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Epstein-Barr virus associated lymphomas

Eva Klein

Microbiology and Tumor Biology Center (MTC) Karolinska Institutet, Stockholm 171 77, Sweden

Four viruses are involved in the genesis of leukemias and lymphomas in humans, EBV, HTLV-1, HHV8 and HCV.

Since its discovery in 1965, thousands of papers have delt with the epidemiology, molecular biology, cell biology and disease related aspects of EBV. It is ubiquitous in the human population with a long history of coexistence, during which the mutual adaptation resulted in a largely non-pathogenic interaction.[5]

The virus was first seen in cultured cells of an African Burkitt lymphoma, BL. It was a new human herpesvirus, antigenically distinct from all previously known herpesviruses. With the exception of Marek disease herpesvirus, that causes epizootic lymphomas in chickens, all animal leukemogenic viruses known were retroviruses. The demonstration that EBV can transform and immortalize human B lymphocytes in vitro created much interest, since it suggested that it is a transforming agent that induces BL. However this idea was soon abandoned, when it was shown that the virus is neither necessary nor sufficient for tumor development. EBVs relationship with B lymphocytes is known best. In contrast to B cells, T and NK cells cannot be infected with EBV although some of the derived malignancies may carry the viral genome.

Life long consequence of EBV infection

In developing countries and in low socio-economic groups infection is usually acquired in childhood, most often without specific symptoms. When infection is delayed, it can cause a benign self limited lymphoproliferation, infectious mononucleosis (IM). The severity of the clinical picture is highly variable. Independently of the disease manifestation, primary infection is always followed by a symptomless carrier state. Prolonged disease, designated as *chronic active EBV infection*, occurs only rarely. In a rare hereditary condition, X linked lymphoproliferative disease, the infection can be either fatal (in half of the cases) or leads to lymphoma development[3] XLP patients have a functional defect in cell mediated immunity, due to the mutation of a gene that codes for an SH2 containing 128aa protein, SAP, that is part of the lymphocyte activation system.

In healthy virus carriers the virus persists in a small fraction of memory B-cells. They maintain EBV specific humoral and cellular immunity. Immunity to the virally encoded proteins is responsible for the harmless EBV carrier state.[11] In B lymphocytes EBV can establish different latent programs. It can be carried as a harmless passenger, influence the maturation pathway and it can induce proliferation. Cell type dependent differences in the expression of viral proteins determine the fate of the infected cell. These patterns are classified as Type I, Type IIa, IIb and Type III.[5,7,15,16]

Full (Type III) program

The EBV encoded proteins expressed in proliferating non virus producing cells of B cell origin were defined in virally transformed *in vitro* lines, designated Lymphoblastoid Cell Lines, LCL. In these cells the EBV genomes reside as episomes. The expression of the virally encoded proteins is regulated by one of the two alternative viral promoters (designated Wp and Cp). A large message is spliced and translated into 6 nucleus localized proteins (EBNA1-6 or EBNA 1, 2, 3a, 3b, 3c, LP). In addition 3 integral membrane proteins (LMP1 LMP2a and LMP2b) are encoded by the virus. Among the 6 nuclear proteins 5 are required for the induction and maintenance of the transformed phenotype. They interact with each other and with cellular transcription factors. LMP-1 is pivotal for transformation, it can be regarded as an oncogene.[4, 7, 16] Proliferating cells with Type III expression pattern can be found in the malignant post-transplantation lymphoproliferative disease, PTLD. This is due to the immunosuppressive treatment because healthy individuals readily develop T cell responses against peptides of the growth associated proteins and eliminate the Type III cells.[11]

Type I program.

The virus expression pattern in the typical BL cells as it appears *in vivo*, is designated Type I. The same program is used in the B lymphocyte compartment of EBV carrier individuals. The cells express only the EBNA-1 protein.[5,7] Importantly, this program does not induce cell proliferation and such cells are not "seen" by the CD8 positive T cells. The latter is partly due to 2 mechanisms. Firstly, the phenotype of the cell corresponds to non-activated lymphocytes that do not express costimulatory molecules which are pivotal for the interaction with the antigen recognizing T cells, secondly the EBNA-1 protein does not provide HLA class I associated peptides.

Type IIa program.

The Type IIa pattern: EBNA-1 and LMP-1, was first discovered in nasopharyngeal carcinoma, NPC, and was named Type II. Later it was also detected in T and NK lymphomas and in the B lineage H/RS cells in HL. Type IIa B cells occur in PTLD tissues which are heterogeneous with regard of the EBV expression, in angioimmunoblastic T-cell lymphomas, and in MALTomas. The lack of EBNA-2 is attributed to the absence of B lymphoblast specific transcription factors in the T, NK cells and in the particular differentiation window of the B lineage cells in these tissues.

Type IIb program.

The combination of EBNA-1, EBNA-2,4,5,6 without expression of LMP-1 has only recently received attention. It was first seen when B-CLL cells were infected with EBV *in vitro*.[14] Consistently with the role of LMP-1 in EBV induced immortalization, CLL clones are usually resistant to *in vitro* transformation.[13] In B cells EBNA-2 regulates the activation of the LMP-1 promoter. Why the EBNA-2 protein does not function in these cells is not yet known.

Experiments with several B-CLL clones (patients) showed that in vitro infection of the cells occurs regularly with EBNA-2 expression in about 30% of the cells. CLL cells infected with EBV do not enter the cell cycle.[1,9,13] The immediate-early genes, c-myc, ATF-2 and c-Jun, are not induced, and the chromatin remains dense. In accordance, pRb is not phosphorylated and p27 expression does not decline. In normal B lymphocytes these events are induced regularly. The CLL cells are not refractory to activation, as shown by their response to CD40 ligand that induces expression of c-myc and entrance to the cell cycle. Although CD40L overcomes the lack of activation after EBV infection, the cells still do not express LMP-1 and do not give rise to lines. Compared to B lymphocytes, the progress of EBV infection in the CLL cells meets thus 2 roadblocks. The first affects the activation step. This block can be overcome by exposure to CD40L. The second block inhibits LMP-1 expression and immortalization of the cells. It is important to note that B lymphocytes with these 4 EBV expression types can be found in lymph nodes and tonsils of IM patients and also in the EBV positive lymphoproliferative tissues in immunosuppressed patients, PTLD.[10,12]

Burkitt lymphoma

Burkitt lymphoma (BL) has a unique clinical picture and histopathology. EBV associated BL are frequent in regions of hyper- or holoendemic malaria. The driving force in the Type I – EBNA-1 only - expressor BL cells is provided by a reciprocal chromosomal translocation involving chromosome 8 and either chromosome 14, 2 or 22. In such cells the expression of myc on chr. 8 is constitutive due to its juxtaposition to one of the immunoglobulin (heavy, chr 14 or light chains, chr 2 or 22) genes. In addition, multiple antiapoptotic mechanisms concur in permitting the development of the lymphoma.

When BL cells are grown in vitro they can shift their EBV protein expression pattern to Type III. This is accompanied by change of morphology and the social behaviour of the cells. Typical BL cells are small, solitary, round, regular spheres with smooth surface, whereas the LCL-like cells are blasts, with irregular shape and villi. They express activation markers and aggregate.

Hodgkin's lymphoma

The role of EBV in Hodkin's lymphoma (HL) development is unclear.[8] The type II latency pattern does not lead to *in vitro* proliferation and among the EBV positive lymphomas in immunosuppressed patients HL is relatively rare. An association between EBV and HL is suggested by the elevated frequency of HL in individuals whose primary infection was accompanied by IM and by the geographical variation in the fraction of EBV positive HL cases. The majority of HL cases are of B-cell origin, but the H/RS cells lack several B-cell characteristics. B-cells without functional immunoglobulin normally go to apoptosis. The rare HRS cells (about 1% in the granulomatous tissue) express the Type IIa pattern. There are no established lines that would be representative for the EBV-positive HRS. It is likely that the Type IIa EBV protein expression does not induce proliferation. Additional cellular changes and or a contribution of the microenvironment may be necessary for the development of the disease. We have studied the interaction of EBV with cells of a HL derived line. We introduced EBV into KMH-2 cells.[6] Interestingly only EBNA-1 was detected, (Type I). Thus, the *in vitro* established EBV carrier line did not correspond to *in vivo* HRS cells. We attributed considerable

significance to the finding that LMP-1 expression could be induced by exposure to IL-4 and CD40-ligand. This suggests that the contact between HRS and CD4 positive T-cells (and other cells) in the tissue may induce LMP-1 expression by providing IL-4 and other cytokines. However, the function of the LMP-1 protein in the HRS cells remains to be elucidated.

Conclusion: The outcome of EBV infection in B lymphocytes is determined by their maturation state. Among the various types of viral expression strategies, only Type III induces autonomous proliferation. The immune system recognizes these cells readily, therefore only immunosuppressed individuals develop progressively growing immunoblastomas.

EBV is also associated with other malignancies, originating from other cell types. The majority of nasal T and NK lymphomas carry EBV. Most low differentiated or anaplastic nasopharyngeal carcinomas carry EBV. Similarly to BL, these diseases of lymphocyte or epithelial origin also have their EBV negative counterparts. For BL the translocation of the myc gene is shared by the virus positive and negative cases. The common feature in HLs, the activation of the NFkB pathway seems to be the determinant factor. Mutation in the NFkB inhibitor IkBa was identified in some but not in all cases.

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