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Erle S. Robertson *Editor*

Burkitt's Lymphoma

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Preface

The origins of this project began when I was approached by Beverly Griffin, who suggested that I put together a volume on Burkitt's Lymphoma, a problem which is still as dominant in Equatorial Africa as it was 50 years ago. Burkitt's lymphoma was first brought to the spotlight and recognized as a major cancer in the human population in the late 1950s to early 1960s by Dr. Denis Burkitt, a missionary surgeon in Equatorial Africa. The incidence of this disease can vary in different parts of Equatorial Africa, and is epidemic in proportion in this region of the African continent. It is quite concerning and disheartening that this treatable disease is still an epidemic in susceptible African children.

This project aims to bring together a spectrum of ongoing efforts by having a patient-oriented focus from physicians, to diagnostics and clinical implications of the disease as mostly seen in the Equatorial African setting. Importantly, the chapters cover the breadth of studies in Burkitt's lymphoma with some clues for the potential future of studies that can have therapeutic benefits for patients. A volume like this has not been previously completed; so this represents a unique text of its kind.

Additionally, we are grateful for the video documentary on Burkitt's lymphoma that is included in this volume as a compendium to the text. The documentary will give readers a real-life account of the clinicians' and scientists' fight against this deadly cancer, in areas of the world that have less access to first rate medical care. It is still heart breaking to know that in developed countries, where patients have access to the best medical care (if detected early), Burkitt's lymphoma is over 90% curable. However, in countries where access to good medical care is limited or nonexistent, the survival rate is sometimes less than 50%. More tragic is the fact that the time period most affected is during early childhood where most of these patients are from families that are less capable of providing the best medical care. How do we deal with this devastating disease in this setting when we have the ability to enhance care and survival of these young patients? Developed countries in the West have a moral imperative duty to support efforts that substantially minimize and hopefully eliminate this disease in our world.

I'm dedicating this volume, in part, to Dr. Beverly Griffin who has been tireless in her pursuit to improving global exposure to Burkitt's lymphoma. She eventually convinced me that this project should be done, especially with a focus on highlighting the quest of clinicians and researchers in this field which would eventually bring better access to care and greater visibility to this devastating disease.

I would also like to thank the contributing authors who have provided insights and suggestions for topics that should be covered and to take time out of their hectic lives to contribute a chapter. I am grateful to all of them for their tireless pursuit to find therapies and develop vaccines to treat Burkitt's lymphoma.

I suspect that Denis Burkitt would be happy that his initial contribution continues to be pursued, although he may have more immediate questions as to why the available therapies are not available to the population most at risk. I hope that patients, physicians, and scientists are able to use the up-to-date information from this volume, and that it provides a helpful guide to novices including students, residents, and junior investigators who are now thinking about entering this field hoping that they may be able to have an impact.

Finally, a special thanks to Rosemary Rochford for her encouragements, Beverly Griffin for her efforts even during difficult times, and Harald Stein for working with Lorenzo Leoncini in completing their chapter even after having major difficulties which minimized his ability to use his hands. This was admirable and shows the enormous conviction of this group of individuals to one of the world's most devastating diseases affecting mostly children in Africa.

Philadelphia, PA, USA Erle S. Robertson

Contents

Chapter 1
An Introduction to Burkitt Lymphoma

Ian Magrath

The first description of Burkitt lymphoma (BL) was probably that of Albert Cook, the first missionary doctor in Uganda. He founded Mengo Hospital and subsequently Mulago Hospital, initially a center for the treatment of tuberculosis, which eventually became the University Hospital of Makerere University. Cook reported a child with a large jaw tumor who came to Mengo Hospital in 1910, and his illustration of the appearance in his meticulous clinical notes leaves little doubt that this was a case of BL [1]. In the first half of the twentieth century, a number of European pathologists working in equatorial Africa noted the high frequency of jaw tumors, or of lymphomas in children [2–6], but it was Denis Burkitt who provided the first detailed clinical description of the tumor in 1958 [7] while working at Mulago Hospital. He recognized a number of different clinical presentations of tumors in children, including jaw tumors and intraabdominal tumors, that could occur either alone or together, and it was this that led him to believe that many, if not all these children, had the same disease, although up until then girls with ovarian tumors were often diagnosed as having dysgerminomas, while other children were thought to have retinoblastoma, soft tissue, or even bone sarcomas. However, it should be remembered that at the time, pathologists also used the term "lymphosarcoma" such that the title of Burkitt's first paper, *A sarcoma involving the jaws in African children*, in which a brief description of the histopathology was given by Jack Davis, then the head of the pathology department at Mulago Hospital, may not have been as misleading concerning the origin of the tumor cells as would appear to be the case today.

Gregory O'Conor, an American pathologist working quite separately from Dennis Burkitt, recognized, with Jack Davis, around the same time as Burkitt's description that approximately half the cases in the childhood cancer registry that had been established

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in the Mulago Hospital Pathology Department some 7 years earlier were lymphomas [8, 9]. The high frequency of BL observed in Africa, however, was not seen in Europe and the USA, leading to debate as to whether this disease was unique to Africa—many believed that it was, leading to the use of the term "African lymphoma"—but in the mid-1960s, several pathologists described lymphomas in Europe and the USA that were indistinguishable at a histological level, and also, for the most part, clinically, from African BL. This was doubtless because of the selection of children with jaw tumors that resembled those so characteristic of BL in African children, but regardless of this, these observations established that the tumor was not unique to Africa [10–12].

It was not until 1969 that a group of experts in the pathology of hematological neoplasms assembled under the auspices of the World Health Organization decided that the tumor should be defined purely on histological grounds [13]. While seemingly indicating that BL is a single disease, the high incidence in Africa, compared to the USA and Europe, led to the African variety (also common in Papua New Guinea) being referred to as "endemic" BL because of its higher incidence in these two regions. Tumors occurring elsewhere were referred to as "sporadic" although, unfortunately, these terms are often used in different ways such that they are not particularly helpful. In 1984 the observation that HIV infection predisposes to BL [14] led to the inclusion in the subsequently developed World Health Organization classification of hematological malignancies of a third variety of BL—immunodeficiency-related BL (see Fig. 1.1). The histological and immunophenotypic characteristics of BL are are described in detail in subsequent chapters.

Clinical Characteristics

Perhaps the most characteristic feature of BL in equatorial Africa (and New Guinea) is the occurrence of jaw tumors (Fig. 1.2) in young children (less than 5 years, and probably peaking at the age of 3). Why this should be the case is unknown, but the tumors arise predominantly around and within the developing molar teeth (often involving all four jaw quadrants, even if this is not always clinically apparent). Early clinical signs are loose teeth, and the earliest radiological sign is loss of the lamina dura surrounding the developing molar teeth with adjacent lytic lesions, all of which are readily detected by oblique X-rays of the jaws. These tumors tend to grow very rapidly, such that the teeth sometimes appear to be floating on top of the tumor, and although they may be lost, in some patients they settle quite quickly back into their sockets once treatment is begun. At this age, the jaw contains bone marrow tissue, and it is remarkable, therefore, that although the tumor cells infiltrate the marrow of the jaw, it tends not to spread to other marrow-bearing bones and diffuse bone marrow involvement is, therefore, uncommon (less than 10% in most newly diagnosed children). Although orbital involvement is also common at this age, and it has been suggested that orbital tumors arise from the maxilla, they are not necessarily associated with clinical jaw tumors and often do not seriously damage the eye, unless the ophthalmic artery or vein is compressed, or there is direct involvement of the retina—a very rare occurrence.

These characteristic jaw tumors have been described in other countries, even in Europe (at least, in the 1960s or so), and occur at somewhat higher frequency in

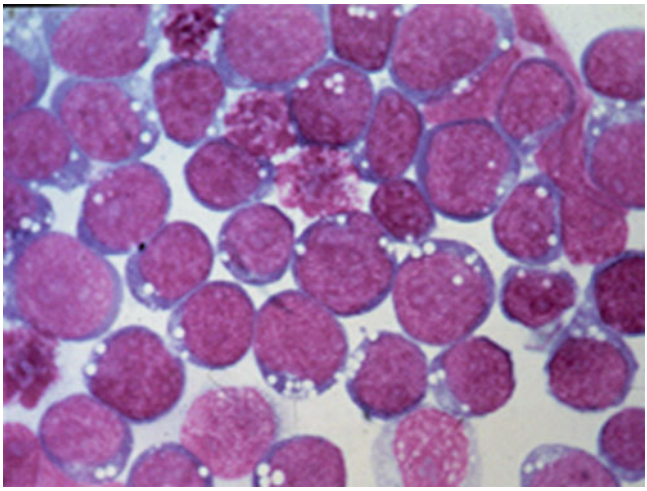

Fig. 1.1 Cytological appearance of BL showing the fenestrated nuclear chromatin with multiple nucleoli, and dark blue cytoplasm containing lipid vacuole. BL is a B cell lymphoma (i.e.,. derived from B cells, which are primarily involved in antibody production). BL expresses surface immunoglobulin, normally IgM, although sometimes IgG. It expresses other B cell markers such as CD20, CD22, CD79 and CD10 as well as proteins associated with very rapidly dividing cells – Ki67 (almost 100% of cells are positive). There is an indistinct dividing line between BL and diffuse large B cell lymphoma, which is reflected in tumors which have a molecular profile (gene expression pattern) that is intermediate between BL and DLBCL. Some of these intermediate tumors are probably derived from follicular lymphomas and have a more complex karyotype, occasionally expressing both the typical BLl MYC/Ig translocations (t8:14) and those found in follicular lymphomas, and some DLBCL, i.e., (t14;18). BL also has a typical gene expression pattern, although once again, intermediate patterns between BL and DLBCL are observed. The BL molecular profile is associated with a good response to intensive combination chemotherapy in countries where this can be given – with a higher survival rate than DLBCL, being in the range of 90–95%

some countries, such as Turkey, or Northern Brazil (and of course, New Guinea, where holoendemic malaria occurs in the river valleys). There is an impression that they were once more common, but today are vanishingly rare outside equatorial Africa and New Guinea. The reason for this is unknown. The high frequency of jaw tumors in young children is not the only difference in the clinical distribution of the tumor in endemic tumors versus tumors occurring elsewhere. In the former, frequent sites of disease include the salivary glands, ovaries, endocrine glands, and retroperitoneal structures, especially the kidneys [15]. Intraabdominal disease is the second most frequent site of involvement in African BL (Fig. 1.2) and the most frequent in all other world regions. Testicular involvement, extradural tumor causing cord compression, and malignant pleocytosis of the cerebrospinal fluid and cranial nerve palsies are also seen in a significant fraction of cases, but interestingly, peripheral lymph node involvement is uncommon as is involvement of the bone marrow or spleen, although splenomegaly is often present because of holoendemic malaria. In Dennis Wright's series of 50 post-mortem cases of BL, the most commonly involved organ was the kidney, and he clearly demonstrated the rarity of significant splenic involvement [16]. It was also possible to demonstrate, at post

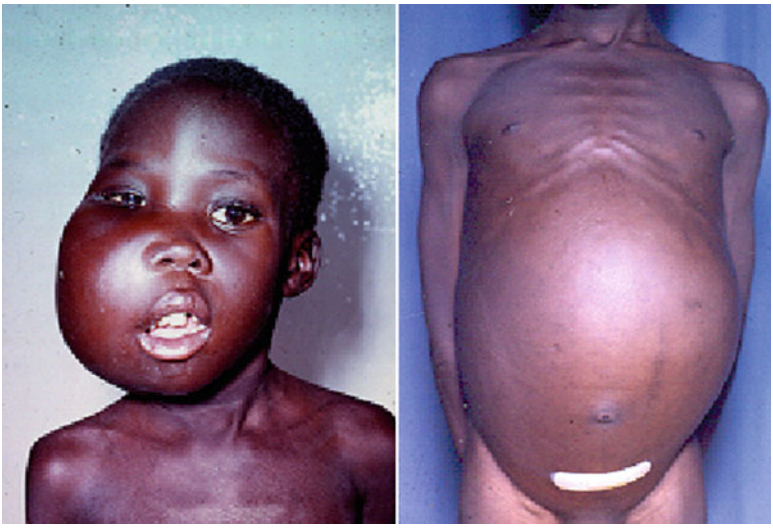

Fig. 1.2 The two most common sites of involvement in African BL—the jaw (*left*) and the abdomen (*right*)

mortem, that cranial nerve involvement was due to infiltration of the nerve by tumor cells—a situation reminiscent of Marek's disease in chickens, a diseases caused by a Herpesvirus. Bowel and mesenteric involvement is frequent (and lymph nodes in the mesentery adjacent to tumor sites in the bowel, most often the ileum may or not be invaded by tumor). Presentation with right-sided abdominal pain, suggesting appendicitis, or acute severe abdominal pain resulting from ileo-ileal intussusception appears to be much more common outside equatorial Africa, although occasional African cases have been described. Interestingly, involvement of the breast occurs particularly in pubertal girls or lactating women [17], suggesting that hormonal or growth factors are involved in creating an appropriate microenvironment in the breast for BL cells—the microenvironment probably also accounts for the high frequency of jaw tumors, and the differences in this respect between equatorial African and children elsewhere may well account for the observed differences in the sites of involvement of BL in different geographical regions. There can be little doubt, however, that at a global level, the abdomen is the most frequent site of involvement, sometimes accompanied by varying degrees of ascites, which can be massive, or involvement of other serous membranes such as the pleura or pericardium. In general, BL occurs particularly in areas where mucosal-associated lymphoid tissue is found and could be considered as a subtype of aggressive MALT lymphoma.

Epidemiology

Early estimates of the incidence of African BL in children (0–14 years) are quite variable, ranging from a few cases per 100,000 to as high as 18 per 100,000, but more recent figures suggest that the incidence in equatorial Africa is similar, in

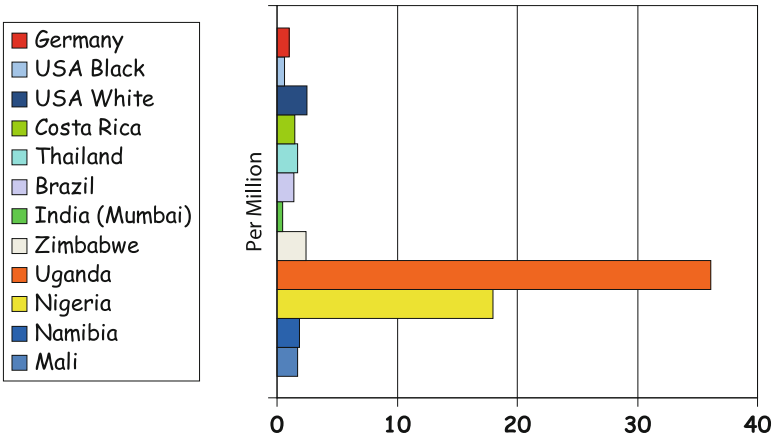

Fig. 1.3 Incidence of BL in selected countries. Data from the International Agency for Research on Cancer (1998)

children, to that of acute lymphoblastic lymphoma—the commonest childhood malignancy in European countries and the USA—probably of the order of 2–6 per 100,000 in children of 0–14 per year. However, case ascertainment is far from reliable, the quality of pathological diagnosis is variable and the incidence often varies within countries, possibly depending upon the local intensity of malarial transmission (see below). Good incidence figures are limited from most world regions, although in the USA and Europe, incidence is probably of the order of 1–3 per million—considerably lower than that of equatorial Africa (Fig. 1.3) [18]. Other world regions appear to have an intermediate incidence, although, once again, the paucity of population-based data, and the variable quality of the data must be taken into consideration in drawing such conclusions. This issue is further compounded by the definition of BL, since the use of microarray techniques does not result in precisely the same dividing lines between diffuse large B-cell lymphoma and BL as does pure histology. Nonetheless, there is no doubt that (a) BL is much more common in equatorial Africa than in other world regions and (b) the incidence varies throughout the world, probably due to differences in environment, and particularly, differences in exposure to particular infectious agents.

Because of its rarity outside Africa, Burkitt was curious with respect to the distribution of the tumor within Africa. He began to indicate on a map places where children with jaw tumors had been seen and sent 1,000 brochures to government and mission hospitals throughout Africa, using the information to plot the "lymphoma belt" shown in Fig. 1.4. Early publications had interested several research organizations in the tumor and Burkitt was given several grants, totaling £700, which enabled himself and two friends, Ted Williams and Cliff Nelson, both missionary doctors, to undertake a safari to define the southern limit of the high incidence zone on the eastern side of Africa. Burkitt and his coresearchers set off from Kampala on October 7, 1961 in a 1954 Ford station wagon and returned 10 weeks later, having visited some 57 hospitals in 8 countries and traveled 10,000 miles. In addition to

Fig. 1.4 The lymphoma belt extends across Africa approximately 15° north and south of the Equator, indicating a temperature and rainfall barrier to the occurrence of African BL, and suggesting that a disease transmitted by an insect vector predisposes to, or is causally related to the occurrence of BL

personal visits he and his colleagues had sent out a large number of leaflets showing pictures of the disease and to ask whether children with large jaw tumors and/or abdominal masses were frequently seen in that region. What came to be known as the "long safari" showed the southern limit of the high incidence region in the eastern part of Africa to be Lourenço Marques in southern Mozambique. As more information became available, it became clear that the "African lymphoma" had a high frequency in a broad band across equatorial Africa. At first, this was thought to be an altitude barrier, but later, it became clear that the height above sea level at which BL occurred became progressively lower as one moved either to the north or south of the equator, and that what appeared to be an altitude barrier was, in fact, a temperature barrier. Alexander Haddow, working in the Entebbe Virus Research Institute, also in Uganda, observed that the distribution was very similar to that of several virus diseases vectored by mosquitoes, such as yellow fever and various Arbor virus diseases, and it seemed quite likely that BL was caused by a virus vectored by an insect [19, 20]. Similar findings were reported by Booth from New Guinea, the other region where BL was known to have a high incidence [21]. However, Dalldorf proposed, in 1964, that malaria may well be implicated in the pathogenesis of the disease, since the distribution of BL corresponded not only to the distribution of malaria (not greatly different from that of other mosquito-borne infections) but also to the intensity of malarial infection [22, 23]. Subsequent observations have confirmed the relationship between the incidence of BL and the intensity of malarial infection

Malaria and BL

Among the many insect-vectored diseases in equatorial Africa, malaria (predominantly *Plasmodium falciparum*, the most severe form) has one particular and unique attribute, which provides a potential mechanism for its ability to predispose to BL—it induces B-cell hyperplasia. Equatorial Africa and New Guinea are holoendemic malarial regions (i.e., regions where essentially the entire population suffers from the disease). In holoendemic regions, >75% of children have splenomegaly and >60% of <5 years olds have parasitemia at any given time. Transmission is throughout the year (as opposed to hyperendemic malarial regions, where transmission may be limited in the dry season) and spleen and parasitemia rates are <70% in children less than 5 years. Most deaths from malaria occur in children <5 years, particularly in the first 2 years of life, and 75% of deaths from malaria occur in Equatorial Africa.

The particularly high frequency and severity of malaria in young children could explain the age distribution of BL in Africa. Malaria causes polyclonal elevation of immunoglobulins, IgM being elevated only in infants, but IgG being persistently raised thereafter, and also an increase in B-cell autoantibodies and an eventual loss of B-cell memory. In fact, malaria initially preferentially activates the B-cell memory compartment via a Plasmodium membrane protein known as cysteine-rich-inter-domain-region 1alpha (CIDR1α), expressed on the red cell surface. It can also induce virus production from such cells [24, 25]. This is almost certainly relevant to the increase in Epstein–Barr virus (EBV)-containing circulating B cells that occur in acute malaria [26, 27], which could be caused either by infection of other B cells by EBV or by inducing replication in the memory B-cell compartment . It is interesting that EBNA1 (see below) is only expressed in replicating memory B cells, not resting cells [28], creating yet another connection between malaria and BL, although by no means providing a definitive explanation for this relationship.

A Role in the Induction of Genetic Change?

In addition to its ability to cause B-cell hyperplasia, which could, on the basis of chance alone, increase the risk of a genetic change leading to BL, it is possible—even probable—that malaria has a direct role in the production of the chromosomal translocations associated with BL. This results from interactions with Toll-like receptors, which are part of the adoptive immune system. Toll-like receptors are expressed on a variety of cell types including monocytes/macrophages and mature B cells and are activated by T-cell independent, highly conserved antigens, such as lipopolysaccharide and CpG-enriched DNA that are present in a large number of microorganisms. The adoptive immune system is linked, via Toll-like receptors, to the adaptive immune systems, since Toll-like receptors are able to induce activation-induced cytidine deaminase (AID) in B cells, an enzyme which causes hypervariable region mutations and class switch recombination as well as B lymphocyte activation [29–31]. TLR9 receptors, for example, are expressed at all stages of

B-cell differentiation and ligand binding has been shown to result in the induction of AID, and in turn, class switching in all such cells regardless of the presence of VDJ joining. TLR9 agonists include hemozoin, produced by malaria parasites from hemoglobin, as well as CpG-enriched DNA. They bind to B cells in the course of acute malaria, leading to B-cell hyperplasia and class switching, regardless of the stage of differentiation of B cells. It is the ability of AID to cause DNA breaks between the heavy chain constant regions, an essential component of class switching, that leads occasionally, via erroneous re-ligation, to the genesis of chromosomal translocations or other genetic defects [32].

In primary B cells, the expression of the catalytically active form of AID has been shown to lead to *MYC*/Ig translocations, similar to those which occur in BL (see below) within a matter of hours [33]. These translocations are normally prevented by the tumor suppressor genes ATM, p19 (ARF) and p53, consistent with the ability of these genes to inhibit progression through the cell cycle and to initiate DNA repair or apoptosis in the presence of DNA damage, although the particular genes that protect against translocations varies with the translocation partner [34]. The development of translocations involving MYC is also inhibited by the pro-apoptotic genes PUMA, BIM, and PKCδ and enhanced by the anti-apoptotic genes BCL-XL and BAFF, while FAS-induced apoptosis is involved in the elimination of cells in which a functional class switch does not result. It is clear that inactivating abnormalities in protective pathways that normally induce cell cycle arrest and apoptosis in the presence of inappropriate regulation could lead to the persistence of chromosomal aberrations, including translocations. In this regard it is interesting that mutations in p53 are common in BL [35, 36]. There is also direct evidence, in mice at least, that the occurrence of MYC/IgH translocations similar to those occurring in B cell tumors is dependent on AID [37].

Finally, there is evidence for the induction of RAG1 and RAG2 in peripheral blood B cells in malaria [38], and although there is no definitive information that these enzymes, responsible for the normal rearrangement—and rearchitecture, e.g. in the case of autoreactivity, of the variable region of the immunoglobulin molecule [39]—are involved in the pathogenesis of BL, they may mediate at least some of the chromosomal translocations, particularly those occurring in the VDJ region of the immunoglobulin gene.

In spite of these experimental observations, there is no direct evidence that malaria is important to the pathogenesis of equatorial African BL. The most suggestive evidence is the correlation between the incidence of BL and the intensity of malaria transmission (Table 1.1) [40, 41]. This was first observed not long after the distribution of BL had been mapped in Uganda [41], and several investigators have confirmed these findings. Of particular interest in this regard are experiments of nature—the absence of BL in arid regions within the so-called "lymphoma belt" running across equatorial Africa, and alterations in the incidence of BL associated with the control of malarial infection. Thus, in the late 1960s, malaria had been essentially eradicated from the Zanzibar archipelago off the coast of Tanzania, and BL too, was noted by Burkitt to be essentially absent. Soon after, the eradication program was halted (it was felt that total success had been achieved), and BL

Table 1.1 BL is an aggressive B cell lymphoma with cells intermediate in size between diffuse large B cell lymphoma (DLBCL) and follicular lymphoma. BL cells typically have fenestrated nuclear

Malarial intensity	BL incidence rate	95% CI
Lake endemic	3.47	1.30–9.30
Endemic coast	1.67	0.56–4.27
Highland	1.22	0.46–3.17
Arid/seasonal	0.58	0.26–1.27
Low risk	–	–

Based on a 10-year retrospective review described in [40]

rapidly returned. Similarly, the administration of chloroquine prophylaxis against malaria to children in the North Mara region in Tanzania was associated with a reduction in the incidence of malaria, and a return to its previous incidence after cessation of the clinical study [42]. Some critics, however, noting a fall in the incidence of BL prior to the introduction of chloroquine have questioned the validity of these findings. More recently, malaria has again been eliminated from Zanzibar, and it would be of great interest to determine whether the incidence of BL has fallen correspondingly. It has also been known for some time that individuals with sickle cell trait and thalassemia are protected against severe malaria, such that it might be expected that individuals with these inherited hematological disorders would also be protected against BL. Although trends in this direction have been noted, statistical significance has not been demonstrable.

Epstein–Barr Virus

The distribution of BL in Africa suggested to Haddow, Burkitt and others that BL could be caused by an insect-vectored virus, a notion that was entirely consistent with the several animal tumors known to be caused by viruses at the time, although no human tumors caused, or even associated with a virus, had been described. In the early 1960s, following a lecture by Denis Burkitt on the African lymphoma in March, 1961 at the Middlesex Hospital in London, Epstein, a young microbiologist at the Middlesex Hospital, discussed with Burkitt the possibility of searching for virus particles in the tumor cells using the then quite recently developed technology of electron microscopy. Although no viruses were observed in fresh tumor cells, the delayed delivery of one particular sample was such that by the time it reached the United Kingdom, the tumor cells were growing as a continuous cell line in the media it had been sent in. When Epstein examined the cloudy medium, which he assumed was a result of infection, he saw that the cloudiness was caused by tumor cells freely floating in the tissue culture medium [43]. He examined the cells, which proved to be able to grow continuously in culture, by electron microscopy, and was able to rapidly establish the presence of an unusual type of Herpesvirus (unusual in that it appeared to be present in only a small percentage of the cells, and that the majority of cells appeared to be healthy).

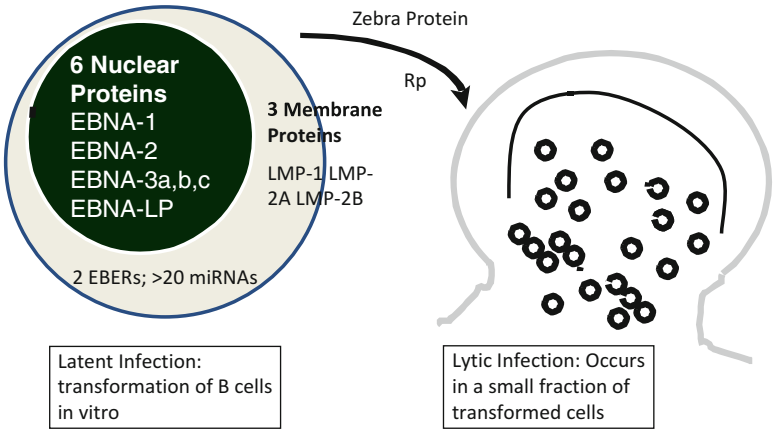

Fig. 1.5 Latent and lytic cycles of EBV showing expression of latent genes (six nuclear proteins and three membrane proteins) and viral non-coding RNAs—2 EBERs and more than 20 microRNAs. Viral structural proteins are not present in the latent cycle, but develop once the lytic cycle is initiated by expression of the Zebra protein, which is necessary and efficient, but is usually accompanied by the expression of other proteins such as "R"

It has subsequently become clear that all BL cells nearly always contain multiple EBV genomes [44] and that essentially all cells in culture express latent genes, i.e. either EBV nuclear antigens (expressed in the cell nucleus) or latent membrane proteins (expressed in the cell membrane) [45, 46]. EBV latent genes (Fig. 1.5) are necessary for the persistence of the virus in B cells (and possibly other cell types) throughout the life of the individual. The primary location of virus persistence is the memory B cell, and the latent genes can be thought of as ensuring that cells containing viral genomes are able to survive in situations in which uninfected cells would not. Normal B cells which do not make high affinity antibody undergo apoptosis when passing through the germinal center of lymphoid tissue, in order to hone the immune response, and ensure that only high-affinity antibody producing cells enter the memory B-cell pool. During this process, a large fraction of normal B cells undergo apoptosis because only those that make high affinity antibodies to the antigen that triggered their proliferation (functioning here as cell surface receptors) receive the necessary viability signals, including antigen and CD40 that are required for them to survive. It seems likely that EBV-infected cells, by virtue of the functions of their latent genes, can avoid undergoing apoptosis even if they do not make high affinity antibody, and thus EBV is assured of entering the B-cell pool, where it can persist in the individual for life. More detailed information regarding the functions of latent EBV genes and their role in virus persistence and the causation of a number of diseases has been published in numerous reviews [28, 46–48].

Persisting in the Population

The persistence of viral genomes in memory B cells is not enough, of course, to ensure survival of the virus in the human population. Virus propagation to other individuals requires the production of virus particles (in the viral *lytic* phase), which are released into the saliva, presumably largely from transformed cells present in pharyngeal lymphoid tissue—cells which may release their virus even as they die as a consequence of detection by T cells sensitized to various EBV latent antigens. Virus that is present in saliva can easily be passed on to other individuals. The switch from latent to lytic phase is triggered by the Z or Zebra gene (Fig. 1.5). It is probable that propagation via saliva leads to the earlier infection of individuals in lower socioeconomic groups in which exchange of saliva is more likely to occur. For example, in African populations, mastication of food by the mother during the weaning process is common, particularly in rural settings, where soft baby foods are not available or are prohibitively expensive. However, the production of virus particles during the "lytic cycle" results in cell death, and is, therefore, incompatible with neoplasia. Even the expression of the full range of latent genes, involved in the "transformation" of normal B cells, inducing proliferation of the infected B cells and expanding the virus pool in the infected individual, is a dangerous proposition since the uncontrolled proliferation of such latently infected cells would be the equivalent of neoplasia. Hence efficient immune responses develop shortly after primary infection against the latent EBV antigens, especially EBNA3a, 3b, and 3c, such that in the absence of mechanisms to overcome the immune response, cells transformed by the gamut of latent genes are rapidly destroyed by T cells [49]. In a number of inherited or acquired immunodeficiency states, the infected cells can, in fact, cause death from the quasi-neoplastic process (which may progress to true neoplasia) that can occur in such circumstances. Evidence has subsequently been acquired that the expression of other viral proteins (particularly the EB nuclear antigen 2) is likely to be detrimental to tumor cells, both because of their immunogenicity and because their expression appears to be inimical to *MYC* overexpression [50] (see below). The pathways to the immunodeficiency-associated neoplasms and classical Burkitt lymphoma appear, therefore, to differ considerably.

After the discovery of EBV, it soon became clear, as the result of a collaboration between Epstein and colleagues, and the virologists Werner and Gertrude Henle, working in Philadelphia, that antibodies to the virus capsid antigen (VCA) of this new virus (which was referred to as EBV by the Henle's, after the cell line in which it was first detected), were ubiquitous in human populations, although it tended to infect individuals of higher social class at a later age than children of low socioeconomic status [51]. A finding of particular importance was the approximately eightfold higher geometric mean titer of anti-VCA antibodies in patients with BL compared to controls [52]. The chance occurrence of infectious mononucleosis in a technician whose blood was frequently used as a negative control for the immunofluorescence tests that the Henle's developed (initially for VCA) led to the observation that the virus was the cause of a high fraction of cases of infectious mononucleosis [53]. Although of great interest, this finding did not shed light on a

possible role for EBV in the genesis of BL, since infectious mononucleosis is nearly always self-limiting. Miller and others subsequently showed that EBV was able to transform circulating B lymphocytes and produce continuously growing cell lines in vitro [54]. This led to the hypothesis that EBV was the causal factor of BL and was responsible for driving proliferation of the tumor cells, although this hypothesis was short-lived for a number of reasons. For example, the latent genes induce a cytotoxic immune response that normally ensures that such an event does not occur. Moreover, the ubiquity of the virus indicated that other factors must be involved in the genesis of BL, since clearly, only a very small fraction of infected individuals develop BL. Further studies also demonstrated that the virus is not transmitted by insect vectors, and that none of the latent genes expressed in B cells transformed in vitro, except EBNA1, were expressed in fresh BL cells, although the majority of cell lines, grown in vitro soon revert to the expression of all six latent viral proteins and three latent membrane protein genes. As information accumulated, it became clear that the nuclear protein, EBNA1, was responsible for the persistence of EBV genomes in the form of intranuclear plasmids, and their equal distribution to daughter cells—thus ensuring the maintenance of the EBV genome in transformed cells. The expression of EBNA1 in BL suggested that the virus is required for the maintenance of the neoplastic state (although it could not be excluded that in some cases of EBV negative BL, the virus could have been lost from the cell after onocogenic genetic changes had occurred, thus rendering the presence of virus-derived molecules superfluous).

In fact, African cases are almost always EBV+ whereas only a small fraction of cases in Europe and the USA contain EBV, and a significant fraction of EBV cases are seronegative for EBV, suggesting that the patient had never been infected by EBV. It also became clear that in addition to EBNA1, in EBV+ BL, small untranslated RNAs including microRNAs from the BART and BHRF1 regions of the genome and the so-called "EBERs" are also present in tumor cells [55]. Thus, it seemed probable that EBV, and particularly early (i.e., at a young age) infection with EBV, predisposes to the development of BL, although it was not clear how. A much greater understanding of the survival strategy of EBV had been gained in recent years, however [47], and Thorley-Lawson, in particular, began to consider the possibility that BL might use its B-cell transforming ability to gain access to the immune system, then switch off all its protein products in order to avoid detection and elimination by T cells such that it could persist throughout the life of the individual "invisible" to the host, or at least, to T cells generated against EBV latent antigens [28]. Whatever the mechanism of avoiding detection by the immune system, it may well be relevant to the pathogenesis of BL. Support for this hypothesis was provided by the initial observation that only EBNA1 could be detected in circulating B cells (it subsequently became clear that some virus containing circulating B cells fail to express any viral antigens), and that EBNA1 is the sole protein expressed when such cells replicate (this would be essential to ensure the persistence of the virus in the cell clone). This pattern of latent gene expression was remarkably similar to that observed in BL, but even EBNA1 is immunogenic, such that its persistence in BL cells could result in elimination of the tumor.

The demonstration that EBNA1 contains a glycine–alanine repeat region which inhibits the expression of EBNA1 in the context of class I major histocompatibility antigens (which are also expressed at low levels in BL cells), and hence makes it difficult to for EBV-infected cells in the B-cell memory compartment to be detected by the immune system—even though EBNA-1 reactive T cells may be present, e.g., generated via antigen presentation after B-cell death by reticular dendritic cells. The blocking of the ability of CD8 cytotoxic T cells to react with EBNA-1 present in BL provided a possible explanation for how EBV-containing B cells can escape immunosurveillance. This has been further bolstered by the recent demonstration of the lack of an intereferon gamma CD4+ T-cell response against EBNA1 in Africa children with BL [56] even though in other circumstances, such CD4+ T cells can be detected [57]. This suggests a rather comprehensive impairment of the ability of immune cells to detect the presence of EBV in the context of BL cells [49] and raises the possibility that variable ability to generate immune responses against EBNA-1 could be one of the factors relevant to whether or not BL develops in a particular individual.

In an attempt to demonstrate that EBV is responsible for the pathogenesis of BL, Geser and colleagues undertook a large study in the West Nile district of Uganda, where they collected serum from some 42,000 children and stored it, assuming that some of these children would subsequently develop BL. In fact, 14 children did develop BL in the course of the next several years and all had higher anti-VCA antibody titers to EBV than did normal controls at the time their first serum was drawn, sometimes a few years prior to the development of BL [58]. This suggested strongly to the authors that EBV was likely to be the causal factor of BL, but although there can be little doubt that it is implicated in a high fraction of tumors around the world—being present, for the most part, in some 95%+ of equatorial African cases and more than half of the cases in most published series from outside the highest income countries (Europe and North America being exceptions), the mechanism whereby EBV predisposes to BL remains unknown.

The presence of typical somatic hypervariable region mutations in the antibody genes of EBV-containing memory B cells [28] strongly suggests that these EBV-containing cells have passed through the germinal center, where such mutations are induced by the enzyme AID [59]. Most probably, the route to the peripheral blood is predominantly via tonsillar or at least pharyngeal lymphoid tissue—the closest to its usual point of entry (via saliva) into the body. It seems probable that the ability of several EBV genes, and potentially untranslated RNAs, to prevent apoptosis ensures that the virus-containing cells are protected while passaging through the germinal center, whether or not they have encountered antigen, and thus avoid diversion into the apoptotic pathway that ensures the elimination of cells which produce lower affinity antibodies to the epitopes to which their immunoglobulin molecules are directed. However, this process would need to be associated with the switching-off of latent genes by the time the cell leaves the germinal follicle, since it would no longer be protected from apoptosis (or, indeed, immune destruction) in the periphery.

Persisting in the Individual

Traversal of the germinal center may be an absolute requirement, as suggested by Thorley-Lawson, for the entry of EBV-containing B cells into the memory cell compartment, where they are sheltered for the life of the infected individual, with only occasional need for replication to maintain the particular cell clone [28, 48]. If so, then the pattern of latent and lytic gene expression might well be: (a) infection of naïve B cells with initial expression of all latent genes, thus transforming the cells, increasing their numbers (and the numbers of virus particles), and protecting them from apoptosis as they pass through germinal follicles in order to enter the memory B-cell compartment (some may become plasma cells, which can produce virus). These cells will eventually reach the memory cell compartment and become small resting lymphocytes, whether or not they have been stimulated by antigen (which can be substituted for by LMP2a). What prompts their occasional replication to maintain numbers is unknown, but it is this replicating memory cell that expresses EBNA1. In (b) infection and transformation of secondary B cells results in cell lysis due to the action of antigen (and epitope) specific T cells, infection of more naïve B cells and also passage of virus into the saliva for transmission to other persons. From the perspective of neoplasia, the point is that the cells are protected from apoptosis whilst in the germinal center, where hypervariable region mutations and class switching occurs, such that aberrant ligations (that can result in tumorigenic chromosomal translocations) do not (always) induce apoptosis and can persist. This hypothesis is supported by the demonstration that several EBV genes, and potentially untranslated RNAs are anti-apoptotic [60].

An Alternative Latent Gene Expression Pattern in BL

Quite recently, a second pattern of latent gene expression in BL was observed by Rickinson and colleagues, who showed that as many as 20% of BLs in Africa express all the latent viral proteins except EBNA2, a gene critical to the transformation of normal lymphocytes [61]. This provides further evidence that EBV infection does not drive proliferation in BL cells, although why this alternative pattern of EBV latent gene expression, which includes the immunogenic proteins EBNA3a, 3b, and 3c should exist is a matter for speculation. Whether or not it is relevant to normal EBV biology is unknown, but the alternative latency pattern in BL also appears to be anti-apoptotic, and can, in the presence of appropriate genetic lesions, give rise to neoplasia [61]. But if EBV is not the driver of neoplastic proliferation, what is? There is little doubt that a major factor in the genesis of BL is the ectopic expression of MYC, caused by a chromosomal translocation resulting from aberrant immunoglobulin gene recombination between *MYC* on chromosome 8 and IgH t(8:14) or, more uncommonly, light chain immunoglobulin genes t(2:8) and t(8:22) or, rarely, other genes. The same pathophysiological impact, however, may, on occasion, be brought about by epigenetic regulation of *MYC* rather than a

translocation, e.g., via the inappropriate expression of specific miRNAs. These gross cytogenetic changes result in a molecular profile that is specific for BL and clearly distinguishes it from diffuse large B cell lymphoma, although some intermediate patterns can also be observed.

AIDS-Associated Burkitt Lymphoma

Ziegler et al. described the increased incidence of non-Hodgkin's lymphomas (NHLs) in homosexual males in 1984 [62], and subsequently of BL [14]. Since then, the relationship between NHL and HIV infection has been confirmed in many parts of the world, including, for example, South Africa. It remains uncertain, however, how much HIV infection predisposes to BL in equatorial Africa. In fact, the relationship is tenuous at best, at least in children, since although a few percent of children with BL are HIV positive, this is similar to the frequency of HIV infection in children in the normal population. Similarly, although HIV infection is more prevalent in adults, the degree to which it predisposes to BL in equatorial Africa is uncertain [63]. HIV is known to alter the immune response to malaria, resulting in increased prevalence and severity [64], and this could, in turn, affect the probable influence of malaria on BL in equatorial Africa, although it would be expected, from the arguments discussed above, to result in an increased predisposition to BL. HIV infection also causes B-cell hyperplasia, and, like malaria, increases the proportion of circulating EBV-containing cells and results in the reactivation of EBV infection, thus increasing the EBV load in HIV-infected individuals [65–67]. However, the memory B-cell population is reduced in HIV infection, and other B cells may become the primary EBV reservoir [65]. Thus, even though HIV+ individuals have a higher EBV load than HIV-persons, the failure to see an obvious and marked connection between HIV infection and predisposition to BL in holoendemic malarial regions, as occurs in non-malarial regions, could possibly arise from the suggestive evidence that B-cell hyperplasia of the memory cell compartment is central to the pathogenesis of BL, and that the differences in the hyperplastic B-cell populations in malaria versus HIV infection could possibly explain differences in the relative proportions of EBV+ and EBV–BL.

It will be of interest to investigate the influence of highly effective antiretroviral therapy on the incidence of BL in Africa. Because BL in HIV+ patients is not associated with severe immunodeficiency, it is even possible that partial immunological reconstitution through the administration of antiretroviral drugs may eventually lead to an increased risk of BL or other forms of aggressive B-cell lymphoma, although this has not been reported to date. Meanwhile, the limited resources in equatorial Africa pose significant difficulties on studies of this kind [68], and it will be important to develop improved pathological diagnosis, better tumor registration as well as facilities for storing human tissues in order to further understand the relationship between HIV infection and BL in equatorial Africa.

Deregulation of MYC

In 1975, a characteristic chromosomal translocation, in which the *MYC* gene is translocated to the immunoglobulin locus, was discovered by Zech et al. [69]. Subsequently, variant translocations, in which the *MYC* gene is translocated to the light chain loci on chromosomes 2 (kappa) or 22 (lambda) were also indentified. Interestingly, *MYC* is not expressed in the majority of cells that reside in normal germinal follicles, indicating that MYC expression is ectopic in BL, assuming that its cell or origin is the centroblast of normal germinal follicles [70]. The presence of hypervariable region mutations in BL strongly suggests that BL cells have passed through the germinal center, as does their pattern of gene expression (at an mRNA level). Further, differences in the average number of hypermutations and small differences between the gene expression pattern of equatorial African and European Burkitt lymphoma suggest that there may be differences in the pathogenesis of these tumors [71, 72], although the most recent miRNA profiling data suggests that the three subtypes of BL are very similar to each other, while clearly differing from diffuse large B-cell lymphoma [73]. Regardless of these findings, there can be little doubt that BL cell proliferation is driven by *MYC* expression, which in turn is usually the consequence of a chromosomal translocation involving immunoglobulin genes (heavy or light) [74] and *MYC*. In fact, it could well be that the rarity of other genetic abnormalities in BL [75] results from the profound effect of ectopic *MYC* expression in germinal center cells. Rarely, an alternative partner to immunoglobulin genes has been identified, and even the absence of a translocation. In such cases, epigenetic lesions or the inappropriate expression of miRNAs) could also lead to ectopic *MYC* expression [76]. In otherwise normal cells, such a major abnormality would almost certainly lead to programmed cell death—indeed, inappropriate *MYC* expression has been known for many years to be capable of initiating the apoptotic pathway in a number of cell types, presumably to avoid inappropriate cellular proliferation, and more recent work indicates that this process is mediated by the pro-apoptotic protein BIM, which can be upregulated by MYC [77]. Point mutations in the *MYC* gene, which has been identified in BL, have been shown to deactivate this pathway, thus inhibiting a mechanism that protects against the genesis of MYC-driven neoplasia [76, 77]. Indeed, protection against apoptosis is required in normal cell types undergoing somatic hypermutation or class-switching, since, as discussed earlier, AID carries a risk of predisposing to chromosomal abnormalities via the production of double-strand breaks. Defects in this mechanism may be relevant to BL pathogenesis, since there is good evidence that AID is involved in the translocations that result in ectopic MYC expression in BL and the potential for MYC-driven neoplastic cell proliferation [78–80]. It has been suggested that the ability of both HIV and malaria to induce B-cell hyperplasia may also counterbalance the tendency of EBV containing B cells bearing *MYC* translocations to undergo apoptosis increasing the likelihood of their emergence as BL [81].

Although there is much to be learned regarding the relationship between potential environmental factors and the pathogenesis of BL, it seems highly probably that the *MYC* translocation is the driver of proliferation, but that other factors, related to

environmental agents such as malaria and possibly HIV, both of which cause B-cell hyperplasia and interfere with immunity and control of the proliferation of EBV, thereby increasing the EBV burden, are important in allowing such genetically damaged cells to persist and may even increase the likelihood of them arising in the first place. The need to rearrange DNA, through a physiological recombinational process in order to generate a tightly binding variable immunoglobulin region and to allow class switching, and the need to prevent apoptosis from occurring during this process, creates a weak point that is likely to give rise on rare occasions to inappropriate recombinations, some of which have the potential to create a neoplastic cell. The experimental evidence supporting the role of AID in mediating chromosomal translocations favors this hypothesis. The details of the pathogenetic events may still need to be worked out, but there is little doubt that the germinal center, a location where apoptosis must be particularly carefully balanced against proliferation, is a critical region for tumorigenesis, precisely because of its importance in the differentiation of B cells. Passage through the germinal center seems to be critical to tumorigenesis in BL and probably also to the genesis of other lymphoid neoplasms, just as it may be to the establishment of a reservoir for EBV. In this case, tumorigenesis can be viewed as an aberration of physiological events, the likelihood of which is increased by the presence of environmental agents such as malaria and EBV, which exploit them for their own purposes, increasing the risk of an aberrant recombination, while removing the defense mechanism that should ensure that cells containing such aberrations (normally a consequence of an abnormal pathophysiological event) are destroyed. Yet the creation of a tumor is not in the best interests of the microorganism, and in comparison with the numbers of people infected with these agents, BL is a rare event indeed, demonstrating the degree to which these parasites have adapted to their human host.

Epidemiology: The Demonstration of Activity of Single Agents

BL was one of the first tumors to be shown to be curable by chemotherapy alone, thus providing critical support to pioneer chemotherapists who, at the dawn of the chemotherapy era, were often maligned for prolonging the misery of patients who were ultimately doomed to die, although occasional cases were cured by radiation or surgery. In the case of African Burkitt lymphoma, surgery was rarely an option although heroic surgery had, from time to time, been attempted when tumor appeared to be localized—for example, to a single jaw quadrant. These attempts invariably met with failure, and usually even more distress to the patient. Radiotherapy was not then available in equatorial Africa (even now, there are very few radiotherapy facilities in this region, and certainly those that do exist are grossly insufficient to provide for the needs of cancer patients), but in any event, radiation was later shown to be of essentially no therapeutic value. By the late 1950s, however, a number of chemotherapeutic agents had become available and several were known to be particularly active in childhood acute lymphoblastic leukemia (ALL). It was clearly of considerable interest to know whether Burkitt's lymphoma responded to chemotherapy in

the same way. Investigators in Africa, such as Burkitt in Uganda, Clifford in Kenya and Ngu in Nigeria, aided by pioneer chemotherapists, including Oettgen and Burchenal from the Sloan-Kettering Institute for Cancer Research in New York, and Alexander Haddow and David Galton from the Chester Beatty Institute in London set out to examine the response of Burkitt's lymphoma to chemotherapy, supported by drug donations from companies such as Lederle, Asta Werke, Eli Lilly and Roche, as well as grants and other support from the Sloan-Kettering and Chester Beatty Institutions, and the National Cancer Institute in Bethesda, Maryland. While clinical trials at the time were performed in a rather haphazard manner, and treatment of most cancers resulted, at best, in transient tumor responses, in the case of BL, tumor regression of significant degree was observed within a matter of days (in fact, changes in the tumor, e.g., less stretching of the skin overlying jaw tumors could even be seen within 24 h).

In the course of the early 1960s, most of the available cytotoxic agents were explored. Although a significant fraction of patients was lost to follow up, the administration of a rather wide range of drugs in the course of time led to the clear demonstration that BL was highly chemotherapy-responsive, and Burkitt in Uganda, Clifford in Kenya and Ngu in Nigeria reported some astonishing apparent cures with minimal therapy (several years of disease-free survival after only one or two cycles of therapy) [82–84], although such impressive responses were much more often seen in patients with localized jaw tumors than extensive tumor, for example, in the abdomen. Of particular note was the rapidity of response—tumors would shrink within days, and in the case of jaw tumors, teeth, although sometimes lost, could even find their way back to the socket from which they had been displaced by tumor.

Much of the data collected in this first era of the chemotherapy of Burkitt's lymphoma was summarized in a meeting that was sponsored by the International Union Against Cancer (UICC) (now, the Union for International Cancer Control) that took place in Kampala, Uganda, in 1966. While diagnosis in the 1960s was not as accurate as today, in equatorial Africa a very high fraction of all lymphomas in children (over 80%) are BL, and the clinical features, particularly the presence of jaw tumor (some of the studies were carried out exclusively in patients with jaw tumors) is generally very distinctive. Thus, there can be little doubt that in the series described the diagnostic error rate was small.

Over the years, Burkitt collected a series of 88 patients with jaw tumors treated in Uganda (two relapsed with separate jaw tumors in other jaw quadrants and were dealt with separately, making a total of 90 jaw tumors). Many of these patients achieved long-term survival with only one or two doses of drugs [85]. A variety of doses and sequences of different drugs were used; for example, one and two doses of cyclophosphamide (60 patients were given 30–40 mg/kg IV, or the same dose given orally over 3–4 days), several days of methotrexate (17 patients received 1 mg/kg daily for 4–5 days), or one or two doses of vincristine (21 patients received 0.07–0.15 mg/kg). The period between drug doses regardless of the drug was 1–3 weeks. Among these patients, 36 had "total or virtually total tumor regression," another 38, "significant but only partial regression" and the remainder "little or no

response." Thus 74, or 82%, of the 90 tumors had a clear response. How many of these patients had disease at other sites in addition to jaw tumor is not clear, although the jaw tumors were classified as small, moderate or large (grades A, B, or C). Complete, durable remissions were observed with all three agents and notably, all 10 patients with small tumors achieved complete remission. Since 16 of 40 patients with moderate tumors and 10 of 40 with large tumors also achieved excellent responses, this early data suggested a relationship between response and tumor size, although it was some years before this was verified, perhaps because some of Burkitt's patients had undetected tumor outside the jaw. Burkitt also noted that recurrent disease, whether at the same or different sites, did not occur after 11 months of remission—now a well-known characteristic of Burkitt lymphoma. Fourteen patients were known to be alive and well a year after treatment and were probably cured, but 38 patients were lost to follow up.

Interestingly, only four patients in Burkitt's series had received more than two doses of therapy and four had received only a single dose. It was also noted, however, that of 12 patients who relapsed after an essentially complete response, 6 developed central nervous system involvement, and many years of empiric approaches to its prevention were required before the predisposition to relapse in the CNS was overcome. In spite of this, as was demonstrated later by Ziegler et al. [86], that almost half of all patients with CNS disease, either at the time of relapse or presentation, could achieve long-term survival. This clearly indicated that CNS disease per se was not necessarily, as was believed in western countries, an obstacle to cure [87].

Ngu also noted that disease extent and site influenced the outcome of treatment. Patients with tumors localized to the facial bones had better responses than those with visceral or CNS involvement—the beginnings of a formal staging system. Patients with CNS involvement, not surprisingly, had a particularly poor response to intravenous cyclophosphamide, although extradural masses were seen to respond. Another observation made by Ngu was that serum uric acid levels were often raised in patients with extensive tumors, and sometimes became even more elevated following therapy. He described a patient who died quite probably from acute tumor lysis (serum uric acid on the day of death was 54 mg per 100 ml), and reported that this and other complications, such as perforation of the bowel, and in one unusual case, of the arch of the aorta, may ensue from rapid necrosis of tumor following therapy [88]. Indeed, in these early series, a significant fraction of patients died before any chemotherapy could be given—due, no doubt, to very advanced disease at the time of presentation, a problem that persists to the present day.

Clifford's observations regarding response to therapy were similar. Among 51 patients 8, 4 treated with cyclophosphamide and 4 with melphalan or orthomerphalan, achieved complete continuous remission, 3 for over 2 years, but 9 patients died from hematological toxicity (7 of these had received methyl hydrazine or mannitol myleran) and 5 from other treatment complications. In a later follow-up, Clifford reported 11 long-term survivors. All had been treated with either cyclophosphamide, or melphalan, sometimes with orthomerphalan in addition [83].

These early results laid the foundation for subsequent studies. Cyclophosphamide, orthomerphalan, melphalan, methotrexate and vincristine were effective drugs, all

capable of inducing complete remission and potentially long-term survival. Chlorambucil, nitrogen mustard, vinblastine and several other drugs were much less promising, at least, at the doses used. Although there appeared to be an association of outcome with disease extent, documentation of disease sites was largely based on clinical examination, supplemented in some patients by bone marrow examination (at least in Clifford's series) and various X-rays, although not in a uniform manner. This may well account for the fact that while Ngu and Burkitt believed that tumor extent was a relevant factor in determining outcome, this was less apparent in Cliffords smaller series. In part, this relationship may have been obscured by the fact that a number of patients died from complications of treatment. Clifford did, however, observe that long-term survival was more likely in stage I patients. Because of the variability of dosage and route of administration, none of the authors were able to draw valid conclusions regarding the relationship between drug dosage and response rate. In part for this reason, as well as the observation of spontaneous remissions, and temporary remissions induced by the infusion of patient serum, an early attempt at immunotherapy, the notion that the host response was critical to success was widely accepted and Clifford, in collaboration with the Klein's and Stjernswärd reported a positive relationship between the presence of serum antibodies reactive with the membranes of BL cells in vitro and response to chemotherapy [89]. This appeared to indicate a "host-versus-tumor" effect, but since the nature of the antibodies is unclear and this data was not confirmed, it is not possible to interpret its significance. Additional studies of immunotherapy using BCG failed to show a clear effect on tumor response, in spite of augmented delayed hypersensitivity [90], and it must be concluded that there is presently no clear evidence of a role for the importance of a host response in the outcome of treatment for BL. In fact, there is considerable information, as briefly mentioned above, that the presentation of foreign antigens to the immune system by BL cells (e.g., EBV antigens) is impaired. With the development of improved staging systems, which probably largely reflect the tumor burden [91], there could be little doubt that outcome correlated with the extent of disease, although the latter, in turn, also influences the ability of the immune system to respond to foreign antigens [92].

The Evolution of Combination Chemotherapy

Denis Burkitt left Uganda in 1967, but his work was continued by means of an agreement between the National Cancer Institute of the USA and the University of Makerere in Kampala, Uganda, to establish the Uganda Cancer Institute. This led to more systematic attempts to improve the results of treatment, although interpretation is difficult in most of these studies because of their small size, limited staging studies and the fact that patients were almost certainly undertreated, such that recurrent disease sensitive to the same or different therapy was extremely frequent. Nevertheless, these early clinical trials were sufficient to extend the observations made previously in Uganda, Kenya, and Nigeria, and it is worth pointing out that even today, few African institutions are able to conduct clinical trials and there is

limited collection of data of any kind. It is probable that most patients die for lack of any therapy, while others have inadequate therapy based only on what they can afford. Supportive care also remains inadequate except in a small number of elite institutions, usually in the private sector.

A number of randomized trials comparing treatment approaches were conducted in the 1960s and 1970s. Unfortunately, the numbers of patients randomized in each trial tended to be very smal, and, coupled to the limitations of the staging system used in early studies (stage III included patients with a broad range of tumor burdens, including some with completely resected disease), these studies left much to be desired. But it must be remembered that these were the early days of clinical trials, and in spite of their deficiencies, a number of interesting observations were made. An excellent response to therapy in patients with recurrent disease was often observed, particularly if the relapse occurred 10 weeks or more after the initiation of therapy (or the previous relapse) [93]. One patient had six relapses, including CNS recurrence, before achieving disease-free survival for 4 years. In these circumstances, the only realistic measure of success was overall survival, which, in spite of the difficulty of interpreting the studies, appeared to be better in patients who had received multiple drugs rather than cyclophosphamide alone, and responses were certainly seen to a combination of drugs including methotrexate, vincristine, and cytarabine (BIKE) in patients who has failed cyclophosphamide [86]. Of note was the fact that corticosteroids were not included in these regimens (which is a standard practice today in most treatment protocols), although there is one major exception—CODOX-M/IVAC, which gives similar results to other intensive regimens. Conversely, doxorubicin is a standard component of childhood B-cell lymphoma regimens, but its value is only now being tested.

In Ghana, similar results were achieved with either cyclophosphamide alone, or a drug combination that included vincristine and cytarabine [94]. The latter regimen, however, proved rather toxic. Among 103 patients who received cyclophosphamide as a single agent (two doses were given initially), 79 (77%) achieved CR. Two more patients achieved CR after VCR and MTX were added. Among those who achieved CR, 42 relapsed—very similar results to those obtained in Uganda. Patients who relapsed were given additional cyclophosphamide; 21 of 40 patients achieved a second CR following CTX alone, rather more among those who relapsed late (12 weeks or beyond) than those who relapsed early. These same 40 patients went on to receive the BIKE regimen designed in Uganda, and 9 achieved long-term survival.

Subsequently, Nkrumah and Perkins used a simultaneous combination of cyclophosphamide, cytarabine, and vincristine in patients with intraabdominal disease but without CNS involvement [95]. All patients received a single dose of intrathecal therapy with each course of combination therapy. Nkrumah and Perkins did not perform a randomized study, but included 42 consecutive patients admitted between April 1973 and September 1975, and compared them with a previous group of 44 patients with abdominal Burkitt's lymphoma treated between January 1969 and March 1973, who received cyclophosphamide alone, given at a dose of 40 mg/kg i.v. at 2–3 weeks' intervals for a total of 2–4 doses, but no intrathecal therapy. Of the patients treated with the three-drug systemic combination, 31 completed the 3 cycles of therapy (11 died