Clinicopathologic implications of Epstein–Barr virus related B cell lymphoma in immunocompromised patients

J A Thomas, D H Crawford, M Burke

Introduction
Immunodeficiency, whether of a primary or an acquired type, carries a well recognised predisposition to the development of B cell lymphoma. In the context of organ transplantation these lymphomas, now generally known as B lymphoproliferative disease (BLPD), constitute a serious post-graft hazard with a mean frequency of about 10% in all transplant groups and mortality rates relating either directly to tumour or its treatment reported in 38–72% of patients.1 Virtually all BLPDs are associated with Epstein–Barr virus (EBV), a ubiquitous human herpes virus which potentiates B cell growth and immortalisation in vitro and is closely implicated in the aetiology of African Burkitt’s lymphoma in vivo. The long term immunosuppression required to ensure transplanted organ survival also serves to impair EBV immunity, engendering uncontrolled B cell proliferation that may manifest clinically as BLPD. This essentially virus-driven tumour exhibits patterns of clinical presentation, histopathological appearance and therapeutic requirements that are different from B cell lymphomas arising spontaneously in the non-immunocompromised host, which are rarely associated with EBV. Conventional lymphoma therapy with cytotoxic agents may be detrimental to the patient, and current first line BLPD treatment aims to restore EBV immunity through reduction or withdrawal of immunosuppression and treatment with antiviral agents. Notwithstanding rapid tumour regression, which may be complete in some cases, this approach is not entirely successful as it jeopardises graft survival; subsequent tumour relapse is not uncommon.

Transplantation now offers a realistic option for chronic organ and haematological disease and, with its increase, the frequency of post-graft BLPD is likely to rise. The diagnostic and therapeutic dilemmas posed by these tumours underscore the need for well defined guidelines on tumour management. Full understanding and definition of BLPD pathogenesis, risk factors, diagnostic criteria, and assessment of disease progress with treatment can only advance through a multidisciplinary approach involving close interaction between the transplant physician, histopathologist, virologist, immunopathologist, and other specialists at ward and laboratory level. This leader will consider BLPD in light of currently available diagnostic parameters, their importance and limitations for successful tumour management, and future directions for improved clinical diagnosis.

Risk factors for BLPD
The risk factors for BLPD in the United States have been analysed by Penn13 from the annual returns to the Cincinnati Transplant Tumor Registry (CTTR). These fall into two broad categories: those associated with an immunosuppressive regimen and those associated with EBV infection. Both cumulative quantity and type of immunosuppression contribute to the increased risk of BLPD in transplant recipients. Thus, patients on T cell immunosuppressive regimens, including cyclosporin A, FK506 and OKT3, have an increased risk compared with those taking other immunosuppressive drugs; and patients taking high levels of immunosuppressive drugs because of recurrent rejection episodes or regrafting are at increased risk of developing BLPD.

EBV is carried as a persistent infection in around 90% of the adult population. Primary infection generally occurs subclinically during childhood although if it is delayed until adolescence, infectious mononucleosis may develop. A number of small scale retrospective studies of transplant recipients have shown that primary EBV infection is associated with the development of BLPD in around 50% of cases, thus strongly suggesting this as a risk factor for the development of BLPD and identifying an at-risk population in those patients, particularly children, who are seronegative at the time of transplant.

Clinical presentation
Two types of clinical presentation of BLPD have been described and, although many patients do not fall conclusively into one or the other, they are useful for descriptive purposes.4 Firstly, children tend to present with BLPD within one year of transplantation with symptoms reminiscent of infectious mononucleosis, including fever, sore throat and generalised lymphadenopathy. Tonsillar inflammation and enlargement are usually present and are often progressive in the absence of treatment. Tonsillectomy is often performed to prevent laryngeal obstruction. This type of presentation is often, but not always, associated with a recent primary EBV infection.

The second type of presentation is more usual in adults who are EBV seropositive at the time of grafting. Onset may be at any time after transplantation, frequently within the first year, although a mean time of 5–3 years was recorded in one study.4 Tumours are usually single and may be nodal or extranodal; com-
mon sites are the brain, the gut and the transplanted organ. Presenting symptoms are those of a lymphoma and depend on the tumour site.

Serology

The majority of immunosuppressed transplant recipients, including patients with BLPD, have normal or high immunoglobulin antibody levels to the EBV viral capsid antigen (VCA) denoting past infection. High IgG anti-VCA antibody levels are usually accompanied by high levels of IgG antibody to the early antigen complex and this reactivity pattern is termed a "reactivated" pattern although in most cases no disease ensues. Thus, serology cannot be used to diagnose BLPD, although it may be possible to document a recent primary infection, particularly if stored serum samples are available. IgM anti-VCA antibodies and/or positive heterophil antibody (as detected by the monospot test) may be detectable. However, these are often transient, delayed or absent. The best evidence of a primary infection is often the demonstration of a seroconversion, although transfused antibody at the time of transplant can lead to confusion. The documentation of a recent primary infection is useful in predicting disease outcome.

Diagnosis of post-graft BLPD

Tumour biopsy is essential for diagnosis of BLPD and, in decreasing order of relevance to clinical management, its examination for morphological appearance and cell phenotype to identify B cell lymphoma, EBV association, clonal organisation, and cytogenetic abnormality.

Histopathology

BLPD shows a wide spectrum of changes that were first categorised by Frizzera et al. in 1981 and concur with a polymorphous B cell proliferation undergoing transition from a polyclonal B cell hyperplasia to a B cell lymphoma containing non-clonal and clonal tumour cell populations. Common features across this spectrum are nodal obliteration, invasion of normal structures, presence of atypical large cells which frequently resemble Reed–Sternberg cells, and varying degrees of necrosis (single cell/coagulative). Categories identified by Nalesnik et al. in 1988 encompassed lesions with overt polymorphic changes, minimal polymorphism, typified by plasma cell or plasmacytoid differentiation, and less frequently occurring monomorphic tumours exhibiting morphological and cytogenetic patterns similar to African type Burkitt's lymphomas. Features of viral lymphadenitis, widely reported in liver allograft recipients as well as other transplant groups, often accompany lymphadenopathic BLPD and an infectious mononucleosis-like illness. These are a prognostically difficult category. That most cases respond well to BLPD therapy (reduced immunosuppression and antiviral agents) is offset by recurrence and progression of these lesions in others and emphasises the need for reliable biological markers to predict tumour behaviour. By conventional morphological classifications, most BLPDs resemble diffuse high grade lymphoma of centroblastic, centrocytic/centroblastic, immunoblastic (or, rarely lymphoblastic) type. Given the therapeutic implications of BLPD and potential for complete regression, the major aim of histopathological diagnosis is to differentiate it from lymphomas occurring in non-immunosuppressed patients and from non-viral changes. This requires a clinicopathological definition involving the detection of EBV in the neoplastic cells within an appropriate clinical and histopathological setting.

Association with EBV

BLPD tumour cells display an activated B cell phenotype and an unrestricted pattern of latent viral gene products similar to B lymphoblastoid cell lines derived from normal B cells immortalised by EBV in vitro. EBV latency is typified by the presence circular/episomal DNA, abundant non-coding small RNA sequences (EBV encoded RNAs (EBERs 1 and 2)) and expression of eight viral antigens: EBV nuclear antigens (EBNAs) 1 to 6 and latent membrane proteins (LMP) 1 and 2, which are crucial to maintaining viral persistence in B cells and are also targets for host immune recognition. EBNAs 2 to 6 and LMP-1 act as targets for virus specific cytotoxic T cell (CTL) activity. Their unrestricted expression in BLPD, together with appropriate cell adhesion molecules, underlies the process of tumour regression after restoring CTL responses by immunosuppression reduction/withdrawal. African Burkitt's lymphoma has a restricted EBN-1 phenotype which probably permits these tumours to escape host immunosurveillance and progress.

Identification of EBV can be made at the tissue level by molecular (non-isotopic in situ hybridisation (NISH)) and immunocytochemical techniques. These are now readily accessible either as a routine histopathology or specialist laboratory service and form an integral part of EBV/BLPD diagnosis. As most individuals are seropositive for EBV and carry low numbers of latently infected normal B cells, it is mandatory that virus involvement is identified within the neoplastic population. At the molecular level, polymerase chain reaction (PCR) will not make this distinction. Analysis using EBER-1 and -2 NISH has now largely superseded EBV-DNA in situ hybridisation and is compatible with conventionally processed histopathological material. While detection of EBNAs using polyclonal or monoclonal antibodies, anticomplement and conventional immunocytochemistry is limited to cryopreserved tissue, this investigation still has an important place in the diagnosis of BLPD. It is rapid and reliable with a result forthcoming within 24 hours of receipt of the biopsy specimen. Wherever possible, collection of fresh biopsy specimens for cryopreservation is recommended in order to analyse the full
tumour cell phenotype with antibodies to formalin sensitive antigens and for protein immunoblotting, if indicated.

Evidence that a high proportion of lesions show restricted ENBA-2 and LMP expression indicates that phenotypic subsets of BLPD can occur and with further analysis may emerge as an important diagnostic parameter and also account for differences in the response of individual tumours to treatment. In light of this, the use of monoclonal antibodies to LMP, reactive with conventionally processed tissue, are inadvisable as a single diagnostic test for EBV and should always be used in conjunction with other markers; negative LMP staining does not exclude BLPD.

**CLONALITY**

Clonal determination is not necessary for diagnosing BLPD but may be useful for assessing malignant progression. By immunoglobulin light chain restriction analysis, tumours may be polyclonal, monoclonal or exhibit indeterminate immunoglobulin expression. Most cases of phenotypically monoclonal tumours are confirmed by molecular analysis for immunoglobulin gene rearrangement. However, over 30% of phenotypically polyclonal lesions contain monoclonal or oligoclonal populations by immunoglobulin genotyping analysis. Many cases of BLPD are oligoclonal or contain mixtures of clonal and non-clonal populations either within a single lesion or multiple tumours occurring simultaneously in the same individual. Clonal patterns often fail to relate closely with lesion histopathology or treatment response. Generally, monomorphic and exclusively monoclonal tumours respond poorly to first line BLPD treatment requiring intervention with cytotoxic agents and occasionally irradiation, whereas polyclonal, polymorphic tumours tend to respond well. However, high grade polymorphic tumours exhibiting polyclonal, oligoclonal or monoclonal features frequently show rapid regression with BLPD treatment which may be complete and long term. Recurrent tumours need to be monitored for changes in immunoglobulin gene rearrangements to identify malignant progression requiring alternative treatment. EBV genotyping, also a clonal marker, has been shown to correspond closely to immunoglobulin gene rearrangement and to tumour behaviour but as yet is not used routinely for diagnosis.

**CYTOGENETICS**

Identification within the BLPD spectrum of Burkitt’s lymphoma-like tumours with typical t(8;14;22;2) chromosome translocations (involving immunoglobulin and c-myc gene rearrangement) has highlighted the importance of cytogenetics as an integral part of diagnosing BLPD. These tumours must be distinguished from other BLPD types as they are rapidly aggressive clinically but respond well to appropriate treatment with intense chemotherapy from the outset (Hunt et al, 1994, manuscript in preparation). Although most BLPD show a normal karyotype or inconsistent chromosome abnormalities, BLPD karyotyping is important to define new tumour subsets and to identify chromosomally aberrant single cells which may be monitored for expansion as an indicator of malignant progression.

**Recommendations for improved BLPD diagnosis and management**

Post-graft BLPD is not easy to diagnose or to manage clinically. Advances in both of these areas will come from concerted efforts to improve diagnostic criteria, methods of assessment, therapeutic strategies, and to understand the underlying pathophysiology of these tumours through clinically applied as well as basic biological research. BLPD is of particular concern in paediatric transplant populations where there is a relatively high tumour risk but overall lack of consensus for diagnosis and treatment. Recent data from the CTTR indicate that the pattern of de novo malignancy in paediatric transplant recipients is different from adult organ graft recipients, with non-Hodgkin’s lymphoma comprising the majority of all cancers (52%) and mainly affecting children with non-renal grafts. Non-Hodgkin’s lymphomas also arise in congenitally immunodeficient children although the incidence, patterns of clinical behaviour and frequency of EBV involvement has not been comprehensively analysed in this group of tumours.

Recognising that well defined guidelines on BLPD management are particularly important to the success of paediatric transplantation, now increasingly undertaken in the United Kingdom, and for evaluating primary immunodeficiency associated B cell non-Hodgkin’s lymphoma, the UK Children’s Cancer Study Group (UKCCSG) has set up a new working party to assess on a nationwide scale current trends in incidence, clinicopathological features, therapeutic responses, and outcome of childhood immunodeficiency associated B cell lymphoma. The programme will undertake a multiparameter analysis of the histopathological, phenotypic, clonal, cytogenetic, and EBV related characteristics of BLPD arising in all paediatric cases registered with the UKCCSG. Full investigation of tumour biopsy specimens will follow a well defined protocol and involve close interaction between clinicians, histopathologists and other specialists at referring hospitals and designated UKCCSG investigation centres. Through this programme, facilities for investigations such as comprehensive testing for EBV, that may not be in place locally, will be readily available. Results will make an immediate contribution to tumour diagnosis and patient management. Systematised co-ordination of data based on shared clinical experiences in different transplant centres and primary disease groups will be used to formulate criteria for accurate diagnosis and consistent histopathological reporting, to produce new and comprehensive protocols for tumour diagnosis and management within the confines of current available therapy. From this
information, recommendations for alternative treatment strategies will be made which aim to be effective in more patients and to minimise graft loss.


