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Review

Co-infections, inflammation and oncogenesis: Future directions for EBV research

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ABSTRACT

Epstein–Barr virus (EBV) is aetiologically linked to a wide range of human tumours. Some arise as accidents of the virus' lifestyle in its natural niche, the B lymphoid system; these include B-lymphoproliferative disease of the immunocompromised, Hodgkin Lymphoma, Burkitt Lymphoma and particular forms of diffuse large B cell lymphoma. Interestingly, HIV infection increases the incidence of each of these B cell malignancies, though by different degrees and for different reasons. Other EBV-associated tumours arise through rare viral entry into unnatural target tissues; these include all cases of nasal T/NK cell lymphoma and of undifferentiated nasopharyngeal carcinoma plus a small but significant subset of gastric carcinomas, a tumour type more generally associated with chronic *Helicobacter pylori* infection. Understanding EBV's involvement in the pathogenesis of these different malignancies is an important long-term goal. This article focuses on two overlapping, but relatively neglected, areas of research that could contribute to that goal. The first addresses the mechanisms whereby coincident infections with other pathogens increase the risk of EBV-positive malignancies, and takes as its paradigm the actions of holoendemic malaria and HIV infections as co-factors in Burkitt lymphomagenesis. The second widens the argument to include both infectious and non-infectious sources of chronic inflammation in the pathogenesis of EBV-positive tumours such as T/NK cell lymphoma, nasopharyngeal carcinoma and gastric carcinoma.

Epstein–Barr virus (EBV), one of eight known human herpesviruses, was first discovered 50 years ago through its association with Burkitt Lymphoma (BL), then a little-known paediatric tumour thought to be restricted to equatorial regions of Africa. Electron microscopic observation of a BL-derived cell line had revealed a small number of cells containing herpesvirus-like particles [1]. At the time, there was profound scepticism about the identity of EBV and the significance of its presence in the cultured lymphoma cells. Ironically, the development of antibody screening assays soon confirmed the unique identity of the virus but at the same time showed that it was a common infection in all human populations worldwide and not, as the epidemiology of BL had implied, restricted to areas where the tumour was first recognized. Even within two years of EBV's discovery therefore, one thing was clear; if the virus was to play any role in BL pathogenesis, other factors must be crucial to the oncogenic process.

As EBV's association with an ever-widening range of human tumours unfolded over the ensuing decades [2], researchers have each time been faced with this same conundrum. What factors act cooperatively with the virus to produce malignancy, and what are the mechanisms that underpin that cooperation? Such questions have been the subject of research by many groups, much of it focusing on the interplay of EBV infection and complementary cellular genomic changes in tumour pathogenesis. That focus, in which the cellular genetics of BL has led the way, is destined to intensify with the advent of cancer genome sequencing. The present review seeks to highlight another area of investigation which forms part of the bigger picture but which has attracted less attention of late. That is the involvement of other infectious agents, or even non-infectious sources of chronic inflammation, in the development of these tumours. Again, it is the Burkitt tumour that gives us the classic example in the form of *Plasmodium Falciparum* malaria, which as a holoendemic infection has long been recognized as a co-factor in the pathogenesis of BL in its high incidence "endemic" form [3]. Yet this is only one of several examples where more research is needed to understand how other infections and/or chronic inflammatory stimuli might impact on EBV-associated oncogenesis. To see the

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issues in context, let us consider what we already know about the biology of EBV infection.

1. An overview of EBV biology

EBV is the best known member of the gamma-1 herpesviruses, a genus with the classical herpesvirus characteristic of persistence in the immune host as a latent infection but with additional features that mark it as distinct from other herpesvirus genera. These features are a restriction to primate hosts, a pronounced tropism for latent infection within B lymphocytes, and the capacity (apparent both in vivo and in vitro) for B lymphocyte growth transformation through the coordinate expression of the so-called virus latent genes [4]. The restriction to primate hosts has long been a barrier to studying gamma-1 herpesvirus infections experimentally in vivo. Although new animal models are now beginning to overcome that barrier [5,6], present views of gamma-1 herpesvirus biology are still largely dependent on inference from studies of EBV infection in the human host, both in health and disease. Fig. 1 summarizes the basic framework of EBV infection as it is currently understood, with further detail given in relevant reviews [4,7,8]. The main features, in chronologic order, are (i) initial replication of orally transmitted virus in permissive cells in the oropharynx; such "lytic" infection, most likely involving squamous epithelial cells and possibly also locally-infiltrating lymphocytes, leads to high level virus shedding in the throat; (ii) colonization of the host through growth-transforming latent infection of B cells in oropharyngeal lymphoid tissues; (iii) down-regulation of growth-transforming gene expression in at least some of these transformed cells; how this occurs is not known but one possible route is by virus-induced entry into and transit through a germinal centre reaction [7], i.e. the physiologic route whereby antigen-stimulated B cells proliferate and mutate their immunoglobulin (Ig) genes such that progeny cells expressing the best-fit somatic Ig gene variants can be selected into the long-lived memory pool; (iv) lifelong virus persistence within the re-circulating memory B cell pool as a silent infection, with latent antigen expression extinguished; such EBV-infected but quiescent memory cells are then subject to the rules normally influencing memory cell behaviour in the B cell system, including the possibility that a chance encounter with cognate antigen will drive a memory cell towards plasma cell differentiation and/or through a further germinal centre reaction; (v) such plasma cell differentiation may be one of the cues triggering the occasional latently-infected B cell into lytic cycle; where this occurs at a mucosal surface, the virus particles thus produced could seed new foci of virus replication at oropharyngeal sites and at the same time initiate new growth-transforming B cell infections. As discussed elsewhere [8,9], both the lytic and growth-transforming latent infections are a rich source of viral antigens that induce strong cell-mediated responses in the immunocompetent host. These responses, involving natural killer (NK) cells, $CD4^+$ T cells and, in particular, $CD8^+$ T cells, act collectively not just to bring the primary infection under control but also, in the longer term, to limit virus reactivation from the memory B cell reservoir and the re-emergence of virus-transformed B-lymphoproliferative lesions.

2. An overview of EBV-associated tumours

Though EBV infection is asymptomatic in the vast majority of individuals, the virus has oncogenic potential and is aetiologically linked to a remarkably wide range of tumours [2,4]. These tumours, of B cell and non-B cell origin, are listed in Table 1. Shown alongside in each case are the strength of their EBV association (as percentage of tumours that are EBV genome-positive) and, where known, the identity of co-factors involved in their pathogenesis.

(i) Tumours of B cell origin: all EBV-positive B cell tumours can be considered as rare accidents of EBV's normal lifestyle in the B cell system. Furthermore, almost all display somatically-mutated Ig genes and therefore derive from cells that have been through a germinal centre reaction at some point. The main B cell tumours are:

B-lymphoproliferative disease (B-LPD) of the immunocompromised: The most direct proof of EBV's oncogenic potential comes in the form of EBV-positive B-LPD lesions that arise in bone marrow and solid organ transplant recipients within the first year post-transplant when their T cell-suppression is most intense (often called "early onset post-transplant LPD") [10,11]. An identical tumour also arises in late-stage AIDS patients when T cell impairment becomes most profound; it is best classified as AIDS-LPD (to distinguish it from other AIDS-lymphomas), but in the literature is usually reported either as immunoblastic lymphoma or as CNS lymphoma, the latter because many cases present in the central nervous system [12].

Hodgkin Lymphoma (HL): EBV is also linked to many cases of classical HL worldwide, particularly those presenting with the mixed cellularity or lymphocyte-depleted histology. These are the majority subtypes of HL as seen in many developing countries, where up to 80–90% tumours are EBV-positive. In the West, subtype distribution is skewed by a young adult peak of nodular sclerosing HL that is <20% EBV-positive, thereby reducing the overall percentage of EBV-positive HL cases in those countries down to 30–35% [13]. Note that HIV-positive patients in the West are at increased risk of developing HL; such AIDS-HL cases are almost all EBV-positive and most frequently of mixed cellularity or lymphocyte-depleted subtype [14].

Burkitt Lymphoma (BL): Essentially all cases of the "endemic" high incidence form of BL, as seen in equatorial Africa and in Papua New Guinea, are EBV genome-positive. BL does occur in children outside of those areas in a lower incidence "sporadic" form. Incidence rates and EBV association rates for the sporadic tumour vary with geography but in ways that are still poorly defined. For example, tumour incidence in affluent Western societies is 100-fold lower than endemic levels and only 10–15% tumours are EBV-positive, whereas data from northern Brazil suggest that there sporadic BL is more common and up to 85% tumours are EBV-positive. This prompted the idea that there is a low base-line rate of BL developing independently of EBV in all populations worldwide and that any increases above that baseline reflect the additional impact of the EBV-associated tumour [16]. However there is a third form of BL that occurs in HIV-infected adults in the West at an incidence that is much higher than the endemic disease, yet only 30–40% of AIDS-BL tumours are EBV-positive [15]. All three forms of BL share essentially the same histology with the tumour cells resembling germinal centroblasts, one of several features identifying BL as a tumour of germinal centre origin.

Other B cell lymphomas: EBV is also found in association with a number of other, less common, B cell tumours, many of which are classified histologically as subtypes of "diffuse large B cell lymphoma (DLBCL)". However, all arise in particular circumstances and are distinct from DLBCLs as seen in the general population. Thus all cases of "pyothorax lymphoma", arising in individuals with chronic lung inflammation caused by pneumothorax surgery, are EBV-positive [17], as are >50% DLBCLs arising in elderly patients, possibly reflecting age-dependent impairment of T cell surveillance [18], and >50% of the B cell lymphomas appearing as "late-onset post-transplant tumours" in solid organ recipients who remain on low level immunsuppression for life [19]. In addition, three rare B cell tumours peculiar to AIDS patients also have associations with EBV. One of these, an AIDS-DLBCL of centroblastic type, is EBV-positive

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