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# Burkitt's Lymphoma Variant of Post-transplant Lymphoproliferative Disease (PTLD)

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**The occurrence of posttransplant lymphoproliferative disorder (PTLD) in solid organ allograft recipients can be quite varied in clinical presentation, histopathological characteristics and frequency. A variety of lymphomas can develop as a PTLD although some types appear infrequently and remain poorly understood in this clinical setting. In this report, we describe two cases of Burkitt's lymphoma presenting as a PTLD following liver transplantation. The recipients were 12 and 44 years of age and displayed gastrointestinal involvement by the tumors several years following transplant. The tumors displayed the typical histological features of Burkitt's lymphoma and were markedly positive for EBV. The tumors displayed similar immunophenotypic characteristics by flow cytometry and had rearrangements of the immunoglobulin J-H heavy chain. The tumors required aggressive chemotherapy and a cessation of immunosuppressive therapy. This report demonstrates that Burkitt's type lymphomas can develop in the posttransplant setting and that these tumors contain morphologic, cytofluorographic and molecular features identical to Burkitt's lymphomas that occur in non-transplant patients. Our experience is that these PTLD- Burkitt's lymphomas behave aggressively and require intensive chemotherapeutic intervention.** (Pathology Oncology Research Vol 8, No 2, 105–108, 2002)

*Keywords:* PTLD, Burkitt's lymphoma

## *Introduction*

Posttransplant lymphoproliferative disorder (PTLD) is a well-recognized complication of the long-term immunosuppression used in solid organ transplantation. According to previous studies, approximately 0.8 to 1.9% of adult orthotopic liver recipients will develop a PTLD in the six years following transplant.[1,2] By comparison, children appear to be more susceptible to development of PTLD after liver transplant with occurrence rates of 11.4 to 13% and an average post-transplant onset of 10.1 to 2.1 months.[1,3]

PTLD may manifest itself clinically as a lymphadenopathy, systemic illness or lymphomatous mass.[4] Histologically, PTLD can be classified into four categories: early lesions, including reactive plasmacytic hyperplasia; polymorphic PTLD, including polyclonal and monoclonal variants; monomorphic PTLD, including B and T cell lymphomas; and a fourth category that encompasses T-cell rich lesions and Hodgkin's disease.[5] B cell proliferations, normally of donor origin, make up 85% of PTLD cases. Another 14% of PTLD cases are of T cell origin and have the worst prognosis. Approximately 1% of PTLD's are of a null cell origin.[6,7]

Risk factors for the development of PTLD include treatment with potent immunosuppression (e.g., cyclosporine and OKT3) and infection with the Epstein-Barr virus (EBV).[6] EBV is associated with a high proportion (89%) of posttransplant B cell PTLD.[8] The virus acts to immortalize the lymphocyte by interacting with the BCL-2 gene to prevent apoptosis. In particular, pre-transplant EBV seronegativity followed by posttransplant seroconversion has been shown to be a major contributing risk factor for developing PTLD.[9] The pediatric population's increased susceptibility to PTLD may be partially due to the higher

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rate of pre-transplant seronegativity in children as compared to adults.

Burkitt's lymphoma is a rarely observed entity as a PTLD.[5] This tumor is defined as an undifferentiated, monotonous, malignant growth of lymphoreticular cells with moderate nuclear and cytoplasmic variations. Burkitt's lymphoma is categorized as a type of mature (peripheral) B-cell neoplasm that encompasses an immunodeficiency-related subtype.[10] The typical presentation involves multifocal, rapidly growing extranodal masses in the retroperitoneum and/or abdominal viscera as well as other sites. Histologically, the tumor cell nuclei appear round or slightly uniform with a prominent nuclear membrane and slight nuclear indentation. Nucleoli are prominent and mitotic activity is high.[11]

## *Materials and Methods*

*Routine Histology* – The tissue was fixed in 10% buffered formalin and routinely processed for paraffin-embedding. The biopsies were cut at 3.0 μm and stained with hematoxylin and eosin.

*Flow cytometry* – Several combinations of monoclonal antibodies were used for four-color immunophenotyping: (A) CD45-PerCP (peridinin chlorophyll) /CD11c-APC (allophycocyanin) /CD23-PE (Phycoerythrin) /CD10-FITC (fluorescein isocytothiocynate); (B) Isotypic controls: mouse $IgG_1$-PerCP/mouse $IgG_1$-APC/mouse $IgG_1$-PE/mouse $IgG_1$-FITC; (C) CD14-PerCP/CD38-APC/CD13-PE/CD64-FITC; (D) CD3-PerCP/CD4-APC/CD56-PE/CD8-FITC; (E) CD42a-PerCP/CD34-APC/CD7-PE/CD33-FITC; (F) CD20-PerCP/CD5-APC/CD22-PE/TCR-alpha/beta-FITC; (G) HLA-DR-PerCP/CD69-APC/CD25-PE/CD2-FITC; (H) (2-color) CD19-PE/kappa-FITC; (I) (2-color) CD19-PE/lambda-FITC. (PerCP, FITC, PE and APC are fluorochromes with non-overlapping spectra, thereby allowing 4-color analysis.)

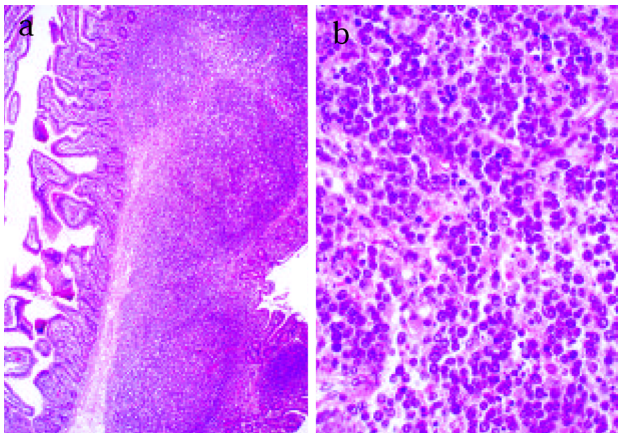


***Figure 1.*** *Histological features of gastrointestinal (ileal) Burkitt's Lymphoma (H&E; A = 100x, B = 400x).*

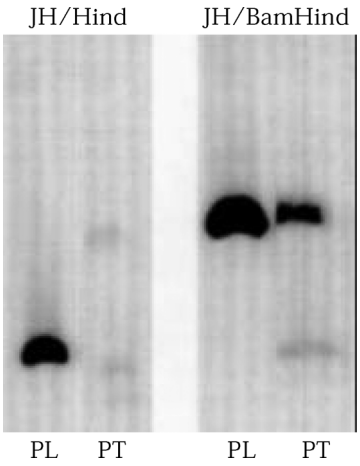


***Figure 2.*** *Southern blot analysis of ileal lesion for the heavy chain (J-region) with two restriction enzymes. Patient shows rearrangement of the germline band consistent with monoclonal B cell population. PL = placental germline; PT = patient.*

Gating was performed on lymphocytes (with characteristic forward and side scatter features and high CD45 staining) or blast-sized cells. The designation of positive cells was based on values compared to cells stained with irrelevant isotype controls and conjugated to the same fluorochrome. The antibodies were attached to the cells isolated from the tissue after a washing step and incubated at 4 degrees Celsius for 30 minutes, then washed twice. The cells were fixed with 2% paraformaldehyde and analyzed in less than 2 hours after staining on a flow cytometer (FacsCaliber Beckman Coulter). Listmode data on 5,000 gated cells was collected with 1024 channel resolution and was analyzed using Cell Quest software version 2.0 (Becton Dickinson). Backgating of listmode files was utilized.

*Southern Blot Analysis* – This procedure was performed for immunoglobulin and T cell receptor rearrangement analysis as previously described (12).

*In Situ Hybridization* for EBV (EBER) – This was performed on paraffin-embedded tissue as previously described (13).

## *Case Reports*

Two cases of Burkitt's Lymphoma (PTLD) after liver transplant occurred at our institution. The first case is an Epstein-Barr virus (EBV) positive 11-year-old male who received a liver transplant in 1995 at the age of 6 for Alagille syndrome. His post-transplant medications included steroids and tacrolimus (FK506). Five years following transplantation, he presented with bowel obstruction. An exploratory laparotomy revealed a tumor in the mesentery

*Table 1.* **Immunophenotypic and DNA profile of Burkitt's type PTLD.**

| *Patient No.* | *Age* | *Surface Antigen Profile1* | *DNA Characteristics* |
|---|---|---|---|
| 1 | 11 | CD19+, CD20+, CD22+, CD23-, Kappa+, CD10+, CD38+, CD79a+ | Diploid, S-phase=39% |
| 2 | 44 | CD19+, CD20+, CD22+, CD23-, Kappa+, CD10+, CD38+ | Diploid, S-phase=43% |

1. Flow cytometric analysis of the tissue lesions was performed as described in the Materials and Methods.

and adjacent small bowel for which he underwent a segmental small bowel resection. A biopsy of the orthotopic liver demonstrated no liver involvement.

The histomorphological features were consistent with a Burkitt's lymphoma (*Figure 1*) and sent for further study that confirmed rearrangement of the joining segment of the heavy chain gene (*Figure 2*), pointing to a monoclonal B-cell lymphocytic population. In addition, in situ hybridization for Epstein-Barr encoded RNA (EBER) was positive in the tumor cells. Cytofluorographic analysis by flow cytometry demonstrated that the lesion had the immunophenotypic and DNA characteristics consistent with Burkitt's *lymphoma (Table 1*). At that time, the patient's immunosuppressive therapy was discontinued, and he was treated with Rituximab (chimeric anti-CD20 monoclonal antibody). Acute rejection followed requiring treatment with steroids and rapamycin. While the lymphoma was responsive to subsequent chemotherapy, complications including tumor lysis syndrome, intestinal obstruction and respiratory failure ensued, and the patient expired six months after being diagnosed with the lymphoma. Residual mesenteric lymphoma was present at autopsy.

In a second case, a 44-year-old white male had a liver transplant for hepatitis C and was also taking tacrolimus post-operatively. He presented four years later with constipation, abdominal tenderness and adenopathy on CT scan. An exploratory laparotomy was done in which a mesenteric mass was removed. This mass proved to be a malignant lymphoma, small non-cleaved cell type, consistent with Burkitt's lymphoma. Gene rearrangement studies showed rearrangement of the J-H heavy chain and flow cytometric analysis showed an immunophenotype and DNA profile (*Table 1*) compatible with Burkitt's lymphoma. In situ hybridization for EBV (EBER) revealed numerous positive lymphoid cells.

## *Discussion*

The Epstein-Barr virus works by gaining entry into B-lymphocytes and epithelial cells of the oropharynx via the CD21 antigen. While the EBNA-1 (Epstein-Barr Nuclear Antigen) maintains the virus' latency, the cell is immortalized by interaction of the BCL-2 (B-cell lymphoma) gene with LMP-1 (latent membrane protein) that prevents apoptosis, thereby promoting indefinite cell proliferation.[14] Typically, T-cells recognize and attack these EBV-associated membrane proteins, thereby keeping B-cells from uncontrolled proliferation. Since transplant patients have diminished T-cell function, they tend to have an impaired capacity to modulate EBV transformation in B-cells. Therefore, PTLD's represent an escape of EBV-infected B-cells that are typically controlled.[15] Some of the B-cell PTLD's are of the large cell lymphoma type and initiate as a polyclonal proliferation of B-cells.

It becomes important to identify Burkitt's type PTLD from other types when deciding upon a course of treatment. Other types of PTLD are likely to respond well to a decrease in immunosuppression. However, Burkitt's lymphoma is not likely to be affected by this change in therapy and characteristically requires adjuvant chemotherapy or radiation.[16]

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