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Early virological and immunological events in Epstein–Barr virus infection

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Epstein–Barr virus (EBV) is a γ-herpesvirus which establishes a chronic yet asymptomatic infection in humans. This saliva transmitted virus has a tropism for B lymphocytes, in which it establishes a latent infection, and epithelial cells where the virus replicates to produce infectious particles. Although the majority of infections are apparently benign, primary EBV infection can be associated with an acute febrile syndrome, infectious mononucleosis, while infection is also associated with the development of malignancies of B lymphocyte and epithelial origin. A better understanding how the virus replicates initially in the host and its control at this stage will lead to the development of rationally targeted interventions which potentially would prevent infection or modify infection associated disease.

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Introduction

Epstein–Barr virus (EBV) is one of the eight known human herpesviruses and has developed a highly successful strategy of colonising its human hosts leading to its global distribution with up to 90% of the adult population infected with this virus. This success comes from its long evolution with humans and their ancestors allowing acquisition of mechanisms to manipulate infected cells to suit its replication strategy. Thus the virus infects B lymphocytes initially causing a growth transforming infection of these cells which eventually results in the virus being maintained latently in these cells [1]. The virus periodically reactivates from these cells and is amplified in epithelial cells which appear to be a major site of virus production [2]. The vast majority of EBV infections are silent, however infection may cause several diseases such as infectious mononucleosis (IM). This syndrome, observed mostly in adolescents undergoing primary EBV infection, is characterised by a self-limiting acute febrile illness where there is the global expansion of the CD8+ T cell compartment with high frequencies of activated EBV-specific T cell responses. Importantly EBV is associated with the development of at least 200 000 cases of cancer per year. These occur in the form of either B cell malignancies which include post-transplant lymphoma, Burkitt lymphoma and Hodgkin lymphoma, or epithelial cell malignancies which include nasopharyngeal carcinoma and some gastric carcinomas.

The ability to study early events in EBV infection has been severely limited by the asymptomatic nature of most infections and the lack of availability of animal models which recapitulate features of EBV infection. Most of what we currently know comes from the study of IM patients, as the symptoms they show are a convenient marker of primary infection. However careful epidemiological studies of such patients indicate they were probably exposed to EBV 4–6 weeks before the development of symptoms [3,4]. Consequently much less is known about how the virus colonises the host and how the immune response constrains replication at this stage of infection. Here we discuss some of the issues arising early in infection with EBV from virological and immunological points of view.

Virological events in early EBV infection

Beginning with the host's initial encounter with the virus, most infections are transmitted via saliva contact. The virus has a tropism for epithelial cells and B lymphocytes, which is dictated by the set of surface glycoproteins the virus expresses. Thus EBV uses the gp350 glycoprotein to attach to CD21 expressed on B lymphocytes, but also requires a second viral glycoprotein, gp42, to interact with its ligand MHC class II to efficiently infect these cells (reviewed in [5]). For epithelial cell infection, these cells do not express CD21 or MHC class II and so an alternative receptor–ligand set is used, probably involving binding of a cellular integrin by the virus [6]. Interesting in this context is that virus produced in B lymphocytes has low levels of gp42, thought to be a consequence of this protein being trapped on MHC class II molecules on the productively infected B lymphocyte as the virus buds from the cell. Conversely virus produced in MHC class II negative cell lines have higher levels of gp42 and, using *in vitro* models, epithelial cell derived virus has a preferential tropism for B lymphocytes [7]. Virus found in saliva, that is, virus being shed, is high in expression of gp42 and

probably then of epithelial origin and may preferentially target B cells. The virus may first infect B cells trafficking through the oral mucosa or within the lymphoepithelial structures such as those seen in tonsillar crypts, where the epithelia covering this tissue is thin and in close apposition to B lymphocytes. However direct evidence for this is lacking and potentially epithelial cells may be the first recipient of incoming virus. Interesting in this context are *in vitro* studies using organotypic epithelial rafts infected with EBV. Here the virus can infect such cells and enter into lytic cycle replication, efficiently spreading within the stratified structure [8]. Nevertheless emerging evidence from EBV infection *in vitro* [9] and other γ-herpesvirus infections *in vivo* suggests that efficient infection of cells is complex and may require interaction between two cell types [10].

Understanding how the virus replicates and disseminates after this first exposure is also not well understood. The best studied experiments come from prospective analysis of EBV seronegative donors who are routinely assessed for virus infection [11••]. Analysis of samples from such donors before the development of infectious mononucleosis has found that the majority do not appear to shed virus, arguing against extensive initial lytic replication in the oropharynx. Similar analysis of matched blood samples have shown that the majority of do not show evidence of virus genome levels in this compartment until just before symptom development. Again this indicates that there is no dramatic expansion of EBV-infected B lymphocytes during the prodromal phase and perhaps suggests that there is a vertical replication of EBV within infected B lymphocytes which may require 4–6 weeks before becoming detectable. To better describe virus colonisation during these early phases of infection, studies using rhesus lymphocryptovirus infection of rhesus monkeys may be applicable [12]. This virus has an identical repertoire of genes to EBV, can establish a latent infection of naïve hosts after oral inoculation and in some cases, animals develop mononucleosis like symptoms (reviewed in [13]).

Immune response to EBV in early infection

The immune response is clearly critical for the control of EBV as is evident from primary immunodeficiencies which affect natural killer cells (NK) or T lymphocytes. The asymptomatic nature of initial virus infection with EBV limits the study of immune control and additionally, most studies have examined EBV-specific immunity in the peripheral circulation rather than relevant tissues, which can show contrasting pictures [14]. However animal models of infection, particularly the humanised mouse model of immunodeficient mice reconstituted with CD34+ hematopoietic progenitor cells, are identifying roles different immune effectors might play in early infection.

NK cells are probably among the first circulating immune effectors to encounter EBV infected cells. These are a diverse population of cells which use multiple activating or inhibitory receptors to interrogate cell surface ligands and the balance of these signals can evoke effector responses. Subsets of these cells are differentially distributed within anatomical compartments: the peripheral circulation contains mostly the more differentiated $CD56^{dim}$ and CD16+ subset of NK cells which have rapid cytotoxic and cytokine secretion effector function. The other population of NK cells found in blood is the less differentiated $CD56^{bright}$ CD16− NK cells. This population is enriched in lymphoid tissues, can produce large amounts of cytokine upon stimulation and can acquire cytotoxic potential after prolonged stimulation. Analysis of blood populations of NK cells has shown that during the asymptomatic phase preceding the development of IM, these cells show little if any expansion [11••]. However upon symptom development expansions of these cells are seen in the periphery and in some [11••] but not all studies [15], the number of NK cells has been shown to correlate with blood virus load.

Although NK cell numbers are not expanded in the periphery before the development of symptomatic infection, this does not preclude a role in the early control of EBV infection. Less mature $CD56^{bright}$ NKG2A+ tonsillar NK cells have been shown to restrict B cell transformation by EBV *in vitro* through an interferon-γ mediated mechanism, suggesting a potential role [16,17]. More compelling evidence comes from the humanised mouse model of EBV infection where depletion of NK cells from mice subsequently challenged with EBV leads to the development of an infection resembling infectious mononucleosis [18••]. Such mice show expansions of CD8+ T cells, increased virus loads and increased levels of serum inflammatory cytokines. These features were thought to be consequences of the loss of either NK control of lytic antigen expressing infected cells, or NK mediated cytotoxic restriction of activated T cells. Interestingly, EBV challenge of humanised mice leads to a transient increase in NK cells with increased frequencies of NKG2A+ NK cells in the blood; a phenotype reminiscent of the subset capable of restricting transformation of B cells *in vitro*. Furthermore IM patients expand a similar population of $CD56^{dim}$ NKG2A+ KIR− NK cells and these preferentially respond to EBV lytic infected cells, suggesting they may be important in controlling EBV lytic infected cells [19]. This population of NK cells decreases with age, making an attractive hypothesis that this subset may control EBV replication early and their loss with age leaves the host unable to efficiently control lytic EBV infection leading to the development of IM.

Another cell population with innate like characteristics are invariant NK T cells (iNKT), which recognise lipid antigens in the context of CD1d molecules. Patients with deficiencies or low numbers of this population are extremely sensitive to EBV infection although such patients may also have defects in other immune effectors [20,21].