Posttransplantation Lymphoproliferative Disorder Associated with OKT3 and Decreased Antiviral Prophylaxis in Pancreas Transplant Recipients

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Between September 1994 and October 1995, we diagnosed and treated four cases of early onset posttransplantation lymphoproliferative disorder (PTLD) occurring within 62 days of pancreas transplantation. The development of PTLD was associated with both a significantly higher total muromonab-CD3 (OKT3) dose and a lack of ganciclovir/acyclovir prophylaxis, but it was not associated with the total dose of antithymocyte globulin or cytomegalovirus serostatus. All four patients were treated aggressively and survived without evidence of recurrent PTLD more than 1.5 years later. We conclude that the use of a high total dose of OKT3 puts pancreas transplant recipients at increased risk for early onset PTLD, while ganciclovir/acyclovir prophylaxis may help to prevent this disorder; however, if early onset PTLD does occur in these patients, aggressive therapy can lead to a favorable outcome.

Posttransplantation lymphoproliferative disorder (PTLD) results from Epstein-Barr virus (EBV)-associated proliferation of B lymphocytes in the immunosuppressed host [1, 2]. The spectrum of disease associated with PTLD ranges from relatively benign polyclonal plasmacytic hyperplasia to malignant immunoblastic lymphoma or multiple myeloma [1–3].

The incidence of PTLD in solid organ transplant recipients appears to depend upon both the type of organ transplanted and the immunosuppressive regimen used. While the observed incidence of PTLD is in the range of 1% for renal transplant recipients, it can be as high as 9% for heart/lung transplant recipients and 12% for pancreas transplant recipients [4–6]. Moreover, the incidence of PTLD has been shown to increase significantly following the administration of antithymocyte agents including muromonab-CD3 (OKT3) to cardiac transplantation patients or administration of OKT3 and antilymphocyte globulin to kidney transplant recipients [7, 8].

We similarly noted a dramatic increase in the incidence of PTLD in pancreas transplant recipients at our center following the initiation of a protocol that included antithymocyte globulin (Atgam; Upjohn Pharmaceuticals, Kalamazoo, MI) for induction immunosuppression and OKT3 treatment for early rejection. This PTLD differed from classic PTLD in that it occurred in EBV-seropositive recipients within 62 days following transplantation (early onset PTLD). The correlation of development of PTLD with total dose of either agent was therefore analyzed.

Methods

Patient Population

One hundred and four simultaneous pancreas and kidney transplantations and six pancreas transplantations alone were performed at the University of Maryland Hospital (Baltimore) between 1 July 1991 and 1 October 1995. All four cases of PTLD occurred among 56 patients who received transplants since the initiation of a protocol using antithymocyte globulin induction with OKT3 treatment for early rejection, begun 1 January 1994. Forty-nine of these 56 patients were evaluable for risk factors associated with the development of PTLD; the 7 patients who were excluded from analysis included 1 who received only a pancreas transplant and lost his graft because of thrombosis within 24 hours of transplantation and 6 who did not have documented EBV infection (as determined by seropositivity of the donor or recipient). The underlying disease in each of the 49 evaluable patients was insulin-dependent diabetes mellitus.

Immunosuppressive Regimens

After 1 January 1994, all patients received antithymocyte globulin for induction immunosuppression at an initial dosage of 15 mg/kg q.d. for 4–15 days; in specific cases, this dosage was adjusted upward to 30 mg/kg q.d. on the basis of the total CD3 cell count. Thirty-six (73.5%) of the 49 evaluable patients also received OKT3 either for early rejection or to complete induction immunosuppression if therapeutic CD3 levels were not produced by antithymocyte globulin; the initial dosage was 5 mg q.d. iv for a 4–14 day course. Eight of these patients received an additional course of OKT3 within the first 60 days post-transplantation for continued evidence of rejection.
All patients also received azathioprine (2.5–3.0 mg/kg iv preoperatively, followed by 1 mg/kg q.d. iv until oral medication was possible, and then 2 mg/kg q.d. po) and a tapered regimen of prednisone (from 2 mg/kg q.d. initially to 0.3 mg/kg q.d. by postoperative day 15). In addition, 43 patients received cyclosporin A (10 mg/kg q.d. po in two divided doses) and 22 patients received tacrolimus (FK506; Fujisawa USA, Deerfield, IL) (0.2 mg/kg q.d. po in two divided doses) as soon as oral medication was possible and the serum creatinine level was ≤2.5 mg/dL. Sixteen patients received both agents (sequential); cyclosporin A was replaced with FK506 because of rejection.

**Antiviral Prophylaxis**

Pancreas transplantation patients at known risk for cytomegalovirus (CMV) disease (i.e., the recipient, donor, or both were seropositive for CMV) routinely received antiviral prophylaxis for a total of 6 months post-transplantation. The group considered to be at highest risk (8 CMV-seronegative recipients of organs from CMV-seropositive donors) received ganciclovir (5 mg/kg b.i.d. for 10–14 days, followed by 5 mg/kg q.d. for 14 days) and then acyclovir (800 mg po t.i.d.) for a total of 6 months of therapy; this group also received intravenous immunoglobulin every other week for the first 8 weeks. CMV-seropositive recipients received ganciclovir (5 mg/kg b.i.d. for 10–14 days and then 5 mg/kg q.d. for up to 14 days) followed by acyclovir (800 mg po t.i.d.), for a total of 6 months of therapy. However, ganciclovir therapy was terminated and acyclovir therapy was begun for the seropositive patients upon discharge from the hospital, so that few of them received >14 days of ganciclovir therapy initially. In addition, all patients at risk for CMV disease resumed high-dose ganciclovir therapy at the time antirejection therapy was begun; the total amount of ganciclovir received by these patients therefore varied greatly, with all patients at risk for CMV disease receiving an average total of 28.4 ± 7.3 days (range, 8–62 days) of ganciclovir therapy during their initial hospitalization.

Patients who were CMV-seronegative recipients of organs from CMV-seronegative donors were all seropositive for herpes simplex virus before transplantation, and were therefore given a lower dose of acyclovir alone (200 mg po t.i.d) for 6 months.

**Pathological Studies**

Hematoxylin and eosin–stained histologic sections were prepared from fixed, paraffin-embedded sections. Each case of PTLD was confirmed by biopsy of lymph nodes or other involved organs. Immunohistochemical staining, including staining for the latent membrane protein-1 of EBV, and in situ hybridization for EBV-encoded (EBER) RNA of EBV were performed on fixed paraffin-embedded sections. Lesions were classified according to the scheme of Knowles et al. [2].

**Statistical Analysis**

Factors considered for their possible association with the development of PTLD in the pancreas transplantation patients who received antithymocyte globulin induction included race, gender, age, use of FK506 vs. cyclosporin A for immunosuppression, total OKT3 dose, total antithymocyte globulin dose, EBV serostatus of the recipient, CMV serostatus of donor and recipient, and ganciclovir/acyclovir prophylaxis.

The association of total dose of antithymocyte globulin or OKT3 with PTLD was analyzed by Wilcoxon’s rank-sum test. The association of PTLD with FK506 vs. cyclosporin A, positive or negative CMV serostatus, and ganciclovir/acyclovir prophylaxis was analyzed by two-tailed Fisher’s exact test. A P value of <.05 was considered to indicate statistical significance.

**Results**

**Development of PTLD Associated with Immunosuppressive Regimen**

Four of the 49 patients developed PTLD an average of 45 days (range, 34–62 days) after transplantation, for an overall incidence of 8.2%. Three of the 4 had received simultaneous pancreas and kidney allografts (as compared with 46 of the 49 total patients), while 1 received only a pancreas transplant (as compared with 3 of the 49 total patients). The patients who developed PTLD were similar to patients who did not develop PTLD with respect to age, race, and gender (table 1). Similarly, all four patients who developed PTLD and all 45 patients who did not were EBV-seropositive before transplantation, thus eliminating recipient EBV serostatus as a parameter for risk factor analysis.

Analysis of the total cumulative dose of OKT3 received within the first 2 months post-transplantation (and prior to development of PTLD) indicated that EBV-seropositive recipients with PTLD had received significantly higher total doses of OKT3 (mean, 95.0 ± 33.0 mg; range, 45–130 mg) than patients who did not develop PTLD (mean, 45.4 ± 38.0 mg; range, 0–125 mg) (Wilcoxon’s rank-sum test, P < .001) (figure 1 and table 1). The higher cumulative dose of OKT3 was given because of rejection in each case.

In comparison, the total dose of antithymocyte globulin was similar between the two groups (mean of 210.9 ± 111.4 mg/kg and range of 50–540 mg/kg for patients who developed PTLD, vs. mean of 228.8 ± 196.7 mg/kg and range of 20–540 mg/kg for patients who did not develop PTLD; P = .78). One of the 4 patients with PTLD had received cyclosporin A (as compared with 42 of the 49 patients), while 3 had received FK506 (as compared with 19 of the 49 patients; some patients received both agents); however, possibly because of the small number of patients involved, no significant difference in risk for PTLD was associated specifically with FK506 (two-tailed Fisher’s exact test, P = .28).
Table 1. Characteristics of pancreas allograft recipients (n = 49) at known risk for posttransplantation lymphoproliferative disorder (PTLD) (University of Maryland, 1 January 1994 to 1 October 1995).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (n)</th>
<th>Who did not develop PTLD (n)</th>
<th>Who developed PTLD (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>28 (57)</td>
<td>26 (58)</td>
<td>2 (50)</td>
</tr>
<tr>
<td>Female</td>
<td>21 (43)</td>
<td>19 (42)</td>
<td>2 (50)</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>43 (88)</td>
<td>40 (89)</td>
<td>3 (75)</td>
</tr>
<tr>
<td>Nonwhite</td>
<td>6 (12)</td>
<td>5 (11)</td>
<td>1 (25)</td>
</tr>
<tr>
<td><strong>Age (y), mean ± SD</strong></td>
<td>33.7 ± 5.8</td>
<td>33.5 ± 6.2</td>
<td>37.0 ± 2.3</td>
</tr>
<tr>
<td><strong>Type of transplantation undergone</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPK</td>
<td>46 (94)</td>
<td>43 (96)</td>
<td>3 (75)</td>
</tr>
<tr>
<td>PTA</td>
<td>3 (6)</td>
<td>2 (4)</td>
<td>1 (25)</td>
</tr>
<tr>
<td><strong>Immunosuppressive regimen</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>43 (88)</td>
<td>42 (93)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>FK506</td>
<td>22 (45)</td>
<td>19 (42)</td>
<td>3 (75)*</td>
</tr>
<tr>
<td>OKT3 (total mg), mean ± SD</td>
<td>49.5 ± 37.2</td>
<td>45.5 ± 38.0</td>
<td>95.0 ± 33.0</td>
</tr>
<tr>
<td>Antithymocyte globulin (mg/kg), mean ± SD</td>
<td>212.4 ± 118.1</td>
<td>210.9 ± 111.4</td>
<td>228.8 ± 196.7³</td>
</tr>
<tr>
<td><strong>Recipient/donor CMV serostatus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/0</td>
<td>13 (27)</td>
<td>10 (22)</td>
<td>3 (75)§</td>
</tr>
<tr>
<td>+/-, +/+</td>
<td>36 (73)</td>
<td>35 (78)</td>
<td>1 (25)§</td>
</tr>
<tr>
<td><strong>Antiviral prophylaxis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCV/high-dose ACV</td>
<td>40 (82)</td>
<td>39 (87)</td>
<td>1 (25)§</td>
</tr>
<tr>
<td>Low-dose ACV</td>
<td>9 (18)</td>
<td>6 (13)</td>
<td>3 (75)§</td>
</tr>
</tbody>
</table>

NOTE. ACV = acyclovir; CMV = cytomegalovirus; GCV = ganciclovir; PTA = pancreas transplantation alone; SPK = simultaneous pancreas and kidney.

* Sixteen patients received both cyclosporin A and FK506; P = .28, Fisher’s exact test.
1 P = .78, Wilcoxon’s rank-sum test.
² P = .08, Fisher’s exact test.
³ P = .036, Fisher’s exact test.

Development of PTLD Associated with Lack of Prophylaxis with Ganciclovir/High-Dose Acyclovir

Three of the four patients who developed PTLD were CMV-seronegative recipients of organs from CMV-seronegative donors (CMV –/–); these three patients had not received the ganciclovir/high-dose acyclovir prophylaxis given to CMV +/+ or +/+, and –/– patients but were receiving low-dose acyclovir prophylaxis (200 mg po t.i.d.) when they developed PTLD. The fourth patient was CMV-seropositive before transplantation and had received prophylaxis with intravenous ganciclovir during both induction and treatment of early rejection, followed by high-dose oral acyclovir in each case.

Because four of the 13 CMV –/– patients had nonetheless received ganciclovir prophylaxis during both induction and OKT3 treatment of early rejection, we were able to analyze the possible association of CMV serostatus or use of ganciclovir/high-dose acyclovir prophylaxis with development of PTLD. All patients given ganciclovir/high-dose acyclovir prophylaxis initially received 6–28 days of ganciclovir, followed by high-dose oral acyclovir for 5–6 months (or until treatment for rejection or the development of PTLD); all other patients received low-dose oral acyclovir for 6 months (or until treatment for rejection or the development of PTLD). The development of PTLD overall was significantly correlated with a lack of prophylaxis with ganciclovir/high-dose acyclovir (two-tailed Fisher’s exact test, P = .036), while CMV seronegativity for both donor and recipient approached but did not achieve a significant correlation (P = .08) (table 1).

Combined Effect of Immunosuppressive Regimen and Lack of Prophylaxis with Ganciclovir/High-Dose Acyclovir on Development of PTLD

Eleven of our 49 patients received high-dose OKT3 (defined as >75 mg), which has been associated with development of PTLD in heart transplant recipients [7]. Three of the 11 patients who received high-dose OKT3 developed PTLD, and therefore the use of high-dose OKT3 alone increased the risk of PTLD from 8.2% overall to 27.3%. Similarly, three of nine patients who did not receive ganciclovir/acyclovir prophylaxis developed PTLD, increasing the risk of PTLD to 33%.
lymph nodes in the other). Immunosuppression was discontinued, and the kidney and pancreas allografts were removed from both patients (numbers 1 and 2), who were also treated with high-dose intravenous acyclovir and intravenous gammaglobulin; IFN-α therapy was added for the patient with more extensive organ involvement (patient 3). Both of these patients also remained disease-free >1.5 year following the diagnosis of PTLD.

Patient 4, who received a pancreas transplant alone, had monomorphic B cell PTLD that would be classified as malignant lymphoma, large-cell immunoblastic type. The atypical cells invaded the pancreas, liver, and bone marrow, and there was extensive necrosis in the pancreas. Her immunosuppression was also discontinued, her allograft was removed, and treatment with chemotherapeutic agents was given. Almost 2 years have passed since the diagnosis of her PTLD, and she remains free of disease.

Discussion

With the use of more potent immunosuppressive agents, the incidence of PTLD appears to be increasing in solid organ transplant recipients [6–9]. A dramatic increase in PTLD in pancreas transplant recipients at our institution followed the initiation of antithymocyte globulin induction with OKT3 treatment for early rejection; development of this early onset PTLD within 62 days of pancreas transplantation appears to be related to high cumulative doses of OKT3. Although primary EBV infection has been noted to be an independent risk factor for PTLD in recipients of other solid organs [10], the relative lack of pretransplantation seronegativity for EBV in our patient population precluded an analysis of this variable as a risk factor for our pancreas transplant recipients.

A similar correlation between PTLD and high cumulative doses of OKT3 was previously noted in heart transplant recipients [7], while the combination of OKT3 and antilymphocyte globulin appeared to predispose kidney transplant recipients to PTLD [8]. Whether it is the cumulative dose itself, the number of days of administration of OKT3, or the mechanisms of OKT3 activity that actually cause B cell proliferation is unknown. It is also possible that the increased use of OKT3 may have been a marker for another process that could affect EBV replication and/or B cell proliferation, rather than a cause of PTLD. For example, early PTLD may have been associated with rejection or misdiagnosed as rejection during its development, since infiltrating T cells can be seen with both conditions; either this association or the misdiagnosis might then have led to additional immunosuppression with OKT3, which would result in correlation of a higher OKT3 dose with PTLD.

Furthermore, in the current study, as in the previous studies that suggested a specific correlation of PTLD with OKT3 use or dosage, it is likely that the cumulative degree of immunosuppression rather than the use of one or two specific agents is the underlying risk factor for PTLD. However, statistical analyses

Histopathology, Treatment, and Outcome

The diagnosis of PTLD was confirmed by immunohistochemical staining of atypical lymphocytes for the latent membrane protein-1 in all 4 cases, plus the demonstration of EBER RNA of EBV by in situ hybridization in 3 of the 4 cases. With use of the classification system of Knowles et al. [2], the diagnosis for one of our patients was established as the least aggressive form of PTLD (plasmacytic hyperplasia), which appeared to be confined to the pancreas allograft alone. Following removal of the pancreas and a decrease in overall immunosuppression, she was treated with high-dose intravenous acyclovir (the equivalent of 15 mg/kg q.d. for normal renal function) and was able to retain her kidney allograft; she remained disease-free 19 months following the diagnosis of PTLD.

Two of our patients were found to have an intermediate form of PTLD (polymorphic B cell hyperplasia/lymphoma) that involved the transplanted pancreas and other organs (kidney, lymph nodes, gall bladder, and liver in one; kidney and lymph nodes in the other). Immunosuppression was discontinued, and the kidney and pancreas allografts were removed from both patients (numbers 1 and 2), who were also treated with high-dose intravenous acyclovir and intravenous gammaglobulin; IFN-α therapy was added for the patient with more extensive organ involvement (patient 3). Both of these patients also remained disease-free >1.5 year following the diagnosis of PTLD.
indicated that the cumulative dose of another potent immuno-
suppressive agent (antithymocyte globulin) did not differ be-
tween the patients who developed PTLD and those who did not
(in both the pancreas and heart transplant [7] patients).

Similarly, the use of FK506 was not significantly correlated
with the development of PTLD in our patients, although this
may have been because of the small number of patients who
developed PTLD and the sizable percentage of patients treated
sequentially with both FK506 and cyclosporin A. Development
of a method for quantifying the absolute degree of immunosup-
pression in each patient is necessary to analyze its influence
on the development of PTLD.

The possible protection against development of PTLD as a
result of antiviral therapy (ganciclovir followed by acyclovir)
was previously suggested by a retrospective analysis of kidney/
pancreas and liver transplant recipients in whom the incidence
of PTLD was lower than previously recorded when EBV status
and the use of antilymphocyte preparations were taken into
account [11]. Whether the protection was afforded by intra-
venous ganciclovir, high-dose oral acyclovir, or the combina-
tion of the two agents is currently unknown. Both agents inhibit
the proliferation of EBV in vitro. However, we suspect that
ganciclovir was the agent primarily responsible for protection
against PTLD, for the following reasons. All of the patients
who developed PTLD did receive oral acyclovir therapy (albeit
3 of the 4 received a lower dose); 6 of the 8 patients who
received >75 mg of OKT3 but did not develop PTLD received
ganciclovir (but not acyclovir) for 21–50 (mean, 34.8) of the
first 62 days post-transplantation; and ganciclovir can inhibit
the replication of many EBV strains at concentrations achiev-
able in vivo (IC₅₀ = 0.05–125 μM).

The only patient to receive ganciclovir/high-dose acyclovir
and develop PTLD also received a relatively low dose of
OKT3; this patient was found to have recently become EBV-
seropositive (within 6 months before transplantation), and his
infection may therefore have been more analogous to a primary
EBV infection with a relatively increased risk for development
of PTLD. This patient also received an extremely high total
dose of antithymocyte globulin (540 mg/kg), further suggesting
that the degree of total immunosuppression may also be a risk
factor for PTLD.

We attribute the long-term survival of all of our patients
with PTLD to an aggressive approach to diagnosis and therapy.
As advocated by Starzl et al. [12], we discontinued all immuno-
suppression in three of the patients (patients 2, 3, and 4), contin-
uing only relatively low-dose prednisone therapy in patient 1
(who also received a kidney allograft and whose disease ap-
peared to be confined to the pancreas allograft). High-dose
therapy with intravenous acyclovir was immediately instituted
for the three patients who did not have immunoblastic
lymphoma; intravenous immunoglobulin therapy was added
for the two patients with polymorphic B cell hyperplasia/
lymphoma, and IFN-α therapy was added for the patient who
had multiorgan involvement. The only patient with immuno-
blastic lymphoma was treated aggressively with chemothera-
peutic agents. All four of these patients have survived and
remained disease free a minimum of 18 months following the
diagnosis of PTLD.

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