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EBV primary infection in childhood and its relation to B-cell lymphoma development: A mini-review from a developing region

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Mini Review

In most underdeveloped countries, the initial contact with Epstein Barr virus (EBV) usually happens in the first decade of life and results in an asymptomatic infection, whereas in developed areas, primary infection in adolescence or adulthood is accompanied by infectious mononucleosis in 50% cases. Although it is generally a harmless passenger, in some individuals, it is associated with B-cell lymphoma. In Argentina, EBV primary infection shows the classical pattern observed in developing populations, given that nearly 70% of patients are seropositive by the age of 2 years. However, EBV association with pediatric Hodgkin and Burkitt lymphoma resembles that observed in developed regions. Concerning diffuse large B-cell lymphoma, our series demonstrated higher EBV association than other adult ones from either developed or underdeveloped countries. Interestingly, the early EBV primary infection observed, characteristic of an underdeveloped population, together with the statistically significant EBV association with patients ≤10 years old demonstrated in all types of lymphoma studied, suggest a relationship between low age of EBV seroconversion and B-cell lymphoma development risk.

Epstein Barr virus (EBV) is a double-stranded DNA virus with a genome of 172 kb, belonging to the γ herpes virus family. During acute infection, EBV primarily infects and replicates in the oropharynx. EBV infection of B lymphocytes is thought to occur in the oropharyngeal lymphoid organs. Primary EBV infection elicits a strong cellular immune response that then brings the infection under control,[1] as the primary infection resolves, effective CD8+ and CD4+ T cell responses become established and the virus host homeostasis is achieved.[2] The final step is the establishment of an EBV latent infection in circulating memory B cells.

In most underdeveloped countries, the first contact of an individual with EBV usually happens in the first decade of life and results in an asymptomatic infection, resulting in the establishment of viral persistence in young children. In contrast, in more developed areas, primary infection by EBV occurs mainly in adolescence or adulthood and it is accompanied by infectious mononucleosis (IM) in about half of the cases. The reasons for the different course of primary infection in childhood and adolescence are not fully understood.[3]

Long-term EBV infection coexists with most human hosts without overt serious consequences.[4] The nature of the long-term latent infection *in vivo* has been subject to considerable speculation, but it is likely that the expression of a limited number of proteins in a B-cell pool represents a phenotype that is poorly recognized by cytotoxic T lymphocytes (CTL). Thus, the virus appears to have evolved in such a way that most individuals suffer no consequences from carrying a small nucleus of latently infected B-cells, which appears to be resistant to CTL recognition and which can, under certain circumstances, be reactivated to release relatively low levels of virus into the oral cavity. This seemingly elegant balance that ensures the long-term survival of the virus is subject to error on certain occasions resulting in the emergence of EBV-driven B-cell malignancies.[5] Among these, EBV-associated lymphomas include Burkitt lymphoma (BL), Hodgkin lymphoma (HL), post-transplant lymphomas and AIDS-associated lymphomas.[6]

EBV Primary Infection in Argentina

As EBV epidemiology shows different features depending on the developing status of the community, it is interesting to determine its epidemiological pattern in a paediatric cohort of our geographic region. EBV infection was assessed in 164 paediatric pre-operative serum samples by indirect immunofluorescent assay for IgM and IgG antibodies against virus capsid antigen (VCA). Patients' median age was 5 years. All patients were negative for IgM anti-VCA; therefore, EBV acute infection was rejected.[7]

IgG antibodies anti-VCA prevalence in the entire population was 72% (118/164 cases). The age group ranging from 6

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to 12 months showed the lower VCA-IgG prevalence value (26%), although in this age group the contribution of maternal antibodies could not be ignored. The older children displayed a VCA-IgG prevalence that increased along the infancy and adolescence, as observed in Table 1. A significant difference in the number of seropositive individuals was observed when comparing the 1–12 months of age ranges with the remaining groups, indicating a high rate of primary infection at these ages (Table 1 and Fig. 1*a*).[7] Concerning distribution by sex, in this paediatric cohort, EBV positive serology was equally distributed in males and females, 72% *versus* 71%, respectively.[7]

Table 1. EBV positivity discriminated by age groups

Age	EBV-positive n/total (%)[1]
1–6 months	0/12 (0)
7–12 months	5/19 (26)
13–24 months	15/20 (75)
25–36 months	16/18 (89)
4–6 years	36/43 (84)
7–10 years	23/28 (82)
11–15 years	23/24 (96)
Total	118/164 (72)

[1]EBV was assessed by detection of VCA-IgG by indirect immunofluorescence (IFI). In patients younger than 6 months, false positive results due to presence of maternal IgG antibodies were counter by determining VCA-IgM antibodies by IFI. Ref. 7.

It was reported that children in developing countries, such as Argentina, acquire the infection in the first few years of life, and universal seroconversion is often seen by ages 3–4 years, whereas infection in developed countries is often delayed until adolescence.[8] Our results confirm that this series epidemiologically behaves like an underdeveloped or developing population. Poor socioeconomic conditions have been associated with early primary EBV infection, whereas late primary EBV infection is seen in populations of high socioeconomic status. Low income and crowded family conditions have also been found to increase the likelihood of being EBV seropositive in children from several geographic locations. Exposure to infected saliva, either directly or via, for example, unclean toys or fingers, is believed to explain differences related to the socioeconomic condition among transmissions,[8] and it is also assumed to be the major route of transmission in younger children.[9]

EBV primary infection at an early age in children from developing regions is usually asymptomatic, whereas in

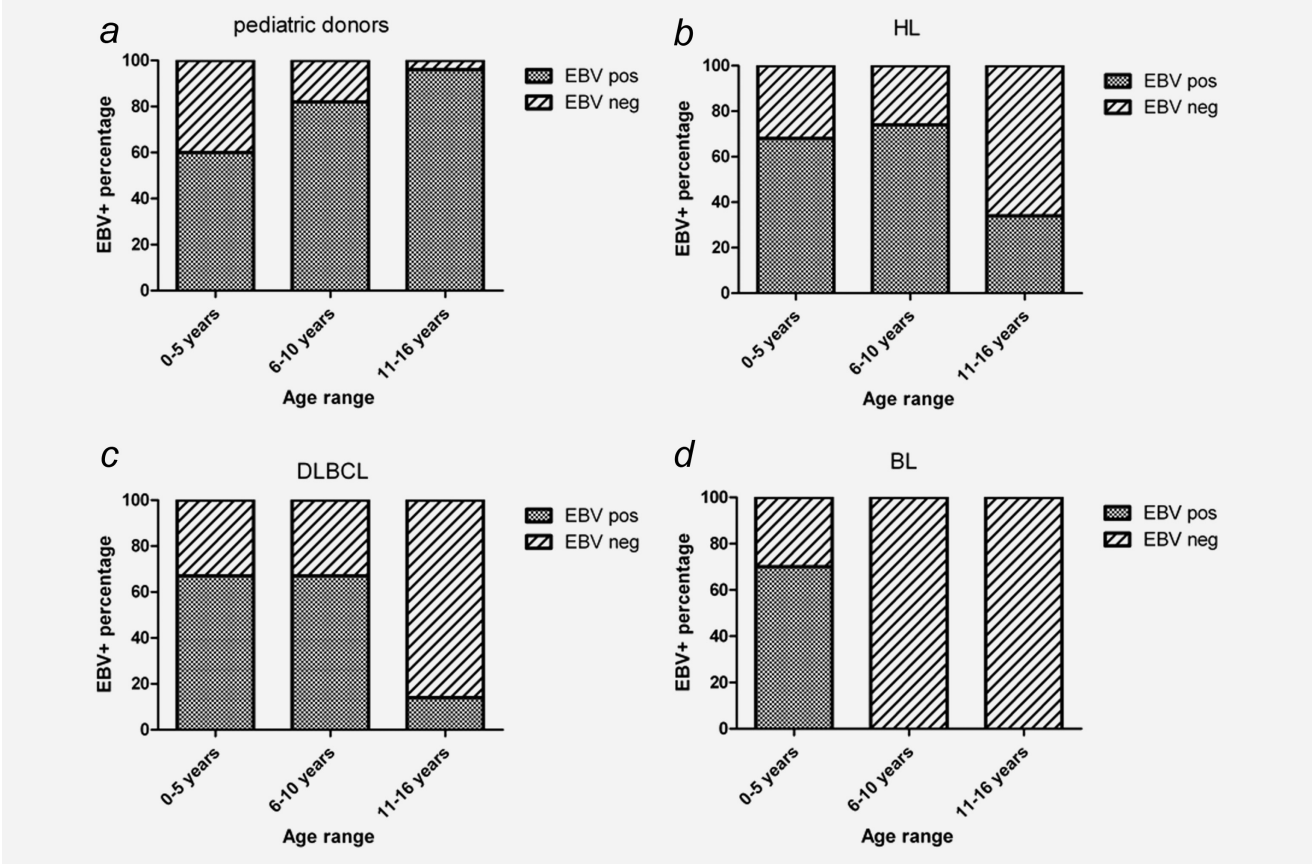

Figure 1. EBV distribution according to age ranges in (*a*) paediatric healthy donors, (*b*) Hodgkin lymphoma (HL), (*c*) Diffuse large B-cell lymphoma (DLBCL), (*d*) Burkitt lymphoma (BL).

young adults from developed economies, it presents mild to severe clinical manifestations of the lymphoproliferative disease, IM.[10] This fact renders the variable features of EBV primary infections an interesting enigma in viral biology. Risk factors for developing IM rather than a subclinical primary EBV infection have not been fully elucidated; however, it has long been suggested that the transmission of a large amount of virus is important in the pathogenesis of IM. In this regard, some studies suggest that IM is most likely to occur when a seronegative individual is infected with a large amount of virus that results in an enhanced ability to stimulate B-cell proliferation. Thus, differences in the initial viral inoculum influences whether primary EBV infection is silent or manifests as IM. At this point, because the symptoms of IM are T-cell mediated, the initial viral load must determine the level of T-cell response and thereby determine whether seroconversion is silent or manifests as IM.[11,12] Furthermore, it has been demonstrated that the viremia was related to the clinical severity of primary EBV infection, but that the amount of EBV in the oral wash fluid was not. Thus, the viral load in whole blood samples is a surrogate marker for the severity of primary EBV infection in young adults.[13]

There is a hypothesis which proposes that the clinical presentation variability related to the age of EBV primary infection is linked to the different magnitude of the viral dose received by a child or a young adult through salivary contact. In a study carried out by our group including 15 paediatric patients from Argentina with mild IM symptoms related to primary EBV infection—confirmed by antibodies against VCA-IgM detection—it was demonstrated that only 8 of 15 patients showed detectable viral load in plasma at diagnosis, and indeed all patients were negative during convalescence (at 1 and 3 months after diagnosis).[14] These findings support the idea of a rapid immunological control of EBV active replication in children who are assumed to receive a lower viral inoculum and consequently milder or absent clinical symptoms. In contrast, another report showed comparable virus loads in acute IM and asymptomatic primary EBV infection in adults. However, the same report showed limited T-cell expansion in those undergoing asymptomatic primary infection as compared with IM, and this fact corroborates that more severe disease is linked to a more marked immune response.[15]

Although EBV epidemiology in Argentina reflects a typical pattern of a developing country with primary infection in early childhood, the epidemiology of EBV-related lymphomas differs from that observed in developing countries. Therefore, in Argentina, EBV infection shows a complex epidemiological pattern.

EBV Association With Paediatric HL

As previously described by Harris, there are three epidemiological patterns of HL according to the level of socioeconomic development. In pattern I, seen in underdeveloped countries, HL incidence shows an early childhood peak, and the predominant HL subtype is mixed cellularity (MC). Pattern II, observed in developing or transitional economies, displays both childhood and second decade peaks, and equal frequency of MC and nodular sclerosis (NS) subtypes. Finally, in pattern III, observed in developed countries, HL shows a third decade peak and a predominance of NS over other subtypes.[16] In the United States and most European countries, HL shows an annual incidence of 4.5–6.0 per million and its incidence increases with age and peaks between 15 and 30 years. In contrast, in developing countries, it shows a high incidence in children exceeding 7 per million per annum and 70% of cases occur below the age of 10 years.[17]

First evidence that EBV might be involved in the pathogenesis of HL was provided by the detection of raised antibody titers to EBV antigens in HL patients when compared with other lymphoma patients, and furthermore, that these raised levels preceded the development of HL by several years.[18] EBV rates in HL from North America and Europe, as well as other developed populations, have been shown to vary between 20% and 50%, whereas much higher rates are observed in underdeveloped countries, suggesting a contribution of socio-economical factors to the pathogenesis of EBV-positive cases of HL.[17] The increased incidence of EBV-positive HL in underdeveloped countries could be due to the existence of an immunological background resembling that observed for African BL in a malaria-infected population. This is supported by higher EBV-positive rates in HL from HIV-infected patients.[16] Specifically in children, classical HL (cHL) is more likely to be EBV-associated than is HL among young adults and shows a significant prevalence of male gender and MC (30%–35%) in comparison with cHL in young adults or adults.[19]

In Argentina, our group has previously reported an epidemiological pattern II together with an EBV association of 31% (25/81 adult HL cases) for adult HL, which rose up to 54% (60/111 paediatric HL cases) in paediatric patients.[20] Therefore, this frequency of EBV associated to HL resembles the one observed in developed countries. However, in the paediatric HL group, EBV association was particularly present in children younger than 10 years, in males and in MC subtype[21] (Fig. 1*b*), typical of an underdeveloped or developing population. These two paradoxical epidemiological characteristics make this population a quite interesting group for analysis. Furthermore, EBV+ cases had a median age of 8 years, *versus* a median of 12 years observed in EBV-cases ($p = 0.006$, Mann-Whitney test) (own unpublished data). In contrast, even though in Southeast Brazil EBV association with paediatric cHL was 44.8%[22,23] and primary infection showed the same pattern observed in Argentina,[24] EBV was not significantly related to patients younger than 10 years, or MC subtype.[22,23] Conversely, paediatric cHL from Northeast Brazil showed EBV association in about 87% of cases together with a prevalence of MC subtype,[25] which provides a typical underdeveloped population pattern. Other Latin

American countries, such as Mexico (70%)[26] and Peru (94%),[27] also showed this high frequency of EBV-HL association.

An alternative explanation for the increased incidence of EBV-positive HL in underdeveloped countries could be the timing of EBV infection, which occurs earlier in these populations.[16] Our observations are in line with this idea, since we demonstrated both EBV infection at early childhood together with an EBV association with HL in the group of children younger than 10 years, despite the EBV association of 54% characteristic for developed countries. Furthermore, it supports the assumption of EBV seroconversion age as pathogenic factor for paediatric EBV-positive HL development, specifically in children younger than 10 years. This could be linked to the immunological control of primary EBV infection, which has a critical impact on HL development risk. It has been proposed that genetic variation in host antiviral immune responses related to HLA polymorphisms might be an important contributor to the development of virally induced malignancies. In fact, a strong association of specific HLA-A alleles with susceptibility to EBV+ cHL in Dutch, English and Scandinavian patients was demonstrated.[28,29] Furthermore, some groups suggested the possibility that the association between EBV-related HL and prior IM simply reflects shared genetic susceptibility.[29] EBV-specific CTL response, restricted through HLA class I, plays a key role in development of EBV-related HL. However, it should be kept in mind that most findings have been described in adult IM and HL patients. Only one Latin American group analyzed immunological characteristics in paediatric HL and demonstrated differences with the adult counterpart, since paediatric EBV+ cases were characterized by a more intense T-cell infiltrate, exhibiting a cytotoxic/Th1 profile and higher numbers of CD3+, CD8+ and TIA1+ on HL microenvironment. They suggested that age-related changes of the immune system may also modulate the tumor microenvironment in paediatric HL.[22] It is still unresolved if specific HLA alleles might be involved in the immunological response to subclinical primary EBV infection in paediatric patients. This genetic background could be related to the susceptibility to develop paediatric EBV+ HL, especially in patients younger than 10 years, providing the basis for this particular HL microenvironment.

EBV Association With Paediatric B-Cell Non-Hodgkin lymphoma (B-NHL)

NHL represents approximately 8%–10% of all malignancies in children between the ages of 5 and 19 years. Over 95% of childhood NHL has an intermediate to aggressive pathological subtype by the World Health Organization (WHO) classification.[30] The majority of paediatric NHLs derives from B cells (B-NHL) and represents primary high-grade lymphomas. Among NHLs, the three most prevalent entities are BL (43%), Diffuse large B-cell lymphoma (DLBCL) (13%) and B-lymphoblastic lymphoma (7%).[31]

EBV association with BL has been deeply described since first description by Burkitt in 1958 in African children from areas holoendemic for malaria.[32] There are three epidemiologically distinct forms of BL related to EBV association. The high-incidence "endemic" form, typically in children from areas of equatorial Africa and Papua New Guinea where malaria is holoendemic, is 100% EBV genome–positive. Elsewhere, BL occurs in "sporadic" form, again mainly in children, at intermediate to low incidence and with different degrees of EBV association depending on the area. Western countries show the lowest BL incidence rate and the weakest virus association, with only 15%–20% tumors being EBV+.[33] By contrast, BL appears to be more common in other underdeveloped locations, for example, equatorial areas of Brazil, and EBV-association rates are correspondingly higher. Remarkably, a third form of the tumor, "AIDS-BL," proved to be very common among HIV-infected adults and often appears as one of the first symptoms of AIDS. Thirty to 40% of these AIDS-BL carry EBV.[34]

Concerning DLBCL, EBV association has been recently investigated and is mainly restricted to patients older than 50 years.[35] This fact prompted a study in Asia which specifically included patients >50 years and described 8%–10% of EBV association with DLBCL in this age range.[36] It was hypothesized that this linkage was due to a defective immune surveillance for EBV secondary to a senescence of the immune system inherent to the aging process.[37] On the basis of these studies, EBV-positive DLBCL of the elderly was included in the 2008 WHO classification of Tumours of Hematopoietic and Lymphoid Tissues, as a new provisional entity.[38] The frequency of this elderly subtype among DLBCL in Western populations was lower than that seen in Asia; whereas in Mexico this prevalence was similar (7%) to that previously described in Asian patients.[37] Although there were several association revisions in adult groups, paediatric DLBCL is an unexplored field.

In a series of paediatric B-NHL from Argentina, we found 40% (16/40 B-NHL cases) of EBV expression among the studied cases (Figs. 2*a* and 2*b*). On the whole, EBV presence was more prevalent in DLBCLs (47%, 8/17), with a cut-off value of more than 20% of Epstein Barr encoded RNAs (EBERs) positive tumor cells, than in BLs (35%, 8/23), but this difference was not statistically significant.[39] Specifically in DLBCL regarding EBERs positive tumor cell proportion, three cases showed 20%–49%, three cases 50%–80% and two cases >80%. Four DLBCL patients were immunocompromised, three of them had primary immunodeficiency and one was HIV+, whereas three BL patients were also HIV+. When taking into account the immunological status of the patients to assess the EBV association, the immunocompetent ones showed EBV+ in 31% (4/13) DLBCLs and 25% (5/20) BLs, whereas EBV was present in all immunocompromised cases. These findings confirm that T-cell immunocompromised patients are at high risk of developing B-cell lymphomas. Finally, it is very interesting to consider EBV frequency

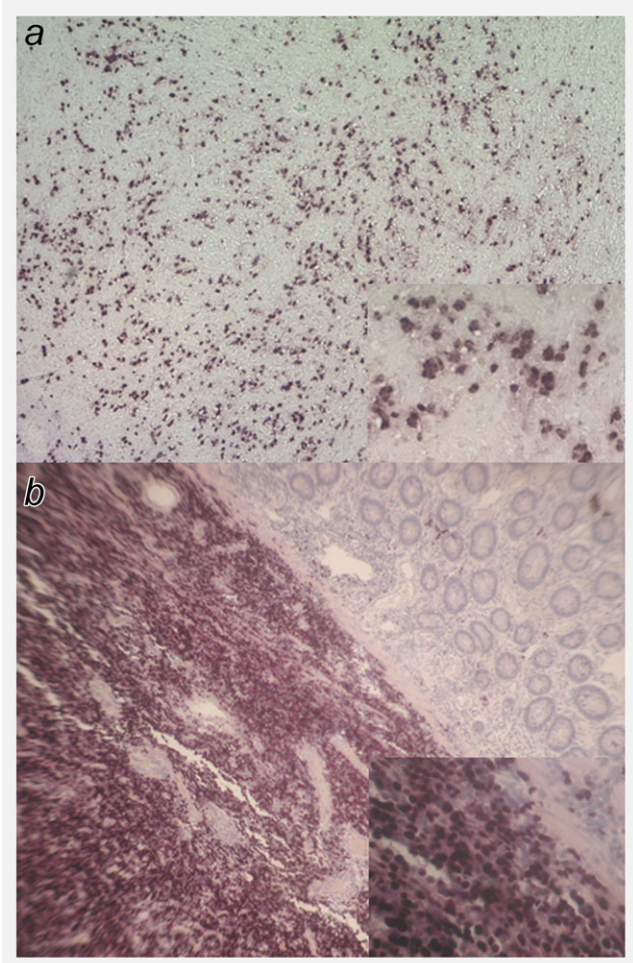

Figure 2. EBERs *in situ* hybridization for EBV shows positive staining in the nucleus of neoplasic cells in a (*a*) DLBCL and (*b*) Burkitt lymphoma lymph node biopsies, original magnification 10×, the insets show 40× magnification. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

in different histological types of lymphoma within the immunocompetent children group. Although EBV expression in the studied BL series showed the typical sporadic pattern observed in Western developed populations, we found a notably more elevated EBV association with DLBCL, which in turn is higher than that previously described for both adults younger than 50 years and elderly populations.[35–37] This fact indicates that EBV might have an important role in paediatric DLBCL development which requires further investigation.

In our paediatric series for BL and DLBCL, a significant association between EBV infection and younger individuals was observed. In fact, we confirmed that in paediatric B-NHL, EBV expression is statistically associated with patients ≤10 years (Figs. 1*c* and 1*d*). Furthermore, all EBV+ BL patients were younger than 5 years, with a median age of 3 years, *versus* a median of 8 years for EBV-negative ones ($p = 0.0027$, Mann-Whitney test) (Fig. 1*d*) (own unpublished data). In line with this observation, EBV+ DLBCL cases showed a median age of 6 years, *versus* a median of 13 years observed for EBV cases ($p = 0.0411$, Mann-Whitney test) (Fig. 1*c*) (own unpublished data). The finding of a relationship between low age of EBV seroconversion and BL risk was already suggested in our geographical region, since Hassan *et al.* described a high frequency of EBV+ BL in younger children from Brazil.[40] Our findings support their observations concerning BL and extend them to paediatric DLBCL. Finally, this high frequency of EBV-associated B-NHL in younger children confirms that, as in HL, EBV may be an important cofactor in B-cell lymphoma development in this age group, perhaps arising as a late complication of EBV primary infection.

It has been proposed that the risk of developing an HL after IM in the young adult age group suggests that its tendency may simply result from the combination of age and time since IM rather than from other particular mechanisms related to HL development in younger adults.[41] It is therefore tempting to speculate that a similar association may exist between primary EBV infection and HL risk at any age, for example, childhood, which could explain the predominance of EBV-positive HL in this age group. Furthermore, based our findings, this assumption could also be extended to paediatric B-NHL from our geographical region. However, molecular mechanisms of EBV involvement in lymphomagenesis in both paediatric HL and B-NHL in patients with low seroconversion age from developing populations remains to be determined.

B-Cell Lymphoma Origin and EBV Contribution

EBV-associated B-cell lymphomas have their origin from cells that have differentiated through germinal centre (GC). Naïve B cells that encounter their cognate antigen migrate to the GC, where they undergo somatic hypermutation and class switch recombination. Early in the course of primary infection, EBV infects B lymphocytes, possibly within the epithelium of the naso and oropharyngeal mucosa. It is not clear whether naïve, memory or GC B cells are the target of initial infection. In asymptomatic carriers, EBV resides in memory B cells that provide the long-term reservoir for EBV. If memory B cells are activated and differentiated into plasma cells, EBV might switch to the lytic life cycle, producing many new virus particles. Some of these might infect new naïve B cells, thereby replenishing the pool of virus-infected cells.[42]

Characteristics of BL cells seem to point to a GC origin. They phenotypically resemble centroblasts, expressing high levels of BCL6 and show signs of somatic hypermutation, a common characteristic of GC B cells.[43] However, the cell of origin may also be a post-GC or memory B-cell re-entering the GC, and indeed, it may differ in EBV-positive and -negative tumours.[44,45] If that as it might, EBV contribution to BL might involve GC reaction, *via* Epstein Barr Nuclear antigen 1 (EBNA1) protein and the EBERs transcripts expressed in EBV+ BL, both implicated in the prevention of apoptosis

rather than as growth-promoting agents.[34] In line with this, GC transit is also altered in HL development, since HL tumor cell, Hodgkin Reed Sternberg (HRS) cells, are likely to be derived from pre-apoptotic GC B cells. The rescue from apoptosis is a key event in the transformation process, leading to HRS cell generation. In EBV+ cases, three viral proteins are expressed, namely EBNA1, Latent membrane protein 1 (LMP1) and LMP2A. They could play an important role in the pathogenesis of HL by rescuing EBV-infected GC B cells with disadvantageous mutations from apoptosis.[46] Conversely, EBV role in DLBCL is still uncertain. As previously mentioned, immunological senescence has been suggested to be involved in the development of EBV+ DLBCL of the elderly, but this scenario is still not elucidated for patients younger than 50 years, including paediatric patients. It has been described that nearly 50% of adult DLBCL derived from GC and the rest from post-GC cells,[47] but Oschlies *et al.* reported that paediatric DLBCL from a developed population might represent a more uniform group, given that 91% of the paediatric cases were originated from GC.[48] Interestingly, our paediatric studied series of DLBCL from a developing region and showed a post-GC origin in 61% of cases (own unpublished data). Since DLBCL is an understudied entity with different features depending on the analyzed group, a comprehensive characterization concerning histological and virological aspects, especially in children, would be necessary.

The hypothesis that proposes an active role of EBV in facilitating cell avoidance from apoptosis during GC transit in BL[34] and HL[46] would not be extensive for other B-cell lymphomas, particularly non-GCB type DLBCL. In this regard, the assessment of EBV latent protein expression pattern in DLCBL is still pending. Viral latency proteins interaction with cellular proteins playing a key role in cellular cascades needs further investigations to clarify the pathogenic role of EBV in DLBCL, especially in immunocompetent patients.

Conclusions and Future Directions

Childhood is a phase of continuous changing, development and maturation. It is conceivable that these predefined processes display windows of accessibility and vulnerability to extrinsic influences at certain stages of development.[49] Particularly, differences observed in childhood social environment between developed and developing populations may affect the maturity of the immune system. Given that early microbial exposure, such as EBV infection, is of importance for immune development and that malignant lymphomas arise from immune system cells, childhood social environment may influence the risk of immune-related disorders.[50]

Even though EBV presence in paediatric HL and BL from this series displays frequencies observed in developed regions, EBV-positive cases are almost restricted to patients younger than 10 years; this fact suggests a relationship between low age of EBV seroconversion and B-cell lymphoma risk. The question about the potential immunological and environmental differences between children living in developed regions and those living in underdeveloped conditions still remains to be answered. This concern in turn would help to understand why EBV infection specially plays a crucial role in B-cell lymphomagenesis in children younger than 10 years within disadvantageous socio-economical conditions.

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