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Pathology of Epstein-Barr virus-related lymphomas in HIV

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Acquired immunodeficiency syndrome-related non-Hodgkin lymphomas (AIDS-NHL) represent a significant source of morbidity and mortality among HIV-infected individuals.[1] According to body location and histologic criteria, the pathologic spectrum of AIDS-NHL includes systemic AIDS-NHL, primary central nervous system lymphoma (AIDS-PCNSL), plasmablastic lymphoma of the oral cavity (AIDS-PBL) and primary effusion lymphoma (AIDS-PEL).[2] Systemic AIDS-NHL are histologically classified into AIDS-related Burkitt lymphoma (AIDS-BL) and AIDS-related diffuse large B-cell lymphoma (AIDS-DLBCL). All AIDS-PCNSL are histologically represented by DLBCL. Despite their clinicopathologic heterogeneity, most AIDS-NHL derive from B-cells that have experienced the germinal center (GC) reaction[3] and, therefore, have been exposed to the mechanism of somatic hypermutation (SHM) which normally targets the immunoglobulin variable region (IgV). In particular, AIDS-BL and a fraction of AIDS-DLBCL and AIDS-PCNSL closely reflect the GC profile, whereas the remaining fraction of AIDS-DLBCL and AIDS-PCNSL are composed of post-GC B-cells maturing toward the plasma cell pathway of differentiation and are classified as AIDS-related immunoblastic lymphoma (AIDS-IBL).[3] Hodgkin lymphoma has also been reported in HIV-infected patients (AIDS-HL).[4] All AIDS-NHL are characterized by extreme clinical aggressiveness and display a predilection for extranodal sites. In particular, meningeal or parenchimal brain involvement has been variably reported as a main feature of extranodal disease.[5]

The pathogenesis of AIDS-NHL as other lymphoproliferative diseases is regarded as a multistep process involving factors provided by the host as well as alteration intrinsic to the tumor clone.[6] Examples of host factors may include chronic antigenic stimulation associated with reduced immunosorveillance, whereas tumor clone alteration are mainly represented by genetic lesions of cancer-related genes and infection of potentially oncogenic viruses. Infection by Epstein-Barr virus (EBV) is common among AIDS-NHL and AIDS-HL and occurs with different rates according to the pathologic subtype, ranging from 30% in AIDS-BL to 50-60% in AIDS-DLBCL, to 75-85% in AIDS-IBL and to virtually in all cases of AIDS-PCNSL and AIDS-HL.[3-7]

The EBV is a human gammaherpersvirus widespread in the world population. Although healthy individuals carry EBV as an asymptomatic latent infection of the resting memory B lymphocytes, a role for EBV in the pathogenesis of malignant diseases has been suggested by *in vitro* and *in vivo* studies.[8] Natural EBV isolated display a certain degree of genetic variability and viral variant are frequently distinguished according to sequence polymorphism in subgenomic regions. Several EBV polymorphisms have been investigated in malignant diseases including those of the EBV nuclear antigen 1 (EBNA-1), EBNA-2, EBNA-3c, EBV-encoded latent membrane protein 1 (LMP-1).[9-12] The current model of persistent asymptomatic EBV infection holds that during primary infection EBV positive proliferating blasts are pushed to differentiate into resting memory B cells that shut down the expression of viral protein and elude immune surveillance. This restriction of the virus in the blood to resting memory B cells is maintained in immunosuppressed patients, in whom the number of virus-infected cells is 20 to 50 times as high as in healthy persons.[13] Periodic cycles of lytic replication are sufficient to maintain the viral infection and permit EBV transmission among the population. Reactivation (a switch from latency to a productive lytic cycle) producing an elevated and increasing EBV viral load preceded the development of EBV-associated non-Hodgkin's lymphomas in patients with HIV infection, suggesting that an impaired cytotoxic T-cell response permits uninhibited growth of EBV-infected cells. But the situation is not so simple. In fact, latently infected B cells grown *in vitro* do not spontaneously undergo lytic

replication suggesting that the virus itself may exert some control over the switch from latency to lytic infection. In particular several studies indicate that EBV BamHI fragment Z (BZLF1) plays a pivotal role in the reactivation of EBV. 14 The regulation of this gene and then of viral lytic cascade is due to different extracellular and intracellular inducers that bind cis-elements in a short zone of BLZF1 promoter (Zp) (from -221 to +12).[14] Recently Gutierrez *et al.* identified three variants of Zp: Zp-P, considered the prototypical sequence of B95.8 strain, Zp-V3 and Zp-V4. These variants seem to segregate with malignant vs non-malignant diseases.

Based on these premises, we decided to explore the sequences of the major promoter regulatory elements (Zp) of BZLF1 to determine whether the regulatory regions of this gene promoter differ in EBV positive AIDS-NHL as compared with EBV positive HIV+ patients. In particular we have studied 74 EBV positive AIDS-NHL (40 AIDS-PCNSL and 34 systemic AIDS-NHL) and 70 EBV positive controls (30 healthy subjects and 40 HIV+ patients). Control cases have shown Zp-P, Zp-P/Zp-V3 and only one HIV+ patient Zp-V3. On the contrary 28% of AIDS-PCNSL and 21% of AIDS-NHL resulted Zp-V3 ($p<0.0001$, O.D 26.17, 95% C.I. 3.23-212.14 and $p<0.0001$, O.D. 17.89, 95% C.I. 2.10-152.35, respectively). In addition, we found a new variant Zp-V5 in 6 cases of AIDS-PCNSL. Finally we found that the Zp-V3 significantly correlates with EBV type B.

In conclusion, we herein demonstrate that polymorphisms in the regulatory sequences of BZFL1 are differentially distributed among various subtypes of AIDS-NHL and nonmalignant cells, a phenomenon that is not due to a peculiar distribution of EBV strains in Italy and that may contribute to the pathogenesis of EBV positive AIDS-NHL.

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