Epstein-Barr Virus, Infectious Mononucleosis, and Posttransplant Lymphoproliferative Disorders

MICHAEL A. NALESNIK AND THOMAS E. STARZL
University of Pittsburgh Medical Center

INTRODUCTION

Posttransplant lymphoproliferative disorders (PTLDs) are a family of lesions that straddle the borderland between infection and neoplasia. The term PTLD is best used to refer to those lymphoid growths which occur in organ transplant patients and in which evidence of the Epstein-Barr virus can be demonstrated. Accordingly, PTLDs can be considered to represent phases of infectious mononucleosis which are rarely seen in immunocompetent hosts. Specifically, destructive lymphoid infiltrates, gross tumor formation and clonal proliferations of lymphoid cells are major components of these progressive forms of IM. In some cases, true malignancy may also occur.

Our understanding of this disease advanced significantly during the 1980s when it was recognized many PTLDs could regress consequent to withdrawal of immunosuppression. This knowledge curtailed the preemptive use of chemotherapy in these disorders and focused later attention on biological response modifiers as possible therapeutic agents. However, experience has also shown that a subset of tumors do not respond to such immunomodulation. A present challenge is to distinguish this subset of tumors, which may require antineoplastic chemotherapy, from the majority of PTLDs, which do not.

PTLDs must be distinguished from sporadic lymphomas or non-EBV-associated lymphadenopathies which may also be seen in the transplant population. In this review the discussion focuses on those lesions in which the presence of EBV has been demonstrated. Selected aspects of the EBV-B-cell interaction and of host control mechanisms utilized during EBV infection are also considered, since these topics deal with the host-parasite system from which PTLDs emerge. Additional EBV-related posttransplant tumors such as spindle-cell tumor and Hodgkin's disease-like proliferations are briefly considered at the end of this discussion.

NORMAL RESPONSE TO INFECTION WITH EBSTEIN-BARR VIRUS

Epstein-Barr virus is a double-stranded, enveloped DNA gammaherpesvirus with a host specificity restricted to humans and nonhuman primates. The virus is ubiquitous and infection (or infection) exists in 90% of individuals worldwide. Approximately 100,000 cases of IM occur annually in the United States. Active infection is most often initiated by salivary contact. EBV may infect oropharyngeal epithelial cells via interaction between the external viral glycoprotein 350/220 and a CR2 (complement receptor type 2)-like receptor on the host cells. The identity of this cellular receptor is a current issue of debate. Following cell penetration, the virus initiates a productive infection which in turn facilitates infection of recirculating B lymphocytes in this region. The oropharynx is considered to represent a major repository of the virus, and viral shedding can be detected in up to 100% of infected individuals with appropriate techniques. However, one group has recently questioned this sequence of events, since they were unable to find evidence of lytic EBV infection within oropharyngeal epithelium during acute mononucleosis by the use of sensitive in situ hybridization procedures.

The B-lymphocyte EBV receptor (CD21) is also the physiologic CR2 receptor, and a receptor for the B-cell protein CD23 as well as for IFN-α. Once within the B lymphocyte, the virus ultimately circularizes into an episomal form. B-cell proliferation and plasma cell differentiation follow. This induced behavior of infected B cells may be one source of antibodies, including autoantibodies, characteristic of IM. Additionally, such antibodies may be due to antigenic similarities between the virus and host.

The B-cell lymphoproliferation evokes a powerful host regulatory response. Studies have consistently shown increased numbers of natural killer (NK) cells and cytotoxic (CD8+ T) cells during the early stages of acute IM. NK cells (large granular lymphocytes) mediate cell killing in a non-HLA-restricted fashion and represent an important first line of defense. In one study the absence of these cells was associated with a more severe clinical course. However, another study found a transient decrease in NK function.
despite increased numbers of these cells, at the time of acute IM diagnosis. Both CD8+ (suppressor/cytotoxic) T lymphocytes constitute the primary effector cell in this disorder. Both CD4+ and CD8+ T-cell subsets express the activation marker CD45RO (UCHL1), but only CD8+ T cells mediate specific cytotoxicity in an HLA Class I-restricted fashion.

In some animal studies, noncytolytic CD4+ and CD8+ T cells have been shown to be capable of causing regression of tumors. One study used activated cells from tumor-draining lymph nodes to prevent subsequent metastatic disease in mice of the same strain inoculated with the same tumor. It was found that gamma-interferon (IFN-γ) was an important mediator of this effect, apparently due to its antiproliferative activity. Further, this effect could be inhibited by antibodies to IFN-γ.

Earlier studies referred to the putative viral targets recognized by cytotoxic T cells as LYDMAs (lymphocyte-determined membrane antigens). More recent studies have shown that both EBNA (EBV nuclear antigens) and LMPs (EBV latent membrane proteins) may serve as preferential targets, dependent upon host HLA type. For example, cells derived from HLA-A11+ individuals preferentially react with experimental target transfectants containing the EBNA 3b and 3c gene products, while they remain unresponsive to cells expressing EBNA 3a. In contrast, HLA A2.1-positive cells preferentially recognize LMP2 and less frequently recognize EBNA 3b. Effective viral control is most likely maintained by recognition of different menus of viral antigens in different individuals.

The T lymphocyte proliferation, which accounts in large part for the clinical "mononucleosis" associated with acute IM, is usually regarded as polyclonal or reactive to the B-cell process. In some cases a more restricted (oligoclonal) proliferation has been observed. This may correspond to restricted usage of T-cell receptor subtypes (Vβ6.1-3, Vβ7) which has been reported in one study of patients with acute IM. Increased expression of cells bearing γδ T-cell receptors has also been noted during this period. Such cells normally comprise only a small number of all T cells and it has been suggested that they may help to mediate non-MHC-restricted cytotoxicity.

Transient cutaneous anergy can occur during acute infectious mononucleosis. This may be related to the activation of suppressor T-cell activity during this stage of the disease. In addition, recent reports suggest that direct interference with T-cell receptor-mediated signals also occurs. In one study of cells from patients with IM, signals delivered through the T-cell receptor pathway failed to result in normal T-cell responses despite normal reactions of these same cells to other forms of stimulation. The rapid expansion of T cells ultimately abates as the acute infection subsides. This is mainly due to apoptosis, which occurs primarily in CD45RO T cells according to one in vitro study. These authors observed that the affected T cells could be rescued from apoptosis by the administration of interleukin 2 (IL-2), IL-5 or IL-6, but not IL-1 or IL-4. From this they suggested that continued local secretion of the appropriate cytokines in vivo might serve to maintain the viability of specifically reactive T cells, and that removal of this microenvironment would result in programmed cell death (apoptosis).

A characteristic antibody response to EBV infection begins with production of IgM antibody against the EB viral capsid antigen (VCA). This isotype disappears rapidly and it is replaced by IgG anti-VCA which remains elevated for life. Most patients also develop antibody to an antigen of the early lytic cycle, EA(D), for a short period of time. Antibodies to EBNA do not develop until late in active infection but persist for life in routine cases.

A variety of cytokines and other molecules undergo upregulation during acute IM. IFN-γ, IL-2 and IL-1-α levels are elevated, as is neopterin, a marker of IFN-γ-induced monocyte activation. Perforin levels are also elevated in acute IM. Levels of this molecule may correlate to the extent of cytotoxic activity in vivo. No increases of IL-1-β, IFN-α, or IL-6 were observed in one study of acute IM.

Following control of the acute infection over a 1–3 month period, cytokine levels return to baseline. Around this time the infection enters a latent stage. Persistence of virus in both blood cells and oropharyngeal washings has been demonstrated, but the relative importance of these sites as the main reservoir of latent virus remains to be determined. The infection remains under the control of immunosurveillance mechanisms of which cytotoxic T cells comprise the major effectors. Cells with specificity for both latent and lytic EBV antigens persist in the host.

In some patients, a chronic active form of EBV can develop, characterized by prolonged or repeated bouts of viral symptoms of IM. This may rarely culminate in a lymphoproliferative disorder of large granular lymphocytes presumed to be NK cells. In one case EBV was not observed in the cells, but in a second case clonal EBV was detected, suggesting a causative role of the virus in this abnormal proliferation.

Molecular aspects of EBV-B cell interaction

The major viral glycoprotein, gp350/220, interacts with the C3d receptor (CD21,CR2) on the surface of B lymphocytes. These receptors are expressed mainly in resting B lymphocytes, and may have a role to play in the activation of these cells. The viral glycoprotein/CR2 interaction may also contribute to activation of the alternative complement pathway. In vitro infection can be inhibited by addition of soluble recombinant CR2, leading to the suggestion that this molecule may have therapeutic application in EBV infection.

The virus is then incorporated into the cytoplasm via endocytosis. Another viral glycoprotein, gp85, mediates fusion with cellular endocytic vesicles and this process releases the virus into the cytoplasm. The virus may associate with vimentin and is then carried into the nucleus. It circularizes and proliferates within the cell to exist as multiple episomal copies.
A limited number of viral gene products are expressed during latent infection. These include six EBV nuclear antigens (EBNAs), two membrane-associated proteins (latent membrane proteins or LMP1,2) and two nontranslated "early" RNAs (EBER 1,2). Many of these proteins interact to induce B-cell activation: EBNAs 2,3A,3C, LMP1 and EBNA LP have been shown to be essential for such transformation of the host cell. EBNA-2 upregulates a specific viral promoter (latency C promoter) which in turn leads to production of viral latent membrane protein (LMP 1). This molecule in turn upregulates production of vimentin, associates with this protein, and induces various surface proteins on the cell including the transferrin receptor and the adhesion molecules LFA-1, LFA-3 and ICAM-1. EBNA-3D, which protects the cell from apoptosis, is also induced. LMP1 cooperates with EBNA 2 to increase expression of the B-cell activation marker CD23. Another membrane protein, LMP2A, colocalizes with LMP1 and has been shown to diminish the calcium mobilization associated with cross-linking of B-cell surface immunoglobulins. The purpose of this is unknown; it has been suggested that it may serve to regulate EBV-associated cell proliferation or it may diminish the likelihood of lytic cycle induction, which is associated with increased intracellular free calcium.

B-cell activation by the virus bypasses the normal cell membrane signaling pathway in which inositol phospholipids are cleaved. EBV thus resembles the tumor-promoting phorbol ester TPA in this regard. As a consequence, there is a decreased requirement for calcium in EBV-induced cellular DNA synthesis.

The exact circumstances which determine whether an individual B lymphocyte will undergo blast transformation upon EB viral infection are unknown. Some studies have suggested that the resting B lymphocyte is the primary target cell which undergoes these changes. Crain et al.

Evidence suggests that lymphotoxin, IL-1, IL-5, IL-6, thioreredoxin, and soluble CD23 may all act as autocrine growth stimulators in this regard. In addition, monocyte derived products, including IL-6, may contribute to stimulation of EBV-infected B-cell lines in vitro. In one study, a synergistic effect of recombinant IL-4 with supernatant from activated monocytes on proliferation of EBV-transformed B cells was demonstrated. Not all investigators have obtained identical results, and Jochems et al.

Increased production of IL-10 has been observed in B cells in vitro following EBV infection. The authors of this study reported that this action enhanced the establishment of transformed cell lines. In addition, they suggested that this cytokine might inhibit the antiviral response of the immune system in vivo. It is interesting that the form of IL-10 found in these cells was the human and not the EB viral IL-10 analog; the latter molecule is expressed during the lytic cycle of infection.

Three forms of virus latency have been described, each expressing a different complement of viral proteins. The different latencies are transcriptionally distinct, and use different viral promoter sequences. All infected cells express EBNA-1 protein, which binds to DNA, is required for viral episomal maintenance and may also function to distribute the episomes during cell division. In type 1 latency, which is seen in Burkitt lymphoma cells, this is the only viral protein expressed. This form of latency utilizes the viral Fp promoter only. Latency Type 2 is seen in EBV-infected Reed-Sternberg type cells as well as in nasopharyngeal carcinoma cells and shows expression of EBNA 1, LMP1 and possibly LMP2. In this form the Fp promoter is again used and other proteins are expressed using their individual promoters. Latency type 3 was initially described in vitro in EBV-infected lymphoblastoid cell lines and shows expression of EBNAS 1-6 and LMPs 1 and 2. In this case, Fp is not used and one of two other promoters, Cp or Wp, are utilized in addition to specific LMP promoters. All three types of latency also express a high copy number of nontranslated, polyadenylated RNAs (EBERs) as well as other transcripts which likely play an important function in maintaining latency. Lymphoblastoid cells with type 3 latency express a variety of B-cell activation markers, in contrast to down-regulation of these molecules in Burkitt lymphoma cells. Such down-regulation is thought to contribute to the ability of Burkitt cells to evade host T-cell immunosurveillance mechanisms.

An increase in intracellular calcium due to cell surface receptor cross-linking has been shown to be associated with the induction of the lytic stage of the viral life cycle in vitro. In addition, lytic infection has been associated with more mature stages of differentiation in both lymphoid and epithelial cells. The switch from viral latency to productive infection is initiated by expression of the viral "Zebra" protein, which in turn transactivates other lytic cycle proteins. The lytic cycle results in production of mature viral particles and infection of additional cells within the host. Of the proteins produced during this phase of the viral life cycle, the major glycoprotein of the viral capsid antigen is particularly dominant and leads to the production of neutralizing antibodies.

Two different forms of EBV isolates, termed EBV-1 and EBV-2 have been described. EBV-1 strains are more efficient than type 2 strains in their ability to transform lymphocytes in vitro. Despite differences in several genes, it appears that differences in EBNA 2, which may reflect a recent evolutionary event, are most important in conferring this advantage upon EBV-1 strains.
PATHOLOGIC ASPECTS OF INFECTIOUS MONONUCLEOSIS IN LYMPHOID TISSUE

The characteristic morphology of lymphoid tissue undergoing active EBV infection reflects the exuberant lymphoproliferation which is a mechanism common to both the infection and its control by the host. In most cases, a recognizable paracortical expansion occurs and total architectural effacement is not seen. This proliferation results in a population of lymphocytes ranging from small to large in size. Large, atypical cells with features of Reed-Sternberg cells have been described. These cells have been suggested to be the precursors of the true Reed-Sternberg cells of Hodgkin’s disease and recent studies have shown these cells to (a) contain EBV and (b) have phenotypes similar, but not identical to, Reed-Sternberg cells. However, the activated background precludes the diagnosis of Hodgkin’s disease and the exact relationship of these cells to cases of EBV-positive HD remains problematic. Necrosis, increased prominence of postcapillary venules, and frequent mitoses also occur in acute IM. One study applied in situ hybridization to show that EBV was present in occasional endothelial cells and sinusoidal lining cells in lymphoid tissue from patients with acute IM. In a separate study, in situ hybridization of tonsil sections from cases of acute IM demonstrated EBV within interfollicular areas, but viral presence within tonsilar epithelium was not found.

We have recently applied in situ hybridization for EBER to a series of 100 unselected tonsils from children undergoing routine tonsillectomy. Twenty per cent showed evidence of EBV-positive cells and in 5% the frequency was substantial. It is probable that these cases represent early infectious mononucleosis, although a high viral carriage state cannot be excluded. These tonsils showed follicular hyperplasia with both randomly scattered paracortical EBV+ cells and rare individual follicles in which the majority of lymphoid cells were EBER-positive (Yunis E and Nalesnik M., unpublished observations). Rare follicles with prominent EBER positivity have been recently reported in cases of acute IM.

In one study of normal or reactive lymph nodes obtained from immunosuppressed patients in an area of high EBV infection, scattered EBV-positive T and B lymphocytes were seen in approximately 50% of cases. This may reflect persistent latent virus in these patients and is not associated with any specific pathological changes.

IATROGENIC IMMUNODEFICIENCY OF ORGAN TRANSPLANT PATIENTS

To date, the goal of allograft-specific tolerance has not been met, and prophylaxis and treatment of organ rejection rely primarily upon blockade of lymphocyte activation. Within this context, different categories of drugs exert their effects by different means. Theoretically, these may impact upon the host:EBV interaction and lead to differing manifestations of PTLD. The difference in onset time between PTLDs occurring under azathioprine regimens and those occurring under CsA or FK506-based regimens provides circumstantial evidence suggesting a more profound interference with EBV control mechanisms with the latter two drugs. Conversely, most posttransplant central nervous system lymphomas have been seen in azathioprine-based series. The reasons for these apparent differences are unknown.

CsA and FK506 are both considered to be prodrugs which acquire their immunosuppressive properties after binding to cytoplasmic immunophilins. Several isoforms of CsA-binding proteins (cyclophilins) and FK506-binding proteins (FKBP) are known, most having wide tissue distribution. The drug-immunophilin complexes result in new functional compounds with calcineurin-binding capability. Despite differences in their surface topographies, both CsA-cyclophilin and FK506-FKBP complexes share this feature.

Calcineurin is a Calmodulin dependent phosphatase. Its activity is blocked by the drug-immunophilin complex and this obstruction may contribute to the immunosuppression induced by these drugs. Both CsA and FK506 interfere with a nuclear factor of activated T cells (NF-AT), which is a transcription factor essential for expression of early T-cell activation genes. NF-AT consists of two subunits, one nuclear and common to many cell types, the other cytoplasmic and presumed specific to T cells. It is hypothesized but not proved that this cytoplasmic factor requires dephosphorylation in order to be translocated to the nucleus and to combine with the nuclear subunit to form functional NF-AT. If one presumes that this dephosphorylation is directly or indirectly mediated by calcineurin/calmodulin, a logical site of interference by CsA of FK506 is derived.

In keeping with this hypothesis, these drugs have also been shown to interfere with Ca²⁺ dependent signals within B lymphocytes such as response to anti-immunoglobulins, while largely sparing Ca²⁺ independent responses such as those to lipopolysaccharide, IL-4, or EBV. Azathioprine (Imuran) is a 6-mercaptopurine analog which is cleaved in vivo into its active form, thioguanosine acid. The latter interferes with purine biosynthesis and leads to decreased cell-mediated hypersensitivity. This drug has been associated with some apparently temporary chromosome abnormalities in humans.

Glucocorticoids bind to cytoplasmic receptors and are then transported into the nucleus where they affect gene transcription and inhibit lymphocyte proliferation. IL-2 and IL-1 production have been shown to be down-regulated by these compounds.

OKT3 and related antibodies block T-cell cytotoxicity by interfering with the interaction between CD3 and the T-cell receptor. Following initial administration, a cytokine release syndrome can occur, characterized by increases in circulating TNF-α, IFN-γ, IL-6, and IL-10.

EBV INFECTION IN TRANSPLANT PATIENTS

Ho et al demonstrated a 77% seroconversion rate in pediatric liver transplant patients who were seronegative for EBV at the time of transplant. Seropositive patients demonstrated evidence of posttransplant reactivation infection in...
48% of cases. In both instances the mean onset time was 60 days posttransplant. In adult recipients of solid organs primary posttransplant infection occurred in 82% and reactivation infection in 33% of cases. Yao et al demonstrated that the salivary shedding of virus is increased in EBV-positive patients following immunosuppression for renal transplantation. Their data suggest a relationship between pre- and postimmunosuppression viral shedding load, i.e., there may be constitutionally "high" and "low" shedder status. Preiksaitis demonstrated increased oropharyngeal shedding of EBV after the first posttransplant month in heart or kidney allograft recipients. Patients with primary infection shed more virus than those who were seropositive at the time of transplant, and heart transplant recipients had higher levels of peak shedding than did renal allograft patients. High-dose intravenous acyclovir or ganciclovir eliminated detectable shedding, but shedding again rose to previous levels after the drugs were stopped.

The donor organ itself may be the source of EBV infection. In the elegant study of Cen et al, a single organ donor provided a kidney to one patient and a heart-lung block to another. One recipient was seronegative before transplant and had serologic evidence of primary posttransplant infection. The seropositive patient had evidence of "reactivation" infection. Both patients developed PTLDs in their allograft organs. However, the identity of the virus isolated from the tumors was that of the donor in both instances. This suggests that some reactivation infections in organ transplant patients may actually represent new primary infection with a separate EB virus and this virus may be responsible for PTLD development.

The method of detection of EBV is important. Marchevsky et al used PCR to detect EBV in allograft lung biopsies. Despite the presence of the virus by this technique, several patients had histologic evidence of acute cellular rejection and responded to treatment for the rejection. This underscores the fact that EBV infection persists for life and that sensitive techniques such as PCR may detect EBV even in those cases in which the virus is clinically irrelevant. Such a situation is not unexpected, since nonimmunosuppressed EBV-positive individuals carry the virus in 1 in 10^6 to 10^7 B lymphocytes and the theoretical sensitivity of PCR approaches 1 copy in 10^6 cells.

Serology has usefulness in the evaluation of EBV infection, but the transplant population may show atypical or absent antibody patterns. In particular, diminished antibody to EBNA may be observed despite persistence of anti-VCA. Howard et al have stressed the importance of rising anti-EBV titers in the particular situation of suspected rebound rejection following OKT3 use. In this scenario, continued antirejection therapy could have disastrous consequences.

POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDERS

Frequency and Risk Factors

PTLD frequency has generally been reported as the total number of affected patients divided by the total number of transplant patients for a given period of time. This calculation can only provide an approximation of the true risk of disease since (a) it does not censor patients who die from other causes and thus overestimates the number of patients at risk, and (b) it does not calculate the actuarial risk of developing PTLD over time. Raw figures from our series have yielded annual PTLD frequencies of approximately 2.2%. Of these, the frequency is 1.0% for renal transplant patients, and 3.3, 2.7 and 3.8% for recipients of heart, liver, and heart-lung transplants. Several PTLDs have also occurred in recipients of small bowel or multivisceral organ allografts at our institution. Bone marrow transplant recipients have a very low frequency of PTLDs unless HLA-mismatched, T-cell-depleted allografts are used, in which case the frequency approximates 24%. Chao et al reported a unique case in which fulminant PTLD developed in a recipient of autologous bone marrow.

Armitage et al re-examined the cardiothoracic transplant population at our institution. After removing patients who did not survive past 30 days posttransplant from consideration, they calculated a 3.4% frequency of PTLD in heart, and 7.9% frequency in lung recipients.

Amitrak P examined a cohort of 132 pediatric liver allograft recipients from the Pittsburgh series and found the actuarial risk of developing PTLD to be 2.8% per year. This stabilized at a cumulative risk of approximately 20% by 7 years, although the study was ended shortly thereafter.

Shelley analyzed the Australia and New Zealand renal transplant population and found that lymphomas constituted 40% of all non-skin malignancies after 10 years but only 12% after 20 years. This was due to the late occurrence of other types of cancer in this population. His data showed that 64% of patients had some form of malignancy after 20 years and that cancer accounted for 34% of deaths in patients with functioning grafts after 10 years.

The duration of immunosuppression, dosage of the agents used, and the number of agents used have all been felt to contribute to the risk of posttransplant lymphomas, including PTLDs. Recently, an increased risk of PTLD was found in one series when prophylactic OKT3 was used in heart transplant patients in conjunction with triple therapy consisting of cyclosporine, azathioprine, and prednisone. Cumulative doses of OKT3 under 75 mg were associated with a 9.2% frequency of PTLD, whereas doses in excess of this figure were associated with a 37.5% frequency. Others have suggested that total immunosuppression rather than a single drug may be a more relevant factor. Altrey et al have observed early and aggressive PTLD which they felt was associated with antirejection OKT3 use in their series. We have not observed an increased incidence of PTLD as a complication of prophylactic OKT3 use in our cardiac transplant series.

Primary EBV infection is also associated with a higher frequency of PTLD than is reactivation infection. Ho et al documented a 10.5% frequency of PTLD in a series of 95 seronegative children who had a primary EBV infection following transplant. This compared to 0% frequency in seropositive children with reactivation infection.
Corresponding figures for seronegative and seropositive adult recipients in this series were 4.9% and 1.6%, respectively. 

Preiksaitis et al.109 have shown that patients who subsequently develop PTLD have a higher antecedent viral load than those who do not. Randhawa et al.28 demonstrated that EBER positivity was demonstrable in 71% of liver biopsies taken from patients who subsequently went on to develop PTLD. This may reflect a higher viral load in these patients; however, this retrospective study needs to be supplemented by prospective studies in order to determine the predictive value of this approach.

Clinical Manifestations
The time from transplant to onset of PTLD was analyzed in a series of renal transplant patients treated with an Azathioprine-based regimen.24 In these patients, two types of presentations were seen. The first resembled infectious mononucleosis and occurred an average of 9 months after transplant. The second presentation was that of a localized tumor mass, seen on average after 6 years posttransplant.

In our cyclosporine-based series, clinical presentations included (a) an infectious mononucleosis-like syndrome with variable lymphadenopathy, (b) allograft dysfunction, (c) solid tumors, often of extranodal sites, or (d) fulminant disease.3,107,119,120,129

The time interval between transplant and PTLD was reduced in our patients relative to non-CsA-treated patients and the median time to onset was 4.4 months.2 Although many of the early lesions resemble IM, this is not invariably the case. Late PTLDs also occur in our patients and these typically are localized tumors. Others have also noted early and late onset PTLDs and have stressed the worse prognosis of late arising tumors.119,130

Patients with infectious mononucleosis-like syndromes may have a preceding lymphopenia for variable times. At the time of clinical presentation, atypical lymphocytosis may be observed. Cervical lymphadenopathy and tonsillitis are typical and may be life-threatening. Some patients also have generalized lymphadenopathy and may have a maculopapular skin rash.

Allograft dysfunction often raises the clinical suspicion of rejection111,115 and may present as tenderness over the involved site, often with fever. Lymphadenopathy may be present or the allograft may be affected in an isolated fashion.

Solid tumors may be single or multiple. They most commonly occur in extranodal locations and frequently involve the gastrointestinal tract129,131 or allograft organ.5,119 Particularly in the gut, rapid tumor growth may lead to considerable morbidity due to perforation. Skin involvement is an uncommon but recognized form of this disorder and may occur in isolated form (Lee E. et al., in preparation). Involvement of the CNS usually takes the form of solid tumors, but a meningeal infiltrate with CSF pleocytosis may also occur.

A leukemic picture has been described in some patients.93 This appears to represent an unusual and advanced form of this disease. The exact relationship of this to the more common forms of PTLD remains undefined.

Occasional patients present with systemic signs and symptoms due to widespread lymphoproliferation.120 This may present as or evolve to multiorgan disease leading to multiorgan failure. Individual tumors occur in multiple sites and lymphadenopathy is common. These patients differ from others in that they tend to have concurrent opportunistic infections, perhaps reflecting a profound immunosuppression.

Pathology
The histology of PTLD reflects the lymphoid proliferation associated with EBV infection and the modification of this proliferation by an impaired host response.

A range of mononuclear cell forms was observed in tissues from a series of five renal transplant patients who developed lymphoid tumors following transplantation.2 This prompted the introduction of the term "polymorphic" to distinguish these growths from other forms of lymphoma. Two forms of polymorphic lymphoid growths, termed polymorphic diffuse B-cell hyperplasia and polymorphic B-cell lymphoma were observed. Both contained a mixture of large and small lymphoid cells and differed from other reactive lymphoid conditions by the presence of tissue invasion. The polymorphic lymphomas also contained areas of necrosis and large immunoblasts with atypical features ("atypical immunoblasts"). It was noted that such cells could resemble Reed-Sternberg cells.

The clinical courses of these patients could not always be inferred from a histological perspective.3 For instance, one patient with polymorphic hyperplasia died with disseminated disease, whereas another patient with polymorphic lymphoma was alive at four months following a reduction of immunosuppression and administration of Acyclovir. Additionally, one patient with polymorphic hyperplasia was found to have a cytogenetic abnormality within the lesion. Despite this, she was alive 26 months thereafter, following a temporary reduction of immunosuppression.

We found a similar inability to precisely distinguish the clinical behavior of PTLD patients based on these histologic features, and felt that the polymorphic nature of the infiltrate was the predominant histologic feature of note.3 Although necrosis and atypical immunoblasts were observed in individual cases, such patients in our CsA-treated series did not behave differently than those who lacked these features. In contrast, a smaller subset of patients had tumors which lacked the polymorphism found in the majority of patients. These "monomorphic" tumors most closely resembled non-Hodgkin's lymphomas and appeared to augur a poorer prognosis. Hence our approach stressed the distinction between polymorphic and monomorphic PTLD and did not incorporate the term "lymphoma" into either category. Craig et al.32 have also recently commented on the usefulness of this simplified approach.
Over time, additional histologic categories were introduced into the literature to accommodate patient specimens which could not be included within existing categories. For example, atypical lymphoid hyperplasia was the term applied to cases in which a polymorphic lymphoid proliferation was observed within the paracortex of lymph nodes, but in which invasion characteristic of polymorphic hyperplasia or polymorphic lymphoma was not observed.\textsuperscript{133} Atypical polymorphic B-cell hyperplasia was used for cases with features intermediate between polymorphic hyperplasia and polymorphic lymphoma.\textsuperscript{133} We introduced the term "minimal polymorphism" to refer to those lesions in which a minor degree of variability was seen among benign-appearing cells of plasmacellular appearance.\textsuperscript{5} Others, however, have observed pure plasmacellular differentiation of PTLDs in extranodal sites such as skin or testis\textsuperscript{134} and have considered these lesions to most closely resemble plasmacytomas.

In retrospect, the main achievement of these classifications has been to recognize that posttransplant lymphoproliferations associated with Epstein-Barr virus are fundamentally different from sporadic non-Hodgkin's lymphomas. Some cases are histologically identical to infectious mononucleosis, while others show exaggeration of individual features, such as necrosis or Reed-Sternberg-like cells, that may be seen in IM. Most monomorphic tumors and tumors with a predominance of Reed-Sternberg-like cells (or atypical immunoblasts) probably represent the emergence of neoplasias resembling non-Hodgkin's lymphomas or Hodgkin's disease, respectively. Beyond that, the emphasis on histopathologic subclassification diversifies attention from the fact that these lesions are primarily manifestations of uncontrolled or poorly controlled infectious mononucleosis. In this regard, it may be impossible to set a dividing line between "infectious mononucleosis" and "PTLD" in the transplant patient. Many histologic features of PTLDs can be seen in IM. In addition, involvement of solid organs may occur in IM, raising the question of distinguishing between IM and PTLD at these sites as well.

For instance, EBV hepatitis in the transplant recipient may be histologically similar to that described in immunocompetent patients.\textsuperscript{135,136} The presence of nodular masses, usually beginning in portal tracts, and associated with atypical lymphoid cells, merits the diagnosis of PTLD in our opinion. There is, however, no sharp histologic cutoff point between these two diagnoses.

In the kidney, PTLD may present as an infiltrative or tumorous lesion with a similar nosological dilemma. Gut lesions present as ulceronodular masses of lymphoproliferation which appear to begin in the submucosa and rapidly infiltrate the entire wall. The use of EBER staining has allowed the detection of EBV within a Peyers patch in an otherwise normal bowel biopsy in one of our patients, indicating that a presently unappreciated subclinical latent infection may also exist in this organ.

In the skin, epidermal and adnexal necrosis may occur. Deep dermal infiltration characteristic of other lymphomas is observed and atypical lymphoid cells may be seen. CNS lesions are rare in our series,\textsuperscript{137} despite the known association between EBV and CNS lymphomas in immunocompromised patients.\textsuperscript{138}

PTLD in lungs is suspected when significant plasmacellular infiltration is found in this organ. The lesions tend to present as multiple nodules. PTLD of the allograft heart is exceedingly rare but does occur (personal observations). The process resembles Grade 3-4 rejection but with frequent blast cells and plasmacytic infiltrate. An association between EBV and Quilty lesion has been postulated, but we have not found evidence of this in our series. We consider the differential diagnosis of Quilty lesions and endocardial-based rejection to be of more importance.

In closing, we believe that the distinction of "IM" from "PTLD" is of less clinical importance than is the distinction of those growths which will from those which will not respond to host immunomodulation with supportive surgical intervention. In our opinion, tumor monomorphism or abundance of Reed-Sternberg-like cells or atypical immunoblasts remain the major histologic criteria which suggest a poor response to this therapy. Areas of monomorphism within otherwise polymorphic lesions and the presence of rare large atypical cells remain of questionable significance at present, since both of these features may be seen in IM in nonimmunocompromised patients.

Phenotypic Analysis of PTLDs and In Situ Detection of Epstein-Barr Virus

Studies have shown that the B-cell phenotype of PTLDs resembles that of EBV-infected lymphoblastoid cell lines rather than that of Burkitt lymphoma.\textsuperscript{139} Thus, expression of various EBNA proteins, latent membrane protein, and cell adhesion molecules ICAM-1, LFA-1 and LFA-3 were observed in these studies. However, not all cases demonstrated the presence of all antigens tested. This may represent a technical artifact or it may indicate that PTLD cells are not strictly equivalent to in vitro lymphoblastoid-cell lines. Cen et al provided recent data to show that PTLDs downregulate EBNA-2 expression and that they also show a variable LMP expression.\textsuperscript{114} It is tempting to speculate that residual host selection pressure may favor growth of clones in which an optimal profile of protein expression produces the maximum degree of cell proliferation with the minimum amount of host cytotoxic recognition. In this regard Alfrey et al\textsuperscript{138} found evidence to suggest that good DR matching was a risk factor for the development of PTLD. They hypothesized that expression of identical HLA types on proliferating B cells might similarly allow the cells to evade immune surveillance mechanisms.

The past decade has seen significant advances in our ability to detect EBV genes and proteins within routinely processed pathologic specimens. First-generation detection kits utilized in situ hybridization with probes to internal repeat portions of the EBV genome. The use of probes to high copy number EBV DNA (EBER)\textsuperscript{140} along with the development of rapid tissue hybridization protocols\textsuperscript{141} has increased the usefulness
of this assay in the clinical setting. In addition, several commercial suppliers provide antibodies to EBV latent membrane protein which can be applied to routinely processed tissues. We have found that microwave pretreatment of tissue also allows the application of anti-EBNA 2 antibodies to paraffin sections. In practice, we use anti-LMP 1 antibodies to detect EBV in clinical specimens and we support this with in situ hybridization for EBER when necessary.

Studies at our institution show significant numbers of infiltrating cells within PTLDs (Wu T et al, MS in preparation). These cells appear to be more frequent in polymorphic than in monomorphic cases. Combined phenotypic and EBER staining allows the distinction between infected and noninfected cells. We have found CD3+ cells are generally free of EBV markers in the cases studied to date. In addition, significant numbers of macrophages are present within PTLDs in our experience.

Infected cells usually carry B-cell markers. In most cases, CD30+ cells are extremely rare. The only exception is a case resembling HD, in which large EBER+ cells were CD15+, LeuM1, LN1, LN2, CD30+, EMA, LCA; a phenotype which resembles that of RS cells. CD15+ cells are rarely seen in PTLDs except near areas of necrosis.

**Molecular Biologic Analyses of PTLDs**

**Clonal Studies**

The polyclonal (reactive):monoclonal (malignant) dichotomy has been replaced by molecular studies which have demonstrated a gradation of clonal alterations within PTLDs. These studies are largely based upon the behavior of immunoglobulin genes, which rearrange uniquely within the maturing B cell. All progeny of a B cell which has already rearranged its immunoglobulin genes will, by and large, carry that same rearrangement. This characteristic is exploited to detect clonal and nonclonal populations of B lymphocytes.

Using these techniques, it has been shown that PTLDs can either be polyclonal (reactive) or they may contain clonal components which may comprise a small to large proportion of the cell population. We have graded these clonal components as 1 to 3, based on relative proportion, and have found some correlation with PTLD behavior. Lesions with no evidence of gene rearrangements are felt to represent virus-induced hyperplasias, despite the fact that some may show invasive tendencies. PTLDs with major clonal components were felt to indicate active neoplasia and were less likely to regress following a reduction of immunosuppression. Conversely, lesions with a minor clonal subpopulation presumably reflected either an emerging tumor or a clone with a restricted growth potential.

It has also been demonstrated that multiple concurrent PTLDs may each have unique clonal rearrangements. This suggests an environment which facilitates the outgrowth of selected B lymphocytes, or a tendency for small numbers of B cells to spontaneously proliferate. We prefer the term "clonal" PTLD over "monoclonal" PTLD in order to describe this condition. Alternate terms for this phenomenon include oligoclonal or multiclonal PTLDs. We prefer the term "monoclonal" for (a) a single tumor comprised primarily of one clone of cells, with no evidence of tumor elsewhere, or (b) multiple tumors, each with an identical clone of cells as demonstrated by immunoglobulin gene rearrangement analysis. In a practical sense, a monoclonal tumor is a fully developed neoplasm with a tendency to produce metastatic disease. Multifocal disease may represent multiple, potentially reversible clonal outgrowths facilitated by the disordered physiology consequent to the interplay between virus infection and immunosuppression.

In addition to these clonal patterns, some PTLDs may have evidence of more than one clone of cells within a single tumor. The terms oligoclonal or multiclonal PTLD have also been applied to this type of lesion.

In the usual PTLD, rearrangements of the T-cell receptor cannot be found. However, individual cases of T-cell posttransplant tumors have been described, and in these cases T-cell rearrangements are seen in the absence of immunoglobulin gene rearrangements.

**Viral Analysis**

Using molecular probes for specific viral sequences, it has been found that almost all abnormal lymphoproliferations in transplant recipients contain EBV. This has led us to use the term "PTLD" synonymously with "EBV-associated posttransplant lymphoproliferative disorder" and we consider any EBV-negative lymphoproliferation in these patients as representative of a different syndrome.

Certain features of the virus allow study of additional variables. Probes to the viral terminal repeat region can distinguish active infection, in which the termini are nonfused, from latent infection, in which the episomal virus demonstrates joining of the two terminal regions. Further, when a single virus fuses its two terminal ends to produce the episomal form, a fixed number of terminal repeat segments are retained, and some are lost. This number may vary among different individual virus particles in a single infection. However, all the progeny episomes that derive from a single episomal virus will retain that same number of terminal repeat segments. Hence, in a manner analogous to the evaluation of immunoglobulin gene rearrangements, it is possible to determine a "polyclonal" from a "monoclonal" population of viruses within a given specimen.

This feature has been used to analyze the question of whether a single virus is present at the initiation of PTLD, or whether the cell proliferation provides a desirable target for viral superinfection. In the former case, a single clone of cells would be expected to carry a single clone of virus. In the latter case, one clone of cells may contain multiple viral forms. The results indicate that the virus is present prior to the development of PTLD, providing further evidence for the importance of this virus in the development of PTLD.

It is generally assumed that latent virus infection plays a major role in this disorder. We detected linear, replicating EBV in a minority of PTLDs using Southern blot procedures.
Subsequently, Katz et al. examined a series of 13 patients with EBV lymphoproliferations occurring under a variety of immune deficient states, including organ transplantation, and they found evidence of replicative virus in 40% of lesions. Recent in situ hybridization studies have shown lytic transcripts in 21 of 22 PTLDs.

**Cytokine Analysis**

Cytokines provide one major mechanism by which cells "talk" to one another. Like individual words, each cytokine has its own identity. However, the "language" of cells combines these words into sentences which can impose different contextual messages supersed ing the isolated definitions of the component structures.

Two such "sentences" are constituted by separate combinatorial patterns of cytokines referred to as Th1 and Th2. These patterns, originally defined in mice, reflect microenvironments conducive to providing help for cytotoxic T-cell activity (Th1) or for primarily B-cell-mediated immune responses (Th2). The Th1 pattern is characterized by upregulation of IL-2, IL-3, GM-CSF, and lymphotakin, while the Th2 environment shows the presence of IL-4, IL-5, IL-6, IL-10, and GM-CSF. It has recently been demonstrated in a model system that IL-12 (NK-cell stimulatory factor), which is produced by macrophages, can shift naive T cells to a Th1 pattern. IL-4 effectively pushes cells into a Th2 pattern, and both IL-4 and IL-10 can inhibit IL-12 production by human monocytes.

Shapiro et al. analyzed 5 bone marrow transplant recipients for the presence of markers of Th1 versus Th2 cytokine patterns in serum. Their results support the presence of a Th2 environment in these patients. These authors have recently expanded their series to include 4 patients with solid organ transplant and PTLD, with similar results. Burke et al. reported a sequential rise of IL-2, followed later by IL-4 elevation and loss of detectable IL-2, in a patient with disseminated PTLD.

We have recently looked at PTLD specimens themselves for mRNA messages encoding IL-4 and cellular IL-10 as markers of Th2 and messages for IL-2 and IFN-\(\gamma\) as indicators of Th1 status (Nalesnik, M. et al, submitted). In every case the microenvironment was consistent with a Th2 pattern, suggesting that these signals play a role in sustaining B-cell growth and proliferation. In addition, Tosato et al. recently reported the presence of IL-6 in cells derived from these tumors and showed that production of this cytokine was dependent upon the presence of adherent, non-B cells, probably macrophages.

**Karyotypic Studies**

Available data suggest that no single pattern of clonal karyotypic abnormality is seen in PTLD. Individual tumors may have a normal genotype or may contain unique cytogenetic anomalies. One study of six patients showed such abnormalities in three cases. Two of these patients had polyclonal disease by phenotypic analysis, and the latter had monoclonal disease by this method. Immunoglobulin gene analysis of clonality was not performed. Had this methodology been available at that time, it may be that minor clonal populations would have been detected in the two polyclonal tumors. Two other patients had polyclonal disease and a normal karyotype. Finally, one patient had a normal karyotype in cells from two separate tumors, one polyclonal and one monoclonal. These data suggest that in some cases of PTLD clonal outgrowth may be possible without corresponding cytogenetic abnormalities.

In our series, one patient with a monomorphic PTLD demonstrated a t(8;14) in conjunction with other clonal cytogenetic abnormalities. The tumor was monomorphic and was recalcitrant to therapy, although it remained localized for several years before leading to the death of the patient. This tumor also represented one of three specimens that demonstrated c-myc gene rearrangements. Karyotype analysis was not available for the other two specimens.

In situ hybridization with chromosome specific probes has provided an alternative means for karyotypic analysis in recent years. In some instances, archival paraffin-embedded materials may be used. We have found one example of a heavily chimeric PTLD by this method. The patient, a female liver transplant recipient received her allograft from a male donor. She later developed an EBV-positive PTLD in an axillary node. Approximately 10% of cells within the tumor displayed a male phenotype, as demonstrated by in situ hybridization using a fluorescent probe specific for a portion of the Y chromosome. Other PTLDs have shown extremely rare donor cells in sex-mismatched cases (Nalesnik, M. and Demetris, A., unpublished observations). This is compatible with the concept of chimerism as recently demonstrated in solid organ transplant recipients and is not a finding specific to PTLDs. There may be an intersection in some cases between acute GVHD and PTLD which remains to be defined.

In a different vein, Spiro et al. used PCR analysis of DNA polymorphic loci to conclude that a lymphoma occurring in the porta hepatitis of a transplanted liver was of donor origin. The authors did not comment on EBV involvement in this case.

**Prevention and Treatment of PTLDs**

Minimization of immunosuppressive therapy and prophylactic use of antiviral drugs represent a rational approach to reducing the risk of PTLD development. Unfortunately, neither of these measures are entirely effective or even possible in individual cases, and a high level of suspicion for this disorder remains essential. It is likely that other unknown factors may impinge upon the virus:host interaction. For instance, one randomized study of nonimmunosuppressed IM patients found that those treated with aspirin actually had a more prolonged disease course than those given placebo. It is possible that such observations may provide hints applicable to the transplant population as well.

Vaccination against EBV might represent a means of preventing PTLD by eliminating the major factor
predisposing to this condition. As such, it would be expected to prove most useful in the pediatric population, since these patients are more often seronegative at time of transplant. It is also possible that a vaccination of seropositive patients may serve to boost immunity to the virus, leading to lower viral burden posttransplant and a lower risk of PTLD. However, this remains highly speculative at present.

Several vaccine preparations are under evaluation. Most target the major envelope glycoprotein which interacts with the cellular EBV receptor. Epstein et al have shown that vaccination of nonhuman primates prevents lymphoma formation upon subsequent challenge with EBV. Phase I trials may begin within 1-2 years if current trends continue.

Reduction of immunosuppression together with Acyclovir/Ganciclovir and surgical management of complications remains the mainstay of therapy for PTLDs. The overall rate of total remissions in PTLD cases has been reported to approximate 31% in a composite series of 323 patients, according to the Cincinnati Transplant Tumor Registry. Responses may be dramatic over several days, or may proceed more slowly over 1-2 months. (In IM in nonimmunosuppressed individuals, lymphadenopathy for more than 1 month is unusual.)

The time course for evaluation of the efficacy of reduced immunosuppression is influenced by the clinical condition of the patient. A mild degree of rejection may be tolerated, but this must be balanced against the need to assure continued function of a vital organ such as the heart. Malatack has recently outlined the approach of his group to the issue of reduced immunosuppression. Following resolution of the disease, we usually reiter immunosuppression to maintain stable allograft function at the lowest level of drug. Others have reported switching from CsA to Azathioprine-based immunosuppression, also with acceptable allograft maintenance. Retransplantation is also possible.

The utility of antiviral medication in these patients is unclear. In acute IM, the use of acyclovir results in a temporary marked reduction of salivary viral load, but has no effect on peripheral blood cell virus load. Occasional transplant patients at our institution have developed PTLD despite the use of prophylactic antiviral therapy (M. Green, personal communication). Nevertheless, recent evidence of a lytic component of virus within PTLDs suggests that these drugs may have some efficacy in preventing continuing infection.

The major complication associated with reduced immunosuppression is organ rejection. At present, there is no way to predict who will and who will not reject their allograft. Whereas some patients exhibit a vigorous rejection response, others never require reinstatement of immunosuppression. In our patient population, those with PTLD appear to be disproportionally represented among those who have successfully been weaned from immunosuppressive medication (Reyes G. et al, MS in preparation). However, it is not known whether this is related to the pathophysiology of this disease or represents a selection artifact due to the universal reduction of immunosuppression in this population.

Active immunomodulation has been attempted by administration of cytokines in selected patients. Shapiro et al used IFN-α together with intravenous immunoglobulin in 5 patients with EBV-associated lymphoproliferations. Two of these patients had received bone marrow transplants and the other three had constitutional immunodeficiencies. One posttransplant patient each had monoclonal or polyclonal disease. Both underwent complete remission, although the patient with monoclonal disease expired several weeks thereafter due to CMV infection. In a more recent report, this group reported partial to complete remissions in 4 evaluable immunodeficient patients with EBV-associated tumors treated with recombinant IFN-α alone. Successful use of IFN-α and intravenous immunoglobulin in PTLD following bone marrow transplant has also been reported in two cases by Trigg et al. Other cytokines, such as TNF α, may also have a role to play in future immunomodulation of these lesions.

Fischer et al reported on the usefulness of monoclonal antibody therapy using a combination of antibodies to CD21 and CD24. Sixteen of 18 patients with oligoclonal disease had complete remission and the other two patients had partial remission with persistence of CNS disease. In some cases remissions occurred in patients who had not responded to reduced immunosuppression. Remission was not observed in seven patients who had monoclonal disease, despite the presence of the antigens on tumor cells.

It has recently been reported in the lay press that transfer of genetically altered heterologous lymphocytes has been successfully employed to eradicate a PTLD arising in a bone marrow transplant recipient. The patient, a woman, received an infusion of her brother's T lymphocytes which had been modified to contain a marker gene and a "suicide" gene. The transfused cells then attacked the tumor and in turn were eliminated by iatrogenic activation of the "suicide" gene. We eagerly await a detailed report of this novel therapeutic approach.

In the absence of any appreciable response to treatment with conservative measures one must consider the use of antineoplastic radiotherapy, chemotherapy, or both. We have previously tabulated the use of chemotherapy and radiotherapy in our patient population. We find that the tumors often respond to such treatment and the major life-threatening complications are concurrent infection and tumor lysis syndrome. Late-arising monoclonal tumors which are either monomorphic or resemble Hodgkin's disease represent the most frequent scenarios under which chemotherapy is employed at our institution. Lien et al reported a case of monoclonal PTLD which arose 22 months following renal transplant and had a monomorphic appearance resembling Burkitt lymphoma. Complete remission was induced utilizing a ProMACE-CytaBOM chemotherapeutic regimen. The patient became tolerant to her graft, and this was attributed to the effect of chemotherapy. However, since we have seen the same phenomenon in some of our patients treated with reduced immunosuppression, we consider the exact cause of the tolerance to be undefined. Barkholte et al described a case...
of allograft-based PTLD which occurred 7 months following liver transplant. Following two weeks of combination chemotherapy the patient refused additional drugs. The mass was then removed surgically and was found to be entirely necrotic. She has remained in complete remission during the three year follow-up. The rapid and complete response of this tumor to "suboptimal" chemotherapy suggests that protocols designed for standard lymphomas may be too aggressive in this disorder and that the optimal regimen remains to be defined.

OTHER EBV-ASSOCIATED POSTTRANSPLANT TUMORS

T-cell lymphoproliferations

In one study of non-Hodgkin's lymphomas arising in nonimmunosuppressed patients, EBV was more frequently associated with peripheral T-cell lymphomas (10% of cases) than with B-cell lymphomas. The virus can infect T-cells in vitro, but this has not yet been demonstrated in vivo. We have recently observed a case of clonal T-cell lymphoproliferation in a patient who had a previous PTLD. The patient is currently under treatment for this condition and studies are currently underway to identify the EB virus-infected cell type, i.e., T cell or other reactive cell (Wu et al, MS in preparation). In a separate reported case EBV was localized to tumor cells of a T-cell lymphoma arising in a renal transplant patient. Several examples of non-EBV-associated T-cell lymphomas in bone marrow or solid organ recipients have also been reported. Hodgkin's disease and "Recurrent" PTLDs

Occasional cases of Hodgkin's disease in transplant recipients have been reported. In our series there have been two such cases. In one patient, no EBV was found and there was a family history of leukemia. This patient received standard antineoplastic therapy and ultimately died of his tumor. In the second case, the lesion was related to EBV. This latter case is also interesting because it represents an example of recurrent EBV-related tumor in a transplant patient. This young female liver recipient developed a polymorphic PTLD following her transplant. Several B-cell clones were found, and at least two clones of EBV were present within the tumor. Immunosuppression was reduced and the tumor regressed. She remained well for about 2 years, when she presented with fever and night sweats. Evaluation showed involvement of spleen, liver, and lymph nodes by a lymphoproliferation most consistent with Hodgkin's disease. EBER staining showed the Reed-Sternberg-like cells to be uniformly positive for the virus. Clonal analysis again revealed a B-cell clone; however this clone was different from that found in the first tumor. In addition, a separate clonal form of EBV was also found. The patient received chemotherapy for Hodgkin's disease and continues to do well without evidence of tumor 16 months after the initial diagnosis.

This case shows that clinically recurrent tumor cannot be assumed to represent pathologic recurrence of the original tumor in these patients. In different cases we have seen histologically similar recurrence, clinical recurrence of more aggressive appearing tumors, or clinical "recurrence" of EBV-associated tumors involving an entirely different cell type (Wu et al, MS in preparation). It may be that EBV infection places these patients at risk for the development of a number of separate tumors, dependent upon the behavior of the individually infected cells within the immunosuppressed host. This concept may also underlie the spindle-cell lesions described below. In passing it is noted that EBV-positive Hodgkin's disease has been rarely observed as a sequel to non-Hodgkin's lymphoma in nonimmunosuppressed patients as well.

Posttransplant Spindle-cell tumor

Three cases of spindle-cell tumors within pediatric transplant recipients have recently been observed within our series. One of these patients also had separate PTLD. EBV in situ hybridization was positive in all three cases. One patient has had her spindle-cell tumor controlled by excision. A second has had recurrent disease, and the third died of metastatic EBV-positive spindle-cell tumor. Molecular analysis of one case has shown clonal virus which appears to be integrated within the genome (Lee E et al, submitted). Actin and desmin stains suggest that the lesion represents a tumor of smooth muscle cells. It is of interest that spindle-cell tumors diagnosed as leiomyosarcomas have also been observed in AIDS patients. In some cases this is associated with mycobacterial infection. No evidence of this agent was seen in any of our patients. The possibility of EBV participation in AIDS spindle-cell tumors, as well as in other reported cases of posttransplant leiomyosarcoma and fibrosarcoma, remains to be explored.

POSTTRANSPLANT LYMPHOMAS UNRELATED TO EBV

Occasional EBV-negative lymphomas have been reported within transplant patients. These include T-cell lymphomas, Hodgkin's disease, and non-Hodgkin's lymphomas (personal observation). The relationship of these tumors to PTLDs remains problematic and at present it may be best to evaluate and treat these as de novo malignancies. Nevertheless, we know of at least one case in which a PTLD which was negative for EBV by molecular analysis did regress following a reduction of immunosuppression. True lymphomas in solid organ transplant recipients most likely derive from host lymphocytes. Rarely, a donor-derived lymphoma may occur. Meduri et al reported a case of EBV-negative donor-derived non-Hodgkin's lymphoma arising in a renal transplant patient. Antineoplastic therapy was successful in eradicating disease for the 18 months of follow-up.

THE SCID MOUSE IN THE STUDY OF EBV-ASSOCIATED LYMPHOMAGENESIS

The development of the severely immunocompromised C.B-17 ICR scid/scid mouse (SCID) has provided a nonprimate model for the analysis of EBV-associated lymphomagenesis in human lymphoid cells. In this system, the inoculation of peripheral blood lymphocytes from
EBV-positive individuals precipitates the development of EBV-positive B-cell tumors. The tumors may be monoclonal or oligoclonal, and generally display a phenotype similar to that of lymphoblastoid-cell lines with Type 3 latency. Direct EBV infection of peripheral blood cells transferred into SCID mice from seronegative donors results in polyclonal tumors, again mimicking a condition seen in some cases of PTLD.

SUMMARY

Hypothetical pathogenesis of PTLDs

PTLD may be considered as an "opportunistic cancer" in which the immunodeficiency state of the host plays a key role in fostering the environment necessary for abnormal lymphoproliferation. The following discussion reflects our own current thoughts regarding events which may result in PTLD and its sequelae. Many of the individual steps have not been rigorously proved or disproved at this point in time.

Following transplantation and iatrogenic immunosuppression, the host:EBV equilibrium is shifted in favor of the virus. Most seronegative patients will become infected either via the graft or through natural means; seropositive patients will begin to shed higher levels of virus and may become secondarily superinfected via the graft. There is a "grace" period of approximately one month posttransplant before increased viral shedding begins. PTLD is almost never seen during this interval. In many cases infection continues to be silent whereas in rare individuals there is an overwhelming polyclonal proliferation of infected B lymphocytes. This is the parallel of infectious mononucleosis occurring in patients with a congenital defect in virus handling (X-linked lymphoproliferative disorder). It is possible that transplant patients with this presentation also suffer a defect in virus handling. In other cases excessive iatrogenic immunosuppression may paralyze their ability to respond to the infection.

With CsA and FK506 regimens, individual tumors may occur within a matter of months following transplant. The short time of incubation suggests that these are less than fully developed malignancies. It may be that local events conspire to allow outgrowth of limited numbers of B-lymphocyte clones. A cytokine environment favoring B-lymphocyte growth may be one factor and differential inhibition by the immunosuppressive drugs of calcium-dependent and independent B-cell stimulation may be another. In addition, there is some evidence that CsA itself may inhibit apoptosis within B cells. Since most patients do not develop PTLDs, an additional signal(s) for B-cell stimulation may be required. Indeed, it is possible that the virus may simply serve to lower the threshold for B-cell activation and/or provide a survival advantage to these cells. The ability of individual cell clones to evade a weakened immune system may set into play a Darwinian type of competition in which the most rapidly proliferating cells with the least number of antigenic targets predominate. In this regard, differences in host HLA types may determine the repertoire of viral antigens which are subject to attack.

Since these tumors are a consequence of disordered physiologic growth, restoration of a normal milieu may lead to a dying back of the process despite the presence or absence of clonal outgrowth. This is clinically evidenced as tumor regression in the face of immunomodulation. However, a subclinical latent infection of lymphoid and nonlymphoid cells may predispose to accumulation of individual cytogenetic errors in rare cells and may lead to a fully malignant phenotype. This may be more likely to occur in those patients harboring larger numbers of the virus. Tumors arising from these cells would be expected to occur later in time than the majority of PTLDs and would be more likely to be resistant to immunomodulation therapy.

In conclusion, the story of PTLDs is an evolving one. As more details of the biology of EBV infection become understood we will progress to a staging system for IM which incorporates and explains both the infectious and neoplastic aspects of this host:parasite system. Until that time, the ongoing dissection of this family of lesions will continue to provide new surprises in the study of virus-associated human carcinogenesis.

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