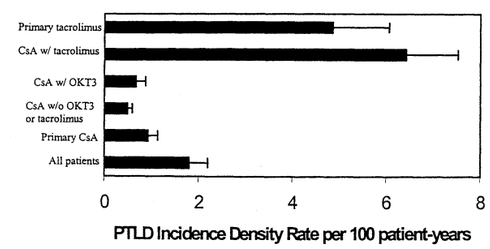

FIGURE 1. PTLD incidence density rate per 100 patient years in all patients. Primary CsA, CsA without OKT3 or tacrolimus, CsA with OKT3, CsA with tacrolimus, and primary tacrolimus group, respectively.

rate of all groups examined in this study were those individuals who received only CsA (0.49±0.1 per 100 patient-years). In contrast, the incidence density in those patients treated with CsA and OKT3 was slightly higher (0.67±0.2 per 100 patient-years), but not statistically different when compared to those who received only CsA ($P>0.05$).

Patients who were initially treated with CsA and subsequently rescued with tacrolimus had an incidence density (6.42±1.1 per 100 patient-years) that was 12-fold more than those who received only CsA ($P<0.001$). Moreover, the incidence density of PTLD in those patients receiving only tacrolimus (4.86±1.2 per 100 patient-years) was 10-fold higher than individuals whom were treated with only CsA (0.49±0.1 per 100 patient-years, $P<0.001$). Figure 1 demonstrates PTLD incidence density rate in the above mentioned groups.

Incidence of ductopenic rejection. In the primary CsA-treated group the incidence of ductopenic rejection was 27.8%, and 3% in the tacrolimus group ($P<0.05$). Three patients in this group with PTLD, had ductopenic rejection after stopping the immunosuppresive agent.

Age and time to PTLD. The median age at the time of PTLD was 25 months (range 5 months to 14.5 years) in the CsA group and 14 months (range 7 months to 9 years) in the tacrolimus group. The mean time from transplant to the development of PTLD was 49.7±20.7 months in the CsA group and 12.6±5.1 months in the primary tacrolimus group. In those individuals rescued by tacrolimus, the mean time to the development of PTLD was 9.8±3 months after the conversion to tacrolimus (Table 1). These data demonstrate that the mean time to develop PTLD is more than 3-fold longer in those individuals treated only with CsA when compared to those treated with tacrolimus used as either primary or rescue therapy.

To investigate the role of the patient's age in the development of PTLD, we stratified patients by age at the time of transplantation, and compared the incidence density of PTLD for both forms of therapy (Fig. 2). Among those patients treated with tacrolimus the incidence density of PTLD was similar in all age groups examined (range 0–1, 1–2, 2–3, and more than 3 years of age). However, among patients treated with CsA, the incidence density was slightly higher (1.25±0.38 per 100 patient-years) in the older group (>3 years of age), when compared to the patients less than 1 year of age (0.72±0.33 per 100 patient-years). $P<0.05$ overall and for the 0–1 and >3 year age groups. Probably this occurred because of the shorter follow-up time in the 1–2 and 2–3 age strata. Of note that the average age, time to PTLD, and follow-up is significantly different between the two groups overall based on the t tests. The CsA group is substantially older with three times more follow-up time and a much longer time to the development of PTLD.

Kaplan-Meier analysis of PTLD-free period is displayed in Figure 3 and includes a follow-up time of 159 and 40 months in the CsA and tacrolimus groups, respectively. In this univariate analysis, the data demonstrate that the percent of patients free of PTLD in the CsA group was 99% at 1 year, 98% at 2 year, 95% at 4 year, and 94% at 13 year. In contrast, in the tacrolimus group, the percent of PTLD free patients was 91% at 1 year, 86% at 2 year ($P<0.001$). Kaplan-Meier analysis was not performed for the CsA rescued with either tacrolimus or OKT3 because the insufficient number of patients per follow-up period would not lend itself to statistical analysis.

EBV serology. Pretransplant serology for EBV was documented in 195/342 patients (57%). Of these, 120 patients were EBV positive pretransplant (63%). Pretransplant EBV serology was documented in 25/30 patients (83%) with PTLD, of which 18 (72%) were EBV negative pretransplant, emphasizing the association between primary EBV infection and the development of PTLD. Until recently we did not follow our patients prospectively with regular repeated EBV serologies or EBV PCR to determine the number of children who developed infection after liver transplantation.

Pathology and management of PTLD. The pathological findings in the different groups are displayed in Table 2. All except one patient (93%) in the tacrolimus group had early stage polymorphic, polyclonal B cell proliferation. The patient, who had monoclonal B cell proliferation in this group, also received OKT3 induction, which was an isolated incident. There were more patients diagnosed with lymphomas in the primary CsA group. One patient in the rescue group was diagnosed based on the clinical presentation without histological diagnosis. There was one patient with Burkitt's lymphoma, one with Hodgkin's, and a third with non-Hodgkin's lymphoma in the primary CsA group.

Immunosuppressants were discontinued at the time the diagnosis of PTLD was established. In all cases evidence of EBV infection was present and patients were started on intravenous acyclovir/ganciclovir. Since 1995 we have been following our high-risk patients with EBV-PCR from the peripheral blood (*21, 22*). Chemotherapy using conventional protocols was applied to three patients: one with Burkitt's and one with Hodgkin's lymphoma who survived and one who died with disseminated monoclonal disease. Interferon α (80,000 U/kg s.c. for 2 weeks) and high doses of IVIG (500 mg/kg for 3 days) were unsuccessful in two patients. Anti-B cell monoclonal antibodies were not used.

Mortality was 20%. Three patients died in each group including one patient with a previous history of PTLD in the tacrolimus group who died secondary to bronchiolitis obliterans but no autopsy evidence of PTLD. In three patients it was necessary to allow rejection of their grafts to achieve PTLD remission. Two patients had PTLD recurrence on reinstitution of the immunosuppresive agents, two others were later found to have PTLD involving the eye.

TABLE 1. Characteristics of patients in primary CsA and primary tacrolimus groups

	Primary CsA	Primary tacrolimus	*P*
Median age	25 mo (range 5 mo–14.5 yr)	14 mo (range 7 mo–9 yr)	<0.05
Mean time to PTLD	49.7±20.7 mo	12.6±5.1 mo	<0.05
Mean time to PTLD in rescue after conversion	9.8±3.0 mo	N/A	
Mean follow-up time	6.4±2.3 yr	2.1±0.7 yr	<0.05
EBV status pretransplant	Neg (8); Pos (2); N/A (4)	Neg (10), Pos (5)	
Etiology of liver disease			
Biliary atresia	9	7	
Hepatoblastoma	1	0	
α1 anti-trypsin deficiency	1	2	
Tyrosinemia	1	0	
Autoimmune hepatitis	2	0	
Fulminant liver failure	1	2	
Familial cholestasis	0	2	
TPN cholestasis	0	1	
Cryptogenic cirrhosis	0	1	

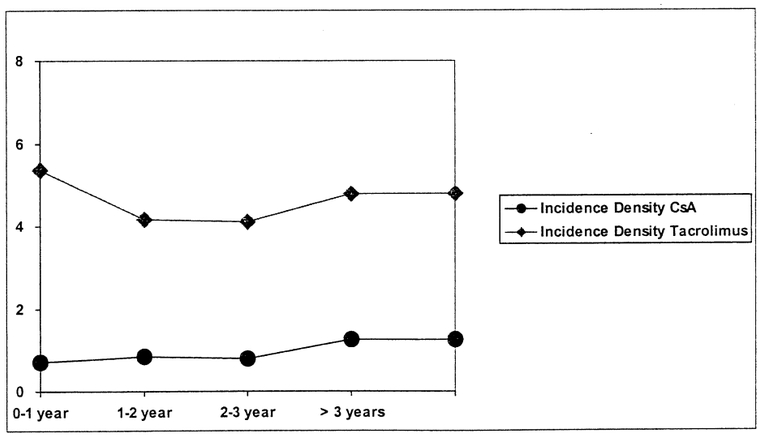

FIGURE 2. Incidence density rate per 100 patient years in CsA and tacrolimus groups stratified by age.

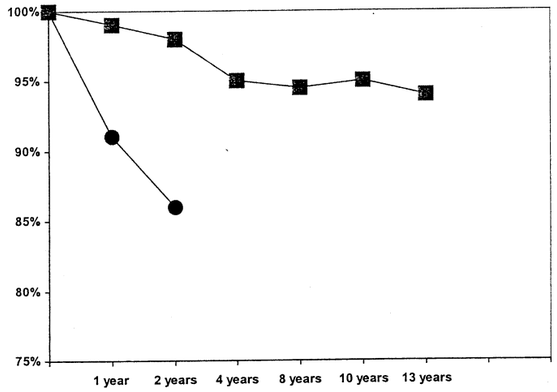

FIGURE 3. PTLD free Kaplan-Meier survival curves in CsA, tacrolimus groups.

TABLE 2. Histological characteristics of PTLD in CsA, CsA with tacrolimus rescue and in tacrolimus groups

	CsA	CsA/ tacro	Tacrolimus	*P*
Polymorphic polyclonal B cell prolif.	2	3	14	<0.05
Monomorphic monoclonal	4	2	1	<0.05
Hodgkin's lymphoma	1	0	0	
Non-Hodgkin's lymphoma	1	0	0	
Burkitt's lymphoma	0	1	0	
Sites of PTLD				
Gastrointestinal	1	3	5	
Liver	0	1	3	
Lymph nodes (cerv, med, abd)	4	5	8	
Tonsils and adenoids	2	0	2	
Spleen	1	1	1	
Lung	0	0	1	
Bone marrow	1	0	0	
Eye	0	0	2	

DISCUSSION

In this retrospective analysis of 392 pediatric liver transplant patients, we assessed the characteristics and risk factors that were associated with PTLD. We determined that patients treated with primary tacrolimus had a significantly higher risk of developing PTLD when compared with those primarily treated with CsA. Furthermore, most of the patients who developed PTLD had a primary EBV infection after transplantation. We found that the use of OKT3 for treatment of steroid-resistant rejection was not associated with a higher incidence rate of PTLD when compared to the cohort of patients treated with only CsA. We could not confirm a difference in the incidence density of PTLD for patients treated with tacrolimus in the different age groups. However, more polyclonal B cell proliferation was found in patients treated with the more potent immunosuppressive agent tacrolimus, compared with CsA-treated children.

The reported incidence of PTLD in transplant patients is quite variable and is a function of the type of organ transplanted, age at the time of transplantation, and the amount and form of immunosuppression. With the introduction of CsA as an immunosuppressive agent in solid organ transplantation, the reported risk for developing PTLD increased (*9, 11–16*). The prevalence of PTLD in our CsA patients who were not treated with either OKT3 or tacrolimus was 4.9%. Ho et al. (*11*) reported the prevalence of PTLD to be 0.8% in adult and 4% in children. Similarly, Malatack et al. (*23*)

reported 12 children with PTLD in 132 pediatric liver recipients in a 3-year period, with a prevalence of 9%.

An unexpected finding from our study was that the incidence density of PTLD was similar between those individuals who received only CsA when compared with those on CsA who were rescued with OKT3. Others have reported that the addition of the monoclonal antilymphocyte antibody increased the prevalence of PTLD (*9, 11–16*). More specifically, in a large review of multiorgan adult transplant patients, the prevalence of PTLD was 3-fold higher after the use of OKT3 and CsA when compared with CsA alone (*16*). Similar data in adult cardiac transplant patients identified a 9-fold increase prevalence of PTLD after the use of OKT3 when compared with CsA alone (*24*).

This increased prevalence of PTLD with the use of OKT3 together with CsA has also been confirmed in the pediatric transplant population. In one study, the prevalence of PTLD in pediatric patients treated with OKT3 and CsA was reported to be 14%; nearly 3-fold higher when compared with CsA therapy alone (*25*). In a pediatric cardiac transplant program, the use of OKT3 and tacrolimus was associated with a 6-fold higher incidence of PTLD when compared with tacrolimus alone (*15*). How can we explain why OKT3 was not associated with PTLD in our pediatric population? What can account for the apparent discrepancy between our analysis and these other studies? Because several groups failed to report the dose of OKT3 that was administered, it is difficult to determine if we used OKT3 at a lower dose and/or for a shorter length of time. However, one of the consistent differences between our study and several of the others is that our use of OKT3 was limited mainly to steroid resistant acute allograft rejection, although other studies appear to have used it for induction therapy.

Previous reports gave inconsistent results about the incidence of PTLD in children treated with either primary tacrolimus or CsA. Although one study made no clear distinction about either the age of patients, or the type of organ transplanted, they reported a surprising low (0.7%) incidence of PTLD in patients treated with primary tacrolimus (*26*). However, the follow-up time was exceedingly short (mean of 10.5 months). More importantly, as with most previous studies in the field, they failed to control for the disparate length of therapies of the two treatment groups. To control for various lengths of treatment, incidence density must be used to perform a fair comparison.

When we controlled for the various lengths of therapy, the incidence density rate of PTLD associated with either primary or rescued tacrolimus was 10-fold higher than the group treated with only CsA. Actually, this is not surprising, because tacrolimus is 10-fold more potent in vivo and 100-fold more potent in vitro than the immunosuppressive agent CsA (*27–30*).

Previous reports suggested that children are at increased risk for the development of PTLD, primarily because they are more likely to be sero-negative for EBV before transplantation, and tend to acquire primary infection posttransplantation (*11, 14*). In our study, we were able to confirm that primary EBV infection occurred posttransplant in 72% of children who developed PTLD. Interestingly, in the tacrolimus group, we failed to document an association between PTLD and the younger age groups. The increase prevalence of PTLD in children younger than 3 years of age was not statistically different. Others have reported that PTLD was especially common in patients less than 5 years of age when compared to those older than 17 years (*14*). In our study, we stratified the younger patients to those older than 3 years and compared them to various younger age groups (0–1, 1–2, 2–3 years of age). Interestingly, with the exception of a higher incidence density in the older (>3 year) group compared to those between 0–1 year of age in the CsA group, the density of all other groups were similar.

The intensity of clinical surveillance may have introduced an important artifact when attempting to compare statistics from different eras in comparing PTLD incidence in the two groups. And the fact that more polyclonal PTLDs were found in the tacrolimus group support this possibility, as polyclonal B cell proliferation can be considered as an early stage in the PTLD spectrum (*10*). How many CsA-treated patients with polyclonal B cell proliferation were not diagnosed with PTLD is unclear.

Restoring the host's immunocompetence to allow the specific reactivation of cytotoxic T cells directed against EBV is the main stay of PTLD management (*31*). This approach allowed complete resolution in 80% of our patients. Acyclovir/ganciclovir were always started intravenously. We used chemotherapy in only three patients two of whom died (*10, 32*). We used high doses of IVIG and interferon twice without success (*33*). We have no experience with the use of monoclonal antibody therapy directed against the CD21 or CD24 receptors expressed on the surface of EBV-infected cells (*34*).

Since 1995 we have been following our high-risk liver transplanted patients with serial measurements of EBV-PCR in the peripheral blood lymphocytes (*19, 21*). In liver/intestinal transplant patients, Green (*21*) demonstrated the promising hope of decreasing the risk of PTLD by using preemptive therapy with antivirals. Although ganciclovir is virostatic, and decreases only the lytic phase of the viral replication by blocking the viral DNA polymerase, it maybe particularly useful in controlling primary infection.

Our present strategy to reduce the incidence of PTLD is to combine serial EBV-PCR monitoring of peripheral blood lymphocytes with prophylactic ganciclovir treatment in the high-risk patients. Rising EBV-PCR titers in the absence of clinical evidence of EBV disease prompts aggressive lowering of immunosuppression. Initial results with this practice, appear to be reducing PTLD incidence (*22*).

In conclusion, this study shows that the more potent the immunosuppressive agents used in younger liver transplant patients the higher the risk they have to develop PTLD. An increased awareness of this entity, appropriate reduction in long-term immunosuppression and preemptive treatment with antiviral and decreased immunosuppression at the first evidence of EBV infection or reactivation may help contain this problem in the future.

REFERENCES

1. McDiarmid SV, Busuttil RW, Ascher NL, et al. FK506 (tacrolimus) compared with cyclosporine for primary immunosuppression after pediatric liver transplantation. Results from the U.S. Multicenter Trial. Transplantation 1995; 59: 530.
2. The U.S. Multicenter FK506 Liver study Group. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression in liver transplantation. N Engl J Med 1994; 331: 1110.
3. Morrison VA, Dunn DL, Manivel JC, Gajl-Peczalska KJ, Peterson BA. Clinical characteristics of post-transplant lymphoproliferative disorders. Am J Med 1994; 97: 14.
4. Dambaugh T, Wang F, Hennessy K, et al. Expression of the Epstein-Barr virus nuclear protein 2 in redent cells. J Virol 1986; 59: 453.
5. Diller J, Kallin B, Ehlin-Henriksson B, et al. Charaterization of a second Epstein-Barr virus determined nuclear antigen associated with BamHI WYH region of EBV DNA. Int J Cancer 1985; 35: 359.
6. Mann KP, Staunton D, Thorley-Lawson DA. An Epstein-Barr virus encoded protein found in the plasma membranes of transformed cells. J Virol 1985; 55: 710.
7. Wang D, Leibowitz D, Kieff E. An EBV membrane protein expressed in immortalized lymphocytes transforms established rodent cells. Cell 1985; 43: 831.
8. Seiden MV, Sklar J. Molecular genetic analysis of post-transplant lymphoproliferative disease. Hematol Oncol Clin North Am.1993; 7: 447.
9. Penn I. Posttransplant malignancies in pediatric organ transplant recipients. Transplant Proc 1994; 26: 2763.
10. Rustgi VK. Epstein-Barr viral infection and posttransplantation lymphoproliferative disorders. Liver Transplant Surg 1995; 1: 100.
11. Ho M, Jaffe R, Miller G, et al. The frequency of Epstein-Barr virus infection and associated lymphoproliferative syndrome after transplantation and its manifestation in children. Transplantation 1988; 45: 719.
12. Lamy M, Favart AM, Cornu C, et al. Epstein-Barr virus infection in 59 orthotopic liver transplant patients. Med Microbiol Immunol 1990; 179: 137.
13. Sokal EM, Caragiozoglou T, Lamy M, Reding R, Otte JE. Epstein-Barr virus serology and Epstein-Barr virus associated lymphoproliferative disorders in pediatric liver transplant patients. Transplantation 1993; 56: 1394.
14. Cox KL, Lawrence-Miyasaki LS, Garcia-Kennedy R, et al. An increased incidence of Epstein-Barr virus infection and lymphoproliferative disorder in young children on FK506 after liver transplantation. Transplantation 1995; 59: 524.
15. Newell KA, Alonso EM, Whitington PF, et al. Posttransplant lymphoproliferative disease in pediatric liver transplantation. Interplay between primary Epstein-Barr virus infection and immunosuppression. Transplantation 1996; 62: 370.
16. Alfrey EJ, Friedman AL, Grossman RA, et al. A recent decrease in the time to development of monomorphous and polymorphous posttransplant lymphoproliferative disorder. Transplantation 1992; 54: 250.
17. McDiarmid SV, Wallace P, Vargas J, et al. The treatment of intractable rejection with tacrolimus with FK506 in pediatric liver transplant recipients. J Pediatr Gastroenterol Nutr 1995; 20: 291.
18. Esquivel CO, So SK, McDiarmid SV, et al. Suggested guidelines for the use of tacrolimus in pediatric liver transplant patients. Transplantation 1996; 61: 847.
19. Martinez OM, Villanueva JC, Lawrence-Miyasaki LS, et al. Viral and immunologic aspects of Epstein-Barr virus infection in pediatric liver transplant recipients. Transplantation 1995; 59: 519.
20. Clayton D, Hills M. Statistical models in epidimiology. Oxford: Oxford University Press, 1993.
21. Green M, Reyes J, Jabbour N, et al. Use of quantitative PCR to predict onset of Epstein-Barr viral infection and post-transplant lymphoproliferative disease after intestinal transplantation in children. Transplant Proc 1996; 28: 5:2759.
22. McDiarmid SV, Wallace P, Vargas J, et al. Prevention and preemptive therapy of post- transplant lymphoproliferative disease in pediatric liver recipients. Transplantation 1998; 66: 1604.
23. Malatack J, Gartner J, Urbach A, Zitelli B. Orthotopic liver transplantation, Epstein Barr virus, cyclosporine and lymphoproliferative disease: a growing concern. J Pediatr 1991; 11: 667.
24. Swinnen LJ, Costanzo-Nordin MR, Fisher SG, et al. Increased incidence of lymphoproliferative disorder after immunosuppression with the monoclonal antibody OKT3 in cardiac-transplant recipients. N Engl J Med 1990; 323: 1723.
25. Renard TH, Andrews WS, Foster ME. Relationship between OKT3 administration, EBV seroconversion, and the lymphoproliferative syndrome in pediatric liver transplant recipients. Transplant Proc 1991; 23: 1473.
26. Nalesnik MA, Demetris AJ, Fung JJ, Starzl TE. Lymphoproliferative disorders arising under immunosuppression with FK 506: initial observations in a large transplant population. Transplant Proc 1991; 23: 1108–1110.
27. Tocci MJ, Matkovich DA, Collier KA, et al. The immunosuppressant FK506 selectively inhibits expression of early T-cell activation genes. J Immunol 1989; 143: 718–726.
28. Yoshimura N, Matsui S, Hamashima T, Oka T. Effect of a new immunosuppressive agent, FK506, on human lymphocyte responser in vitro, II: inhibition of the production of IL-2 and gamma interferon, but not B-cell stimulating factor 2. Transplantation 1989; 47: 356.
29. Sawada S, Suzuki G, Kawase Y, Takaku F. Novel immunosuppressive agent, FK506. In vitro effects on the cloned T cell activation. J Immunol 1987; 139: 1797.
30. Morris RE, Wu J, Shorthouse R. Comparative immunopharmacologic effects of FK506 and CyA in in vivo models of organ transplantation. Transplant Proc 1990; 22: 110.
31. Starzl TE, Nalesnik MA, Porter KA, et al. Reversibility of lymphomas and lymphoproliferative lesions developing under cyclosporine-steroid therapy. Lancet 1984; 1: 583.
32. Swinnen LJ. Treatment of organ transplant-related lymphoma. Hematol Oncol Clin North Am 1997; 11: 963.
33. Shapiro RS, Chavenet A, MucGuire W, et al. Treatment of B-cell lymphoproliferative disorders with interferon-alpha and intravenous gammaglobulin. N Engl J Med 1988; 318: 1334.
34. Fischer A, Blanche S, Le Bidois J, et al. Anti-B-cell monoclonal antibodies in the treatment of severe B-cell lymphoproliferative syndrome following bone marrow and organ transplantation. N Engl J Med 1991; 324: 1451.

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