H. Wolf
C. Bogedain
F. Schwarzmann

Institute of Medical Microbiology
and Hygiene, University of
Regensburg, FRG

Epstein-Barr Virus and Its Interaction with the Host

Summary

Epstein-Barr virus (EBV) as a member of the herpesvir
family persists lifelong in the human body and causes diseas
associated with virus replication (infectious mononucleosi
oral hairy leukoplakia) as well as neoplastic conditions such
nasopharyngeal carcinoma, B-cell lymphoma, Hodgkin's di
ease associated with viral latency. This complex biolo
relates to a highly regulated control of the persisting viru
Still, EBV is lytically produced in certain compartments of tl
human body. Epithelial cells were found to be of key impo
tance for this. Various routes (cell fusion, IgA recepto
mediated uptake) were described for EBV to enter epitheli
cells in the absence of CR2 receptor. Viral entry into cel
however, via CR2 receptor fusion or IgA mediated was n
found to be sufficient for viral production. The molecul
mechanisms for the lack of viral production in most targ
cells are primarily the presence of silencer activities and t
early elimination of cells entering the lytic cycle. Only term
nally differentiated epithelial cells are capable of supporti
an efficient lytic cycle of EBV replication. EBV-mediated su
pression of apoptosis as well as down-regulation of cellular a
viral gene products, such as HLA molecules, which medi
recognition by the immune system, are important contribu
ing factors to the development of these neoplasias where vi
genes, possibly via interaction with anti-oncogenes, such
p53, in context with genetic and environmental factors pla
key role. Novel diagnostic tools and a vaccine have been dev
oped which could help to control EBV-related diseases.

H. Wolf, PhD
Institute of Medical Microbiology and Hygiene
University of Regensburg
Franz-Josef-Strauss-Allee 11
D–93042 Regensburg (FRG)

When Dennis Burkitt approached Epstein in 1961 for help in search for a common principle involved in the generation of clinically very divergent neoplasias, a new field in virology started. Several achievements and discoveries, sometimes favored by luck, identified the first virus related to human neoplasia. Epstein's and Pulvertaft's groups succeeded for the first time in growing lymphoid cell lines out of lymphoma tissue [Epstein and Barr, 1964a; Pulvertaft, 1964]; Epstein's group detected herpesvirus-like particles in such cells [Epstein and Barr, 1964b], Werner and Gertrude Henle identified this virus as a new species of the herpesvirus family [Henle and Henle, 1966] and V. Diehl, when working with the Henles, found, when following up the seroconversion of a lab technician, that this new virus, now named after its first observers Epstein-Barr, was the cause of infectious mononucleosis (IM) [Henle et al., 1968]. Pope et al. [1967] were able to demonstrate that virus from IM patients can readily immortalize peripheral B lymphocytes. The newly developed technique of nucleic acid hybridization resolved the puzzle, that many cell lines did not produce any virus or early or late antigens related to viral replication. However, Epstein-Barr virus (EBV) DNA could be regularly demonstrated in DNA from cell lines and tumor biopsies. When sera from patients with nasopharyngeal carcinomas (NPC) were used as control to Burkitt lymphoma and other era, the regular and dramatically elevated antibody levels to EBV-related antigens suggested a causative involvement of EBV also in this malignancy [Old et al., 1968], where epithelial cells are the proliferative cell type. Is the presence of EBV an epiphenomenon introduced into the tumor tissue by infiltrating lymphocytes, or is it really in the epithelial tumor cells? A refinement of nucleic acid hybridization technique allowing the detection of specific nucleic acid in tissue sections was developed to answer this question [Wolf et al., 1973]. The linkage of EBV with IM as primary disease and B-cell lymphomas and NPC [Wolf et al., 1973] raised many questions to the molecular biologists, e.g.:

– What does EBV do to the human host?
– How does EBV enter the body and its host cells?
– Why does the presence of efficiently infectable target cells not kill all such cells and produce fatal disease and still ensure spreading of the virus by lifelong shedding of EBV?
– How does the immune system control EBV-infected cells?
– How does EBV immortalize and transform its host cells?
– What are the mechanisms permitting persistence of EBV?
– How can we control serious EBV-related diseases?

The number of diseases related to EBV has grown during the years. Whereas the primary infection has a tendency to milder symptoms in younger children, the typical picture is IM with the characteristic features fever, high leukocyte counts (from 20,000 up to 80,000) and sore throat. There is a tendency to more chronic courses lasting several months, in particular for older patients. The reason for rare fatal cases where virus replication has been detected in the liver is unclear [Deutsch et al., 1986].

A clear gentic factor is involved in Duncan syndrome where a locus of the X chromosome determines a fatal outcome of EBV infection [Purtilo et al., 1975; Skare et al., 1987]. 75% of males die from fatal IM, the remainder die from a variety of EBV-related conditions, mostly of B-cell lymphomas; 70% die by 10 years of age, 100% are dead by 40 years.

Whereas so far consequences of primary EBV infection were discussed, oral hairy leukoplakia is a secondary disease related to EBV replication [Greenspan et al., 1985] which occurs in discrete areas of the tongue, due to activation of EBV in maturing epithelial cells

Table 1. Cell proliferation-related conditions

1 B-cell lymphoma
   – Polyclonal
   – Monoclonal: Burkitt's lymphoma

2 T-cell lymphoma
   – AILD angioimmunoblastic lymphadenopathy
     with dysproteinemia [Weiss et al., 1992]
   – Lethal midline granuloma [Harabuchi et al.,
     1990]

3 Hodgkin's lymphoma (nodular sclerosing)
   [Weiss et al., 1987, 1989]

4 Nasopharyngeal carcinoma

in the absence of an intact cellular immune system [Becker et al., 1991].

More than lytic infection, induction of cellular proliferation is a characteristic feature of EBV. Besides Burkitt's lymphoma, where EBV is present in about 25% of worldwide sporadic cases, it is present in more than 90% of endemic cases in Africa. Because chromosomal translocations involving the myc oncogene are invariably present regardless of the EBV status [Taub et al., 1982; Erikson et al., 1982], EBV is seen by many as a favoring rather than in the strict sense causative factor. Considerable speculation has been published to this point [Lenoir and Bornkam, 1987], but the apparently high load of EBV in children, who later develop Burkitt's lymphoma (BL) in Africa [de The et al., 1978] might suggest that EBV gives some additional growth advantage to already immortalized cells or even may be involved in disturbing the chromosomal rearrangement, which through the activity of myc alters the immunologic features of the cells and makes them less recognizable by immune mechanisms. The latter function would explain why cells of the BL type have not been established by infection in vitro of EBV-negative peripheral or stem cells, where in vitro class switch in the immunoglobins is usually

not observed. Furthermore, in vitro the right selective pressure by specific T cells is not exerted.

For other neoplastic diseases with EBV association, even less is known on the mechanisms of development (table 1).

For NPC exclusive epidemiological studies suggest a genetic component, possibly related to the HLA family and environmental factor involving ingredients of certain medicinal herbs, voltaile nitrosamines from preserved food and low vitamin C content in the diet [Zeng et al., 1988, 1993; Poirier et al., 1987; Lu et al., 1990; Bouvier et al., 1991; Hubert al., 1993].

How Does EBV Enter Its Host and Its Host Cells?

A usually pleasant event, kissing, is the best established route of infection, although transplantation and blood transfusion are also very efficient in virus transmission, other routes including sexual intercourse are possible, due to demonstration of EBV in vaginal fluid, but are not definitely proven [Sixbey et al., 1986]

For the cellular level we know less about pleasure, but infection of lymphocytes via the cellular receptor CD21 (CR2) for complement C3d ends in an immortalizing liaison [Frade et al., 1985; Nemerow et al., 1987].

Is CD21 mandatory for infection? If so, how can epithelial cells or T cells be infected. EBV-infected B cells entering a lytic cycle viral replication have been shown to efficiently fuse to a wide variety of cells [Bayliss and Wolf, 1980, 1981]. Therefore, the entry problem of EBV into cells does not seem to be a limiting factor. Recently, an IgA-mediated entry via a polymeric IgA receptor on polarized epithelial cells has been described as an additional entry mechanism [Sixbey and Yao,

Epstein-Barr Virus and
Host Interaction

Table 2. Overview of various subsets of latent EBV gene products

Type of latency	Promoter usage	Expressed gene products	Tissue or cell
Lat I	Fp	EBNA1￼ EBER	Burkitt's lymphoma biopsy and fresh cell lines
Lat II	Fp (LRS)	EBNA1 LMP1, LMP2B LMP2A EBER BARFO transcripts	nasopharyngeal carcinoma
Lat III	Wp < 24 h	EBNA1–6 LMP1, LMP2B	lymphoblastoid cell lines passaged Burkitt's lymphoma cell lines
	Cp	LMP2A EBER	
Lat IV	Wp	LMP2A	peripheral blood B lymphocytes

1992]. As EBV-specific IgA antibodies are transiently present upon primary infection, this could in rare events lead to epithelial cell infection. The high IgA antibody levels in NPC patients, however, are a consequence rather than a cause of NPC.

Why Does the Presence of Efficiently Infectable Target Cells Not Kill All Such Cells and Produce Fatal Disease and Still Ensure Spreading of the Virus by Lifelong Shedding of EBV?

After entry, the virus apparently undergoes limited replication of its DNA [Yates and Guan, 1991]. It persists usually as multiple copies of closed circular DNA. For persistence during cell division the expression of EBNA1 is mandatory. It binds to the BamC origin of plasmid replication (oriP). Furthermore, nuclear matrix attachment regions have been identified [Janklevich et al., 1992]. Dependent on the cell type, various subsets of latent EBV gene products are expressed. Ta-

ble 2 gives an overview. Whereas in BL tumor cells the stage Lat I with only EBNA1 as viral gene product is favored, in lymphoblastoid cell lines (LCL's) (EBV-immortalized non-malignant B cells) Lat III with the whole set of EBNA and LMP expression is established.

If a cytolytic virus would efficiently replicate in a readily accessible and easily infectable cell type, this could potentially kill the host. And indeed, EBV replication is strongly down-regulated in B-lymphoblastoid cells as well as in less differentiated epithelial cells. Only terminally differentiated epithelial cells seem to be capable of efficiently supporting the EBV lytic cycle. This can be seen in biopsies from parotid glands [Wilmes and Wolf, 1981; Wolf et al., 1984] and oral hairy leukoplakia [Becker et al., 1991] and is supported by experimental evidence [Marschall et al., 1991]. The mechanisms for this virus host cell interaction are subject of detailed studies.

Transcription of BZLF1, a major immediate early gene of EBV, is negatively controlled by more than one silencer region up-

Fig. 1. a Band shift experiment with subcloned fragments (*Bam*HI/*Bal*I = Z200 and *Bal*I/*Sph*I = Z120) of the distal BZLF1 promoter. The DNA fragments were incubated with nuclear protein extracts and the resulting protein-DNA complexes analyzed on a native polyacrylamide gel. Competitor DNA was added to identify specific and unspecific binding of proteins. Protein extract from EBV-negative BJAB cells (BL), EBV-positive latently infected Raji- and TPA-treated (EBV replication induced) Raji cells (BL). Specific (Z120, Z200) and unspecific (pUC18) competitor DNA was added as indicated. The arrow indicates a specific protein-DNA complex with both promoter fragments (lanes 3, 5, 10, 12) that does not form in TPA-treated lymphoid cells, where lytic replication of the virus is induced. **b** Drawing of negative and positive regulatory elements in the promoter of BZLF1.
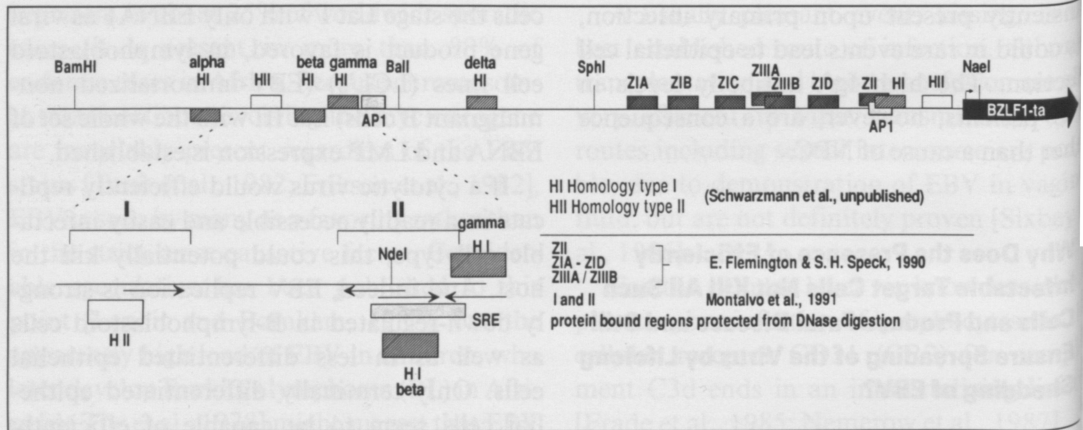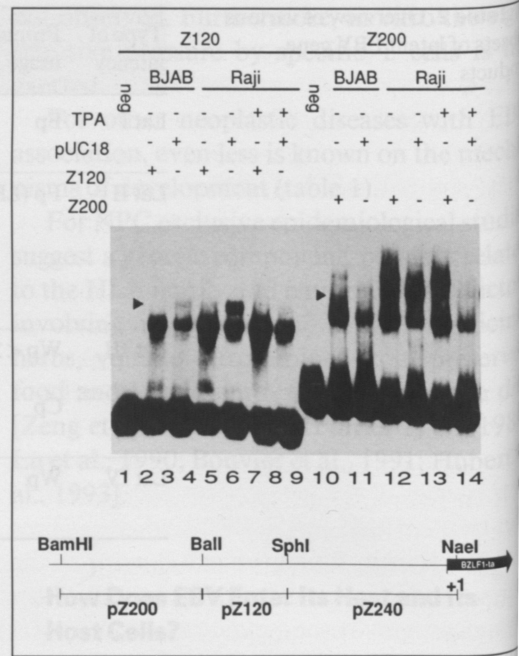
stream of the promoter (fig. 1b). Two types of silencer elements have been identified so far in this region by testing the regulative modules in functional assays [Flemington and Speck, 1990; Montalvo et al., 1991]. A consensus binding site for a negative regulative cellular factor, YY1, has been described [Shi et al., 1991]. We have now found another kind of reg-ulatory element (HI element, consensus sequence: 5'-(CATN)ACAGAT/GGA-3') in the distal promoter region, which does not directly confer negative regulation of a heterologous promoter but does interfere with flanking positive regulatory sequence motifs [Schwarzmann et al., unpubl.]. Further, we could demonstrate that in this region of the promoter the protein

Wolf/Bogedain/Schwarzmann

Epstein-Barr Virus and
Host Interaction

binding pattern changes with the ability of EBV to switch to the lytic cycle (fig. 1a).

Even if the transactivator Zta (also termed BZLF1, ZEBRA, Z, EB1) is translated from the BZLF1 reading frame, it is not sufficient in all cells to induce a full lytic cycle. While it does so in BL-type cells and fully differentiated epithelial cells, it is inactive in LCL's. Whereas Zta alone induces lytic proteins in BL cells, it is not able to induce important lytic gene products in LCL's, among them the lytic transactivator Rta (also termed BRLF1, R) [Bogedain et al., unpubl.] (fig. 2, 3). Rta is assumed to be crucial for the onset of the lytic cycle in lymphoid cells (LCL) [Zalani et al., 1992].

For the BSLF2/BMLF1 regulatory upstream region, a silencing function was detected in latently infected B-lymphocytes which was shown to be inefficient in EBV-negative lymphoid and epithelial cells [Marschall et al., 1990]. Using teratocarcinoma cells as an in vitro differentiation model, the down-regulation of EBV promoter activity was demonstrated for unstimulated immature cells, whereas specific transcriptional responses became obvious during proceed-
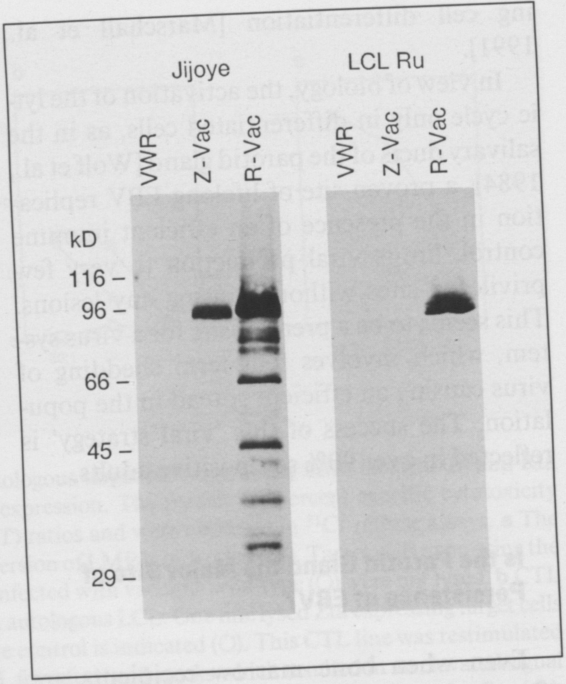

Fig. 2. Western blot analysis of each one BL cell line (e.g. Jijoye) and LCL. The cell lines were infected with vaccinia wild-type (strain WR, VWR), recombinant vaccinia viruses Z-Vac and R-Vac for expression of Zta and Rta, respectively, as given in the figure. Blots were screened for expression of Rta using a polyclonal rabbit antiserum. Rta has a size of 96/92 kD and is induced by Zta in the BL line, but not in the LCL.

Fig. 3. Schematic illustration indicating gene regulation events of the early phase of the lytic cycle in BL cells and LCLs observed following expression of the transactivators Zta and Rta with recombinant vaccinia viruses. The arrows give the activities exerted by the transactivators in Zta (Z) and Rta (R) on the gene products of the reading frames BSLF2/BMLF1 (M) and BALF2 (p138) of the early group.
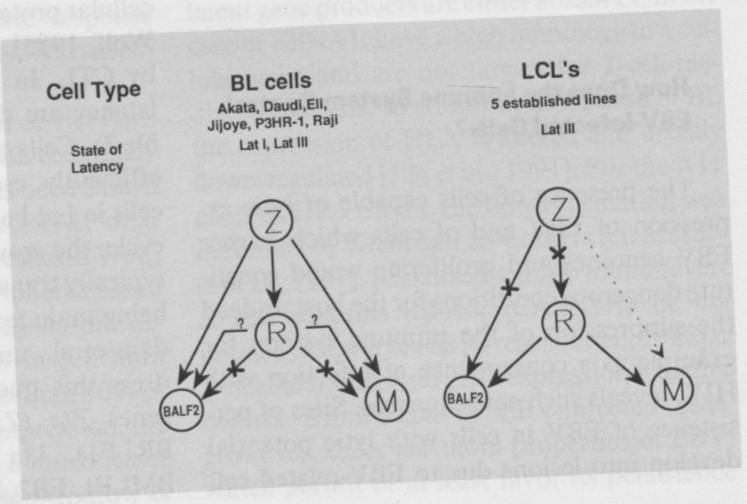
ing cell differentiation [Marschall et al., 1991].

In view of biology, the activation of the lytic cycle only in differentiated cells, as in the salivary ducts of the parotid gland [Wolf et al., 1984], a proven site of lifelong EBV replication in the presence of an efficient immune control, limits viral production to very few privileged sites without causing any lesions. This seems to be a prerequisite for a virus system, which involves long-term shedding of virus causing an efficient spread in the population. The success of this 'viral strategy' is reflected in over 90% seropositive adults.

Is the Parotid Gland the Major Site of Persistence of EBV?

Even when bone marrow recipients are treated by radiation, oral shedding of virus continues for several months, but eventually declines [Gratama et al., 1988, 1992]. Therefore, one has to assume a dynamic process with the salivary gland being a major site of EBV production and persistence, which needs to be occasionally replenished with virus from lymphoid cells of LCL or possibly memory cell type with their very restricted replication-supporting potential.

How Does the Immune System Control EBV-Infected Cells?

The presence of cells capable of lytic expression of EBV and of cells which harbor EBV genomes and proliferate would constitute dangerous conditions for the host. Indeed the suppression of the immune system, for example as a consequence of infection with HIV, reveals such consequences. Sites of persistence of EBV in cells with lytic potential develop into lesions due to EBV-related cell

lysis (oral hairy leukoplakia) [Greenspan al., 1985]. LCL-like peripheral blood lymph cytes develop into polyclonal lymphom which are likely targets for the control throu the immune system. Because most EBV po tives have no antibodies to the group of ea antigens, typically consisting of a group replication-related enzymes, elimination cells entering a lytic cycle likely occurs at earlier phase of the viral life cycle, so ea antigens are not synthesized and therefore not available antigens. Because latently fected cells are not eliminated from the bo altogether, these cells seem to follow a co mon strategy to escape immune control. Fr the panel of latent antigens (table 2), sev of them were identified as targets for cy toxic T lymphocytes. The long (spliced) v sion of LMP, which is preferentially pressed in nonmalignant LCL-like cells, is r ognized by cytotoxic T lymphocytes (CT [Markert et al., unpubl. data] (fig. 4a). Thi in contrast to malignant BL cells, which press the nonimmunogenic (fig. 4a) trunca form [Modrow and Wolf, 1986]. In additi EBNA2, 3a, 3b, and 3c were identified CTL targets [Murray et al., 1990; Burrow al., 1990; Gavioli et al., 1992]. Howe EBNA1, possibly because of its homology cellular proteins [Heller et al., 1982; Seibl a Wolf, 1985], was found not to be recogni by CTL. In BL cells several stages of v latency are defined [Rowe et al., 1987] ble 2). Cells persisting in stage Lat I sho efficiently escape immune recognition. cells in Lat I are able to directly enter the l cycle, the group of immediate early prote typically transactivators, meet well criteria being main targets for T-cell response [Bo dain et al., unpubl. data] (fig. 4b, c). To dress this question, the lytic transactiva genes Zta (Z, BZLF1, ZEBRA), Rta BRLF1), I'ta (BI'LF4), and M (BSL BMLF1, EB2, MS-EA) have been cloned i
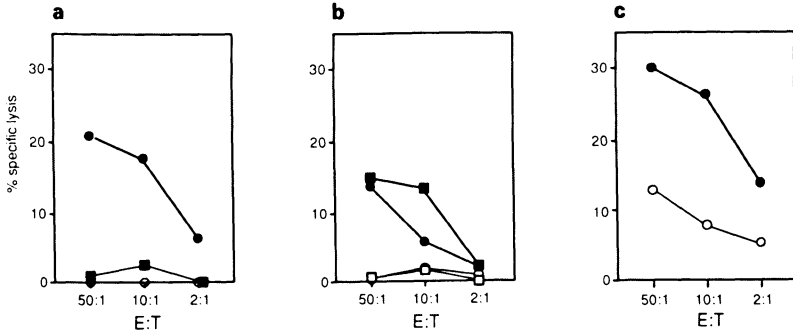
Fig. 4. CTL response against autologous target cells expressing LMP (**a**), Zta (**b**) and Rta (**c**) by recombinant vaccinia virus expression. The results are percent specific cytotoxicity observed at three effector:target (E:T) ratios and were obtained in ^{51}Cr-release assays. **a** The response obtained against the long version of LMP is indicated (●). Target cells expressing the truncated version of LMP (■) and infected with vaccinia wild-type (○) were not lysed. **b** CTL lines were stimulated for weeks with autologous LCL. One line lysed Zta expressing target cells (●). The vaccinia wild-type negative control is indicated (○). This CTL line was restimulated with Z-Vac-infected autologous LCL for a further 2 weeks which resulted in an elevated signal (■). □ = Vaccinia wild-type negative control. **c** CTL response against cells expressing Rta (●). ○ = Vaccinia wild-type negative control.

vaccinia virus and used to infect EBV ge-nome-positive B cells to serve as targets for syngeneic T cells, which have been expanded with IL-2 in the presence of induced and irra-diated B cells. A clear cytotoxic response could be seen for the immediate early transac-tivators Zta and Rta (fig. 4b, c).

The incomplete elimination of carrier lym-phocytes of the LCL type and of BL cells likely has more complex reasons. It has been shown that BL cells either do not produce LMP or at most in a truncated form [Modrow and Wolf, 1986]; in addition, certain peripheral blood cells have been identified, which do not ex-press LMP. Furthermore, even in cell lines known to express LMP, only less than 10% of cells show a high level of LMP expression, the rest expresses LMP at most to a much lesser extent [Modrow and Wolf, 1986; Modrow et

al., 1987]. It has been shown by several groups that absence of LMP would allow such cells to escape specific killing [Jilg et al., 1988, 1989; Thorley-Lawson and Israelsohn, 1987]. Other latent gene products are either absent or, in the case of EBNA1, have a high homology to a cel-lular gene and are not targets for T-cell-me-diated cell lysis. In addition, in the case of BL the expression of HLA is altered and usually down-regulated [Jilg et al., 1991]. For the A11 allele of HLA class I, the down-regulation was described by Klein and co-workers [cf. Masuc-ci et al., 1987]. Rickinson's group found sever-al cell adhesins (LFA3, ICAM1) to be ex-pressed at lower levels [Gregory et al., 1988].

Cellular and viral gene expression together control elimination of EBV-infected cells. However, there are more properties of EBV, which permit or at least favor its persistence

Table 3. Properties of EBV: mechanisms important during establishment of persistence

---

1 EBNA1 is not immunogenic (related to host protein) [Seibl and Wolf, 1985; Heller et al., 1982]

2 LMP1 absent or truncated in BL cells [Modrow and Wolf, 1986]

3 Burkitt's lymphoma cells (not a direct effect of EBV): down-regulation of:
   – HLA class II [Masucci et al., 1987]
   – HLA class I [Jilg et al., 1991]
   – LFA3, ICAM1 [Gregory et al. 1988]

4 BCRF1 gene product has homology to IL-10: block of IL-2 and IFN-γ [Hsu et al., 1990] Enhancement of CTL and NK cells [Stewart and Rooney, 1992]

5 EBER transcripts inhibit IFN-induced 'ds-RNA activatable phosphokinase' (p68) [Clarke et al., 1990]

6 EBNA2 blocks induction of IFN

7 a) LMP1 induces cellular bcl2: suppression of apoptosis [Henderson et al., 1991]
   b) BHRF1 homology to bcl2: suppression of apoptosis? [Reed et al., 1989; Lee and Yates, 1992]

8 Virus production in privileged sites [Sixbey et al., 1986; Wolf et al., 1984; Greenspan et al., 1985; Becker et al., 1991]

---

in the host. These additional mechanisms (table 3) might be particularly important during the establishment of infection where unspecific defense mechanisms of the host, such as interferons [Clarke et al., 1990], could otherwise result in fast and complete clearance of infecting EBV from the receiving organism already at the primary target organ, the tonsils [Wilmes and Wolf, 1987]. Some similar 'viral strategies' have been suspected for other viruses. The abundant virus-associated transcripts of adenoviruses for example may as well block interferon, permitting adenoviruses to persist in adenoids and elsewhere in the human body.

How Does EBV Immortalize and Transform Its Host Cells?

Little is known about the immortaliz[ing] activity of EBV. LMP has been shown [to] change growth behavior of rodent cells [Wa[ng] et al., 1985]. Experiments with human ce[lls] did induce reduced serum dependence wh[en] transfected into type I BL cells [Henderson [et] al., 1991]. In BL this gene is usually n[ot] expressed. The same is true for EBN[A] which has interesting transactivating prop[er]ties including activation of bcl2 [Henders[on] et al., 1991], which blocks apoptosis, and CD23, the low-affinity IgE receptor. It w[ill] have to be tested whether strong viral trans[ac]tivators such as BZLF1 in analogy to ade[no] EIA could play a much more important r[ole] than presently appreciated. The observat[ion] that BZLF1 might be transcribed and tra[ns]lated in NPC biopsies by Patton et al. [19] might point in that direction.

Even though there is quite a way to [go] before we have a coherent picture of even [the] most prominent activities of EBV, we hav[e to] do our best to control the most import[ant] EBV-related diseases.

Of special interest is IM, the second m[ost] frequent disease of young adults (numb[ers] available only for the US and Denm[ark] [Evans and Niedermann, 1989].

Increasing attention is directed to case[s of] virus activation in transplant recipients wh[ere] as many as 23% of apparent cases of reject[ion] crisis may be caused by CMV and EBV ra[ther] than by tissue incompatibility. Altho[ugh] numbers may be relatively low, the fatal [out]come of EBV infection in patients with [a] constitution is an urgent problem when E[BV] infection needs to be excluded or contro[lled]. Of course, the control of EBV-related m[alig]nancies, such as NPC, is a long-term goal [that] will also and finally clarify the causal relat[ion]ship of EBV with these neoplasias.
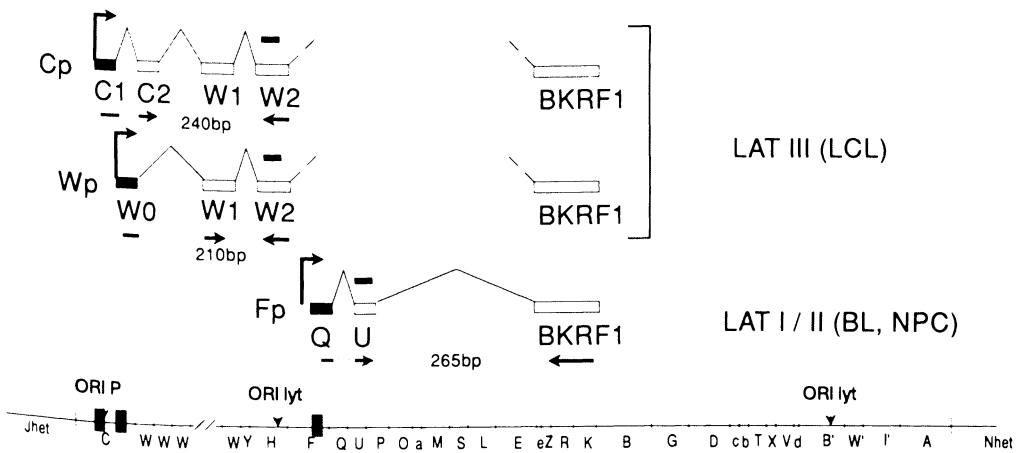
Fig. 5. Drawing of the different promoters used for expression of latent EBNA genes. Detection of these transcripts is possible by RNA-PCR with 5'-upstream primers that are specific for the first exons C1, W0 and F/Q [Kerr et al., 1992]. All three promoters splice to the same coding exon for EBNA1 in the *Bam*HK fragment up to 100 kbp downstream. Promoters Cp and Wp are giving rise to all latent EBNA transcripts (LAT III) whereas the Fp promoter only enables EBNA1 transcription (LAT I).

In definable high risk groups a vaccine may be highly desirable. For other conditions, precise early diagnosis followed by specific therapy will be the way to go. For transplant recipients this becomes quite evident. Considerable evidence suggests that even antiviral therapy at optimal doses might not be good enough to control a virus-induced rejection crisis; additional substitution with specific immunoglobulins has been described as a successful procedure. Whether a similar procedure would help persons susceptible to XLP is yet unclear.

Early diagnosis alone has been proven to be very successful in areas with extremely high levels of NPC. Rather than detecting 2 and 25% of cases in stages 1 or 2, serological controls using detection of IgA antibodies to early antigen has pushed these numbers to 40 and 45%, respectively [Zeng et al., 1988,

1993]. In combination with conventional radiation therapy, early detection dramatically decreases the death toll of NPC patients. More efficient test systems based on recombinant proteins facilitate this approach and allow better diagnosis of acute infection [Wolf et al., 1985; Gorgievski-Hrisoho et al., 1990]. For lymphomas, clonality and type of proliferating cells are of major importance, whereas clonality of virus can be tested via Southern blots using restriction enzymes, which cut once in the terminal repeats and hybridize with appropriate probes [Raab-Traub and Flynn, 1986]. A definition of cell types might be very useful. The restricted transcription pattern and promoter usage of the EBNA gene family is of particular interest in this context (fig. 5). For Hodgkin's lymphomas, in situ hybridization with EBER

probes are used increasingly often [Pallesen et al., 1991].

A vaccine is also in sight now. After extensive work with virus-derived antigens in an experimental infection system [Shope et al., 1973; Werner et al., 1975; Wolf et al., 1975] by Epstein, conducted by Morgan and co-workers [cf. Epstein et al., 1985], the first human vaccine trial gave very encouraging results [Gu et al., 1993]. The study was performed in China, where only 10–20% of 1-year-old, but 90% of 3-year-old infants are positive for EBV due to natural routes of infection. This is an excellent condition for evaluation of efficacy for induction of immunity by a vaccine. Two candidate vaccines for human use have been developed by our group: one is based on purified major membrane protein gp 350/250 (BLLF1 MA) secreted from transfected Chinese hamster ovary cells [Motz et al., 1987]. After the sequence encoding the membrane anchor of MA has been genetically removed. The viral gene w. stably integrated into the Chinese hamst ovary cells via a transfer vector with dihydr folate reductase as a selective marker. T other vaccine uses recombinant vaccinia vir (strain Tien Tan), which expresses under t 11k vaccinia promoter the same viral mer brane antigen, in this case including the mer brane anchor. Other vector systems used i cluded yeast with discouraging results, va cella virus, baculovirus and other vaccin based constructs not approved for human u. The latter life vaccine is already under evalu tion for efficacy in China. 60% of vaccinat children have been protected after the first months from EBV infection, all control children were infected with EBV through n ural routes, as detected by appearance of ant bodies to EA and VCA. Further studies us the purified antigen will follow to identify least harmful but most effective vaccine.

References

Audouin J, Diebold J, Pallesen G: Frequent expression of Epstein-Barr virus latent membrane protein-1 in tumor cells of Hodgkin's disease in HIV-positive patients. J Pathol 1992;167:381–384.

Bayliss G, Wolf H: An Epstein-Barr virus early protein induced cell fusion. Proc Natl Acad Sci USA 1981;78:7162–7165.

Bayliss GJ, Wolf H: Epstein-Barr virus-induced cell fusion. Nature 1980;287:164–165.

Becker J, Leser U, Marschall M, Langford A, Jilg W, Reichart P, Gelderblom H, Wolf H: Expression of proteins encoded by Epstein-Barr virus trans-activator genes depends on the differentiation of epithelial cells in oral hairy leukoplakia. Proc Natl Acad Sci USA 1991;88:8332–8336.

Bogedain C, Alliger P, Schwarzmann F, Marschall M, Wolf H, Jilg W: The Epstein-Barr virus transactivators BZLF1 and BRLF1 act differently on the expression of lytic cycle gene products in Burkitt lymphoma cells and lymphoblastoid cell lines (unpubl. data).

Bouvier G, Poirier S, Shao YM, Malaveille C, Ohshima H, Polack A, Bornkamm GW, Zeng Y, De-The G, Bartsch H: Epstein-Barr virus activators, mutagens and volatile nitrosamines in preserved food samples from high-risk areas for nasopharyngeal carcinoma. IARC Sci Publ 1991;105:204–209.

Burrows SR, Sculley TB, Misko IS, Schmidt C, Moss DJ: An Epstein-Barr virus-specific cytotoxic T cell epitope in EBV nuclear antigen 3 (EBNA 3). J Exp Med 1990;171:345–349.

Clarke PA, Schwemmle M, Schick J, Hilse K, Clemens MJ: Bindi Epstein-Barr virus small R EBER-1 to the double-stra RNA-activated protein kinase Nucleic Acids Res 1990;19: 248.

de The G, Geser A, Day NE, Tuke Williams EH, Beri DP, Smith Dean AG, Bornkamm GW, Fe P, Henle W: Epidemiologica dence for causal relationshi tween Epstein-Barr virus and kitt's lymphoma from a Uga prospective study. Nature 197 756–761.

Deutsch J, Wolf H, Becker H, Fu Goriup U, Grubbauer HM, tean W, Popow-Kraupp T, Stu D: Demonstration of Epstein virus DNA in a previously he boy with fulminant hepatic f Eur J Pediatr 1986;145:94–9

Epstein MA, Barr YM: Cultivation in vitro of human fibroblasts from Burkitt's malignant lymphoma. Lancet 1964;i:252–253.

Epstein MA, Barr YM: Virus particles in cultured lymphoblasts from Burkitt's lymphoma. Lancet 1964;i:702–703.

Epstein MA, Morgan AJ, Finerty S, Kandle BJ, Kirkwood JK: Protection of cottontop tamarins against Epstein-Barr virus-induced malignant lymphoma by a prototype subunit vaccine. Nature 1985;318:287–289.

Erikson J, Finan J, Nowell PC, Croce CM: Translocation of immunoglobulin Vh genes in Burkitt's lymphoma. Proc Natl Acad Sci USA 1982;79:5611–5615.

Evans AS, Niedermann J: Epstein-Barr virus; in Evans AS (ed): Viral Infections of Humans, Epidemiology and Control, ed 3. New York, Plenum Press, 1989, pp 265–292.

Flemington E, Speck S: Identification of phorbol ester response elements in the promoter of Epstein-Barr virus putative lytic switch gene BZLF1. J Virol 1990;64:1217–1226.

Frade R, Barel M, Ehlin-Henriksson B, Klein G: gp140, the C3d receptor of human B lymphocytes, is also the Epstein-Barr virus receptor. Proc Natl Acad Sci USA 1985;82:1490–1493.

Gavioli R, De Campos-Lima PO, Kurilla MG, Kieff E, Klein G, Masucci MG: Recognition of the Epstein-Barr virus-encoded nuclear antigens EBNA4 and EBNA6 by HLA-A11-restricted cytotoxic T lymphocytes: Implication for down-regulation of HLA-A11 in Burkitt lymphoma. Proc Natl Acad Sci USA 1992;89:5862–5866.

Georgievski-Hrisoho M, Hinderer W, Nebel-Schickel H, Horn J, Vornhagen R, Sonneborn HH, Wolf H, Siegl G: Serodiagnosis of infectious mononucleosis by using recombinant Epstein-Barr virus antigens and enzyme-linked immunosorbent assay technology. J Clin Microbiol 1990;28:2305–2311.

Gratama JW, Oosterveer MAP, Zwaan FE, Lepoutre J, Klein G, Ernberg I: Eradication of Epstein-Barr virus by allogeneic bone marrow transplantation: Implications for sites of viral latency. Proc Natl Acad Sci USA 1988;85:8693–8696.

Gratama JW, Lenette ET, Lonnqvist B, Oosterveer MA, Klein G, Ringden O, Ernberg I: Detection of multiple Epstein-Barr viral strains in allogeneic bone marrow transplant recipients. J Med Virol 1992;37:39–47.

Greenspan JS, Greenspan D, Lenette ET, Abrams DI, Conant MA, Petersen V, Freese UK: Replication of Epstein-Barr virus within the epithelial cells of oral 'hairy' leukoplakia, an AIDS-associated lesion. New Engl J Med 1985;313:1564–1571.

Gregory CD, Murray RJ, Edwards CF, Rickinson AB: Down-regulation of cell adhesion molecules LFA-3 and ICAM-1 in Epstein-Barr virus-positive Burkitt's lymphoma underlies tumor cell escape from virus-specific T cell surveillance. J Exp Med 1988;167:1811–1824.

Gu SY, Huang TM, Ruan L, Miao YH, Chu CM, Motz M, Wolf H: Immunogenicity in human volunteers of a recombinant vaccinia virus expressing Epstein-Barr virus membrane antigen; in Tursz (ed): EBV and Human Disease. Paris, Inserm and John Libbey Eurotext, 1993.

Hamilton-Dutoit SJ, Pallesen G: A survey of Epstein-Barr virus gene expression in sporadic non-Hodgkin's lymphomas. Detection of Epstein-Barr virus in a subset of peripheral T-cell lymphomas. Am J Pathol 1992;140:1315–1325.

Harabuchi Y, Namanaka N, Kataura A, Imai S, Kinoshita T, Mizuno F, Osato T: Epstein-Barr virus in nasal T-cell lymphomas in patients with lethal midline granuloma. Lancet 1990;335:128–130.

Heller M, Henderson A, Kieff E: Repeat array in Epstein-Barr virus DNA is related to cell DNA sequences interspersed on human chromosomes. Proc Natl Acad Sci USA 1982;79:5916–5920.

Henderson S, Rowe M, Gregory C, Croom-Carter D, Wang F, Longnecker R, Kieff E, Rickinson A: Induction of bcl-2 expression by Epstein-Barr virus latent membrane protein 1 protects infected B cells from programmed cell death. Cell 1991;65:1107–1115.

Henle G, Henle W: Immunofluorescence in cells derived from Burkitt's lymphoma. J Bacteriol 1966;91:1248–1256.

Henle G, Henle W, Diehl V: Relation of Burkitt's tumor-associated herpestype virus to infectious mononucleosis. Proc Natl Acad Sci USA 1968;59:94–101.

Hsu DH, De-Waal-Malefyt R, Fiorentino DF, Dang MN, Vieira P, De-Vries J, Spits H, Mosmann TR, Moore KW: Expression of interleukin-10 activity by Epstein-Barr virus protein BCRF1. Science 1990;250:830–832.

Huber A, Jeannel D, Tuppin P, de The G: Anthropology and epidemiology: A pluridisciplinary approach to environmental factors of nasopharyngeal carcinoma; in Tursz (ed): EBV and Human Disease. Paris, Inserm and John Libbey Eurotext, 1993.

Janklevich S, Kolman JL, Bodnar JW, Miller G: A nuclear attachment region organizes the Epstein-Barr viral plasmid in Raji cells into a single DNA domain. EMBO J 1992;11:1165–1176.

Jilg W, Voltz R, Markert-Hahn C, Mairhofer H, Munz I, Wolf H: Expression of class I major histocompatibility complex antigens in Epstein-Barr virus-carrying lymphoblastoid cell lines and Burkitt lymphoma cells. Cancer Res 1991;51:27–32.

Jilg W, Mairhofer H, Markert C, Wolf H: Generation of cytotoxic T-cells against EBV-infected lymphocytes; in Ablashi DV, Faggioni A, Krueger GR, Pagano JS, Pearson GR (eds): Epstein-Barr Virus and Human Disease. Clifton, Humana Press, 1988, pp 219–222.

Jilg W, Modrow S, Volz R, Markert C, Mairhofer H, Münz I, Wolf H: Target structures for cellular immune mechanisms on the surface of Epstein-Barr virus infected malignant and non-malignant lymphocytes; in Koldovsky et al (eds): Lymphocytes in Immunotherapy on Cancer. Berlin, Springer, 1989, pp 66–73.

Kerr BM, Lear AL, Rowe M, Croom-Carter D, Young LS, Rookes SM, Gallimore PH, Rickinson AB: Three transcriptionally distinct forms of Epstein-Barr virus latency in somatic cell hybrids: Cell phenotype dependence of virus promoter usage. Virology 1992;187:189–201.

Lee MA, Yates JL: BHRF1 of Epstein-Barr virus, which is homologous to human proto-oncogene bcl2, is not essential for transformation of B cells or for virus replication in vitro. J Virol 1992;66:1899–1906.

Lenoir GM, Bornkamm GW: Burkitt's lymphoma, a human cancer model for the study of the multistep development of cancer: Proposal for a new scenario. Adv Viral Oncol 1987;7:173–206.

Lu S, Day NE, Degos L, Lepage V, Wang P-C, Chan S-H, Simons M, McKnight B, Easton D, Zeng Y, de The G: Linkage of nasopharyngeal carcinoma susceptibility locus to the HLA region. Nature 1990;346:470–471.

Marschall M, Schwarzmann F, Leser U, Oker B, Alliger P, Mairhofer H, Wolf H: The BI'LF4 trans-activator of Epstein-Barr virus is modulated by type and differentiation of the host cell. Virology 1991;181:172–179.

Marschall M, Schwarzmann F, Alliger P, Wolf H: Transregulation of the early MS gene of EBV and its depencence on the host cell; in Ablashi DV, Faggioni A, Krueger GR, Pagano JS, Pearson GR (eds): Epstein-Barr Virus and Human Disease. Clifton, Humana Press, 1990, pp 111–115.

Masucci MG, Torsteinsdottir S, Colombani J, Brautbar C, Klein E, Klein G: Down-regulation of class I HLA antigens and of the Epstein-Barr virus-encoded latent membrane protein in Burkitt lymphoma lines. Proc Natl Acad Sci USA 1987;84:4567–4571.

Modrow S, Wolf H: Characterization of two related Epstein-Barr virus-encoded membrane proteins that are differentially expressed in Burkitt lymphoma and in vitro transformed cell lines. Proc Natl Acad Sci USA 1986;83:5703–5707.

Modrow S, Jilg W, Meerwarth I, Mairhofer H, Haus M, Wolf H: Differential expression of EB-viral and cellular surface markers on Burkitt lymphoma and lymphoblastoid cells; in Levine PH et al (eds): EBV and Its Diseases. Clifton, Humana Press, 1987, pp 407–411.

Montalvo EA, Shi Y, Shenk TE, Levine AJ: Negative regulation of the BZLF1 promoter of Epstein-Barr virus. J Virol 1991;65:3647–3655.

Moss DJ, Burrows SR, Khanna R, Misko IS, Sculley TB: Immune surveillance against Epstein-Barr virus. Semin Immunol 1992;4:97–104.

Motz M, Deby G, Wolf H: Trucated versions of the two major Epstein-Barr virus viral glycoproteins (gp250/350) are secreted by recombinant Chinese hamster ovary cells. Gene 1987;58:149–154.

Murray RJ, Kurilla MG, Griffin HM, et al: Human cytotoxic T-cell responses against Epstein-Barr virus nuclear antigens demonstrated by using recombinant vaccinia viruses. Proc Natl Acad Sci USA 1990;87:2906–2910.

Nemerow GR, Mullen JJ, Dickson PW, Cooper NR: Soluble recombinant CR2 (CD21) inhibits Epstein-Barr virus infection. J Virol 1989;64:1348–1352.

Old LJ, Boyse EA, Geering G, Oettgen HF: Serologic approaches to the study of cancer in animals and man. Cancer Res 1968;28:1288–1299.

Pallesen G, Sandvej K, Hamilton-Dutoit SJ, Rowe M, Young LS: Activation of Epstein-Barr virus replication in Hodgkin and Reed-Sternberg cells. Blood 1991;78:1162–1165.

Patton DF, Venable E, Ribeirio, R: Detection of *Bam* W-Z rearrangements consistent with het-DNA (defective EBV) in human disease. 4th Int Symp on Epstein-Barr Virus and Associated Diseases, Taiwan, 1990.

Poirier S, Bouvier G, Malaveille C, Ohshima H, Shao YM, Hubert A, Zeng Y, de The G, Bartsch H: Volatile nitrosamine levels and genotoxicity of food samples from high risk areas for nasopharyngeal carcinoma before and after nitrosation. Int J Cancer 1989;44:1088–1094.

Pope JH, Achong BG, Epstein MA, Biddulph J: Burkitt lymphoma in New Guinea: Establishment of a line of lymphoblasts in vitro and description of their fine structure. J Natl Cancer Inst 1967;39:933–945.

Pulvertaft RJV: Cytology of Burkitt's tumor (African lymphoma). Lancet 1964;i:238–240.

Purtilo DT, et al: X-linded recessive progressive combined variable immunodeficiency. Lancet 1975;i:935–940.

Raab-Traub N, Flynn K: The structure the termini of the Epstein-Barr virus as a marker of clonal cellular proliferation. Cell 1986;47:883–889.

Reed JC, Haldar S, Cuddy MP, Croce Makover D: Deregulated BCL2 expression enhances growth of a human B cell line. Oncogene 1989;1123–1127.

Rowe M, Rowe DT, Gregory CD, Young LS, Farrell PJ, Rupani H, Rickinson AB: Differences in B cell growth phenotype reflect novel patterns of Epstein-Barr virus latent gene expression in Burkitt's lymphoma cells. EMBO J 1987;6:2743–2751.

Schendel DJ, Reinhardt C, Nelson, Maget B, Pullen L, Bornkamm G, Steinle A: Cytotoxic T lymphocytes show HLA-C-restricted recognition of EBV-bearing cells and allorecognition of HLA class I molecules presenting self-peptides. J Immunol 1992;149:2406–2414.

Seibl R, Wolf H: Mapping of Epstein-Barr virus proteins on the genome by translation of hybrid-selected RNA from induced P3HR1 and induced Raji cells. Virol 1985;141:113.

Shi Y, Seto E, Chang LS, Shenk T: Transcriptional repression by YY1, human GLI-Krüpper-related protein, and relief of repression by adenovirus E1A protein. Cell 1991;67:377–388.

Shope T, Dechairo D, Miller G: Malignant lymphoma in cotton-top marmosets following inoculation of Epstein-Barr virus. Proc Natl Acad Sci USA 1973;10:2487–2491.

Sixbey JW, Lemon SM, Pagano JS: second site for Epstein-Barr virus shedding: The uterine cervix. Lancet 1986;ii:1122–1124.

Sixbey JW, Yao QY: Immunoglobulin A-induced shift of Epstein-Barr virus tissue tropism. Science 1992;255:1578–1580.

Skare JC, Milunski A, Byron KS, Sullivan JL: Mapping the X-linked lymphoproliferative syndrome. Proc Natl Acad Sci USA 1987;84:2015–2018.

Stewart JP, Rooney CM: The interleukin-10 homolog encoded by Epstein-Barr virus enhances the reactivation of virus-specific cytotoxic T cell and HLA-unrestricted killer cell responses. Virology 1992;191:773–782.

Taub R, Kirsch I, Morton C, Lenoir G, Swan D, Tronick S, Aaronson S, Leder P: Translocation of the c-*myc* gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. Proc Natl Acad Sci USA 1982;79:7837–7841.

Thorley-Lawson DA, Israelsohn ES: Generation of specific cytotoxic T-cells with a fragment of the Epstein-Barr virus-encoded p63/latent membrane protein. Proc Natl Acad Sci USA 1987;84:5384–5388.

Voltz R, Jilg W, Wolf H: Modification of HLA expression as a possible factor in pathogenesis of Burkitt's lymphoma. Haematol Blood Transfus 1989;32:289–292.

Wang D, Liebowitz D, Kieff E: An EBV membrane protein expressed in immortalized lymphocytes transforms established rodent cells. Cell 1985;43:831–840.

Weiss LM, Strickler JG, Warnke RA, Purtilo DT, Sklar J: Epstein-Barr viral DNA in tissues of Hodgkin's disease. Am J Pathol 1987;129:86–91.

Weiss LM, Movahed SA, Warnke RA, Sklar J: Epstein-Barr viral genomes in Reed-Sternberg cells of Hodgkin's disease. N Engl J Med 1989;320:502–506.

Weiss LM, Jaffe ES, Liu XF, Chen YY, Shibata D, Medeiros LJ: Detection and localization of Epstein-Barr viral genomes in angioimmunoblastic lymphadenopathy and angioimmunoblastic lymphadenopathy-like lymphoma. Blood 1992;79:1789.

Werner J, Wolf H, Apodaca J, zur Hausen H: Lymphoproliferative disease in a cottontop marmoset after inoculation with infectious mononucleosis derived Epstein-Barr virus. Int J Cancer 1975;15:1000–1008.

Wilmes E, Wolf H: Der Nachweis von Epstein-Barr-Virus-Genomen in der Ohrspeicheldrüse. Laryngol Rhinol Otol 1981;60:7–10.

Wilmes E, Wolf H: Nachweis von Epstein-Barr-Viren in der Tonsilla palatina bei infektiöser Mononukleose. Klinische Konsequenzen? Laryngol Rhinol Otol 1987;2:278–283.

Wolf H, Werner J, zur Hausen H: EBV-DNA in non-lymphoid cells of nasopharyngeal carcinoma and in a malignant lymphoma obtained after inoculation of EBV into cottontop marmosets. Cold Spring Harb Symp Quant Biol 1975;39:791–796.

Wolf H, zur Hausen H, Becker V: EB viral genomes in epithelial nasopharyngeal carcinoma cells. Nature New Biol 1973;244:245.

Wolf H, Haus M, Wilmes E: Persistence of Epstein-Barr virus in the parotid gland. J Virol 1984;51:795–798.

Wolf H, Motz M, Kühbeck R, Seibl R, Bayliss GJ, Modrow S, Fan J: Selection and production by gene technological methods of medically relevant EBV antigens; in Levine PH, Ablashi DV, Pearson GR, Kottaridis SD (eds): Epstein-Barr Virus and Associated Diseases. Boston, Nijhoff, 1985, pp 485–494.

Yates JL, Guan N: Epstein-Barr virus-derived plasmids replicate only once per cell cycle and are not amplified after entry into cells. J Virol 1991;65:483–488.

Zalani S, Holley-Guthrie EA, Gutsch DE, Kenney SC: The Epstein-Barr virus immediate-early promoter BRLF1 can be activated by the cellular Sp1 transcription factor. J Virol 1992;66:7282–7289.

Zeng Y, Deng H, Zhong J, Huang N, Li P, Pan W, Huang Y, Li Y, Wang P, de The G: A 10-year prospective study on nasopharyngeal carcinoma in Wuzhou city and Zangwu country, Guangxi, China; in Tursz (ed): EBV and Human Diseases. Paris, Inserm and John Libbey Eurotext, 1993.

Zeng Y, Wolf H, Takada K, Arrand JR, de The G: Detection of EBV-specific IgA antibodies to EA, MA and EBNA-1 recombinant proteins in NPC patients and controls; in Ablashi DV, et al (eds): Epstein-Barr Virus and Associated Diseases. Clifton, Humana Press, 1988, pp 309–313.