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Epstein Barr virus — a tumor virus that needs cytotoxic lymphocytes to persist asymptomatically

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Epstein Barr virus (EBV) is a human γ-herpesvirus that was discovered in Burkitt's lymphoma more than 50 years ago. Since then it has been proposed as the causative agent of up to 2% of all tumors worldwide, mainly lymphomas and epithelial cell carcinomas. Surprisingly, persistent EBV infection is at the same time found in more than 90% of healthy human adults. Even the very same programs of EBV infection that are found in the associated malignancies are continuously present in healthy EBV carriers. We will discuss primary immunodeficiencies and immune compartment changes during the first decade of human life that give us insights into how tumorigenesis by persistent EBV infection and Hodgkin's lymphoma predisposing infectious mononucleosis is prevented during primary infection. These insights should allow identifying individuals at risk to develop EBV associated malignancies, who would benefit from EBV specific vaccination.

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Introduction on EBV associated tumors

Epstein Barr Virus (EBV) is arguably the most successful human pathogen with more than 90% of the human population persistently infected. Despite this stalemate of viral persistence without symptoms, EBV is also one of the most growth transforming viruses and the only one that can readily immortalize human cells, namely B cells, into continuous growth as lymphoblastoid cell lines (LCLs) after infection in vitro. Indeed it was discovered in 1964 by Antony Epstein and colleagues as the first human candidate tumor virus in tumors of sub-Saharan children, called Burkitt's lymphomas [1]. Since then it has been found to be associated with a broad variety of human malignancies, ranging from smooth muscle tumors, nasopharyngeal carcinomas and a subset of gastric carcinomas to lymphomas of primarily B cell, but also less frequent of T and natural killer (NK) cell origin [2]. Indeed, by now around 1-2% of human tumors are thought to be EBV associated and in the order of 200′000 EBV associated malignancies are newly diagnosed every year [3•]. These numbers range only threefold below the incidence of human papilloma virus (HPV) associated tumors and make EBV, similarly to HPV, an attractive vaccination target. However, it is surprising that EBV does not cause malignancies more frequently. I would argue that the human immune system has during its co-evolution with EBV nearly perfectly adapted to control this tumor virus. In order to understand how this nearly perfect immune control works, which is required to prevent disease despite persistent infection, I have to discuss, what the immune system can see in healthy EBV carries.

EBV is thought to be primarily transmitted via saliva exchange. EBV in saliva is prone to infect B cells at submucosal sites [4], to which it is thought to gain access via transcytosis or in between epithelial cells with incomplete tight junction networks in the tonsillar crypts [5]. Naïve submucosal B cells express all eight latent EBV proteins (6 nuclear or EBNA and 2 membrane or LMP proteins) [6]. These proteins drive B cell proliferation mainly via EBNA2 and LMP1 and rescue proliferating B cells from programmed cell death mainly via EBNA3A, 3C and LMP2 [7]. This so called latency III EBV infection program can also be found in LCLs. B cells that are activated in this fashion via EBV infection are then thought to enter the germinal center reaction according to normal B cell physiology. In such centroblasts and -cytes only three EBV proteins are found to be expressed in the so-called latency II pattern (EBNA1, LMP1 and LMP2) [6]. These gene products ensure the survival of EBV infected B cells to differentiate further and persist then finally as memory B cells without EBV protein expression (latency 0) [8]. Homeostatic proliferation of these memory B cells requires transient EBNA1 expression (latency I), which ensures replication of the viral genome and distribution to daughter cells during proliferation [9]. Plasma cell differentiation from this memory B cell pool induces lytic EBV replication [10], which can then infect epithelial cells from the basolateral side for another amplification of viral particles through lytic replication in epithelial cells and shedding into saliva for transmission [11]. Latencies I, II and III can be found in EBV associated malignancies, like Burkitt's lymphoma,

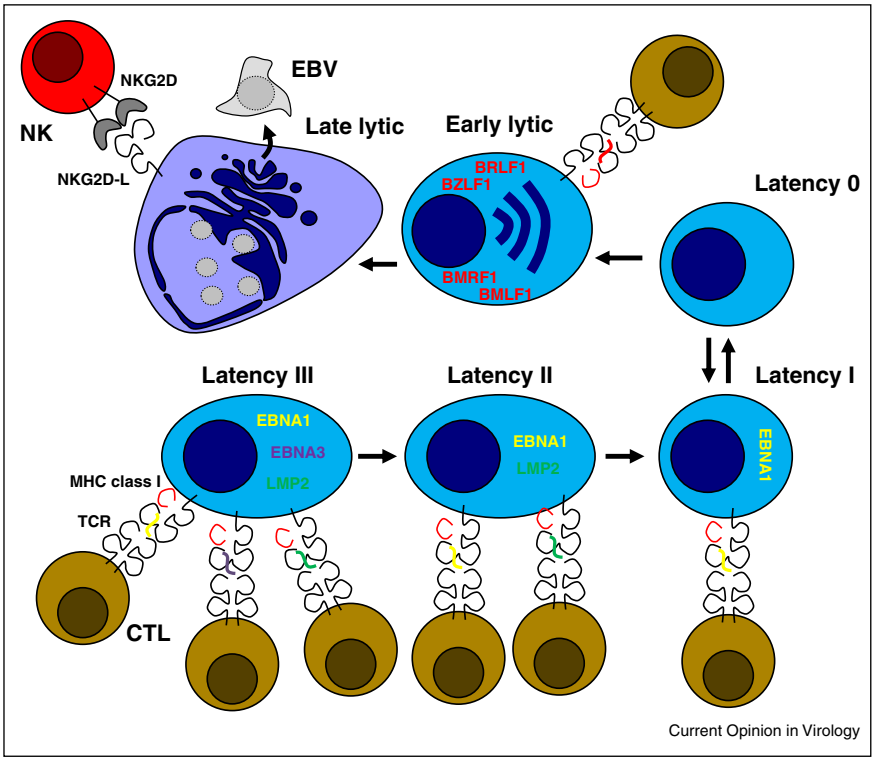
Hodgkin's lymphoma and immunoblastic lymphomas, respectively. However, with decreasing EBV antigen expression the tumor cells require compensatory mutations that maintain their proliferation and/or cell death resistance. Some of these are thought to be induced by the somatic hypermutation machinery of germinal center B cells, which is in part also induced by latent EBV antigens directly [12••]. Thus, the gene expression programs of EBV associated tumors are already present in healthy EBV carriers and even the machinery for additional tumorigenic mutations is in part induced by EBV infection. In this review, we will discuss the immune components that stand between this tumor anlage and the realization of EBV associated malignancies.

Primary immunodeficiencies resulting in loss of EBV specific immune control

Genetic lesions that predispose for infectious diseases have identified essential pathways for the immune control of the respective causative pathogens [13,14]. Susceptibility to uncontrolled EBV infection is no exception. Chronic active EBV infection (CAEBV) was found in individuals with mutations in perforin and two vesicular fusion proteins for cytotoxic granules Munc18-2 and 13-4 [15•,16•]. Munc18-2 and 13-4 are involved in the fusion of perforin and granzyme containing vesicles with the cell membrane during the release of cytotoxic granules, and perforin assembles into pores in the cell membrane of target cells to allow secreted granzymes to get access to the cytosol and initiate programmed cell death. These deficiencies identify the cytotoxic effector function of the immune system as a crucial parameter in EBV specific immune control.

This cytotoxic function is mainly mediated by $CD8^+$ T cells and NK cells (Figure 1). Accordingly, primary immunodeficiencies that compromise the differentiation of these cytotoxic lymphocytes affect EBV specific immune control. Indeed, the first primary immunodeficiency, causing susceptibility to herpesvirus infections, including EBV, was found to be caused by mutations in the GATA binding protein 2 (GATA2) [17,18]. GATA2 deficiency, however, affects many hematopoietic lineages, including B cells, dendritic cells, monocytes and neutrophils in addition to T and NK cells [19]. Nevertheless, CAEBV and virus associated smooth

Figure 1



The cytotoxic lymphocytes $CD8^+$ T cells and NK cells share the work to target all EBV infection programs. $CD8^+$ T cells target latent EBV infection, while lytic EBV replication in differentiated plasma cells is recognized by NK cells. With decreasing EBV antigen expression in the B cell differentiation from EBV latency III to I less cytotoxic T lymphocyte (CTL) antigens can be recognized by $CD8^+$ T cells. The immunodominant EBNA3 and LMP2 CTL antigens are lost during this differentiation and EBNA1 remains the only target antigen in latency I. Plasma cell development is associated with lytic EBV replication. Early lytic EBV antigens are still well recognized by $CD8^+$ T cells (e.g. BZLF1, BRLF1, BMRF1 and BMLF1). However, with successive MHC class I down-regulation and up-regulation of NKG2D ligands (NKG2D-L) late lytically EBV replication cells become NK cell targets.