Post-transplantation lymphoproliferative disorders: advances in diagnosis, prevention and management in children

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Abstract

With improving operative and postoperative survival after pediatric thoracic transplantation, attention has appropriately begun to focus on complications of long-term immunosuppression. One important complication is post-transplantation lymphoproliferative disorders. Much has been learnt about this spectrum of disorders over the last 15 years, including the pivotal role of primary Epstein–Barr virus infection in the etiology of most cases. Despite these advances, nomenclature remains confusing for the clinician, prediction of outcome is imprecise and treatment strategies are poorly defined. Indeed, no treatments have been subjected to comparative prospective clinical trials. Only recently has attention focussed on strategies for prevention. This article will review the current state of knowledge of post-transplantation lymphoproliferative disorders, with emphasis on recent advances in diagnosis, prevention and management. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Etiology: importance of primary Epstein–Barr virus (EBV) infection

Post-transplantation lymphoproliferative disorders (PTLD) are a spectrum of conditions that straddle the borders between infection and malignant neoplasia. It has long been recognized that normal immune surveillance is essential for control of viral infection and for prevention of development of neoplasms. Lymphomas may arise in congenital (e.g. X-linked lymphoproliferative disease) as well as acquired (e.g. Acquired Immunodeficiency Syndrome) immunodeficiency syndromes. The association between therapeutic immunosuppression, as occurs in the transplant recipient, and the development of lymphoid tumors was first recognized in 1968 [1]. Recognition of an association between EBV infection and X-linked lymphoproliferative disease stimulated a successful search for this virus within PTLD lesions [2]. Subsequently, a large literature has accrued demonstrating the pivotal role of Epstein–Barr virus in most cases of PTLD in pediatric solid organ transplant recipients. Evidence comes both from epidemiological observations [3], as well as from demonstration of EBV genome within lesions [4]. We have found that approximately 80–90\% of cases in pediatric thoracic recipients are EBV driven, including all cases arising in the first 3 years after transplantation. This is broadly comparable to findings reported for other organ transplants and in
adult recipients. In our experience, EBV negative cases usually arise late after transplantation, generally beyond 5 years and their etiology remains an enigma. We have searched for other viruses that are known to cause lymphoma or other malignancies in Acquired Immunodeficiency Syndrome, such as Human Herpes Viruses 6 and 8, but have not found evidence for these in EBV negative PTLD in the small number of patients that we have evaluated.

The source of EBV infection is not always clear. What is apparent is that most cases of PTLD occur in patients who are seronegative for EBV at the time of transplantation and who subsequently develop primary EBV infection [3]. Since the vast majority of adults are seropositive at the time of transplantation, it is not surprising that PTLD occurs at a much lower frequency in adult recipients. Approximately 5–15% of all cases in pediatric thoracic transplantation have arisen in recipients who were seropositive at the time of transplantation. In our own series, we found that 18/22 cases of PTLD arose in patients who were seronegative at transplantation (Table 1) [5]. Zangwill and colleagues noted that 12 of their 13 cases of PTLD in pediatric heart recipients arose in patients who were seronegative at the time of transplantation, and post-transplantation seroconversion was documented in all 12 cases [6]. The strong association between the acquisition of primary EBV infection after transplantation and the risk of PTLD appears to be true for all types of solid organ transplantation with the exception of intestinal transplantation. In the latter setting, EBV seropositive children frequently develop PTLD [7]. This may reflect the intensity of the immunosuppressive regimens used, and the high load of lymphoid tissue that is contained within the intestinal allograft.

In the seronegative thoracic recipient, primary EBV infection could come from several potential sources, including the donor organ(s), perioperative use of blood products, or from subsequent community acquisition of the virus. In our own series, 23% of patients in the primary mismatch group (donor EBV seropositive/recipient seronegative) went on to develop PTLD. When both recipient and donor were seronegative, 16% of recipients developed PTLD. The demographic features of PTLD differed between these two groups. In the ‘primary mismatch’ group, the median time to onset of PTLD was 4 months after transplantation, with all cases occurring within 1 year of transplantation. In the group with seronegative donors, a much broader range of time to onset was observed (4 months–7 years). These observations provide indirect evidence that the donor may be an important source of infection in many patients in the primary mismatch group. The low incidence of EBV seroconversion and PTLD in the early months after transplantation in seronegative recipients with seronegative donors suggests that perioperative blood transfusion is an unlikely source of EBV infection in pediatric thoracic recipients. Interestingly, a small number of studies have now used molecular techniques to unequivocally demonstrate that EBV was of donor origin [8,9]. In one of these studies, a single donor provided kidney and heart–lung block to two different recipients, one being EBV positive pre-transplantation and the other being EBV negative. Both developed PTLD with EBV of donor origin [8]. These observations suggest that some cases of PTLD associated with ‘reactivation’ may in fact represent reinfection with a separate strain of EBV.

### 2. Frequency and risk factors

It is clear that acquisition of a primary EBV infection post-transplantation is the most important risk factor for the development of PTLD. It remains to be explained, however, why the majority of patients who develop a primary EBV infection do not develop PTLD. Clearly, there must be interplay with other risk factors. These factors have not yet to been fully elucidated, but undoubtedly will include overall intensity of immunosuppression. In some studies use of OKT3 as well as other lympholytic agents have been associated with increased risk of development of PTLD [10,11]. Heart–lung and lung recipients receive higher doses of immunosuppression than patients receiving an isolated heart allograft. It is likely that this increased level of immunosuppression, and perhaps the higher lymphoid load transmitted with the allo-

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**Table 1**

Occurrence of EBV-associated PTLD based on donor and recipient EBV serologies at time of transplantation*

<table>
<thead>
<tr>
<th>EBV status</th>
<th>D+ / R−</th>
<th>D− / R−</th>
<th>D+ or − / R+</th>
</tr>
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<tbody>
<tr>
<td>Number of patients</td>
<td>26</td>
<td>31</td>
<td>88</td>
</tr>
<tr>
<td>Number developing PTLD (%)</td>
<td>6 (23%)</td>
<td>5 (16%)</td>
<td>4 (4.5%)</td>
</tr>
</tbody>
</table>

*Data from pediatric thoracic transplant program at Children’s Hospital of Pittsburgh, PA, USA. Notes: Seven additional cases of PTLD occurred among recipients with unknown donor serologies (total 22 cases among 210 recipients). All seven were EBV seronegative at time of transplantation. Abbreviations: D, donor; R, recipient; +, seropositive; −, seronegative.
graft (and thus higher EBV inoculum in the seropositive donor), contributes to the greater frequency of PTLD in heart–lung and lung recipients.

Currently, there is considerable controversy as to whether tacrolimus based therapy is associated with a greater frequency of development of PTLD than cyclosporine-based regimens. Recent reports suggest this may be the case for pediatric liver [12] and for renal [13] transplantation, though not all studies have confirmed this observation [11]. It should be noted, however, that no pediatric randomized trials comparing tacrolimus and cyclosporine therapy have been performed and these observations are based on historical controls or registry data. Among pediatric heart recipients, we have noted comparable frequencies of PTLD between cyclosporine and tacrolimus treated patients up to this time [14], and our incidence in tacrolimus-treated patients is similar to that reported by the Columbia [6] and Stanford University [15] groups using cyclosporine based therapy.

It is currently unclear, whether age per se is a risk factor for the development of PTLD or whether this merely reflects donor and recipient EBV status. Younger age will be associated with lower risk of pre-transplantation seropositivity, but also with lower risk of the donor being EBV seropositive since younger recipients are more likely to receive organs from young donors to avoid excessive donor/recipient size mismatch. The Loma Linda group have observed a relatively low incidence of PTLD in infant heart recipients (11 of 255 recipients, 4.3%; R. Chinnock, personal communication). Although this may reflect the low frequency of donor/recipient EBV mismatching, it is of interest to speculate whether maternal–fetal transmission of anti-EBV antibodies might protect the young infant during the period of greatest immunosuppression early after transplantation. The precise role of antibody (as opposed to cytotoxic T cell) responses in the protection of the patient against the development of PTLD is unknown, though several reports have suggested that lack of antibody response to EBV nuclear antigens (EBNA) may increase the risk of developing PTLD [16]. These observations are important as prophylactic use of intravenous antibody preparations containing high titers of anti-EBV antibodies has been suggested as a possible means of preventing PTLD (see below).

Several problems in the definition and analysis of PTLD cases have led to inconsistencies in the reported ‘incidence’ of this complication. Although the risk is highest in the first year after transplantation, the patient remains at risk indefinitely. Thus, the frequency in any series will depend on post-operative survival and length of follow-up, as well as on case definition. Patients dying soon after transplant are ‘at risk’ for only a very short period. Some reports have, therefore, quoted the frequency among 30-day survivors. A more appropriate method of analysis for a time related event is to use Kaplan–Meier or similar ‘survival analysis’ techniques. An overall ‘frequency of occurrence’ of PTLD of 4–10% for pediatric heart recipients [6,15,17,18] and approximately 10–20% for pediatric heart–lung and lung recipients have been reported [18,19]. None of these studies used ‘survival analysis’ methodology.

A second, and perhaps more important, limitation is the method of case definition of PTLD used within any given center. Most centers have not included infectious mononucleosis and other EBV-associated viral syndromes as cases of PTLD, though they represent part of the continuum of EBV driven pathology. When histological findings (e.g. of enlarged lymph nodes, tonsils or adenoidal tissues) reveal only benign lymphoid hyperplasia with architectural preservation, many centers do not consider the tissue to represent a PTLD. The importance of this variability in case definition is illustrated by the Columbia group, who found that inclusion of such cases resulted in an approximate doubling of cases of PTLD [6]. Inclusion of these more ‘histologically benign’ cases will also result in improved overall clinical outcomes since this group of patients (see below) carry a very favorable prognosis. Thus, variation in the case definition will result in problems in comparing both ‘incidence’ and outcomes between centers.

3. Clinical presentation and diagnostic evaluation

EBV is increasingly recognized to be associated with a wide range of disease manifestations in transplant recipients. This includes a non-specific viral syndrome, mononucleosis, and PTLD including EBV-associated malignant lymphoma (e.g. Burkitt’s lymphoma). Although this classification is useful, it is important to note that EBV presents as a continuous spectrum of illness, and benign manifestations can evolve to more serious syndromes within individual patients. Furthermore, non-PTLD viral syndromes are not always benign, and fatal viral sepsis may occur in the absence of mass lesions.

The diagnosis of EBV disease in pediatric thoracic recipients is based on clinical history and physical examination in combination with laboratory confirmation. The most important factor in making this diagnosis is maintenance of a high index of suspicion at all times. In our experience, earlier diagnosis appears to have correlated with more successful outcomes. A critically sick child with disseminated disease involving multiple extranodal sites was seen on a number of occasions in our early pediatric transplant...
experience. These patients frequently died within days or weeks of presentation, often from infection with secondary pathogens. Such cases are now exceedingly rare, suggesting that earlier diagnosis and improved management may be responsible for the changing pattern of presentation in this population.

In contrast to these severe manifestations, a history of lethargy, malaise, weight loss and fever are now common presentations of PTLD. An additional history of vomiting and/or diarrhea (which may be guaiac positive) is suggestive of gastrointestinal involvement, which has been increasingly recognized to be a common site of disease in pediatric thoracic recipients. Intestinal hemorrhage, obstruction and perforation may also be seen in patients with intestinal PTLD. Surprisingly, the highest risk period for the later may be during therapy when necrosis of transmural lesions may develop. Pulmonary disease is also very common in cardiac recipients and is almost invariably present in lung recipients (Fig. 1). Pulmonary presentation ranges from asymptomatic nodule(s) on routine chest radiograph to life threatening pulmonary dysfunction in the lung allograft. The latter may resemble lung rejection on chest radiograph with rather diffuse consolidation without clearly defined mass lesions. This may lead to inadvertent augmentation of immunosuppression with severe consequences [19]. Other less common presentations include persistent sore throat, adenopathy, and cutaneous nodules, as well as seizures, headaches and focal neurological lesions with CNS disease. We have even diagnosed PTLD following elective removal of enlarged tonsils and adenoids in a child developing airway obstruction during conscious sedation for routine endomyocardial biopsy. Interestingly, the heart is the only organ transplant in which there is not a strong tendency for the disease to involve the allograft. In most other solid organ transplants, allograft dysfunction may be a manifestation of PTLD and may mimic acute or chronic rejection. We have not seen cardiac dysfunction in a child due to direct involvement with PTLD, though cardiac involvement has been reported [20].

Although physical examination may not reveal specific findings, there will frequently be evidence of pallor, weight loss, peripheral adenopathy, and hepatosplenomegaly. A full physical examination is essential and should include thorough neurological examination. Examination of the entire skin and sites of all lymph nodes is warranted. Careful examination of the oropharynx is required and should include evaluation by an ENT surgeon if there is clinical evidence of tonsillar or adenoidal hypertrophy.

An overview of the laboratory evaluation for the diagnosis of PTLD is given in Table 2. Initial evaluation should include a complete blood count with white cell differential and platelets. Leukopenia, often in association with atypical lymphocytosis, as well as thrombocytopenia are frequent findings. Anemia is common and may be normocytic and normochromic or may demonstrate findings of iron deficiency when occult gastrointestinal bleeding is present. Stools should be tested for blood. End organ function (liver, kidney) should be evaluated. Elevations in uric acid and lactate dehydrogenase are common and should also be sought on blood chemistry testing. Immunglobulin levels may be elevated. This is particularly true of IgE, perhaps due to a particular cytokine environment present with PTLD (so-called T helper type 2 predominance). Serum protein electrophoresis should also be performed. Although many patients do not demonstrate a monoclonal or oligoclonal gammo-

Fig. 1. Chest radiographs of a 5-year-old girl 3 years out from heart transplantation. She presented with low-grade fevers and mild respiratory symptoms. The right-sided pulmonary infiltrates were treated as a pneumonia with oral antibiotics. Failure of clinical response ultimately led to a diagnosis of EBV-positive polymorphic PTLD, which also involved the tonsils and adenoids. This case demonstrates that the diagnosis may be difficult unless high index of suspicion is maintained. There was rapid resolution with reduction of immunosuppression (right-hand panel).
Table 2
Diagnostic evaluation of patient with suspected PTLD

<table>
<thead>
<tr>
<th>Routine</th>
<th>Selected patients</th>
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<tbody>
<tr>
<td>CBC; platelets, WCC with differential</td>
<td>GI endoscopy</td>
</tr>
<tr>
<td>Serum electrolytes, calcium, BUN and</td>
<td>Bone scan</td>
</tr>
<tr>
<td>creatinine</td>
<td></td>
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<tr>
<td>Liver function tests</td>
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<tr>
<td>Uric acid</td>
<td></td>
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<tr>
<td>Lactate dehydrogenase</td>
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<tr>
<td>Quantitative immunoglobulins</td>
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<tr>
<td>Serum protein electrophoresis</td>
<td></td>
</tr>
<tr>
<td>EBV serologies (anti-EBNA, VCA and EA)</td>
<td></td>
</tr>
<tr>
<td>EBV viral load by quantitative PCR</td>
<td></td>
</tr>
<tr>
<td>Stools for occult bleeding</td>
<td></td>
</tr>
<tr>
<td>Chest radiograph (AP and lateral)</td>
<td></td>
</tr>
<tr>
<td>CT scan of chest/abdomen/pelvis</td>
<td></td>
</tr>
<tr>
<td>Core needle or excisional biopsy of lesion(s)</td>
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</tbody>
</table>

Pathology, if present this provides an additional means of following the patient’s response to therapy.

A variety of imaging tests are also of help in the evaluation of the thoracic transplant recipient with suspected PTLD. A chest radiograph often reveals evidence of pulmonary nodular disease and/or evidence of mediastinal lymphadenopathy. The most informative diagnostic study is usually computed tomographic (CT) evaluation of the chest and abdomen. Evidence of nodal or extranodal disease will frequently be apparent on CT at one or more sites. In the chest, small pulmonary nodules or enlarged mediastinal lymph nodes may be apparent even in the presence of normal chest radiograph. In the abdomen, disease may be found at normal lymph node sites, within the GI tract, or at extranodal sites, including the liver and kidneys (Fig. 2). Some centers routinely perform CT or magnetic resonance imaging of the brain. These studies should always be performed if there is any clinical suggestion of CNS disease (Fig. 3).

Other studies performed on selected patients as directed by the clinical findings are shown in Table 2. Upper and/or lower gastrointestinal endoscopy should be performed when there are gastrointestinal symptoms or evidence of occult gastrointestinal bleeding.

Evidence of EBV infection has traditionally been sought by serological studies. The presence of antibodies to the viral capsid antigen (VCA), nuclear antigens (EBNA), and early antigens (EA) is frequently assessed. These studies should be routinely obtained when PTLD is suspected and certainly the presence of anti EBV-IgM antibodies suggests an acute infection. Some patients, however, mount a poor, or even absent, antibody response and antibody testing will also generally be non-contributory for the diagnosis of PTLD in the patients who are seropositive pre-transplantation. Confusion may also arise due to passive transmission of antibodies from blood products, or of maternal origin in the infant recipient.

An important advance in the diagnosis of EBV-associated PTLD, and other EBV-associated viral syndromes, is the measurement of EBV viral load in peripheral blood using quantitative or semi-quantitative polymerase chain reaction (PCR). We have recently reviewed the role of EBV PCR evaluation in

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Fig. 2. Abdominal CT of a 6-year-old boy 5 years out from heart transplantation demonstrates the opposite end of the spectrum of EBV-driven lymphoproliferation. He presented with weight loss and rapidly progressive jaw swelling. Histology confirmed Burkitt’s lymphoma. There was diffuse disease throughout the abdomen including the huge renal lesions shown here. Note also the large masses anterior to the left kidney. The facial involvement extended into the floor of the orbit. No residual disease was present after the second course of chemotherapy.
Fig. 3. PTLD of the central nervous system is rare. This 6-year-old boy with relapsed PTLD, re-presented with a sixth nerve palsy. MRI demonstrated five enhancing intracerebral masses with surrounding edema. Stereotactic biopsy showed monomorphic PTLD. These lesions resolved with aggressive reduction in immunosuppression.

the diagnosis, management and possible prevention of PTLD [21]. A growing experience suggests that a markedly elevated EBV viral load is present in all patients with EBV driven PTLD. Within our own program, we use a quantitative competitive PCR technique (QC-PCR) developed by Rowe et al. [22]. Children with latent viral infection demonstrate very low circulating viral loads [generally < 8 EBV genome copies per $10^5$ peripheral blood mononuclear cells (PBMC)]. With PTLD, viral loads are usually extremely high, being greater than $1000$ genome copies/10$^5$ PBMC in most cases, and greater than 200 in all cases that we have studied to date. However, we have found that EBV viral loads are comparable among patients with EBV-associated viral syndromes and those with PTLD. Thus, while evaluation of the EBV viral load is a very useful rapid screening procedure for suspected EBV disease, it cannot replace histologic examination of suspected sites of involvement when the diagnosis of PTLD is contemplated.

4. Pathological findings

The pathological description of PTLD remains confusing for many transplant physicians. This is regrettable since an understanding of the histology and molecular pathology of these disorders is important for determining therapy and prognosis. Fortunately several recent reviews have attempted to clarify this topic [4,23,24]. We have adopted a simple approach to the pathological description of PTLD (Table 3). First and foremost, it should be noted that evaluation should be done by a pathologist with extensive experience in the evaluation of PTLD. The transplant physician should forewarn the pathologist of the impending biopsy of suspected lesions and, ideally, the pathologist should be present to receive the biopsy, which must be submitted fresh, rather than in formalin. Whenever possible, tissue from several involved sites should be obtained since different morphologies may be present at different sites of disease. Core needle biopsies, under CT guidance, or excisional biopsies are generally required for full diagnostic work up. Upper and/or lower gastrointestinal endoscopy also offer opportunities to obtain multiple biopsy samples in patients with involvement at these sites. Occasionally, laparoscopy or mediastinoscopy may be helpful techniques for obtaining tissue.

The diagnosis of PTLD is confirmed by histology [23,24]. The most benign end of the spectrum is represented by nonspecific reactive lymphoid hyperplasia, including mononucleosis lesions. These lesions have been variously labeled as benign lymphoid hyperplasias, plasmacytic hyperplasias and even as ‘infectious mononucleosis-like PTLD’. The central characteristic of this group of lesions is the diffuse proliferation of mononuclear cells of various sizes, many with plasmacytoid features, but with preservation of normal tissue architecture. These lesions tend to occur in lymph nodes, and in tonsils and adenoids rather than in other extranodal sites. These lesions represent one end of the spectrum of PTLD, but it should be noted that many groups have not included them as cases of PTLD when reporting the frequency of this problem in their population of patients.

Polymorphic PTLD also demonstrate lymphoid infiltrates of varying shapes and sizes. The entire range of lymphocyte differentiation may be seen. However, in these lesions there is effacement and/or destruction of normal tissue architecture. Areas of necrosis are frequently present. These lesions may occur in both nodal tissue and at extranodal sites, and may involve any organ. Large, bizarre cells (atypical immunoblasts) may be observed. In monomorphic PTLD, the destructive lymphoid infiltrate has a much more monotonous appearance with most cells appearing to be transformed lymphocytes at one stage of differentiation. These lesions more closely resemble non-Hodgkin lymphomas in the non-transplant patient. It is important to recognize that some degree of polymorphism is often seen, although much less pro-
Table 3
Pathological evaluation of suspected PTLD lesions

<table>
<thead>
<tr>
<th>Technique</th>
<th>Purpose</th>
</tr>
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<tbody>
<tr>
<td>Routine histology</td>
<td>Classify lesions:</td>
</tr>
<tr>
<td></td>
<td>‘benign’ lymphoid hyperplasia</td>
</tr>
<tr>
<td></td>
<td>polymorphic PTLD</td>
</tr>
<tr>
<td></td>
<td>monomorphic PTLD</td>
</tr>
<tr>
<td></td>
<td>malignant lymphoma (e.g. Burkitt’s)</td>
</tr>
<tr>
<td>Immunophenotyping/flow cytometry</td>
<td>Cell lineage and characterization (e.g. T vs. B cell);</td>
</tr>
<tr>
<td></td>
<td>clonality (Ig light or heavy chain expression)</td>
</tr>
<tr>
<td>Immunoglobulin gene rearrangement studies b</td>
<td>Host cell clonality (monoclonal vs. polyclonal)</td>
</tr>
<tr>
<td>In situ hybridization for EBER 1 (or Southern</td>
<td>Presence of EBV genome</td>
</tr>
<tr>
<td>blot or PCR analysis)</td>
<td></td>
</tr>
<tr>
<td>Southern blot analysis of EBV terminal repeat</td>
<td>EBV clonality</td>
</tr>
<tr>
<td>region</td>
<td></td>
</tr>
<tr>
<td>Cytogenetic studies</td>
<td>Structural chromosomal abnormalities</td>
</tr>
<tr>
<td>Molecular studies of proto-oncogenes and tumor</td>
<td>Define structural gene alterations c</td>
</tr>
<tr>
<td>suppressor genes</td>
<td></td>
</tr>
</tbody>
</table>

a Full diagnostic work up requires core needle or excisional biopsy. Needle aspiration cytology is not adequate. Tissue must be submitted fresh, not in formalin.

b For clonal analysis of T cell PTLD, comparable analyses are performed for rearrangements of T cell receptor.
c Studies of oncogenes remain a research tool at the present time.

nounced than in polymorphic lesions. Further confusion in nomenclature can arise when predominantly polymorphic lesions contain localized areas that appear monomorphic. Hence the need for description of the entire lesion, rather than placing undue reliance on short labels. Rarely, other specific histological variants may also be noted including mature plasma cell type predominance and tumors that strongly resemble Hodgkin’s lymphoma.

We attempt to distinguish monomorphic PTLD lesions from specific overt malignant lymphomas such as Burkitt’s lymphoma. In the Knowles’ classification [25], the distinction between monomorphic PTLD and malignant lymphomas is not made, and this group of lesions are all designated as ‘immunoblastic lymphoma’. While we recognize that this distinction is often arbitrary, we have continued to use the term monomorphic PTLD, because a number of these lesions may regress with reduction in immunosuppression, as do most cases of polymorphic PTLD (see below). By contrast, malignant lymphomas such as Burkitt’s disease will invariably progress without urgent chemotherapy. If the term malignant lymphoma is applied routinely to all monomorphic lesions, we believe it may convey the wrong message to the transplant physician, i.e. that chemotherapy is indicated as first line of therapy.

Further work-up of suspected PTLD includes immunophenotyping by immunohistochemistry and flow cytometry. These procedures will demonstrate the cell lineage of the lesions. Almost all PTLD are of B cell origin, though rare cases of T cell PTLD are seen. It should be noted that variable numbers of T cells (and macrophages) are generally interspersed between the B cells populations in polymorphic lesions. Whether these may be ‘reactive’, and offer an improved prognosis if the T cell infiltrates are pronounced is under investigation. Immunophenotyping for immunoglobulin light and heavy chains may occasionally provide information about clonality, when the lesions express these products. Molecular studies offer the most definitive assessment of the clonality of the lesions. The concept of clonality is complex since it is now recognized that a gradation of clonal alterations may be observed. Thus lesions cannot be simply labeled as polyclonal or monoclonal. Sometimes, minor clonal sub-populations may be present in a predominantly polyclonal lesion. In other cases, individual PTLD lesions within the same patient may be monoclonal, yet each lesion is clonally distinct. Furthermore, the concept of clonality can be applied to both the host cell and to the virus separately. Analysis of host cell clonality is based on the behavior of immunoglobulin genes, which rearrange uniquely in the maturing B cell. Progeny of a B cell that has already arranged its immunoglobulin genes, will carry the same rearrangement [23–25]. EBV clonality is assessed by evaluation of the viral terminal repeat region. When a single EBV fuses its terminal ends to produce an episomal (circular) form, a fixed number
of terminal repeat segments are retained, and some are lost. With the use of specific molecular probes for this region, it is possible to determine if the EBV virus within PTLD lesions is itself clonal or polyclonal in nature. Monomorphic lesions are clonal in nature, and analysis of clonality is therefore of little relevance. Polymorphic lesions may be polyclonal, but frequently represent clonal B cell proliferations. Some lesions are clonal by EBV, but not by immunoglobulin gene analysis. Thus, analysis of clonality is most important in polymorphic rather than monomorphic diseases (which are always clonal). However, these studies will primarily remain research tools unless they can be shown to influence therapy and outcome.

There is some suggestion that regression of polymorphic lesions in response to reduction in immunosuppression may be influenced by their clonal pattern, though more work in this area is required [24]. Another attempt to predict progression from the molecular pathological findings involves a search for abnormalities in oncogenes and tumor suppressor genes (e.g. c-myc, N-ras and p53). Polymorphic lesions, even if clonal for EBV and immunoglobulin gene rearrangements, do not usually demonstrate mutations of oncogenes and tumor suppressor genes. By contrast, a proportion of monoclonal lesions will show evidence of mutations of c-myc, N-ras or p53 [4,25]. Such structural gene alterations may portend a worse prognosis in monomorphic PTLD.

Cytogenetic abnormalities are mostly found in malignant lymphomas, such as Burkitt’s disease. Few data exist on the presence and significance of cytogenetic abnormalities in polymorphic or monomorphic PTLD lesions. Where present, it is likely they may also portend a poor prognosis if managed by reduction in immunosuppression alone.

The pathologist must demonstrate whether PTLD lesions contain evidence of EBV. A number of techniques are available including Southern blot analysis (generally combined with analysis of EBV clonality), PCR and in situ hybridization. We, and many programs, use in situ hybridization with the EBER-1 probe which labels EBV-encoded early RNA transcripts in infected cells. This technique is reliable, rapid and is performed on routinely processed paraffin sections. Overall, approximately 80–90% of pediatric thoracic PLTD cases are EBV positive, including all cases arising in the first year or two after transplantation. In our experience, EBV negative cases develop late after transplantation. The spectrum of histopathological findings is similar in EBV positive and negative lesions, though Burkitt’s lymphoma has always been EBV driven.

Finally, it should be noted that non-lymphoid Epstein–Barr induced neoplasia may very rarely develop after solid organ transplantation, especially in children [26]. These tumors are composed of spindle cells with smooth muscle features. We have observed three such cases among over 1300 pediatric solid organ transplants at Children’s Hospital of Pittsburgh from 1982–1995. All showed evidence of clonal EBV infection. Previous PTLD was present in one of our cases.

5. Prevention of PTLD

The ability to prevent PTLD would be of enormous benefit to pediatric transplant recipients. The most logical approach would be to immunize all seronegative recipients prior to transplantation. Several vaccine preparations are under evaluation but progress has been slow, despite early optimism from primate work [27]. This may reflect the low interest from industry, given the low morbidity and mortality associated with EBV infection in the immunocompetent host in developed countries. An alternate strategy would be to avoid transplanting seronegative recipients, especially with a seropositive donor. This approach would effectively exclude many pediatric thoracic recipients from receiving organs, and entails tremendous logistical problems since donor EBV serologies are generally not available at the time of acceptance of the donor organ [5]. Various other preventive strategies have been suggested. Chemoprophylaxis, with short term intravenous ganciclovir, followed by long-term oral acyclovir therapy was not helpful in preventing PTLD in a randomized trial in pediatric liver transplantation [28]. Several studies have also demonstrated rise in EBV viral load by PCR while patients are receiving therapy with ganciclovir or acyclovir.

Use of intravenous gammaglobulin (IVIG) preparations containing high levels of anti-EBV antibody titers has also been suggested. IVIG has been shown to prevent the development of EBV-associated lymphomas in a severe combined immunodeficiency mouse model [29]. A multicenter, randomized trial of prophylactic CytoGam (which contains high anti-EBV titers) vs. a placebo infusion in seronegative liver recipients is currently in progress.

Another appealing preventive strategy is to target patients with evidence of early EBV infection (prior to development of symptoms) for preemptive therapy. This targeted approach avoids treatment of all seronegative recipients. Published experience suggests that EBV PCR levels rise during primary infection, prior to both antibody development and the onset of symptoms. A quantitative or semi-quantitative EBV PCR would, therefore, seem to be an appealing screening test. We currently perform monthly EBV QC-PCR assays on all seronegative recipients for the first 6 months after transplantation, and then at less
frequent intervals. When the PCR becomes positive, increased clinical surveillance is instituted and cautious temporary reduction in immunosuppression is performed for those children whose recent biopsies revealed no rejection. Other potential preemptive strategies include institution of ganciclovir or Cyto-Gam at the time of evidence of primary infection by PCR. All these strategies require investigation by randomized clinical trials.

6. Management of established PTLD

We have recently reviewed the various treatment strategies for histologically confirmed PTLD [28,30]. Treatment options are also summarized in Table 4. In 1984, Starzl et al. reported the reversibility of PTLD by reduction in immunosuppression in cyclosporine-treated patients [31]. This strategy remains the mainstay of therapy for most patients. As stated earlier, we believe this should be the initial approach to patients with both polymorphic and monomorphic disease. However, patients with overtly malignant disease, such as Burkitt’s lymphoma, should be managed with immediate chemotherapy. Some authorities believe that monomorphic PTLD should also be initially managed with chemotherapy, although resolution of such lesions by reduction in immunosuppression has been well documented. As discussed earlier in this review, there is an urgent need for more research to define those clinical, histological and molecular pathological findings that most accurately predict response to reduction in immunosuppression.

The goal of reduced immunosuppression is to allow the host to recover natural immune surveillance and subsequently to regain control over the proliferation of EBV-infected cells. Wide variation in response to this approach has been noted between centers. This variation may be explained by a number of reasons including differences in patient populations, definitions of PTLD, and strategies for reducing immunosuppression. In our experience with pediatric heart transplant recipients, the vast majority (80–90%) of non-malignant lesions will respond to reduction in immunosuppression combined with anti-viral therapy, with most children showing evidence of clinical re-

Table 4
Treatment strategies for PTLD

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First line therapies</strong></td>
<td></td>
</tr>
<tr>
<td>Reduced immunosuppression</td>
<td>Effective in most pediatric heart recipients, especially in polymorphic PTLD</td>
</tr>
<tr>
<td>Antiviral therapy (e.g. ganciclovir)</td>
<td>Widely used but unproven</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>First line for overt malignancy, e.g. Burkitt's lymphoma</td>
</tr>
<tr>
<td><strong>Second line therapies</strong></td>
<td></td>
</tr>
<tr>
<td>Interferon-alpha</td>
<td>May cause severe rejection</td>
</tr>
<tr>
<td>Intravenous immunoglobulins (containing anti-EBV antibodies)</td>
<td>Unproven. Trials in PTLD prevention underway.</td>
</tr>
<tr>
<td>Anti-B cell monoclonal antibodies</td>
<td>Limited experience in PTLD. No randomized trials. Pediatric trial in preparation</td>
</tr>
<tr>
<td>Cellular immunotherapy e.g. autologous or recipient HLA matched EBV-specific cytotoxic T lymphocytes; autologous lymphokine activated killer (LAK) cells</td>
<td>Promising techniques. Limited experience to date. No controlled trials.</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>Established second line therapy for refractory and relapsed PTLD; more often required in monomorphic PTLD and late onset EBV-negative disease</td>
</tr>
<tr>
<td>Surgery/radiation therapy</td>
<td>Reserved for treatment of local compression of critical structures, bowel obstruction, etc. Also for excisional biopsy of localized, easily accessible lesions at presentation</td>
</tr>
</tbody>
</table>
sponse within 2–4 weeks of reduction of immune suppression. Results in heart–lung and lung recipients have been less satisfactory with approximately half showing response to reduced immunosuppression, perhaps due to the less aggressive reduction in immunosuppression that we have used in these patients.

In our own program, all patients are initially taken off tacrolimus or cyclosporine, as well as azathioprine or mycophenolate mofetil (if they were receiving these medications). If a patient is receiving corticosteroid therapy, it is usually continued at maintenance levels. The time at which calcineurin inhibitors are reintroduced varies depending on severity of disease, prior rejection history, time from transplantation and time required for tacrolimus or cyclosporine levels to fall.

We have frequently observed that immunosuppressant levels are high at time of diagnosis and it may take many days for drug levels to become immeasurable. This presumably reflects impaired drug metabolism, often associated with mild liver dysfunction. Careful monitoring is carried out for evidence of rejection. Echocardiograms are performed two to three times per week and an endomyocardial biopsy is routinely obtained 1 week into therapy, and subsequently every 1–2 weeks while a major reduction in immune suppressive therapy is continued. For heart–lung and lung transplant recipients, the chest radiograph, pulse oximetry and pulmonary function tests of the patient are followed carefully and a transbronchial biopsy is performed approximately 7–10 days into therapy.

In addition to the above strategies, we now recommend following the EBV viral load in the peripheral blood by PCR, initially on a weekly basis, following the diagnosis of PTLD. This has proved very helpful in patient management [21,30]. A fall in the EBV viral load is consistent with the development of an immune response against the EBV-infected B-cells and has predicted a good clinical response (often before reduction in size of lesions is observed). Among eight pediatric heart recipients with PTLD (all with very high viral loads at presentation; range 200–>5000 genome copies/10^5 PBMC), seven cleared their viral loads to ≤200 at a median of 2 months following the diagnosis of PTLD (range: 7 days–9 months). The drop in the viral load appeared to be concomitant with resolution of their PTLD. Two of the children who initially cleared their viral loads showed a transient rebound to values greater than 200 without clinical symptoms. Rebound rejection occurred in five of eight cases at a time when the viral load was 8–100 genome copies/10^5 PBL. No rejection was seen at higher viral load values. In one 6-year-old boy, 1 year out from cardiac re-transplantation, we held all immunosuppression for 2 months after he presented with viral ‘septic shock’ and multi-system failure. Persistent elevations in QC EBV-PCR to > 5000 genome copies/10^5 PBMC for approximately 8 weeks prompted us to withhold immunosuppression for this very prolonged period. A precipitous fall in PCR to < 200 occurred at this time and low-dose tacrolimus therapy was successfully introduced without development of rebound rejection. Shortly, thereafter, resolution of multi-system failure occurred and the patient was successfully discharged home. These observations, supported by similar results in our liver recipients [21], suggest that viral load monitoring by PCR may be very helpful in monitoring response to therapy and determining the timing of reintroduction of immunosuppression.

Prior to PCR monitoring, we had no clear indicators of when immunosuppression should be reintroduced, other than when acute rejection developed. Ideally, one wishes to reintroduce immunosuppression before important rejection evolves. It is important to note, however, that acute rebound rejection, if it develops, needs to be treated in a conventional manner (usually with bolus intravenous solumedrol for cardiac 3A rejection). Acute rejection often coincides with resolution of PTLD and PTLD usually does not recur after treatment of acute rejection. We suspect that reports of failure of polymorphic lesions to respond to reduced immunosuppression may frequently be due to overly cautious reduction in immunosuppression, because of fear of the consequences of rebound rejection. For patients showing good response to therapy, we typically re-institute maintenance therapy with low doses of their primary immunosuppressant (typically at half the previous dose). We attempt to use the minimum dose necessary to prevent acute rejection and we accept an increase in surveillance biopsies as the price necessary to achieve this aim. Azathioprine or mycophenolate mofetil are frequently not reintroduced.

In addition to reduction of immune suppression, most centers also use antiviral therapy. Both acyclovir and ganciclovir have been shown to inhibit lytic EBV DNA replication in vitro and would seem to be of value in treating the lytic phase of EBV infections. Pathologic analysis has shown that most EBV infected cells within PTLD lesions are transformed B-cells and are not undergoing lytic infection. Neither acyclovir nor ganciclovir suppress EBV-driven proliferation of transformed B cells in vitro, nor are they active against B-cells that are latently infected with EBV. A few studies have demonstrated, however, evidence of some lytic phase EBV within PTLD lesions. It, therefore, seems reasonable to continue therapy until there is clinical and virologic (e.g. falling EBV viral load) evidence of resolution of EBV/PTLD. We do acknowledge that there is no
clear proof of efficacy of ganciclovir or acyclovir in the management of PTLD.

Beyond reduction of immune suppression and anti-viral therapy, the optimal management of PTLD in solid-organ transplant recipients is controversial. The use of interferon has been described in anecdotal reports as a therapeutic option in the management of PTLD [30,32,33]. There is a lack of prospective, controlled trials to establish the therapeutic role of this agent. We have treated six children (three liver and three thoracic transplant recipients) with interferon. Five of these patients received alpha-interferon 2b while one was treated with gamma-interferon. Three of the six patients (one liver and two thoracic transplant recipients) appeared to have responded to the interferon therapy without significant side effects. However, each of these patients was receiving either a reduced dose or no immune suppression at the time of initiation of interferon. One lung transplant recipient had to prematurely discontinue the use of interferon because of the development of severe rejection. In addition, each of the three pediatric liver transplant recipients treated with interferon developed rejection while receiving this therapy. These observations, and those from the literature, suggest that the role of this agent in the treatment of PTLD is far from established and that rejection will frequently occur in children receiving this therapy.

A potential role for the use of intravenous immune globulin (IVIG) for the treatment of PTLD has also been suggested [32,33]. Several reports have documented an association between loss, or absence, of antibody against at least one of the Epstein–Barr nuclear antigens (EBNA) in EBV infected organ recipients and the subsequent development of PTLD [16]. In addition, Riddler et al. demonstrated a correlation between an increasing level of anti-EBNA antibodies (including those introduced through transfusions) with a decrease in EBV viral load [16]. IVIG has been used in combination with interferon-alpha as treatment for PTLD at the University of Minnesota and other centers [32,33]. As with the use of antiviral agents and interferon, there are no comparative trials evaluating the role of intravenous immunoglobulin preparations in the management of PTLD.

The use of anti-B-cell monoclonal antibodies has recently been suggested as a therapeutic option for patients with PTLD not responding to conventional therapy. Limited experience with the use of anti-CD21 and anti-CD24 monoclonal antibodies in combination, as well as use of an anti-CD22 immunotoxin have been reported [34,35]. The results were most promising for polyclonal disease [34]. These antibodies are not currently available, though an anti-CD20 human/mouse chimeric monoclonal antibody (Rituximab; Genentech Inc. & IDEC Pharmaceuticals) is currently commercially available for treatment of certain CD20-positive B-cell non-Hodgkin lymphomas in adult non-transplant recipients. A multi-center, clinical trial is currently being planned to evaluate this product in the treatment of pediatric PTLD.

Another interesting strategy for the treatment of refractory PTLD is cellular immunotherapy. Nalesnik et al. have used interleukin-2 (IL2) ex vivo-stimulated lymphokine-activated killer (LAK) cells of recipient origin to treat seven patients [36]. Autologous PBMC were obtained by leukapheresis and depleted of monocytes, and then cultured for 10–11 days in the presence of IL2. The cells were then returned to the patient intravenously. The use of this strategy appeared to successfully treat four patients with EBV-associated refractory PTLD, although the patients also were treated with decreased immunosuppression. Three patients with EBV negative disease did not respond. A concern with this therapy is that it may also stimulate anti-donor lymphocytes leading to rejection.

A more logical approach to cellular therapy is to give the patient an infusion of cytotoxic T-lymphocytes (CTLs) directed against EBV-specific antigens. This should result in control of the proliferation of EBV infected B cells, but without the risk of rejection. The use of EBV-specific CTL therapy has already been developed and applied for the management of PTLD in bone marrow transplant (BMT) recipients [37]. In this situation, the donor is often available to provide the CTL, not receiving immunosuppression and will hopefully be EBV seropositive. Application of this technique is more problematic in solid organ transplantation since the PTLD lesions are of recipient cell origin and the recipient will need to be the source of CTL, unless an HLA identical source of CTL is available. Since EBV-associated PTLD occur much more frequently in patients who are EBV seronegative prior to transplantation, pre-existing immunity specific to EBV does not exist. To obtain functional EBV-specific CTLs, one would need to ‘immunize’ and stimulate recipient T-cells against EBV ex vivo. Efforts to do just this are underway in several centers. There is one report in the literature of a pediatric lung recipient with central nervous system PTLD, who received mononuclear cell infusions from an HLA-identical, EBV positive, sibling. The patient did develop significant rejection, but dramatic response of the monoclonal PTLD was seen. The response correlated with in vivo reconstitution of normal EBV-specific cytotoxic activity [38].

Other therapeutic options available for the treatment of PTLD include the use of chemotherapeutic agents, radiation and surgery. The very high viral loads documented in the peripheral circulation of patients with PTLD, emphasizes the systemic nature
of this disease, even when only a solitary lesion is identified. These observations suggest that surgery and radiation are only appropriate for the management of local complications (e.g. gastrointestinal hemorrhage, intestinal obstruction, or local compression of critical structures). Some centers strongly support the use of chemotherapy, especially for monomorphic PTLD. These agents are immunosuppressive in nature and interfere with the recovery of the host's natural immune surveillance mechanisms. They do, however, offer protection to the allograft from rejection while simultaneously treating the PTLD [39]. Thus, they may have a role for treating patients who relapse during reintroduction of immunosuppression necessitated by rejection, or in the rare case of active PTLD with concomitant rejection. Their role in patients with overt malignancy, e.g. Burkitt's lymphoma, has already been discussed. The optimal chemotherapy regimen has not been determined, though most are based on protocols used for treatment of non-Hodgkin's lymphoma. Guidelines on which children should receive chemotherapy, and which chemotherapeutic regimen should be used, are not available at this time.

7. Conclusions

Over the last decade, much has been learnt about the nature of PTLD. The pivotal role of EBV infection in the majority of cases has been established and the pathological description of lesions has entered the molecular era. It seems likely that further understanding of the molecular pathology may lead to greater ability to define optimal treatment regimens and prognosis. Quantitative PCR techniques for EBV hold great promise for enhancing the diagnosis and prevention of PTLD, and for monitoring response to therapy. In particular, this technique appears to help predict when re-introduction of immunosuppression should be instituted. A number of exiting new therapies are on the horizon, including use of monoclonal antibodies against B cell surface antigens and the development of cellular therapies, such as use of infusions of autologous or HLA matched EBV specific CTLs. Such strategies offer the promise of controlling abnormal B cell proliferation without the risk of allograft rejection, since the host alloresponse is not enhanced by these therapies. The place of chemotherapy, and the optimal regimens required, remain to be defined. Our understanding of the etiology, behavior and optimal treatment for EBV negative PTLD remains limited, in part due to the rarity of these lesions. Encouragingly, there is an increasing level of interest in PTLD among clinical and basic investigators, as well as recognition of the need for multi-center trials to define optimal prevention and treatment strategies. A degree of optimism seems warranted!

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