The infectious kiss: Newly infected B cells deliver Epstein–Barr virus to epithelial cells

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Epstein–Barr virus (EBV) is an extremely successful virus in that it infects >90% of the human population. The success of the virus as an infectious agent in the human population stands in stark contrast to our poor knowledge of the life cycle of the virus in immunocompetent individuals *in vivo*. There is consent in the scientific community that B cells represent the primary target cells for infection and establishment of latency and that infection of B cells is usually nonproductive. There is evidence that B cells are necessary to establish an EBV infection (1) and that the hematopoietic system is the only site of viral latency *in vivo* (2). Although the role of the oropharyngeal epithelium as a site of virus replication is firmly established in immunocompromised patients (3), its role for the life cycle of the virus in normal healthy individuals is controversial. Many reports support the view that in normal, healthy individuals the entire viral life cycle takes place exclusively in B cells. An article in a recent issue of PNAS by Shannon-Lowe *et al.* (4) may restimulate the interest in epithelial cells as the site of viral replication in the natural course of infection. The authors found that newly infected primary B cells are an ideal transfer vehicle for infection of epithelial cells. Before we discuss the article in detail, we will briefly outline the current knowledge of the life cycle of EBV.

In the last decade, great progress has been made in our understanding of how EBV uses its transforming capacity to expand the pool of infected B cells *in vivo* and how it exploits normal B cell biology to establish *in vivo* latency in B cells, usually without doing any harm to its host (5). Primary infection with EBV usually takes place early in childhood and remains clinically inapparent, whereas infection of older children and adolescents may lead to infectious mononucleosis, a self-limiting lymphoproliferative disease. Primary targets of EBV *in vitro* and *in vivo* are resting human B cells that are driven into proliferation by the virus. *In vivo*, the burst of virally induced B cell proliferation elicits a very potent T cell response against viral antigens that keeps infected B cells under control (6). Despite this vigorous immune response, the virus is not eliminated. By means not well understood, the viral proliferation program is switched off, allowing infected cells to escape immune recognition and establish lifelong persistence in the memory B cell compartment. How the virus gets access to memory B cells is still a matter of debate (7, 8). As a result, healthy individuals harbor between 1 and 10 latently infected B cells within 10^6 peripheral blood mononuclear cells. Immunosuppression will lead to an increase in the number of latently infected B cells. Over a wide range of virus load, a dynamic stable equilibrium between virus and host may still be achieved (9), and only under severe immunosuppression, overt EBV-induced lymphoproliferative disease (PTLD) ensues.

Infection of B cells is initially nonproductive, i.e., no viral progeny is released. Infection of B cells thus can hardly explain the successful spread of the virus in the human population. Given the well established dual tropism of EBV for B lymphocytes and epithelial cells that is reflected by the association of EBV with Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC), it was long thought that the epithelial cells of the nasopharynx and the parotid glands represent the physiological site of virus production (10, 11). Several modes of viral entry into epithelial cells also have been described (12–14). The concept that epithelial cells replicate and produce EBV gained wide acceptance with the compelling evidence that EBV undergoes massive lytic replication and sheds virus from the epithelium of the lateral part of the tongue in AIDS patients with oral hairy leukoplakia (OHL) (3). It was assumed that what is seen in AIDS patients is an amplification of the situation in healthy non-HIV-infected individuals (2).

The concept that epithelial cells represent the primary site of virus entry and replication has been challenged in the 1990s by pathologists who failed to detect the virus in exfoliated epithelial cells of patients with infectious mononucleosis and normal healthy individuals (15, 16). Because they detected the virus in B cells and in plasma cells, it was suggested that the life cycle of the virus might be restricted to B cells under physiological conditions (15–19). The "B cell-only model" implies that B cells not only represent the site of *in vivo* latency but also play the decisive role in virus replication and transmission (1). Differentiation of B cells into plasma cells was described to promote viral replication (20). A recent study confirmed and extended the link between plasma cell differentiation and virus reactivation in the tonsils of Waldeyer's ring, although only a minority of plasma cells complete the lytic cycle, implying that the actual number of plasma cells that shed virus into the saliva must be small (21).

The article by Shannon-Lowe *et al.* (4) may shift the balance back to epithelial cells as a possibly important site of virus replication in the natural life cycle of EBV. The authors started from the observation that epithelial cells can be readily infected by coculture with virus-producing B cell lines but not, or only poorly, by cell-free virus (22). In addition to using LCLs or BL lines, they used freshly infected primary B cells and EBV-negative cell lines. They found (*i*) that transfer infection using primary B cells is an efficient process leading to infection rates of 5–25% in various epithelial cell lines, (*ii*) that transfer infection is equally effective using a replication-defective BZLF1-knockout virus, (*iii*) that infection efficiency correlates with the level of CD21 expression and virus binding to the B cell surface, and (*iv*) that neither fibroblasts nor endothelial cells could be infected by these means. Fixation of the B cells by glutaraldehyde abolished transfer infection but not virus binding, pointing to an active participation of the donor cell. Time course experiments revealed that an infected B cell is competent to virus transfer ≈6 hours postinfection and that it stays so for up to 2 days. This long competence to transfer the virus correlated with the continuous presence of gp350 and DNA containing virus particles on the B cell surface (23, 24). Once the contact to the epithelial cell is established, virus transfer is completed within 10 min.

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By using deletion mutants for the various glycoproteins, the authors showed that gp350 is required for virus binding as well as transfer infection. As expected, gp42, the ligand for HLA class II, is essential for infection of B cells but not for transfer infection into epithelial cells, whereas gp85 is essential (25). Remarkably, gp350-deficient virus gave rates of direct infection to epithelial cells similar to those seen with wild-type virus using transfer infection, indicating that gp350 is important in cell-mediated, but not in cell-free, EBV infection of epithelial cells. The authors finally show that virus transfer from B cells to epithelial cells involves synapse formation but not cell fusion and that CD21 and gp350 accumulate at the interface between both cell types. The data suggest that the viral ligand for a receptor on epithelial cells is not accessible in free virus and becomes exposed after gp350 binding to B cells.

The data have a number of interesting and thought-provoking implications. It is well known that the virus is transmitted through the saliva and that intimate contact renders transmission of the virus highly efficient. This is the reason why infectious mononucleosis is also known as "kissing disease." Cell-free virus transmitted through the saliva may first infect B cells, and these newly infected B cells may lead to highly efficient transfer infection of epithelial cells. Alternatively, newly infected B cells may be transmitted from one individual to another through intimate contact, and these B cells may directly transfer the virus to epithelial cells. A first wave of virus production in the oral cavity may give rise to virus that is highly infectious for B cells, unable to infect epithelial cells (25), and produced in sufficient quantities to provoke infectious mononucleosis in some patients with delayed primary infection (Fig. 1*A*). This first wave of virus production might be terminated at the end of the incubation period undetectable to pathologists. A second, not mutually exclusive possibility is that infection of epithelial cells represents an amplification step in the viral life cycle after latency in the hematopoietic system has been established *in vivo*. Notably, B cells decorated with surface-bound virus particles not only act as transfer vehicle for epithelial cells, but they also internalize viral antigens by receptor-mediated endocytosis, present them to CD4 T cells, and elicit a vigorous, highly efficient antiviral T cell response (26) (Fig. 1*B*). This finding is compatible with the concept that transfer infection into epithelial cells may be a highly efficient process in immunosuppressed patients (3) and much less efficient in an immunocompetent host.

The experimental model system provided by Shannon-Lowe *et al.* (4) will allow the elucidation of molecular mechanisms involved in the infection of epithelial cells by EBV, including the essential B cell functions and virion proteins, as well as the putative EBV receptor expressed on epithelial cells. Understanding how EBV infects epithelial cells *in vitro* may stimulate research on EBV's highly related cousins in Old World primates *in vivo* and will ultimately provide new insight into how EBV spreads within the human population.

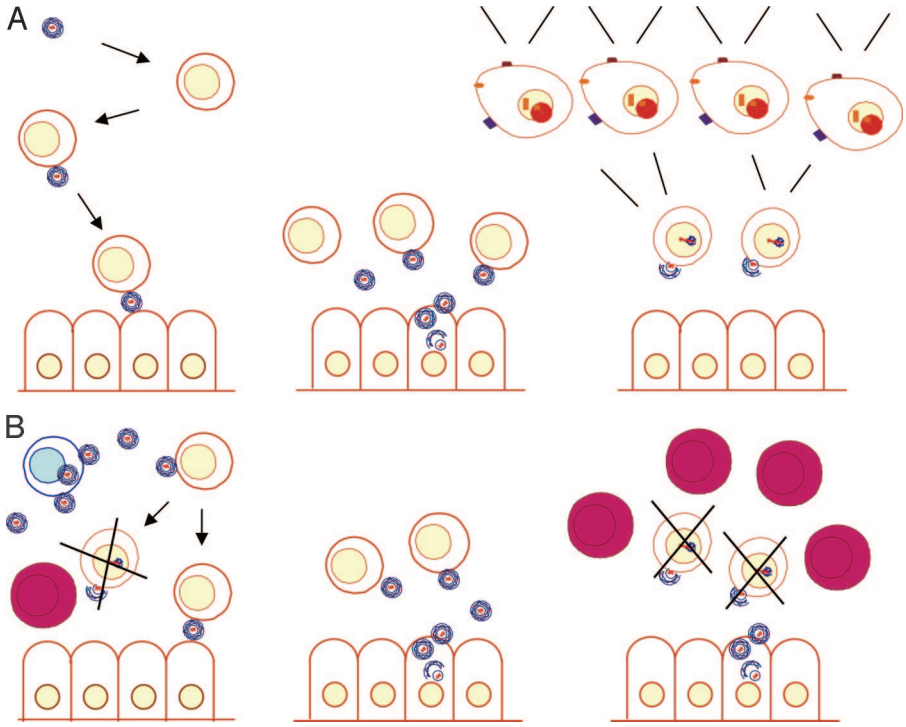

Fig. 1. A possible role for epithelial cells in EBV infection. (*A*) Transfer infection of epithelial cells by newly infected B cells may represent an amplification loop for massive infection of B cells during primary infection. In an EBV-positive healthy individual, latently infected B cells that differentiate into plasma cells (blue) give rise to virus progeny. (*B*) The T cell response to EBV may impair or prevent reinfection of epithelial cells (*Left*), reinfection of B cells after release of virus progeny from epithelial cells (*Right*), or both. T cells are shown in pink.

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