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A Comparative Review on the three variants of Burkitt Lymphoma

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Abstract

Burkitt lymphoma (BL) is a form of non-Hodgkin's lymphoma that begins in the immune cells. This disease is widely recognized for its quick tumor growth rate, rapid fatality when left without treatment, and its rather ambiguous infection mechanism. Despite these challenges, development of BL has been linked to the presence of the Epstein-Barr virus (EBV) and various synergistic disease factors. This review is focused on finding new evidence to differentiate between the characteristics of the three clinical variants of BL: endemic, sporadic and HIV/AIDS associated. Previous reports have shown that the development of endemic BL in African children is likely to be aided by co-infection of cells with *Plasmodium falciparum* and EBV. While sporadic BL is often believed to be independent of EBV infection, alternative detection modalities have demonstrated that removal of viral DNA occurs after genomic instability and constitutive expression has been activated. Lastly, patients with HIV/AIDS are more prone to lymphoma development because of their immunosuppression, characterized by low levels of CD4 cells and EBV-specific humoral responses. We emphasize the differences among the pathogenic profiles of the three clinical variants of BL to aid in treatment development.

Keywords: Burkitt Lymphoma, Epstein-Barr Virus, non-Hodgkin's lymphoma

1. Introduction

Burkitt lymphoma (BL) is a form of cancer centered in the lymphatic system, that specifically affects the B-cells of the humoral immune response [17]. Responsible for secreting antibodies to label foreign invaders, B-cells are created in the bone marrow, and travel to lymphoid tissues for maturation and differentiation. Found in three distinct clinical variations, the purpose of this review is to distinguish between the routes of pathogenesis and synergistic factors that result in BL development. Similar to all three variants, Burkitt lymphoma is characterized by a chromosomal translocation between the intact Myc gene (*c-MYC*) on chromosome 8 and various immunoglobulin loci located on either chromosome 2, 14 or 22 [11]. This translocation is markedly detrimental because it results in the decreased regulation of the *c-MYC* oncogene, which is a regulator of cell proliferation, differentiation, and metabolism [22]. This over-expression of *c-MYC* has further repercussions in that it leads to constitutive expression of at least 27 additional genes ranging from cyclin D2 to nucelolin and fibrillarin proteins [2].

Development of Burkitt lymphoma is closely linked to infection of B-cells by the Epstein-Barr virus, a microbe found globally and known to cause the commonly encountered mononucleosis. Responsible for initiating genomic instability within B-cells, EBV is capable of immortalizing cells through the expression of nine viral proteins [7]. In the latent form, the virus replicates as a circular episome, utilizing the origin of replication, oriP, cooperatively with the essential viral protein EBNA-1. To transition into the lytic cycle, the virus employs a different origin of replication, oriLyt, which causes the genome to become linear [25].

Endemic Burkitt lymphoma (eBL), found primarily in African children, ages 4-7, is caused by EBV infection of cells, and is also highly associated with recurrent infection from *Plasmodium falciparum*, the microbe responsible for

malaria. Alternatively, sporadic Burkitt lymphoma (sBL), has thus far presented no substantial causation between EBV infection and cancer development. We hypothesize that this lack of evidence is due to the conventional EBV screening methods that primarily seek intact viral genomes or measure specific protein expression levels in sporadic tumors. In the third variant, AIDS Burkitt lymphoma, HIV/AIDS patients are more susceptible to developing BL after infection by EBV, likely due to their compromised immune system characterized by low levels of CD4 cells (T-cells). T-cells are responsible for targeting specific invaders that have been previously labeled by B-cells for destruction. In addition, living with HIV/AIDS causes the dysregulation of B-cells by continuous immune activation, which in turn causes a build-up of exhausted B-cells that exhibit high levels of inhibitory receptor gene expression [10].

2. Endemic BL

The link between endemic Burkitt lymphoma and EBV infection has been previously confirmed and represents one of the first examples of a virus-associated human cancer [1]. Although all three variants are characterized by a *c-MYC* translocation with an immunoglobulin heavy chain, the breakpoint is unique to each subform of BL. The DNA from isolated tumor cells show that the eBL translocation results in the greatest distance between the transcriptional start site and the first coding exon, relative to the distances resulting from sBL and AIDS-BL [3]. As demonstrated *in vitro*, the initiation of EBV infection occurs once EBNA-1, a viral homo-dimeric DNA-binding protein, facilitates the necessary chromosomal tethering through its N-terminal AT-hook [4]. From here, replication of the viral genome leads to increased expression levels of viral proteins that has major repercussions in human B-cells. In healthy individuals, B-cells exist in a resting state, which can be quantified by low levels of cellular markers CD23, CD30, CD39, and CD70. When it is time for routine replication, B-cells receive antigenic or mitogenic stimulation, resulting in a significant, yet highly regulated, increase in these cellular markers. Young and Murray found that upon EBV infection, the levels of CD23, CD30, CD39, and CD70 were considerably increased, pointing to the hypothesis that EBV drives the immortalization of B-cells by abusing the same biochemical pathway a healthy cell uses to proliferate [23].

Along with B-cell immortalization, EBV is responsible for inducing chromosomal instability. Genomic instability has been assessed by comparing the overall percentages of metaphases with dicentric chromosomes in both EBV negative and positive cell lines. Dicentric chromosomes are chromosomes that received two centromeres during meiosis, and can be used as an indication for chromosomal abnormality. They found that among EBV negative cells, only 1.1% had dicentric chromosomes, representing the random error that occurs during cell division. Conversely, EBV positive cells expressing latency membrane I proteins had 3.2% dicentric chromosomes and those expressing latency III proteins had 8.7%. Similar proportions were observed for chromosome fragments and chromatid gaps in these different treatment groups [8]. As dicentric cells attempt to replicate, the two centromeres tend to travel in opposite directions which leads to the bridge-fusion-breakage (BFB) cycle, characterized by repeated trials of chromosomal breakage and rearrangement [12]. By combining the ability to evade apoptosis with increased mistakes made during cellular division, EBV exhibits the ability to increase the frequency of oncogenesis.

Despite the virus' worldwide ubiquity, the factors contributing to the unique distribution of eBL in Africa warrants additional study. One supported hypothesis is that co-infection of *P. falciparum*, the parasite responsible for malaria, facilitates lymphatic oncogenesis [15]. The World Health Organization estimates that 88% of all malaria cases are found in sub-Saharan Africa. Development of malaria, in turn, causes a severe polyclonal B-cell activation, characterized by increased levels of autoantibodies, hypergammaglobulinemia, and the loss of B-cell memory capabilities. If this parasite co-infects a cell that is host to a latent EBV genome, it may synergistically aid in the necessary *c-MYC* translocation by reactivating the viral lytic cycle [20]. In the same right, malarial infection causes the reduced ability of B-cells to defend against viral replications. One study examined children between the ages of 5-9 living in areas with high levels of recurrent malaria infections. Overall, they had fewer EBV-specific IFN-γ responses than children of the same age bracket in countries lacking high malarial rates [14]. IFN-γ, a specific type of cytokine, is a signaling protein responsible for interfering with viral replication by activating macrophages and killer T-cells, as well as increasing antigen presentation. Thus, with the ability to reactivate EBV replication, as well as weaken the specific cellular machinery responsible for keeping EBV replication confined, *Plasmodium falciparum* seems to be the hidden piece that explains the unique distribution pattern of eBL across Africa.

3. Sporadic BL

Sporadic Burkitt lymphoma represents 5-10% of clinical BL cases, yet the association with EBV infection has only been linked in 15% of these cases [4]. The *c-MYC* translocation is still present in this variant, however the breakpoint is usually located in the first coding exon or intron of the immunoglobulin gene. Defined as a sporadic disease, sBL naturally sparks the interest of biologists determined to expose the pathogenesis as something more than random chance. Razzouk et al. took on this task by examining the involvement of EBV in nine tumors isolated from Americans. Using conventional EBV detection methods, they had categorized one of the nine tumors to be associated with viral infection. This conventional detection method identifies the presence of Epstein-Barr Encoding RNA (EBER) via *in situ* hybridization because there is often a large number of EBERs within infected cells [21]. However, they demonstrated *in vitro* that the loss of an intact EBV genome can occur after lymphatic oncogenesis has already begun. Alternatively, they employed PCR screening for specific EBV restriction sequences created by *Bam*HI, and found partial EBV genomes in four of the nine original tumors [18]. This result suggests that some mechanism of viral DNA fragmentation and removal exists. Coined a "hit-and-run" strategy, the need for viral DNA after the necessary translocation has occurred may be trivial. This mechanism of initial infection and eventual removal of viral DNA has already been hypothesized in adenovirus carcinogenesis. Nevels et al. demonstrated that the transitory expression of certain adenoviral proteins are necessary to introduce genomic instability and initiate oncogenesis, yet do not need to be maintained during tumor formation [16]. Mainly, these proteins are involved in transcriptional activation and suppression of p53 (an essential tumor suppressor protein), explaining their necessity at the preliminary steps of viral infection [6]. Confirming their hypothesized "hit-and-run" strategy, the tumor cell lines did not contain any viral proteins or detectable DNA sequences at the end of the study.

Another group of researchers looked at the effect of EBV DNA rearrangement in lymphatic tumor cells and found that it causes the constitutive expression of BZLF1, a transcriptional transactivator. This constitutive expression of BZLF1 demonstrates the protein's ability to bind and physically interact with p53, as well as to cause the partial elimination of EBV episomes [24]. Once the carcinogenic mutation has occurred and polyclonal B-cell expansion of these infected cells has been initiated, there is minimal need to maintain the presence of the EBV genome within tumor cells. These findings indicate that, due to insufficient detection methods, it is conceivable for EBV to be implicated in a higher percentage of sporadic cases of Burkitt lymphoma than previously thought.

4. HIV/AIDS BL

The final variant of BL is in immunocompromised individuals suffering from either HIV or AIDS. HIV/AIDS leads to an accumulation of exhausted B-cells in peripheral blood circulation because the continuous immune activation causes their deregulation. Dysregulation is characterized by increased expression of B-cell inhibitory receptors such as FCRL4 and Siglec-6 [10]. In turn, this lack of regulation allows for exhausted B-cells to proliferate without responding to cellular checkpoints. They also fail to carry out their routine antigen detection and binding duties that are pivotal to the humoral immune response [13]. It is this immunodeficiency that leaves patients more susceptible to the development of BL because infected EBV lymphocytes, which are accumulating mutations, can propagate unchecked without fulfilling their humoral role [5]. In addition, as patients live longer with HIV/AIDS, their immune system continually becomes more compromised, which explains why older patients are more at risk to develop BL. Specifically, one study examined the manifestation of HIV/AIDS and showed that it induced the production of cytokines (IL)-6 and (IL)-10, which are signaling molecules that lead to the increased proliferation of B-cells [3].

To further validate this cooperation amongst AIDS-BL and EBV infection, the pathogenic genetic alterations were demonstrated *in vitro* on human B-lymphoblastic cell lines derived from peripheral blood of AIDS patients. The *c-MYC* locus was found to be regulated at its 5' untranslated region (5'-UTR) via a negative feedback mechanism in a tissue-specific manner. Viral alterations due to EBV, whether truncations or mutations, located in the 5'-UTR of *c-MYC* are necessary to cause the constitutive activation of this oncogene [11]. Once mutated and immortalized, these cells have the ability to initiate tumorigenesis. Although immunodeficiency is neither necessary nor required for the development of BL, HIV/AIDS acts synergistically with EBV infection to enhance lymphatic tumor development.

	Endemic BL	Sporadic BL	HIV/AIDS BL
Synergistic disease factors	*Plasmodium falciparum*	N/A	Low levels of CD4 cells due to HIV/AIDS
Translocation position	*c-MYC* breakpoint is greater than 1 kb from 1st coding exon *Ig* breakpoint occurs in V(D)J region	*c-MYC* breakpoint is between exon 1 and 2 *Ig* breakpoint occurs in class switch region	*c-MYC* breakpoint is between exon 1 and 2 *Ig* breakpoint occurs in class switch region
Pattern of initial cell growth in tumor	Cells grow individually	Cells grow in large clumps	N/A
Location of first tumor development	Jaw, kidneys, or facial bones	Abdomen	Lymph nodes and bone marrow
Prevalent geographic region	Malaria belt in Africa and in New Guinea	Worldwide	Worldwide
Age group affected	Children ages 4-7 years old	All ages	All ages, but most at risk is senior patients
General prognosis	If treated with aggressive chemotherapy, 90% of children and 50-60% of adults survive disease-free		

Figure 1. Comparing and contrasting the unique characteristics of the three clinical variants of Burkitt lymphoma [19, 3, 9].

5. Conclusion

While the characteristics of Burkitt lymphoma are strictly defined by a chromosomal translocation of *c-MYC* and an immunoglobulin loci on either chromosome 2, 14 or 22, the clinical variants of this disease differ in pathogenesis and synergistic conditions which increase the probability of developing this fast-acting lymphoma. It is accepted that EBV causes eBL by infecting cells with their viral capsid antigen (VCA) resulting in the immortalization of B-cells that preludes tumorigenesis. More elusive, however, is how malaria contributes to eBL, considering that both diseases share an endemic geographic region, predominantly in Africa. Evidence has been collected demonstrating that malaria can reactivate the EBV lytic cycle, depress T-cell EBV specific immunity (IFN-γ), and promote polyclonal B-cell expansion; all of which interdependently lead to the proliferation and oncogenesis of EBV infected B-cells.

Sporadic BL, often lacking the genetic marker ENBA-1, is also a cancer caused by the EBV virus. Lack of evidence of EBV in sBL tumors appears to be due to the methods of identification because after tumorigenesis has been initiated, the viral genome is dispensable and often unrecognizably rearranged. Rather, the majority of sBL tumors contain partial segments of viral DNA. Although not entirely conclusive, these findings warrant a thorough investigation of multiple aspects of the EBV genome within sporadic tumors.

HIV causes the dysregulation of B-cells through over-expression of B-cell inhibitory receptors including FCRL4 and Siglec 6. In turn, this causes the enhanced replication of exhausted B-cells. Propagation of cells host to EBV is more likely to occur when the primary immune response is compromised. This synergistic relationship between these

diseases leads to the high association of people living with HIV/AIDS and developing Burkitt lymphoma after EBV infection.

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