bjh commentary

Epstein–Barr virus positivity as a defining pathogenetic feature of Burkitt lymphoma subtypes

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Commentary on: Richter J, *et al*. Epstein–Barr virus status of sporadic Burkitt lymphoma is associated with patient age and mutational features. *Br J Haematol*. 2022;**196**:681–689.

Burkitt lymphoma (BL) is the first human cancer to be associated with the Epstein–Barr virus (EBV), the first tumour to exhibit a chromosomal translocation activating an oncogene [MYC proto-oncogene, basic helix-loop-helix transcription factor (*MYC*)], and the first lymphoma to be associated with human immunodeficiency virus (HIV) infection. The World Health Organization (WHO)[1] classification describes three clinical variants of BL: endemic (eBL), sporadic (sBL) and immunodeficiency-related. These variants are similar in morphology, immunophenotype and genetics. While sBL occurs outside of Africa and is rarely associated with EBV infection, eBL arises mainly in Africa and is associated with malaria endemicity and EBV infection. Epidemiological studies have shown that malaria and EBV combined do not fully explain the distribution of eBL in high-risk regions.[2] Malaria and EBV are, in fact, ubiquitous within the lymphoma belt of Africa, suggesting that other aetiological agents may be involved.[3] However, other epidemiological factors and/or possible genetic predispositions still have an unclear role in the genesis of eBL.

Yet, sBL may also occur in Africa, accounting for the rare EBV-negative cases of BL reported from that continent. Dennis Wright, one of the first pathologists to work on BL, commenting on the publication of the 1999 volume of *The World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues*, raised this question in his letter foreseeing that EBV-positive BL is different from EBV-negative BL in cellular biology and pathogenetic mechanism.[4] As no scientific proof was available at the time, he was likely hesitant to assess this hypothesis. To date, there is increasing evidence from different studies that EBV-positive BL may have a separate pathogenetic mechanism from EBV-negative BL; therefore, we have crossed this point, mainly thanks to the work by Abate *et al*.,[5] Grande *et al*.[6] and also due to more accumulating data.[7–12]

In the paper of Abate *et al*.,[5] the eBL mutational landscape was compared with published data on sBL. An almost mutual exclusivity could be demonstrated between EBV presence and mutations in transcription factor 3 (*TCF3*)/inhibitor of DNA binding 3, HLH protein (*ID3*), both well-known driver genes in sBL.[13,14] A hierarchical clustering of both eBL and sBL cases on *TCF3* target genes, previously reported in the article by Schmitz *et al*.,[15] was performed to explore this hypothesis and revealed that the samples could be classified into EBV-positive and EBV-negative BL independently on the specific clinical subtype with an accuracy rate of 96%.

The fact that EBV-positive BL cases owned fewer driver mutations was furthermore extensively confirmed in the paper by Grande *et al*.[6] Namely, an integrative analysis of whole-genome and transcriptome data proved a striking genome-wide increase in aberrant somatic hypermutation in EBV-positive tumours, thereby supporting a link between EBV and activation-induced cytidine deaminase (AICDA) activity. On the other hand, EBV-positive lymphomas had significantly fewer driver mutations, especially among genes with roles in apoptosis. These results suggested that tumour EBV status defines a specific BL phenotype with molecular properties and pathogenic mechanisms that do not account for the geographic origin.

Therefore, from both papers comes forth a dual mechanism of transformation in BL: mutations *versus* virus driven. Thus, EBV positivity should be the defining feature of clinical subtypes of BL and not the epidemiology.

In particular, most EBV-negative BLs (~70%) have an impaired *TCF3*/*ID3* inhibitory heterodimerisation caused by either gain-of-function mutations affecting the *TCF3* gene or mutations disrupting the *TCF3*-negative regulator ID3. This pathological mechanism increases the expression of the B-cell receptor (BCR) genes and activates a tonic form antigen-independent BCR signalling. On the contrary, BCR analysis of

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First published online 2 November 2021
doi: 10.1111/bjh.17922

EBV-positive BL revealed an intense antigenic pressure potentially related to prolonged microenvironment interactions. The chronic stimulation of BCR by specific pathogens could be necessary to promote the clonal expansion of B cells expressing distinctive BCRs and the growth of the neoplastic clone.[16] However, the combination of tonic and extrinsic BCR signalling activation cannot be excluded in a subset of BL.[16]

In this issue of the *British Journal of Haematology*, a large age-overarching cohort of sBL ($n = 162$) was analysed by immunohistochemistry, translocations of *MYC*, B-cell leukaemia/lymphoma 2 (*BCL2*), *BCL6*, and by targeted sequencing.[17] The Authors illustrate an age-associated intertumoral molecular heterogeneity in this disease. Mutations affecting *ID3*, *TCF3*, and cyclin D3 (*CCND3*) were prevalent in paediatric BL, and expression of sex determining region Y-box transcription factor 11 (SOX11) decreased with patient age at diagnosis. In contrast, EBV was mainly detected in adult patients. Regardless of age, EBV-positive sBL showed significantly less frequent mutations in *ID3*/*TCF3*/*CCND3* but more recurrent G protein subunit alpha 13 (*GNA13*) and forkhead box O1 (*FOXO1*) mutations when compared to EBV-negative tumours.[17] These findings suggest that an EBV-positive subgroup of lymphomas increases with patient age, demonstrating distinct pathogenic features reminiscent of EBV-positive eBL, providing further evidence of the differences between EBV-positive and EBV-negative BL in cases out of Africa.[17]

Malaria and EBV are ubiquitous pathogens within the lymphoma belt of Africa. *Plasmodium falciparum* can repeatedly infect African children and may be responsible of chronic antigenic stimulation and consequent proliferation of latently EBV-infected B memory cells that may acquire *MYC* translocation before or when they re-enter the germinal centre.[18–23]

Existing data provide information on how these two pathogens interact to provoke the disease, supporting the emerging concepts of polymicrobial disease pathogenesis.[2,24–26] Out of Africa, sBL is more common in adults and elderly patients where other infectious agents may be involved,[27] such as HIV and other pathogens, possibly related to immune senescence. Certainly, the epidemiology for BL is different among various geographic areas; more specifically in Africa and developing countries BL frequently affects children and is almost always EBV-related, while in western countries it is more prevalent in adults and also widely linked with EBV in the elderly.[17,28] Consequently, based on increasing evidence, the epidemiological differences in BL reflect distinct pathogenesis of the disease,[29] and this well-known aspect could be valid even for other lymphoproliferative processes, like Hodgkin lymphoma, which have the same visible epidemiological diversities.[30–34]

However, EBV can be responsible for more cases than those we currently acknowledge. According to the 'hit and run' theory, EBV plays an initial oncogenic role, but the viral genome can be lost subsequently due to the neoplastic cell's acquisition of stable (epi)genetic changes. This mechanism has been proposed based on anecdotal case reports and cell lines. It has been recently re-proposed by a study that identified 'traces' of EBV infection in EBV-negative BL in primary tumours and several lymphoma cell lines, where the clonal relation to the neoplastic clone could be demonstrated by higher-sensitivity methods (dual scope).[35,36]

To the present day, BL, the 'Rosetta Stone' of cancer, may still hide information that needs to be revealed.

Funding sources

Open Access Funding provided by Universita degli Studi di Siena within the CRUI-CARE Agreement.

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