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REVIEW ARTICLE

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The Epstein-Barr virus: a group 1 carcinogen?

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Abstract The Epstein-Barr virus (EBV) is a human herpes virus with the ability to transform B-lymphocytes in vitro. EBV has been linked to the pathogenesis of a variety of human tumours, including Burkitt's lymphoma, immunosuppression-related lymphomas, Hodgkin's disease, nasal angiocentric T/NK-cell lymphoma and nasopharyngeal carcinoma. Based on the association of the virus with these tumours, EBV has been classified as a group 1 carcinogen by the WHO International Agency for Research on Cancer. In this article, the evidence suggesting that EBV is carcinogenic to humans is briefly reviewed.

Key words Epstein-Barr virus · Latent membrane protein 1 · Post-transplant lymphoproliferative disorder · Hodgkin's disease · T-cell lymphoma · Nasopharyngeal carcinoma

Introduction: classification of human carcinogens

In 1969 the International Agency for Research on Cancer (IARC) established a programme aimed at assessing the carcinogenic risks to humans associated with chemicals. The scope of this programme was later expanded to include physical and biological agents. To this end, the IARC set up working groups to evaluate the evidence regarding the carcinogenicity of an agent and to summarise the findings in the IARC monographs series [20]. For the purpose of these discussions, carcinogens are defined as agents capable of increasing the incidence of human cancers, without considering their carcinogenic potency [20]. Based on these deliberations, the agent under discussion is assigned to one of four groups (Table 1). A more detailed account of these proceedings can be found elsewhere [20]. Following monographs on such agents as hepatotropic viruses, *Helicobacter pylori*, human papillomaviruses, and human immunodeficiency viruses, the possible carcinogenicity of the Epstein-Barr virus (EBV) and the Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8 (KSHV/HHV8) was considered in 1997. As a result, EBV was classified as a group 1 carcinogen, while HHV8 was assigned to group 2A [20].

Table 1 IARC classification of carcinogenic agents [20]

Group 1	Agents carcinogenic to humans
Group 2	Agents probably (2A) or possibly (2B) carcinogenic to humans
Group 3	Agents not classifiable as to their carcinogenicity
Group 4	Agents probably not carcinogenic to humans

The Epstein-Barr virus: biology of the virus

EBV is a B-lymphotropic herpesvirus characterised by its ability to infect B-cells and to immortalise them into permanently growing "lymphoblastoid" cell lines (LCL) [48]. In LCL cells infection is mostly latent, i.e., without production of infectious virions, and the viral genome is present as a circular extrachromosomal episome. In LCL cells, a limited set of latent viral gene products is expressed, which comprises two small nuclear RNA molecules (EBV-encoded RNAs, EBER1 and 2), six nuclear antigens (EBV-encoded nuclear antigens EBNA1, 2, 3A, 3B, 3C, -LP) and three latent membrane proteins (LMP1, 2A, 2B) [48]. The characteristic pattern of EBV gene expression in LCL cells is paralleled by an equally distinctive cellular phenotype characterised by the expression of lymphocyte activation antigens (e.g., CD30, CD39, CD40, CD70), cell adhesion molecules (e.g., CD44, CD54, CD58), and cytokines (interleukin (IL)-5, IL-6, IL-10) [20]. The changes brought about by EBV infection are reminiscent of the phenotypic alterations seen in antigen-activated B-cells, and thus it seems likely that EBV exploits and interferes with similar pathways.

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Table 2 Forms of EBV latency (*BL* Burkitt's lymphoma, *HD* Hodgkin's disease, *NPC* nasopharyngeal carcinoma, *IM* infectious mononucleosis, *PTLD* post-transplant lymphoproliferative disease)

Latency Disease	EBERs[a]	EBNA1[a]	EBNA2[a], -3A, -B,-C, -LP	LMP1[a], -2A[a], -B
I BL	+	+	–	–
II HD, NPC	+	+	–	+
III IM, PTLD	+	+	+	+

[a] Gene products detectable in paraffin sections by in situ hybridisation (EBERs) or by immunohistochemistry (all others) [14, 37, 38]

Of the EBV-latent genes, only EBNA1, EBNA2, EBNA3A, EBNA3C, and LMP1 are indispensable for B-cell transformation [20]. LMP1 can transform rodent fibroblasts in vitro, rendering them oncogenic in nude mice, and is thus a viral oncogene [58]. Expression of LMP1 in B-cells induces a variety of phenotypic changes and, alone or in cooperation with EBNA2, is responsible for most of the phenotypic features of EBV-immortalised LCLs [48]. LMP1 can prevent apoptosis in B-cells, and this is at least partly due to the induction of *bcl-2* expression [48]. Furthermore, there is an increased incidence of B-cell lymphomas in LMP1-transgenic mice [28]. LMP1 interacts with several tumour necrosis factor (TNF)-receptor-associated factors (TRAF1, -2, -3, -5) and the TNF-receptor-associated death domain (TRADD) [21, 50]. This interaction leads to the activation of the NFκB and JNK pathways [10, 25, 56]. Thus, it has been argued that LMP1 functions in the same way as a constitutively active member of the TNF-receptor family and is similar but not identical to CD40 [12, 26]. EBNA2 binds to RBP-Jκ, a nuclear protein, which acts downstream of a cellular receptor called Notch, and EBNA2 is functionally equivalent to activated Notch [51]. Of the other latent viral proteins, EBNA1 is required for maintaining the viral episome in proliferating cells, but a direct role for EBNA1 in the development of lymphomas has also been suggested [59]. EBNA-LP (leader protein) is not strictly necessary for transformation, but improves the outgrowth of transformed B-cells [20]. LMP2A is important for prevention of the switch from latent to replicative infection in response to B-cell activation [31]. A role for LMP2A in B-cell transformation beyond this effect remains a possibility [3, 4].

Studies of LCLs, Burkitt's lymphoma (BL) cell lines and human tumour biopsies have led to the identification of three distinct forms of EBV latency [48] (Table 2). The pattern of EBV gene expression seen in LCLs (see above) has been termed latency III and is characteristic for virus-driven lymphoproliferation. A much more restricted pattern of latency characterised by the expression of the EBERs and EBNA1 only is seen in Burkitt's lymphoma (latency I), while in Hodgkin's disease (HD) and nasopharyngeal carcinoma (NPC) expression of the LMPs has been detected together with the EBERs and EBNA1 but in the absence of the other EBNAs (latency II) [48] (Table 2). The function of the EBERs is still unknown, and it has been shown that they are not indispensable for B-cell transformation [48]. However, they are abundantly expressed in all known forms of EBV latency and therefore represent ideal targets for in situ hybridisation studies. Thus, EBER-specific in situ hybridisation has now become the standard tool for detecting latent EBV infection in tissue sections [20].

Primary and persistent EBV infection

Primary EBV infection usually occurs during childhood and is usually asymptomatic [20]. Delayed primary infection, which is common in most industrialised countries, may induce a benign lymphoproliferative disorder, infectious mononucleosis (IM). Most of what is known of primary EBV infection is derived from studies of IM patients and is based on the implicit, but unproved, assumption that IM is also representative of asymptomatic primary infection. IM is characterised by a proliferation of EBV-infected B-cells in the paracortex of lymphoid tissues such as the tonsils. These B-cells include large activated blast cells, occasionally resembling Hodgkin and Reed-Sternberg cells. In IM, the full range of latent viral gene products characteristic of latency III is expressed [57]. However, analysis at the single cell level has revealed a degree of heterogeneity, with cells expressing latency I, II, and III patterns being detectable [37]. A varying proportion of EBV-carrying B-cells shows evidence of plasmacytoid differentiation, and some of these cells may support virus replication [37]. Cells of other lineages, such as epithelial cells or T-cells, are rarely if ever infected with EBV in IM [37].

The proliferation of EBV-infected B-cells in IM is accompanied by the development of a vigorous cytotoxic T-lymphocyte (CTL) response directed against both latent and lytic viral proteins [48, 55]. Among the latent genes, all EBNAs and LMPs, with the notable exception of EBNA1, may provide CTL target epitopes [20]. This CTL response mediates the transition from primary infection into a state of asymptomatic life-long viral persistence. During EBV persistence, expression of latent genes is restricted to EBNA1 and LMP2A [57]. Present evidence suggests that EBV persists in the B-cell system, but the exact mechanisms mediating viral persistence are uncertain [20]. EBV can clearly infect other cell lineages, as illustrated by the detection of the virus in a variety of human tumours derived from, for example T-cells,

epithelial cells, and even smooth muscle cells. Thus, it is possible that cells of other lineages, in addition to B-cells, contribute to EBV persistence and replication.

EBV-associated malignant lymphomas

EBV is associated with several malignant lymphomas, including B-cell non-Hodgkin lymphomas (NHL), e.g., Burkitt's lymphoma (BL), lymphoproliferations in transplant patients, Hodgkin's disease (HD), and some cases of T-cell NHL, notably nasal T/NK-cell lymphomas [20].

Immunosuppression-related non-Hodgkin lymphomas

Post-transplantation lymphoproliferative disorders (PTLD) are a frequent complication in allograft recipients, occurring in between 0.5% and 30% of patients [20]. High levels of immunosuppression, primary EBV infection after transplantation, and treatment with anti-T-cell antibodies are recognised risk factors associated with an increased PTLD incidence [20]. The vast majority of PTLD cases have been shown to be of B-cell origin and to be associated with EBV infection [20]. Histologically, PTLDs represent a disease spectrum ranging from benign polyclonal, polymorphic lymphoproliferations to frankly malignant monoclonal, monomorphic lymphomas [20]. Characteristically, PTLDs show a type III latency such as is commonly observed in virus-driven lymphoproliferations [20] (Table 2). However, there is considerable variability both between and within lesions [7, 39, 41, 47]. Particularly in monomorphic lymphomas, EBV-latent protein expression may be restricted to latencies I or II [7, 39, 47]. Moreover, even EBN2A$^+$/LMP1$^+$ PTLDs may consist of a relatively small proportion of cells displaying a latency III pattern and others that show latency patterns I or II [37, 41]. A large proportion of PTLDs also supports lytic infection, usually in a small subset of cells [39, 44, 47]. However, if this is sufficient justification for the inclusion of acyclovir in the treatment of PTLD, as is often advocated, is uncertain [6, 44]. It seems likely that entry into the lytic cycle is merely a reflection of the underlying immune defect rather than of pathogenetic significance.

It is generally held that the above-mentioned spectrum of PTLDs is representative of an evolutionary process, which begins as an EBV-driven polyclonal B-cell proliferation [20]. The acquisition of additional genetic changes leads to the emergence of dominant B-cell clones and eventually to fully developed malignant lymphomas morphologically indistinguishable from lymphomas arising in immunocompetent individuals. This concept is based on molecular studies showing that polymorphic PTLDs are polyclonal, while monomorphic large cell lymphomas are monoclonal [30]. In between, there is a group of polymorphic lesions in which minor clonal components are detectable on the background of polyclonal B-cell proliferations [30]. Moreover, only monomorphic, monoclonal PTLDs contain alterations involving oncogenes or tumour suppressor genes, for example, *c-myc* translocations or mutations of the *N-ras* and *p53* genes [8, 27, 33]. Recurrent PTLD may show progression from polymorphic PTLD to frankly malignant lymphoma, while recurrence of a primarily monomorphic PTLD as a polymorphic PTLD has not been observed, further supporting the notion that PTLDs progress from early polymorphic polyclonal lesions to monomorphic monoclonal disease [60]. The importance of immunosuppression in the pathogenesis of PTLD is underlined by the frequent regression of PTLD lesions in response to a reduction or cessation of immunosuppressive therapy [54]. PTLDs developing less than a year after transplantation have a much higher response rate than those occurring later [1], and polyclonal PTLDs appear to respond better than monoclonal lesions. However, this distinction is not absolute, and a trial of reduced immunosuppression has been recommended in patients with monoclonal PTLDs [34]. The role of a failure of the EBV-specific immunity and therefore of the virus in the pathogenesis of PTLD is also underlined by recent successful efforts to prevent or treat PTLD using EBV-specific CTLs generated in vitro [49].

Lymphomas developing in the context of HIV infection are pathogenetically more complex than PTLD. Morphologically, they fall into two large groups: diffuse large B-cell lymphomas and Burkitt's lymphomas [20]. Diffuse large B-cell lymphomas develop in severely immunosuppressed patients. These lymphomas are mostly EBV-associated and express type II or type III latency patterns. While most cases are monoclonal, a few polyclonal lymphoproliferations have been described [20]. Thus, it has been proposed that AIDS-related diffuse large B-cell lymphomas develop according to a similar pathogenetic pathway to PTLDs. AIDS-associated Burkitt's lymphoma, by contrast, develop earlier and in less severely immunosuppressed than diffuse large B-cell NHL [20]. Only a proportion of these cases (up to 40%) are EBV-associated, and thus, AIDS-related Burkitt's lymphoma appears to be pathogenetically similar to sporadic BL (see below). This similarity may also extend to the rare BLs developing in transplant patients [39].

Burkitt's lymphoma

Burkitt's lymphoma (BL) was the first human tumour to be linked to a viral infection, and EBV was first detected in BL cells [48]. Subsequently, the virus has been demonstrated in virtually all cases of endemic BL, while only up to 30% of sporadic cases occurring in western Europe or in North America are EBV associated. All BL, endemic and sporadic, carry a *c-myc* translocation juxtaposing this oncogene to immunoglobulin genes, which leads to a transcriptional deregulation of *c-myc* [20]. In all EBV-positive BL cases, the virus is present in virtually all tumour cells and the viral genomes have been shown to be of monoclonal origin [20]. In contrast to

PTLD, BL develop in the face of an apparently normal EBV-specific immunity. The ability of BL cells to evade EBV-specific CTLs has been linked to phenotypic properties of BL, such as down-regulation of certain adhesion molecules, of MHC class I, and of the co-stimulatory molecules, B7-1 and B7-2 (CD80 and CD86) [20]. Most importantly, the expression of EBV-latent genes in BL is reduced to the EBERs and EBNA1 [20]. Thus, those latent viral proteins that are recognised by EBV-specific CTLs are not present in BL. Whilst allowing BL cells to escape EBV-specific immunity, this feature also raises questions about the role of EBV in the initiation and maintenance of this tumour, since the viral proteins with transforming properties, notably LMP1, are not expressed.

The presence of EBV in only a proportion of sporadic BL indicates that the function of EBV in the pathogenesis of BL may be substituted by other, as yet unidentified, factors. Thus, whilst a role for EBV in the pathogenesis of BL remains probable, the mechanisms by which EBV contributes to the disease are still uncertain. Notably, it has not been established which of the genetic alterations characterising BL (EBV infection and *c-myc* translocation) comes first [20]. It is conceivable that by inducing B-cell proliferation, EBV may simply function to increase the likelihood of a *c-myc* translocation. This notion is supported by the observation that BL cells may lose EBV, suggesting that the virus may not be required once the malignant growth has been established [52]. However, in vitro studies have shown that EBV-negative subclones derived from EBV-positive BL cell lines have lost their oncogenicity and that this may be restored by superinfection with EBV [5, 52]. Thus, these findings seem to suggest an important role for EBV not only in the development of BL, but also for the maintenance of the malignant phenotype.

Hodgkin's disease

Evidence has accumulated implicating EBV in the pathogenesis of Hodgkin's disease (HD). EBV antibody titres are elevated in HD patients prior to the onset of the disease [32], and there is an increased risk of developing HD following IM [20]. Using molecular techniques, EBV has been detected in the Hodgkin and Reed-Sternberg (HRS) cells of up to 50% of HD cases in western countries, and in up to 100% of cases in some developing countries and in paediatric patients [20]. Mixed cellularity HD (HDmc) shows the strongest association with EBV infection, the virus being present in the HRS cells of between 56% and 100% of cases, whereas EBV-carrying HRS cells are detectable only in between 17% and 56% of nodular sclerosis HD (HDns) cases [20]. Very few lymphocyte-depleted HD (HDld) cases have been examined, and their association with EBV has been variable, consistent with their origin from either HDns or HDmc and with the proposed overlap with CD30-positive anaplastic large-cell lymphomas [20]. In nodular lymphocyte-predominant HD (HDlp), EBV infection of HRS cells is at best rare, consistent with the notion that this represents a different entity distinct from the other forms of "classic" HD [20].

EBER in situ hybridisation has demonstrated the virus in virtually all neoplastic cells of EBV-associated HD [16]. Together with the detection of monoclonal EBV episomes in HD, this indicates that the EBV infection has taken place before initiation of the clonal cellular proliferation [16]. In agreement with this notion, it has been demonstrated that in multifocal HD the virus is detectable in all affected tissues and that the virus persists in most cases of recurrent HD [20]. EBV gene expression in HRS cells is restricted to the EBERs, EBNA1, LMP1, LMP2A and LMP2B, consistent with a type II latency [20] (Table 2). There is growing evidence to suggest that EBV, probably through LMP1, modulates the phenotype of HD. Thus, EBV is preferentially associated with the development of HDmc. Moreover, the presence of EBV in the HRS cells has been shown to correlate with an increased expression of lymphocyte activation antigens and with a decreased expression of the CD20 B-cell antigen [17]. LMP1 can induce TRAF1, and this molecule is overexpressed in HRS cells [9]. The presence of EBV also correlates with the expression of cytokines such as IL-10 and IL-6 in the HRS cells [15, 18]. As both IL-6 and IL-10 are up-regulated in LMP1-transfected cells in vitro [11], it is possible that this effect in HRS cells is also mediated by LMP1. LMP1 and LMP2A are known to provide target epitopes for EBV-specific T-cells. Their expression in HRS cells has therefore raised the question as to why these cells are not eliminated by CTLs. HRS cells retain the ability to process and present antigen in an MHC class I-restricted manner and can be lysed by CTLs in vitro [29, 53]. It is unclear which factors prevent this in vivo. Patients with EBV-positive HD do not appear to suffer from a systemic impairment of EBV-specific immunity [13]. It appears more likely that factors within the microenvironment of the HRS cells induce a local suppression of EBV-specific immunity [13]. The expression of IL-10 in HRS cells correlates with the presence of EBV, and this may locally inhibit EBV-specific CTLs [2, 15]. That EBV^+ HD can potentially be controlled by the immune system is illustrated by the observation that HD cases occurring in immunosuppressed individuals are invariably EBV positive and may regress spontaneously upon restoration of the immune system [20]. Thus, although definite proof of an aetiological role of EBV in the pathogenesis of HD has yet to come, the available evidence strongly implicates the virus as a co-factor in the pathogenesis and morphogenesis of a significant proportion of HD cases.

T-cell NHL

EBV is primarily a B-lymphotropic virus and is not normally found in T-cells. Therefore, the detection of the virus in a small sample of peripheral T-cell lymphomas

was unexpected [23]. Subsequent studies of large case series have revealed that EBV is absent from lymphoblastic T-cell NHL but can be detected in peripheral T-cell NHL more frequently than in B-cell NHL [20, 42]. However, in many nodal T-cell NHLs, the virus is present only in a subpopulation of tumour cells [42]. This may indicate either that EBV infection has occurred in an already established malignancy as a secondary event or that the viral genome has been lost from an initially EBV-positive tumour. In any case, this observation raises questions regarding the role of EBV in the pathogenesis of these tumours. Interpretation of this finding is further complicated by the observation that EBV-positive B-cells may proliferate in lymph nodes affected by EBV-negative T-cell NHL [19]. This finding underlines the importance of clearly identifying the phenotype of EBV-infected cells in such situations.

Several entities of T-cell NHL have attracted particular attention. A role for EBV in the pathogenesis of angioimmunoblastic lymphadenopathy (AILD)-type T-cell NHL has been suspected. However, characterisation of the phenotype of EBV-infected cells has identified the virus mainly in B-cells and only in a small proportion of cases in the neoplastic T-cells [20]. Enteropathy-type intestinal T-cell lymphomas, cutaneous T-cell lymphomas and anaplastic large cell lymphomas have been EBV negative with few exceptions [20]. The T-cell lymphoma showing the most consistent association with the virus is nasal angiocentric T/NK-cell lymphoma (so-called lethal midline granuloma). Studies from Asia, North America, and Europe have identified EBV in the vast majority of cases, and monoclonal viral episomes have been detected in virtually all tumour cells [20]. Moreover, LMP1 is expressed in the tumour cells in the context of a type II latency [20].These observations suggest a role for EBV in the pathogenesis of nasal angiocentric T/NK-cell lymphomas.

EBV-associated carcinomas

Nasopharyngeal carcinoma

Nasopharyngeal carcinomas (NPCs) occur with a high incidence in certain parts of Southeast Asia, northern Africa, and some other regions, but are rare in western Europe and North America. In all regions, nonkeratinising undifferentiated NPC represents the most common histological type, with squamous cell NPC being comparatively rare [36]. Independently of the geographic origin of the patients, undifferentiated NPCs are always EBV-positive, and thus the virus appears to be a rate-limiting step in the pathogenesis of this tumour [36]. Moreover, in undifferentiated NPC the virus is present as a monoclonal episome and can be detected in virtually all tumour cells [36]. This indicates that infection takes place early in the pathogenesis, before expansion of the malignant cell clone. This notion is also supported by the detection of EBV in in situ NPC, a presumed precursor lesion of undifferentiated NPC [43]. Moreover, clonal progression from mild dysplasia to invasive carcinoma has been suggested [22]. Expression of LMP1 can be detected at the mRNA or protein level in most undifferentiated NPCs and, as is the case with HD, the expression of this protein appears to affect the phenotype of the tumour cells [36]. Thus, most undifferentiated NPC express the CD70 antigen, which is not normally expressed in epithelial cells but has been induced in an LMP1-transfected keratinocyte line [36]. Moreover, the expression of the B7-1 and/or B7-2 molecules (CD80 and CD86) in NPC directly correlates with the immunohistological detection of LMP1 [36]. In addition to LMP1, LMP2A expression in NPC cells is detectable at the transcriptional level [20]. Since NPC cells possess normal antigen-presenting function and are effectively recognised by EBV-specific CTLs [24], this raises the question as to why these cells are not eliminated by CTLs in vivo. Expression of IL-10 in NPC cells has been reported, but this result requires confirmation [61].

While the association of undifferentiated NPC with EBV has been well documented, the possible association also with squamous cell carcinomas of the nasopharynx has been a subject of controversy [20]. Recent studies have demonstrated that EBV is present in all squamous cell NPCs from areas where NPC is endemic, while the virus is detectable only in a proportion of cases from regions with a low NPC incidence [35]. This is reminiscent of Burkitt's lymphoma and suggests that other factors may substitute EBV in the pathogenesis of squamous cell NPCs. Smoking and infection with human papillomaviruses are likely to be important in this respect [20].

Other carcinomas

The analysis of carcinomas other than NPC has revealed complex and unexpected patterns of EBV association. Interest has focused initially on carcinomas showing morphological features similar to undifferentiated NPCs, so-called lymphoepithelial carcinomas. Analyses of such cases have revealed three different groups. Lymphoepithelial carcinomas of the stomach are EBV-associated in approximately 80% of cases regardless of the geographic and ethnic origin of the patients [40]. Lymphoepithelial carcinomas of the salivary glands, the lungs and possibly the thymus are frequently associated with EBV infection in areas where NPC is endemic, whereas similar tumours arising in Caucasian patients have been shown to be EBV negative [20]. Lastly, there is a group of lymphoepithelial carcinomas that fail to show an EBV association. This group includes tumours of the cervix uteri, the urinary bladder, the skin, and the larynx [20].

The regular detection of EBV in lymphoepithelial carcinomas of the stomach has prompted the analysis of conventional gastric adenocarcinomas, revealing the presence of EBV in the neoplastic cells in between 2% and 16% of cases [40]. Significantly, this association has

been described in different populations from low-incidence areas, such as western Europe and the United States, to high-risk countries, such as Japan [20]. Although EBV has been detected in only a small proportion of gastric adenocarcinomas, this tumour is so common as to make EBV-associated gastric carcinoma a more significant health problem in terms of absolute case numbers than, for example, EBV-associated HD.

In all virus-associated carcinomas, EBV has been detected in virtually all tumour cells using EBER in situ hybridisation, and Southern blot studies have demonstrated clonal viral episomes [45]. This indicates that EBV infection in virus-associated carcinomas occurs before expansion of the malignant cell clones. The expression of EBV-latent genes in virus-associated carcinomas other than NPC has been most thoroughly analysed in gastric carcinomas. These studies have revealed the expression of EBNA1 and LMP2A in the absence of EBNA2 and LMP1 [40]. In contrast, transcriptional analysis of EBV gene expression in salivary gland lymphoepithelial carcinomas has indicated a type II latency, as also seen in NPC [46].

Thus, analysis of various carcinomas has revealed a heterogeneous picture with regards to the EBV association and to the prevailing patterns of EBV latency. This suggests that the contribution of the virus to the pathogenesis of EBV-associated carcinomas may vary.

Conclusions: EBV – a group 1 human carcinogen

EBV is a human herpes virus with the ability to transform resting B-cells into permanently growing lymphoblastoid cell lines. The viral genome encodes several transformation-associated latent proteins. One of these, LMP1, is a viral oncogene with functional similarities to a constitutively active member of the TNF-receptor family. Some of the other latent proteins, notably EBNA2, are also likely to interfere with intracellular signalling pathways.

EBV is associated with a growing list of human tumours, which now also includes leiomyosarcomas in immunosuppressed individuals [20]. Although the mechanistics of EBV transformation of B-lymphocytes and other cells in vitro are at last beginning to emerge, an aetiological role for the virus in the pathogenesis of human malignancies is difficult to establish. Ultimate proof can only come if vaccination against EBV infection leads to a reduction in the incidence of EBV-associated tumours [20]. Nevertheless, there is good evidence implicating EBV in the pathogenesis of some human tumours. In most EBV-associated neoplasms the virus is detectable in all tumour cells and viral episomes are of monoclonal origin. This suggests that EBV infection has taken place before expansion of the malignant clone and places EBV into the appropriate timeframe to play a part in the aetiology of these tumours. With the exception of BL and gastric adenocarcinoma, LMP1 is expressed in the tumour cells, and in some cases a correlation between the expression of LMP1 and the tumour cell phenotype has been established, suggesting that LMP1 may contribute to the malignant phenotype. The most convincing evidence for the oncogenicity of EBV has come from the study of PTLD. These tumours start as EBV-driven polyclonal B-cell lymphoproliferations which, through the acquisition of additional genetic alterations, may develop into fully fledged monoclonal malignant lymphomas. The role of EBV in these tumours is also underlined by the frequent regression of PTLDs once EBV-specific immunity is restored. Thus, there is good evidence to support the classification of EBV as a group 1 carcinogen (Table 1) [20]. However, this statement has to be qualified in several ways. The classification of EBV as a group 1 carcinogen only means that EBV infection increases the risk of developing certain cancers; it does not say anything about its carcinogenic potency. Clearly the vast majority of EBV-infected individuals (over 90% of the adult population world-wide) are asymptomatic and will never develop an EBV-associated cancer. Together with the usually long latency between primary infection and the occurrence of EBV-associated tumours this points to the need for co-factors in the pathogenesis of these malignancies. This is also underlined by the observation that the development of monoclonal malignant lymphoma from polyclonal PTLD requires additional genetic alterations.

The only tumour entity always associated with EBV is undifferentiated NPC, suggesting that EBV is a rate-limiting step in the pathogenesis of this tumour; for all other tumours pathogenetically linked to the virus, EBV-negative cases have been reported, and in some, such as gastric adenocarcinoma, EBV-associated cases represent only a small minority of cases.

This clearly illustrates that whatever the contribution of EBV to the pathogenesis of these tumours may be, it can be replaced by other factors.

The variable patterns of EBV-latent gene expression in different tumours suggest that the contribution of EBV to the pathogenesis of these tumours may also vary. In PTLD, the virus is, at least initially, in the driving seat. In HD and NPC, to name but two instances, LMP1 is expressed, making a contribution of EBV to the tumorigenic process at least likely. In yet other EBV-associated tumours, notably BL, the viral genome is almost silent, and therefore, the significance of EBV for the development and maintenance of such tumours remains uncertain.

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