REVIEW ARTICLE

Posttransplantation Lymphoproliferative Disorders

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Posttransplantation lymphoproliferative disorders include a wide spectrum of diseases ranging from hyperplastic-appearing lesions to frank non-Hodgkin lymphoma. More than 90% of these disorders are Epstein-Barr virus–associated lesions of B-cell origin that arise in the setting of pharmacologic immunosuppression after transplantation. With the increased use of organ transplantation and intensive immunosuppression, posttransplantation lymphoproliferative disorders are becoming more common. The prognosis is often poor, with most patients dying despite receiving treatment. The aim of this review is to report the most recent knowledge about the clinical features, diagnosis, prophylaxis, and treatment of posttransplantation lymphoproliferative disorders, which can be useful to physicians and health assistants dealing with these life-threatening, posttransplantation clinical entities in clinical practice.

Arch Intern Med. 2003;163:1997-2004

Posttransplantation lymphoproliferative disorders (PTLDs) are lymphoid proliferations or lymphomas that develop in a recipient of a solid-organ or bone marrow allograft.[1] Most PTLDs are associated with Epstein-Barr virus (EBV) infection and seem to represent B-cell or, rarely, T-cell proliferations that occur in a setting of decreased T-cell immune surveillance due to immunosuppressive drugs used to prevent graft rejection.[2] Posttransplantation lymphoproliferative disorders occur in 1% to 20% of transplant recipients,[3] but the incidence markedly increases in patients receiving anti–T-cell therapies or T-cell–depleted bone marrow transplants.[4]

PATHOGENESIS

The pathogenesis of PTLDs is complex and probably multifactorial. Drug-induced immunodeficit and chronic antigenic stimulation exerted by the graft play an important role. Other risk factors include the type of transplanted organ, the recipient and donor EBV serologic status, the type of disease leading to transplantation, and, finally, the type, length, and intensity of immunosuppressive drug treatments.[5]

Type of Transplanted Organ and Disease Leading to Transplantation

The incidence of PTLDs is 7% to 11% in small-bowel transplant recipients, 3.4% in heart transplant recipients, 1.8% to 7.9% in lung transplant recipients, 2.2% in liver transplant recipients, and 1.0% in renal transplant recipients.[3] In patients receiving bone marrow transplants, the PTLD incidence is lower than 1%, but it markedly increases to as high as 24% in patients receiving T-cell–depleted transplants.[4] Patients undergoing allogeneic hematopoietic stem cell transplantation have a PTLD incidence of about 1%.[6] In bone marrow and hematopoietic stem cell transplant recipients, the risk of PTLDs is increased for transplants from an unrelated or an HLA antigen–mismatched donor. In unrelated transplants, the National Marrow Donor

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Program reported an overall incidence of PTLDs of 2%.[7] After unrelated donor umbilical cord blood transplantation, the incidence of EBV PTLDs is similar to that observed after transplantation using unrelated bone marrow and compares favorably with unrelated donor T-cell–depleted bone marrow transplantation.[8]

The greater incidence of PTLDs in small-bowel or lung transplant recipients might be a consequence of the high amount of lymphoid tissue that is present in these grafts, whereas the lack of control on EBV latently infected B lymphocytes by T cells may cause the greater incidence of PTLDs in T-cell–depleted bone marrow transplant recipients.

Several studies have shown that the type of disease that had led the patient to transplantation has an important role in the risk of PTLD development. For example, patients undergoing bone marrow or hematopoietic stem cell transplantation for the treatment of immunodeficiency or chronic myeloid leukemia are at higher risk for PTLDs than patients who receive the same type of transplant for different reasons.[6] In liver transplant recipients, a preexisting autoimmune hepatitis or a primary biliary cirrhosis can increase PTLD risk. In these patients, the immune system dysregulation that is the main cause of their hepatic disease could collaborate with the immunosuppressive drugs in causing the appearance of PTLDs.[9] Liver transplant recipients with hepatitis C virus infection have a PTLD frequency as high as 10.5%. In this case, the ability of hepatitis C virus to chronically stimulate the host's immune system can be the main cause of the abnormal lymphoproliferation. Otherwise, in several studies, hepatitis C virus has also been associated with lymphoproliferative disorders in nontransplanted patients.[10]

EBV Infection

Epstein et al[11] discovered EBV almost 40 years ago by electron microscopic studies on Burkitt lymphoma cellular lines. In 1968, EBV was shown to be the etiologic agent of infectious mononucleosis,[12] and in 1970 it was detected in the neoplastic tissue of patients with nasopharyngeal carcinoma.[13] Moreover, in the 1980s, EBV was found to be associated with non-Hodgkin lymphomas and oral leukoplakia in patients with AIDS.[14]

Epstein-Barr virus is a DNA virus belonging to the human gammaherpesvirus family. The viral DNA is encased within a nucleocapsid, which is in turn surrounded by the viral envelope.[15] The genome consists of a DNA linear molecule that encodes nearly 100 viral proteins,[16] which, during viral replication, are important for regulating viral gene expression, replicating viral DNA, forming structural components of the virion, and modulating the host immune response.[15] The virus infects B lymphocytes in regions rich in lymphoid tissue. In vitro, epithelial cell infection leads to an active viral replication,[17] whereas B-cell infection leads to a latent infection with cell immortalization.[15] In vivo, viral replication is spontaneously activated in a small percentage of latently infected B cells. Before the virus enters the B cell, the major envelope glycoprotein, gp350, binds to the viral receptor, the CD21 molecule (the C3d complement receptor), on the surface of the B cell.[18] The major histocompatibility complex class II molecules serve as a cofactor for infection of the B cells.[19]

Infection of humans with EBV usually occurs by contact with oral secretions. The virus replicates in oropharyngeal cells, and nearly all seropositive persons actively shed virus in the saliva.[20] Although earlier studies[17] indicated that the virus replicates in epithelial cells of the oropharynx and that B cells were subsequently infected after contact with these cells, others studies[21] suggest that B cells in the oropharynx may be the primary site of infection. Resting memory B cells are thought to be the site of persistence of EBV within the body.[22]

Of the nearly 100 viral genes expressed during replication, only 10 are expressed in latently infected B cells.[16] Two types of nontranslated RNA (EBV-encoded RNAs [EBERs]), 6 nuclear proteins (Epstein-Barr nuclear antigens [EBNAs]), and 2 membrane proteins (latent membrane proteins [LMPs]) are expressed in these latently infected B cells. By avoiding full gene expression during latency, EBV reduces the number of viral proteins that can be recognized by cytotoxic T cells and, in this way, eludes the host's immune system response.[15]

Epstein-Barr virus LMP type 1 (LMP1) and type 2 (LMP2) act as oncogenes, and expression of these proteins in transgenic mice results in frank B-cell lymphoma.[23] Latent membrane protein type 1 induces a signaling response that mimics a constitutively active form of the B-cell surface molecule CD40.[24] Moreover, LMP1 binds to several tumor necrosis factor receptor-associated factors in vitro and in EBV-positive lymphomas in vivo.[25,26] These activities result in activation of the nuclear factor-κB transcription factor in vitro and in vivo, activation of c-*jun*, up-regulation of cellular adhesion molecules, cytokine production, and B-cell proliferation.[15] In transgenic mice, LMP2 allows nontransformed B cells to survive even in the absence of normal B-cell receptor signaling.[27]

Diseases associated with EBV generally show viral gene expression limited to 1 to 3 patterns of latency.[28] In the first form of latency, typical of Burkitt lymphoma, only EBNA1 and EBER are expressed; in the second form, typical of nasopharyngeal carcinoma, Hodgkin disease, and peripheral T-cell lymphoma, EBNA1, LMP1, LMP2, and EBER are expressed.[15] In the third pattern of latency, seen in infectious mononucleosis, X-linked lymphoproliferative disease, and PTLDs, all the latency genes are expressed. A fourth pattern of latency, expressing EBER, LMP2, and EBNA1, is seen in B cells obtained from the peripheral blood of healthy persons with EBV after infection.[29]

The cellular immune response is more important than the humoral response for the control of EBV infection.[15] In particular, natural killer cells and $CD4^+$ and $CD8^+$ cytotoxic T cells control the proliferation of EBV-infected B cells during primary infection.[30] The ability of EBV to persist despite a potent immune effector response indicates that the virus

has evolved strategies to elude the immune system.[15] Epstein-Barr virus encodes a cytokine and a cytokine receptor that may be important for modulating the immune system, thus allowing persistent infection.[15] The EBV BCRF1 protein shares 70% of its amino acid sequence with interleukin (IL) 10[31] and mimics the activity of IL-10 by inhibiting interferon (IFN) γ synthesis by human peripheral blood mononuclear cells in vitro.[32] The EBV BARF1 protein acts as a soluble receptor for the colony-stimulating factor 1. Because colony-stimulating factor 1 usually enhances the expression of IFN-α by monocytes, BARF1 protein may function as a decoy receptor to block the action of this cytokine. Because IFN-γ and IFN-α inhibit the outgrowth of EBV-infected cells in vitro, BCRF1 and BARF1 proteins may help the virus to evade the host's immune system during acute EBV infection or virus reactivation in latently infected B cells.[15] Moreover, EBNA1 has been shown to block its own degradation by proteosomes.[33] Since viral protein presentation to cytotoxic T cells usually needs the intracellular peptides to be broken down, the ability of EBNA1 to inhibit this process may allow the protein to avoid triggering the activation of cytotoxic T cells.[15]

In patients affected by congenital or acquired immunodeficiency, in particular those with severe combined immunodeficiency, AIDS, and transplants, EBV is associated with lymphoproliferative disorders.[15] Bone marrow and organ transplant recipients, who have a deficit of the cellular immune response as a consequence of the immunosuppressive drug therapies, cannot control EBV latently infected B-cell proliferation. Consequently, they can develop symptoms of infectious mononucleosis or other EBV-related lymphoproliferative diseases, ranging from hyperplastic lesions to frank malignant lymphoma with possible involvement of lymph nodes, liver, spleen, lung, central nervous system, and small bowel.[34,35] Increases in EBV viral load in peripheral blood have been detected in these patients before development of the lymphoproliferative disease.[36] Similarly, EBV DNA was detected in liver biopsy specimens from 71% of patients before development of disease but in only 10% of those in whom the disease did not develop.[37]

Immunosuppressive Drug Treatment

The type, length, and intensity of immunosuppressive drug therapies are considered to be independent factors for the appearance of PTLDs. In the past 20 years, the possibility of preventing transplant rejection has been radically improved by the introduction of new immunosuppressive drugs such as cyclosporine, tacrolimus, muromonab, and mycophenolate mofetil. Ciancio et al[38] investigated PTLD incidence in transplant recipients treated with different types of immunosuppressive regimens during 18 years and observed a greater prevalence of PTLDs in patients treated with new immunosuppressive drugs than in those treated only with corticosteroids. In vitro studies[39] have shown that cyclosporine and tacrolimus promote EBV latently infected B-cell proliferation and block apoptosis phenomena. On the other hand, in a recent study,[40] the use of mycophenolate mofetil, in a corticosteroid-free immunosuppressive protocol administered with antiviral treatment, was associated with a lower incidence of EBV infection reactivation and PTLDs.

Swinnen et al[41] reported a higher prevalence of PTLDs in transplant recipients treated with anti-CD3 monoclonal antibodies (muromonab) as antirejection agents. In their study, a cumulative dose of muromonab greater than 75 mg was associated with a PTLD frequency of approximately 38%, whereas lower doses were associated with a frequency of only 6%. The major frequency of PTLDs in the group of patients treated with higher doses of muromonab has been imputed to an overimmunosuppression state, but the potential role of a cytokine-releasing syndrome has not been investigated, to our knowledge.

Other factors that might be associated with a higher risk of PTLDs are under evaluation, the most important being the lymphocyte phenotype at the time of transplantation, cytomegalovirus (CMV) infection, and the presence of a constitutional polymorphism of the cytokine genes.[5]

CLINICAL FEATURES

Although the disease is extremely variable, some general clinical patterns can be recognized. An infectious mononucleosis–like presentation, with prominent B-cell symptoms and rapid enlargement of the tonsils and cervical nodes, is often the case with PTLDs occurring less than about a year from the time of transplantation (early PTLDs).[42] Highly immunosuppressed patients may have widespread disease and diffuse infiltrative multiorgan involvement and may pursue a fulminant clinical course that is difficult to distinguish from sepsis.[43] Posttransplantation lymphoproliferative disorders that manifest later than about a year after transplantation (late PTLDs) are likely to be more circumscribed anatomically, to manifest fewer systemic symptoms, and to follow a more gradual clinical course. Extranodal disease and visceral nodal involvement are characteristic.[1]

The diagnosis of PTLD requires awareness of the protean appearance of this syndrome (**Table**). Localization of the dysfunction directs the diagnostic evaluation. Gastrointestinal tract involvement is a frequent finding in PTLD; endoscopic evaluation of the gastrointestinal tract may disclose large or small ulceronodular lesions that reflect PTLDs in the organs. In the case of pulmonary involvement, multiple nodular densities may be seen on radiographs. Ultrasonography of the graft is also required, thus the transplanted organ can be affected in up to 20% of cases.[1] Moreover, PTLD staging requires total-body computed tomography.

Several laboratory assays can be used to suggest or support the diagnosis of PTLDs. Badley et al[44] demonstrated the presence of monoclonal gammopathy in 71% and 27% of transplant recipients with and without PTLDs, respectively. A separate study[45] showed that PTLDs developed in 9% of all transplanted patients who had monoclonal gammopathy.

Although serologic tests are readily available in most medical cen-

The Diagnosis of Posttransplantation Lymphoproliferative Disorders

Laboratory Assays	Instrumental Examinations	Histologic Examinations
Blood cell count	Chest radiography	Biopsy of the lesions
Peripheral lymphocyte typization (in case of lymphocytosis)	Graft ultrasonography	Biopsy of the bone marrow
ESR, LDH, β_2-microglobulin	Abdomen ultrasonography	Biopsy of the enlarged lymph nodes
Serum protein concentration	Total-body computed tomography	Cytologic examination of effusions
EBV-specific IgM and IgG antibodies	Digestive endoscopy	
EBV DNA PCR on PBMCs in blood and effusions		

Abbreviations: EBV, Epstein-Barr virus; ESR, erythrocyte sedimentation rate; LDH, lactic dehydrogenase; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction.

ters, they generally are not useful for the diagnosis of PTLDs. In fact, although primary EBV infection in the immunocompetent host can be detected by seroconversion with development of antiviral capsid antigen IgM and IgG and anti-EBNA antibodies, transplant recipients undergoing immunosuppressive drug therapies can fail to produce detectable anti-EBV antibodies.[5] For this reason, the only way to demonstrate EBV infection in patients with PTLDs is to detect high blood levels of EBV DNA by using polymerase chain reaction. Polymerase chain reaction assay for the diagnosis of EBV infection in patients with PTLDs has been used as a consequence of the in vitro evidence of EBV active replication in the peripheral blood mononuclear cells of these individuals. Such replication does not occur in "normal" EBV-positive patients, and patients with PTLDs had elevated numbers of circulating viral genomes. Hanosono and colleagues[46] showed that healthy EBV-positive adults had fewer than 2000 viral genomes per microgram of blood cell DNA, whereas the number of genomes was increased 10- to 100-fold in patients with PTLDs. In a more recent study, Baldanti et al[47] assert that transplant recipients with a quantity of EBV DNA between 1000 and 5000 genome equivalents in blood are at high risk for PTLD development.

The diagnosis and classification of PTLDs are currently based on histologic criteria, and the pathologic examination of tissue is currently the gold standard for PTLD diagnosis. Although excisional biopsy is preferred, fine-needle biopsy is acceptable when larger biopsies are impractical, as in the case of allograft organ biopsy.[5] Clonality can be determined by demonstration of clonal immunoglobulin gene rearrangement or on the basis of clonal EBV in the tumor. The presence of tumor-associated EBV can be determined by DNA analysis or by immunohistochemical staining for EBERs, EBNA, and LMP; finding EBV or a clonal population represents strong supportive evidence for the lesion being a PTLD.[1]

HISTOLOGIC FINDINGS AND STAGING

On microscopic grounds, PTLDs may show a polymorphic or a monomorphic appearance. At molecular analysis, PTLDs can arise either from a variety of unrelated cells (polyclonal PTLDs) or from a single element (monoclonal PTLDs). If a person has several monoclonal PTLDs at the same time, each tumor may stem from one common ancestor cell or, in other cases, each tumor may contain a unique clone. Many of the classification systems proposed in the past have recently been unified in the World Health Organization classification of the tumors of the hematopoietic and lymphoid tissues,[48] which distinguishes 4 main categories of PTLD, each containing some subtypes:

- *Early lesions* are characterized by partial architectural preservation of the involved tissue (lymph node or tonsil and adenoids), polyclonal nature, younger patient ages than with other PTLDs, and usual regression, either spontaneously or after reduction of immunosuppression. These lesions include reactive plasmacytic hyperplasia and an infectious mononucleosis–like presentation.
- *Polymorphic PTLDs* show the full range of B-cell maturation, more frequent monoclonal derivation, and variable response to immunosuppression withdrawal (some regressing and others requiring treatment for lymphoma).
- *Monomorphic PTLDs* correspond to overt monoclonal malignant lymphomas of B-cell or, more rarely, T-cell derivation. B-cell neoplasms include diffuse large B-cell lymphoma (immunoblastic, centroblastic, and anaplastic), Burkitt or Burkitt-like lymphoma, plasma cell myeloma, and plasmacytoma-like lesions. T-cell neoplasms include peripheral T-cell lymphoma (not otherwise specified) and other types.
- *Hodgkin lymphoma and Hodgkin lymphoma–like PTLDs* are mostly observed in patients who underwent allogeneic bone marrow transplantation.

The EBV-associated antigens LMP1 and EBNA2 are detected in the immunoblastic component of most early/polymorphic and monomorphic B-cell PTLDs, respectively.[48] Monomorphic T-cell PTLDs are variably EBV positive, whereas the Hodgkin lymphoma and Hodgkin lymphoma–like forms are virtually all positive.[48] Monomorphic PTLDs could have mutations of the *RAS*, *TP53,* and *MYC* genes, but these abnormalities are rare in polymorphic PTLDs.[48] *BCL6* gene mutations occur in 40% and 90% of polymorphic and monomorphic PTLDs, respectively, and herald a lower sensitivity to decreased immunosuppression.[48] In approximately 50% of cases, multiple organs or sites are involved at the time of presentation.[49] No staging system currently exists for PTLDs. In solid-organ recipients immunosuppressed with azathioprine, PTLDs

usually involve extranodal sites, including the allograft and the central nervous system. Patients receiving cyclosporine or tacrolimus develop PTLDs involving the lymph nodes and gastrointestinal tract but less frequently the central nervous system. Bone marrow allograft recipients tend to have widespread disease at nodal and extranodal sites, including the liver, spleen, gastrointestinal tract, and lungs.[48]

PREVENTION

In the absence of effective therapy for PTLDs, the best strategy for the management of PTLDs is currently focused on prevention. Patients who are at high risk of developing PTLDs should be identified before transplantation. Because primary EBV infection after transplantation is a significant risk factor for PTLD development, EBV serostatus should be determined for all potential transplant recipients.[5] Patients who are also at risk for primary CMV infection or severe CMV disease should be identified because of their increased vulnerability to development of PTLDs. In addition, recipients of lung or small-bowel transplants and patients who receive large doses of immunosuppressive drugs (especially muromonab) for either induction or allograft rejection should be considered to be at a high risk for PTLDs. Such patients should be monitored carefully for clinical and laboratory evidence of EBV infection.[5]

Several studies are investigating the correlation between PTLDs and high EBV DNA loads. In most of these studies,[36,47] high virus loads seem to antedate the manifestation of clinical PTLDs. A standardized monitoring technique for EBV infection in transplant recipients is not available; EBV loads in transplant recipients have been assayed using peripheral blood lymphocytes,[50] whole blood,[51] and serum.[52] The monitoring of EBV loads in patients who are at high risk for PTLDs is a promising technique that would permit preemptive treatment in the form of reduction of immunosuppression or the use of antiviral agents.[5]

Antiviral agents with activity against EBV may be of benefit as prophylaxis for PTLDs. Because CMV disease seems to be a cofactor in PTLD development and ganciclovir has greater activity against EBV in vitro,[53] the use of ganciclovir may be preferable. Historical comparisons of the incidence of PTLDs in patients receiving or not receiving acyclovir or ganciclovir prophylaxis either immediately after transplantation or during antilymphocyte antibody therapy for acute rejection suggest that use of either prophylactic antiviral drug may be of some benefit.[54-56] The role of the passive administration of neutralizing antibodies to EBV through intravenous immunoglobulin therapy is not clear, although results in the animal model of PTLDs are promising.[57] Prospective studies of any of these forms of antiviral prophylaxis for PTLDs are needed.

TREATMENT

The treatment of patients with PTLDs has to be based on the clinical outcomes described in case reports or a limited series of patients. To our knowledge, until now, no controlled trials with therapeutic interventions have been performed.

The most important initial strategy in the management of PTLDs is to reduce and even discontinue, if possible, the immunosuppressive drug therapy, which can lead to regression of the PTLD in 23% to 50% of patients.[43,58] This strategy is efficient for EBV-associated PTLDs occurring within the first year of transplantation, whereas it may have limited application in the setting of PTLDs that occur after that time or in EBV-negative or T-cell tumors.[59] None of these variables—pretransplantation EBV serostatus, clinical presentation, extent of disease, and pathologic features—can definitively predict whether the patient will respond to a reduction in immunosuppression.[5] In 36 cases of PTLD, Cesarman et al[60] demonstrated that *BCL6* gene mutations in transformed lymphocytes are associated with a lower probability of the PTLD to respond to the reduction in immunosuppression.

Surgical resection seems to be useful for the treatment of isolated PTLD lesions, for tumor debulking, or for management of local complications such as gastrointestinal hemorrhage or perforation.[61] Local radiotherapy may also be useful for such lesions, particularly when they occur in the central nervous system.[61]

Because of the associated risk of neutropenia and septic complications, standard cytotoxic chemotherapy is rarely used as first- or second-line therapy for early PTLDs but is frequently used in patients who have failed the more conservative approaches to treatment.[43]

Even if PTLD regression has sometimes been observed after antiviral agent administration, the efficacy of the antiviral therapy (acyclovir or ganciclovir) is under discussion. Antiviral medications may still generally be helpful to prevent recruitment of B cells to the lymphoproliferative process, especially during the early phases of PTLD development. Similarly, immunoglobulin preparations may prevent new infection of cells or may contribute to antibody-dependent cell-mediated cytotoxicity.[5]

Complete long-lasting responses have sometimes been obtained with interferon alfa-2b therapy. The mechanism of action of this drug is not clear, but it is likely that interferon alfa-2b exerts antiviral and antiproliferative effects. The association of T-helper cell type 2 cytokine responses with PTLDs, and their disappearance after PTLD resolution, lends support to the use of interferon alfa-2b to treat these disorders.[62] Indeed, circulating IL-4 and IL-10 levels seem to be useful for monitoring PTLDs and response to therapy, although the specific levels at which therapeutic intervention is appropriate still need to be determined.[63,64] Interferon therapy is associated with a theoretical risk of precipitating rejection because it can up-regulate HLA antigen expression in the renal allograft; however, whether this has any clinical relevance in recipients of other solid-organ allografts or in the setting of profound immunosuppression in patients with PTLDs is uncertain.[5]

The concept of targeted antibodies directed against specific antigens of tumors has intrigued researchers for many years. The

lymphoid malignancies, such as PTLDs, are particularly good candidates for this therapeutic approach owing to identification of the CD molecules that are lymphocyte-specific antigens. The introduction of monoclonal antibodies directed against CD lymphocyte molecules has marked the beginning of a new era in the treatment of lymphoid proliferation.

Monoclonal anti–B-cell antibodies against CD21 and CD24 have been used for the treatment of PTLDs.[65,66] Benkerrou et al[67] recently published the results of an open multicenter trial evaluating the efficacy of these monoclonal antibodies in patients with PTLDs. However, many of the patients treated with these agents had received concomitant or recent therapy with acyclovir, ganciclovir, interferon, or corticosteroids in association with a reduction in immunosuppression. Nonetheless, 20 (65%) of 31 solid-organ transplant recipients achieved complete remission, and only 1 relapsed. However, anti-CD21 and anti-CD24 antibodies are no longer available.

An anti-CD20 antibody (rituximab) preparation approved for use in relapsed low-grade non-Hodgkin lymphoma is currently being tested in patients with PTLDs refractory to reduction of immunosuppression.[5] Rituximab is a chimeric monoclonal antibody directed against the CD20 antigen, which is an excellent target because it is widely expressed on B lymphocytes and does not shed, modulate, or internalize. After binding to the CD20 antigen, the Fc portion of rituximab antibody binds to Fc receptors on effector cells (cytotoxic T lymphocytes [CTLs] and natural killer cells) and triggers a lytic reaction, leading to cell death. The antibody also leads to activation of the complement cascade, resulting in cell lysis. In addition to these mechanisms, which apply to other monoclonal antibodies, targeting the CD20 receptor probably adds a third mechanism of cell death. Although the normal function of this antigen is not completely understood, it is probably involved in regulation of calcium channel activity in the cell membrane. Binding with the anti-CD20 antibody leads to high intracellular calcium levels, which keeps the cell in the G_1 phase and results in maturation arrest and apoptosis. This mechanism of action can explain the synergism seen with anti-CD20 antibody and chemotherapeutic agents.[68] Anecdotal studies[69-71] on the efficacy of this monoclonal antibody in PTLDs have been published. The therapeutic regimens more commonly used consist of doses of 375 mg/m^2 intravenously weekly for a total of 4 weeks. Patients who respond to an initial 4-week course of rituximab can frequently benefit by retreatment with rituximab at the time of progression. In a group of 39 patients with refractory lymphoma who responded initially, the response rate to a repeated 4-week course of rituximab was 40%.[72] The low toxicity and high specificity associated with the use of anti-CD20 make it attractive as a first-line agent for the treatment of PTLDs after reduction of immunosuppression; however, further data regarding its efficacy are required.

Cell therapy represents a recent innovation in the treatment of EBV-related malignancies. Limited data on solid-organ transplant recipients have shown regression of disease or decreased levels of EBV DNA after adoptive transfer of autologous EBV-specific CTLs. The immune response analysis in patients affected by infectious mononucleosis has shown that, in vitro, virus-specific CTLs have lytic activity against lymphoblastoid cellular lines transformed by EBV and that CD8$^+$ cells are responsible for the infection control in vitro. Rooney et al[73] expanded recipient CTLs by culturing them in vitro with recipient lymphoblastoid cellular lines transformed by EBV. In this way, they obtained EBV-specific CTLs, which, after infusion, blocked the viral replication in vivo. The study of more patients and gene marking procedures of the CTL infused could allow definition of the actual efficacy of these agents and the length of the response in vivo. In the bone marrow transplant setting, lymphoblastoid cell lines can be generated in vitro from the bone marrow of the donor and can provide an effective antigen-presenting cell and a source of viral antigen for generation of EBV-specific CTLs. Moreover, most marrow donors have a strong immune response against EBV.[74] Several clinical studies show that T-cell infusions can control life-threatening PTLDs in bone marrow transplant patients where the bone marrow donor is healthy and available to donate a blood sample. In one study,[75] 5 patients with EBV-positive PTLDs, arising after allogeneic T-cell–depleted bone marrow transplantation, received peripheral blood mononuclear cell infusions from their EBV-seropositive marrow donors. Infusions were well tolerated and caused lymphoma regression; however, all recipients experienced severe graft-vs-host disease. Therefore, alloreactive T cells must be removed from peripheral blood mononuclear cells to achieve complete tumor and virus specificity.[75] To determine whether adoptive transfer of donor-derived EBV-specific CTLs can restore the immune response to EBV and prevent EBV lymphoma, Heslop et al[74] administered EBV-specific CTL lines containing gene-marked CD4 and CD8 cells to 14 bone marrow transplant patients. This study showed that transferred T cells not only restored the patients' immune response to EBV but also persisted for as long as 18 months, saving their ability to respond to viral stimulation in vivo. Even if the use of a marker gene allows demonstration of the longevity of infused T cells and their retention of EBV reactivity, it does not permit an estimate of the contribution from endogenous EBV-specific T cells.[74] Further studies have demonstrated that EBV-specific CTLs grown from donor bone marrow can be used to prevent and treat PTLDs after bone marrow transplantation. Rooney et al[76] infused 39 bone marrow recipients with 2 to 4 doses of EBV-specific polyclonal (CD4$^+$ and CD8$^+$) T-cell lines grown in vitro from the bone marrow donors. The prophylactic CTL infusions were administered a median of 3 months after transplantation. Six recipients had high levels of EBV DNA in peripheral blood before CTL infusion, which decreased within 2 to 3 weeks of treat-

ment. None of the patients developed PTLDs during follow-up, whereas historical controls who did not receive CTL prophylaxis had a tumor incidence of 11%.

Alternative immunomodulatory approaches to treat PTLDs include the use of anti–IL-6 antibodies, but currently available data using this approach are limited to patients with myeloma.[5]

CONCLUSIONS

Epstein-Barr virus–positive PTLDs represent a model of virus-associated lymphomagenesis, and a better knowledge of their pathogenesis may allow a deeper understanding of other tumor systems. Because no reliably effective therapy is currently available for this disease, the optimal strategy for the management of PTLDs is prevention. Although anecdotal evidence suggests that several treatments may be useful, they need to be evaluated in more patients. Finally, the definition of a standardized approach to the diagnosis of EBV infection in transplant recipients, perhaps similar to that for CMV infections, is needed.

Accepted for publication November 21, 2002.

This study was supported in part by the Associazione per la Ricerca sulle Malattie Epatiche, Bologna.

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