History and Clinical Features of Burkitt Lymphoma

In 1958, Dr. Denis P. Burkitt reported on a series of 32 children presenting with large malignant tumors of the jaw at Mulago Hospital in Uganda and six other district hospitals.1 The syndrome was notable for starting in the mandible and often spreading to other jaw quadrants, as well as to the adrenals, kidneys, and liver. No involvement of spleen or lymph nodes was detected in these initial 38 patients. Of note, in that initial report, the histopathology was described as “strongly resembling lymphocytes… [and] in some cases the tumor [resembled] a lymphosarcoma.” Definitive classification of this as a lymphoma would await O’Conor and Davies’ description of these and other cases in 1960.2

Of interest, the peculiar distribution of this tumor in the malaria belt of Africa was noted at the outset, with a few cases being reported in North or South Africa, and the bulk of cases coming from tropical areas. To better delineate the regions of endemic tumor, Burkitt and two companions, Drs. Ted Williams and Cliff Nelson, traveled 10,000 miles through ten countries in order to map the tumor endemic areas of Africa.3 They also took this opportunity to measure the extent of other diseases in different areas. The end result of this “geographical biopsy” was a map that showed tumor prevalence only in areas with mean temperatures consistently over 15°C and rainfall over 20 in. per annum. This map very accurately predicted the areas of endemic “Burkitt lymphoma” (BL), as well as intense endemic malaria. This extensive epidemiologic work linking malaria and BL was confirmed in more detailed examinations of differentially affected areas of Africa,4 as well as comparing malaria-protected sickle-cell trait children with controls,5 and in mouse studies of lymphomagenesis.6

Since Burkitt’s original description in 1958, BL has been described outside of the malaria belt of Africa. Interestingly, there are notable clinicopathologic differences between African (or endemic) BL and the sporadic BL described in the developed world.7 Sporadic Burkitt lymphoma represents approximately 1–2% of lymphomas in Western Europe and the USA, although in pediatrics it represents 40% of all lymphomas. Sporadic BL typically involves lymphoid tissue in the terminal third of the ileum or Waldeyer’s ring. Although massive abdominal involvement is not rare in sporadic BL, jaw involvement is distinctly rare. Furthermore, advanced cases of the sporadic type present with bone marrow involvement or circulating lymphoma cells. Previously, these were diagnosed as mature B cell acute lymphoblastic leukemia (ALL) or L3-morphology ALL. The FAB classification of L3 ALL is now considered leukemic presentation of BL in the most recent WHO classification.

More recently, a third clinical subset of BL has been described in patients with immunodeficiency and is most commonly seen in patients with HIV infection.8,9 Immunodeficiency-associated BL is interesting in that unlike other HIV-associated B cell lymphomas it typically occurs in patients with CD4 counts greater than 200 cells per micro-liter. In fact, because HIV symptoms are often absent at this CD4 count, lymphoma can be the AIDS-defining criterion. Compared to other HIV-associated B cell lymphomas, BL patients are typically younger with higher CD4 counts and with a shorter history of HIV infection. Induction of HAART therapy augments chemotherapy in these cases.10 Solid organ transplant patients are another population of patients with immunodeficiency-associated BL. These patients typically present with BL four to 5 years after organ transplantation. Again, restoration of immune function by relieving immunosuppression may augment response to therapy. Stem cell transplant patients are less likely to present with immunodeficiency-associated BL, likely reflecting their lower levels of immunosuppression. Immunodeficiency-associated BL is the subtype to most commonly involve lymph nodes. Of interest, these three clinical subtypes – endemic, sporadic and immunodeficiency-associated – all have similar histologic, immunophenotypic, and molecular presentations, although there are unique features to each.
EBV and Burkitt Lymphoma  
(Also See Chap. 7)

In 1961, soon after Burkitt described this lymphoma, he began collaboration with Dr. M.A. Epstein, a young experimental pathologist. Three years later, Epstein discovered that the endemic BL specimens were all infected by a novel virus that would later be called Epstein–Barr virus (EBV). This was the first virus shown to be associated with a human malignancy. The role of EBV in lymphomagenesis would continue to be unraveled over the next 45 years with active basic research still underway.

Interestingly, although EBV is found in over 95% of endemic BLs, it plays a less significant role in sporadic and immunodeficiency-associated BL. Most series in the literature from Western Europe and North America have consistently shown EBV infection in 5–30% of sporadic BLs, depending on the population in the study. Of note, some developing countries (such as Brazil and Egypt) have shown an intermediate rate of EBV association, with 60–80% of “sporadic” BLs showing EBV infection. Immunodeficiency-associated BL is associated with EBV infection in approximately one out of three cases. These varying rates of infection with EBV in the different clinical subtypes of BL clearly demonstrate that EBV infection is not necessary for transformation to BL. In that case, what is the role for EBV when it is associated with BL?

EBV is a ubiquitous gamma-herpes virus that infects approximately 95% of people in most populations studied and establishes life-long latency in B cells. EBV has evolved a complicated pattern of latency programs, which allows the virus to manipulate B cell differentiation and establish long-term latency in the memory B cell reservoir. There are three patterns of EBV latent infection. Latency III is characterized by expression of all EBV latency genes, including Epstein–Barr nuclear antigens (i.e., EBNA1, 2, 3a, 3b, 3c and LP), latent membrane proteins (i.e., LMP1 and 2), and small noncoding RNAs (called EBERs). This pattern of infection occurs during primary infection of B cells and in EBV+ posttransplant lymphoproliferative disorders (PTLDs). EBV Latency III is associated with B cell activation and proliferation, primarily because of the roles of LMP1 and EBNA2 in B cell transformation. In germinal center B cells, EBV switches to Latency II, expressing EBNA1 and the LMPs. Latency II expression may drive B cell differentiation into memory B cells, the long-term latent reservoir of EBV, in which all latent protein expression may be downregulated. In BL cells of EBV+ BL, only the EBERs and EBNA1 are consistently seen, regardless of clinical subtype. This pattern of expression is considered the Latency I expression pattern of EBV. BLs with LMP2A expression or with EBNA1, 3a, 3b, and 3c expression have been reported, but likely represent a minority of cases.

The precise role of EBV in lymphomagenesis of BL is still debated. The Latency I program of EBV is not associated with immortalization of B cells, and the bulk of evidence indicates that EBNA1 is not transforming. EBNA1 is expressed in all replicating EBV-infected cells and is required for the maintenance and replication of the viral genome. EBV Latency III is associated with immune recognition of infected B cells by cytotoxic T lymphocytes specific for EBV latent proteins and, conversely, immunosuppression may result in the risk of expansion of activated B cells with Latency III EBV expression, as is seen in EBV+ PTLD. On the other hand, Latency I BL cells are immunologically silent and are not recognized by virus-specific cytotoxic T lymphocytes. A Gly-Ala repeat domain within EBNA1 has been shown to inhibit antigen processing by preventing proteasome-mediated degradation. Decreased expression of HLA class I, TAP, and proteasome subunit LMP7 have all been associated with Latency I expression patterns. These factors may play a role in protecting EBV-infected BL cells from immune surveillance and clearance of infected cells by cytotoxic T cells.

EBV infection may also promote cell survival and protect BL cells from apoptosis. Work with the Akata Burkitt cell line has demonstrated a cell survival role for both EBNA1 and the EBERs via virus-induced upregulation of the TCL1 oncogene. EBV-negative subclones of this cell line are more sensitive to apoptosis induction. EBER expression in EBV-negative subclones has been shown to enhance tumorigenicity and resistance of apoptosis. There is evidence that this may occur through inhibition of the IFNα(α)-inducible dsRNA-activated protein kinase PKR. EBV negative subclones have also been shown to increase production of IL-10, a known B cell growth factor.

While these EBV-associated factors may all contribute to lymphomagenesis, none are absolutely required, since most sporadic and immunodeficiency-associated BLs are EBV-negative. In fact, the only required molecular abnormality in BL is abnormal expression of the MYC oncogene. EBV infection, which induces growth and activation of B cells, may produce an environment that is prone to acquiring MYC translocations, which may occur as an error of the somatic hypermutation process during B cell differentiation. Subsequent Myc upregulation may drive the switch from EBV Latency III to Latency I, as well as other events required for evolution to BL. Interestingly, this latter process has been successfully modeled in vitro. Recent experimental evidence supports the hypothesis that a switch from the growth-promoting, immunogenic EBV Latency III to Latency I may be a key event in progression to BL.

The role of malaria infection in the pathogenesis of endemic BL is also not well understood, although it is associated with a 100-fold increased risk of BL. It has been proposed that malarial infection may act as a chronic stimulus of the B-cell system, recruit EBV-infected B cells into germinal centers, and increase the likelihood of MYC translocation.
The malarial parasite *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) contains a cysteine-rich interdomain region 1 alpha (C1DR1alpha), which binds to surface immunoglobulin (IG) and may directly activate B cells. HIV-infection may also serve as a chronic stimulator of B cells, through induction of IL-6 and IL-10. Alternatively, the immunodepressant effects of malaria and HIV infection may allow for the expansion of EBV and the outgrowth of BL cells. None of these hypotheses precisely explains why >95% of endemic BLs are EBV-positive. After over 40 years, it remains a mystery what the precise interplay is between EBV and malaria in the pathogenesis of endemic BL.

**Myc and Burkitt Lymphoma**

In 1972, Manolov and Monolova reported that cases of endemic BL were associated with an additional band on the long arm of chromosome 14. Several years later, George Klein’s group identified that this material was from chromosome 8. In 1982, two separate groups reported that the t(8;14) translocated the *MYC* (c-myc) oncogene to the IG heavy chain gene locus. Since that time, it has been well established that the key molecular event associated with Burkitt lymphoma is translocation of the *MYC* gene on chromosome 8q24 to one of the immunoglobulin gene loci (i.e., *IGH, IGK*, or *IGL*), resulting in the upregulation of *MYC*. The most common translocation associated with BL is t(8;14), resulting in translocation of *MYC* to the IG μ(mu) heavy chain gene (*IGH*) locus on chromosome 14q32. The resulting *IGH–MYC* gene rearrangement is seen in ~80% of BL and has been associated with all forms of BL. Translocation of *MYC* to one of the IG light chain loci may also occur in BL, with ~15% associated with t(2;8) and ~5% with t(8;22), resulting in fusion of the *MYC* gene with the kappa (*IGK*) or light chain (*IGL*) genes, respectively. Although *MYC* translocation is a consistent feature of BL, it is not specific for Burkitt lymphoma and may also be seen in some cases of diffuse large B-cell lymphoma (DLBCL).

Interestingly, although *MYC* gene translocation is associated with all forms of BL, the translocation breakpoints differ in endemic, sporadic, and immunodeficiency-associated BL (see Figure 23.1). In endemic EBV+ BL, the *IGH* breakpoint is within the JH domain and the *MYC* breakpoint is over 100 kb upstream of *MYC*, resulting in fusion of *IGH* control elements upstream of the *MYC* promoter region. In sporadic and immunodeficiency-associated BL, the *IGH* breakpoint is usually within the Sμ(mu) switch domain (occasionally Sγ[gamma] or So[alpha]) and the *MYC* breakpoint is within exon 1 or intron 1, translocating the coding region of *MYC* into the *IGH* locus. In sporadic BL with the variants t(2;8) or t(8;22), the breakpoint is downstream of the *MYC* gene, resulting in the translocation of *IGK* or *IGL* control elements downstream of *MYC*. The use of alternative breakpoints may indicate that *MYC* translocation occurs during different stages of B cell differentiation, and that there may be differential regulation of *MYC* in the different forms of BL. The *IGH JH* breakpoints are not at canonical recombination signal sequences, but are located within J region introns or rearranged VDJ sequences, and are thought to occur during the somatic hypermutation process.

Thus, *MYC* translocation likely occurs as an error of IG somatic hypermutation or class switching, both of which occur in mature germinal center B cells to produce antibody diversity.

The consistent translocation of the *MYC* proto-oncogene to a locus that is upregulated in B lymphocytes suggested soon after its discovery that *MYC* dysregulation is important to the pathogenesis of BL. *MYC* is normally downregulated during cellular differentiation and its upregulation in BL is likely an early event in lymphomagenesis. *Myc* is a transcriptional regulator that affects multiple downstream targets, including genes involved in signal transduction, cell cycle regulation, metabolism, cell differentiation, and apoptosis. The Myc protein is over 430 amino acids and includes several functional domains. The amino terminal transactivation domain contains two conserved Myc family domains, boxes I and II. The carboxy-terminal region includes a helix-loop-helix DNA binding domain and a dimerization domain that binds Max, which is required for Myc transactivation and transforming activity. Myc triggers apoptosis by inducing Bim, which inhibits Bcl-2, and p53 or ARF. Recent studies have identified several microRNA targets of Myc regulation that are dysregulated in BL and may play a role in lymphomagenesis. Although the key downstream genes critical for Myc-induced transformation in BL are not well understood, pathways involved in cell proliferation, downregulation of the immune response, and antiapoptosis are key features of BL, that are likely to play important pathogenic roles.

Interestingly, while wild-type *MYC* is upregulated in many human cancers, in ~20% of BLs, the *MYC* gene is mutated at one of several hotspots in the Box I transactivation domain, including threonine-58 (which is a target for GSK3β phosphorylation). The juxtaposition with IG enhancers may predispose *MYC* to somatic hypermutation. Mutations in the Box I transactivation domain may increase the tumorigenic potential of Myc by abrogating the inhibitory effect of p107, preventing proteasomal degradation, or by preventing activation of Bim and thus inhibiting Myc-mediated apoptosis. Other reported mechanisms of inhibition of Myc-induced apoptosis include mutations in the p53 gene TP53 (which are seen in approximately one third of BLs), alterations in the p53/ARF/MDM2 pathway, and induction of the antiapoptotic kinase PIM-1. Interestingly, BL cells with inactivating mutations in *TP53* have been shown to lack *MYC* Box I domain mutations, suggesting that only one antiapoptotic mechanism may be necessary for the survival of Burkitt lymphoma cells.
Diagnosis of Burkitt Lymphoma

BL has a distinctive histological presentation. At low magnification, the characteristic “starry sky” pattern may be appreciated on hematoxylin and eosin staining. This pattern is composed of a “blue” background of tightly packed, medium-sized, round basophilic nuclei forming the sky on which the “stars” of interspersed tingible-body macrophages are scattered (see Figure 23.2a). At higher magnification, the lymphoma cells are intermediate-sized, monomorphic lymphocytes with scant blue cytoplasm. The nuclei are round with lacy chromatins and multiple small nucleoli (see Figure 23.2b). On touch imprints or aspirate smears, the cells are notable for cytoplasmic lipid vacuoles (see Figure 23.3). There is a rare plasmacytoid variant, that is more common in children and immunodeficiency-associated subtypes. This variant is notable for increased polymorphism with a slightly eccentric nucleus containing a single, central nucleolus. Atypical Burkitt or Burkitt-like lymphoma is a second variant. It is characterized by more pleomorphism in size and shape of the lymphoma nuclei with more prominent nucleoli.

BLs typically express monotypic surface IgM, CD20, CD10, and bcl-6, and have an MIB1/Ki-67 proliferative fraction >95%. They typically do not express TdT, bcl-2, or CD5. The expressions of CD10 and bcl-6 favor a germinal center (GC) origin for BL, which has been confirmed in gene expression analysis of BL cells.59,60 The GC origin of BL is consistent with the finding of somatic hypermutation of the IGH variable region in tumor cells and with evidence that MYC translocation occurs as an error of somatic hypermutation or class switching. This immunophenotype is distinguished from precursor B-cell lymphoblastic neoplasms, in
that BL does not express TdT and shows surface light chain restriction by flow cytometry. While follicular lymphomas are similar to BLs in their expressions of CD10, CD20, and light chain restriction with TdT-negativity, they typically express bcl-2; whereas, BLs do not. Furthermore, the morphology of BL cells is quite distinct from the cleaved cells of follicular lymphoma. Diffuse large B cell lymphomas may be similar to BL, both morphologically and immunophenotypically; however, DLBCLs rarely have an MIB1 proliferative rate >95%.

Occasionally, the sole definitive method to distinguish DLBCL from BL is with molecular techniques (also see Chap. 22). As noted previously, BLs are characterized by MYC translocations – most commonly t(8;14), but also t(2;8) and t(8;22). In lymphomas with morphologic and immunophenotypic features of BL, these translocations are definitive when occurring in isolation. The mainstay of molecular diagnosis of BL is the use of FISH probes which span the MYC locus, allowing for the detection of all breakpoints.70,71 The wide range of breakpoints seen precludes efficient detection of IGH–MYC by conventional PCR, although long-range PCR has been used.72–76 Southern blot hybridization maybe used to detect MYC gene rearrangement, but requires high-quality DNA and multiple probes to detect the various genomic breakpoints.49–51

There have been numerous reports of lymphomas with Burkitt morphology or Burkitt-like morphology that contain a MYC translocation in addition to complex cytogenetics or other translocations, such as the t(14;18) associated with IGH–BCL2 gene fusion that is seen in follicular lymphomas.12 The new 2008 WHO classification of these lymphomas is “large B cell lymphoma with features intermediate between diffuse large B cell lymphoma and Burkitt lymphoma.” These cases may represent a transformation of a low-grade lymphoma, and the MYC-associated translocation generally portends a dismal prognosis.60,77 Likewise, it should also be recognized that there is a group of DLBCLs with MYC gene rearrangement (without a coexistent 14:18 translocation). Whether these lymphomas (i.e., “double-hit” lymphomas and DLBCL with MYC rearrangement) benefit from the Burkitt chemotherapy regimens is still unclear.
Microarray Profiling

As previously discussed, the distinction between BL and DCLBL is not always possible with routine diagnostic techniques and treatment for these two entities is different. Whereas MYC translocation is characteristic of Burkitt lymphoma, it may also be seen in DCLBL and some unusual Burkitt lymphomas lack MYC translocation. Several groups have recently employed microarray analysis to identify genomic or gene expression profiles that may distinguish Burkitt lymphoma from other mature, aggressive B cell lymphomas, including DLBCL (also see Chap. 22).

Hummel et al identified a molecular signature for Burkitt lymphoma (mBL) that was associated with classic and atypical BLs, as well as with several cases that had morphologic features of DLBCL or unclassifiable mature aggressive B-cell lymphomas.60 Most cases with the mBL signature were IGH–MYC positive and lacked other chromosomal abnormalities, including BCL2 or BCL6 translocations. However, a few mBL cases were MYC negative or had MYC translocations to non-IG gene loci. Cases without the mBL signature were more likely to be MYC negative or involve MYC translocation to non-IG loci and to have rearrangement of BLC2, BLC6, and/or other complex chromosomal abnormalities. However, several intermediate cases that lacked the mBL were IGH–MYC positive and lacked other chromosomal changes. Cases with the mBL signature were associated with a better survival rate, regardless of MYC status or the presence of other chromosomal abnormalities, although in multivariate analysis survival could be attributable to young age or early stage.60,78

Dave et al identified a molecular genetic signature for BL that was associated with high level expression of Myc target genes, expression of genes associated with GC B cells, and low level expression of HLA class I genes and NFκB target genes.59 Patients with a Burkitt’s signature had higher target genes. Patients with a Burkitt’s signature had higher free surviving fractions approaching 90% in some series.

Therapy of Burkitt Lymphoma/Leukemia

Key to the improvement in therapy has been the development of shorter intensive courses of chemotherapy with higher doses of alkylating agents combined with intrathecal therapy and careful preventive management of tumor lysis syndrome. The German Multicenter Study Group for the treatment of adult ALL (GMALL) reported results of two protocols for the therapy of adult Burkitt leukemia, that showed 50% (BNHL83) and 71% (BNHL86) disease free survival at 8 and 4 years, respectively. These protocols both implemented a cytoreductive phase to prevent tumor lysis syndrome, followed by six cycles of fractionated cyclophosphamide, methotrexate, and low-dose cytarabine in alternating cycles. CODOX-M/IVAC (cyclophosphamide, vincristine, doxorubicin, high-dose methotrexate/ifosfamide, etoposide, high-dose cytarabine) developed by Magrath is notable for its higher doses of cytarabine and methotrexate, when compared to the BNHL trials. Magrath et al showed that the CODOX-M/IVAC regimen may lead to 2-year event-free survival (EFS) of 75% in children and 100% in adults.79 Unfortunately, the original Magrath regimen was associated with high toxicities, particularly neurotoxicity, mucositis, and severe myelosuppression. Modified regimens with decreased methotrexate and reduced intrathecal cytarabine have decreased the associated toxicities without severely compromising effectiveness (i.e., 2-year EFS of 60% in high-risk patients and 100% in low risk patients, respectively).80

Two other therapy regimens (not based on the BNHL studies) have been used. MD Andersen has reported that hyper-CVAD (high dose cyclophosphamide, doxorubicin, vincristine and dexamethasone alternating with methotrexate and cytarabine) led to a 3-year overall survival (OS) of 49% in an older patient population.81 Subpopulation analysis of patients younger than 60 years old showed a 3-year OS of 77%, which is comparable to the results in the Magrath and modified Magrath regimens. The CALGB regimen (i.e., cytoxenduction with cyclophosphamide and prednisone followed by three cycles of ifosfamide, vincristine, etoposide, cytarabine, methotrexate, and dexamethasone alternating with cyclophosphamide, doxorubicin, vincristine, methotrexate and dexamethasone) was reported to have a 4-year disease-free survival of 50%; however, few patients were able to complete all cycles of therapy, due to severe toxicities, particularly neurologic toxicity. As noted previously, immunodeficiency-associated BL may be treated with high dose intensive chemotherapy, yet it benefits from concomitant HAART therapy in the case of HIV or removal of immunosuppression in solid organ transplant patients.

Autologous bone marrow transplantation (BMT) as consolidation therapy after high-dose chemotherapy has been used with mixed success. One phase II study showed comparable 5-year EFS and OS between standard chemotherapy-only patients and those who received autologous BMT after short intensive chemotherapy (that avoided high-dose methotrexate and cytarabine).82 Nevertheless, the lack of a clear benefit for most patients combined with the additional morbidity from
BMT has prevented the more widespread use of autologous BMT in first line therapy. A European group for Blood and Marrow Transplantation (EBMT) retrospective review of autoBMT, as salvage therapy for refractory or relapsed BL in second or greater remission, showed a 3-year OS of 72% in patients in first complete remission, 37% in chemo-sensitive relapsed patients, and 7% OS in chemo-resistant patients. Allogeneic BMT has had even less critical investigation. Retrospective reports indicate that patients receiving allogeneic BMT did not have a longer OS than those receiving autologous BMT. There have been other case reports, yet no large prospective trials have shown a clear benefit to allogeneic BMT.

New Therapeutic Agents

Rituximab, an anti-CD20 monoclonal antibody that induces apoptosis of B cells, has been added to hyper-CVAD-, CHOP-, and EPOCH-containing regimens with very promising preliminary results. Fayad et al reported that the addition of rituximab to the MD Andersen hyper-CVAD protocol led to a 3-year OS of 89% in BL patients. Epratuzumab is an anti-CD22 monoclonal antibody that in vitro demonstrates a different and synergistic mechanism of inducing apoptosis in B cell lymphomas (than does rituximab). Trials with these and other agents are still underway, but expectations are high for the synergistic actions of immuno-therapy and chemotherapy in BL. Molecular-targeted therapies currently under investigation include histone deacetylase inhibitors, selective serotonin reuptake inhibitors, antisense oligonucleotides to Myc, proteasome inhibitors, and highly active antiretroviral therapy for patients with acquired immunodeficiency syndrome-related Burkitt lymphoma/leukemia. Cancer. 2002;94(5):1492–1499.

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