Glycoprotein gp110 of Epstein–Barr virus determines viral tropism and efficiency of infection

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The Epstein–Barr virus (EBV) genome has been detected in lymphomas and in tumors of epithelial or mesenchymal origin such as nasopharyngeal carcinoma or leiomyosarcoma. Thus, there is little doubt that EBV can infect cells of numerous lineages *in vivo*, in contrast to its *in vitro* infectious spectrum, which appears restricted predominantly to B lymphocytes. We show here that the EBV *BALF4* gene product, the glycoprotein gp110, dramatically enhances the ability of EBV to infect human cells. gp110^{high} viruses were up to 100 times more efficient than their gp110^{low} counterparts in infecting lymphoid or epithelial cells. In addition, gp110^{high} viruses infected the carcinoma cell line HeLa and the T cell lymphoma cell line Molt-4, both previously thought to be refractory to EBV infection. Analysis of several virus isolates showed that the amount of *BALF4* present within mature virions markedly differed among these strains. In some strains, gp110 was found expressed during lytic replication not only at the nuclear but also at the cellular membrane. Heterologous expression of gp110 during the virus lytic phase neither altered virus concentration nor affected virus binding to cells. It appears that gp110 plays a crucial role after the virus has adhered to its cellular target. gp110 constitutes an important virulence factor that determines infection of non-B cells by EBV. Therefore, the use of gp110^{high} viruses will help to determine the range of the target cells of EBV beyond B lymphocytes and provide a useful *in vitro* model to assess the oncogenic potential of EBV in these cells.

Among human viruses that have been etiologically linked to cancer, the Epstein–Barr virus (EBV) is unusual in that it is associated with very diverse tumors, including B and T cell lymphomas, carcinomas of the stomach and nasopharynx, or even sarcomas (for a recent review, see ref. 1). These observations provide solid evidence that the virus can infect various cell lineages *in vivo*. In contrast, evidence for EBV infection of non-B cells in normal individuals is lacking, suggesting that persistence of EBV in non-B cells might be associated with a high carcinogenic risk. This hypothesis is supported by the study of the EBV-related virus-associated hemophagocytic syndromes. In these unusual diseases that follow primary EBV infection, virus-infected T cells are readily detectable in the blood (2). These patients have a high risk of developing an EBV-positive T cell lymphoma during the course of the virus-associated hemophagocytic syndromes or in the following months, suggesting that persistent virus infection of T cells is a key event in carcinogenesis. Investigation of the oncogenic properties of EBV in non-B cells has been hampered by the lack of an appropriate *in vitro* system. Even though primary B lymphocytes are extremely sensitive to EBV infection *in vitro* and readily become immortalized, infection of primary epithelial cells or T lymphocytes with cell-free viral supernatants proved to be much more difficult *in vitro* (1). One exception is the infection of primary gastric cells by EBV (3). Viral infection promoted cellular proliferation and allowed extended passaging of these primary cells in culture, reinforcing the idea that EBV possesses transforming properties in epithelial cells (3). Interestingly, these authors used the virus strain Akata, whereas earlier experiments were generally conducted with the B95.8 strain. EBV strains might therefore differ in their ability to infect target cells, as already suggested (4, 5).

At the molecular level, EBV infection of target cells involves the interaction of viral glycoproteins with cell surface receptors. Virus entry has been shown to require binding of the gp350 viral glycoprotein to the cellular receptor CD21 and fusion of the viral particle with its target cells via the gp85 viral glycoprotein (6–8). Introduction of the CD21 gene in EBV-resistant keratinocyte cell lines restored sensitivity to viral infection, suggesting that the absence of CD21 is the restricting barrier for EBV infection in these cells (5). However, because skin keratinocytes are not physiological target cells for EBV infection *in vivo*, these results might not apply to other epithelial cell types. In addition, a gp350-negative EBV partly retained its infectious potential, suggesting that gp350-independent, and therefore probably CD21-independent, modes of viral entry exist (9). The gp110 glycoprotein encoded by the EBV *BALF4* ORF is expressed during the lytic phase of EBV and has been shown genetically to be essential for virus maturation (10, 11). No direct role in infection could be assigned to gp110 so far. In this paper, we show that gp110 is present within the virus particle and augmented incorporation of gp110 into the virus particle dramatically improves its efficiency to infect B and non-B cells. Moreover, we show that the amount of gp110 incorporated into the mature virion markedly varies among different viral strains. This work identifies gp110 as essential for efficient infection of non-B cells, a crucial step in virus-mediated cellular transformation.

Materials and Methods

Cell Lines. B95.8 is an EBV-immortalized marmoset monkey lymphoblastoid cell line (12), and 293 was generated by transfection of the adenovirus type 5 *E1a* and *E1b* genes into human embryonic epithelial kidney cells (13). Raji, Akata, P3HR1, and BJAB are human Burkitt's lymphoma cell lines (14, 15). RJ2.2.5 is an HLA class II negative mutant of the Raji cell line (16). M-ABA is a lymphoblastoid cell line established with a virus isolated from a nasopharyngeal carcinoma (17). HeLa is a human cervix adenocarcinoma cell line. Molt-4 is derived from a peripheral T cell lymphoma (18). All cell lines with the exception of HeLa cells were grown in RPMI 1640 medium supplemented with 10% FCS. HeLa cells were grown in DMEM/25 mM Hepes medium supplemented with 10% FCS.

Plasmids. The EBV lytic cycle was induced by transfection of the BZLF1 viral transactivator (19, 20). The B95.8 *BALF4* gene, which encodes the gp110 glycoprotein, was inserted into the pRK5 expression plasmid p2670. The B95.8 *BALF4* ORF was amplified by PCR with primers (5′-CATATGACTCGGCGTAGGGT-3′ and 5′-CAATTGAACTCAGTCTCTGCCT-3′), cleaved with *Sma*I, and inserted into pUC19 to yield p2375. After complete sequencing, the *Nde*I/*MunI* fragment of p2375 was

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Abbreviations: EBV, Epstein–Barr virus; PEG, polyethyleneglycol.

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treated with the Klenow fragment of DNA polymerase and ligated into cytomegalovirus-promoter-containing pRK5 that had previously been cleaved with *Eco*RI/*Hin*dIII and treated with Klenow to yield p2650. p2303 encompasses the EBV gp350 gene (9).

Immunostaining. Fixed (5 min, 1% paraformaldehyde) or living cells were incubated for 30 min with a mouse mAb (Chemicon) against gp110 (dilution 1:500 in PBS/5% FCS). After three washings, a Cy5-coupled sheep anti-mouse Ab was applied to living cells for 30 min at 37°C. FITC-labeled donkey anti-mouse Ab was used as a secondary Ab in fixed cells. After repeated washings in PBS, staining was recorded by using a laser scanning microscope (Zeiss).

Infections and Detection of Bound Viruses. The 293 cells containing the EBV recombinant virus (2089) were induced by transient transfection of the *BZLF1* expression plasmid p509 with or without cotransfection of the *BALF4* expression plasmid (21). B95.8, P3HR1, and M-ABA were induced with phorbol 12-tetradecanoate 13-acetate (TPA; 20 ng/ml final concentration) and butyrate (3 mM final concentration), whereas Akata was induced with anti-IgG immunoglobulins (22). Supernatants were harvested 3 days after induction and kept frozen at −80°C. Target cells were infected with 1 ml of filtered (0.8 μm) supernatants containing infectious viruses and kept in culture in cluster plates for 3 days before evaluation of GFP expression by UV microscopy. To detect viruses bound to their target cells (see below), BJAB and 293 cells were incubated with viral supernatants for 3 h or overnight at 4°C, washed twice in PBS, and further used for Western blotting or for Gardella gel analysis. In other experiments, viruses were precipitated in a polyethyleneglycol (PEG)-containing solution (0.5% wt/vol PEG 6000 in 0.5 M NaCl) and collected by centrifugation at 9,000 × *g* for 20 min.

Transfections. Plasmid DNA was introduced into cell lines by using lipid micelles (Lipofectamine, GIBCO/BRL). Cells were seeded into six-well cluster plates 1 day before transfection. For transfection, 70% confluent cells were placed in OptiMEM (Invitrogen) minimal medium for 2 h and incubated with DNA embedded in lipid micelles for 4 h.

Gardella Gel Electrophoresis and Southern Blot Analysis. Viral linear DNA from viruses present in cell-free supernatants or bound to EBV-negative target cells or from lytically induced EBV-positive cells was detected by using the agarose gel electrophoresis described by Gardella *et al.* (23). In this method, PEG-precipitated viruses (from 1 ml of supernatant) or 10^6 lytically induced cells are directly lysed in gel slots to avoid shearing of the viral DNA. Southern blot hybridization has been described (21).

Western Blot Analysis. Protein extracts from three different sources were analyzed in this work. Extracts from 5×10^6 lytically induced cells were generated by resuspending cells in an extraction buffer (final concentration, 50 mM Tris at pH 7.5/150 mM NaCl/0.1% SDS/1% sodium deoxycholate/1% Triton X-100) containing glass powder to shear DNA. After vigorous vortexing for 30 sec, cell debris was spun down at 16,000 × *g* for 5 min and supernatants were directly loaded without denaturation onto an SDS/7.5% acrylamide gel. A similar method was applied to viruses bound on target cells: 5×10^6 EBV-negative cells, e.g., BJAB or 293, were incubated with filtered supernatants and protein extracts were generated. A third assay assessed the presence of viral glycoproteins within the mature virion. Filtered supernatants were ultracentrifuged at 20,000 × *g* for 2 h. Virus pellets were then resuspended in 50 μl of extraction buffer, and debris was discarded after centrifugation at 16,000 × *g* for 5 min. After electrophoresis, proteins were electroblotted onto an ECL-Membrane (Amersham Pharmacia) for 1 h at 500 mA. After preincubation of the blot in PBS/5% dry milk powder containing 0.1% Tween 20, a mAb against gp110 (Chemicon MAB8184 dilution 1:1,000), or gp350 (American Type Culture Collection murine hybridoma 72A1) was added overnight at 4°C. After extensive washings in PBS, the blot was incubated for 1 h with a goat anti-mouse horseradish peroxidase-coupled secondary Ab (Promega, final dilution 1:10,000). Bound Abs were revealed by using the ECL detection reagent (Amersham Pharmacia) with exposure times varying from 10 to 120 sec. Relative amounts of antigen in Western blots or of DNA in Southern blots were estimated by scanning blots on a Heidelberg 1200 scanner at 1,200 dpi followed by analysis on a Macintosh G3 computer by using the public domain NIH IMAGE program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

Results

***BALF4* Overexpression Dramatically Increases EBV Titers.** EBV usually persists latently in infected cells but can be induced to enter the lytic phase in permissive cells. Activators of the lytic phase of EBV include chemicals such as phorbol 12-tetradecanoate 13-acetate or butyrate, immunoglobulins directed against human IgG, and the EBV transactivator BZLF1. The 2089 cell line is a 293 cell clone stably transfected with a recombinant EBV from

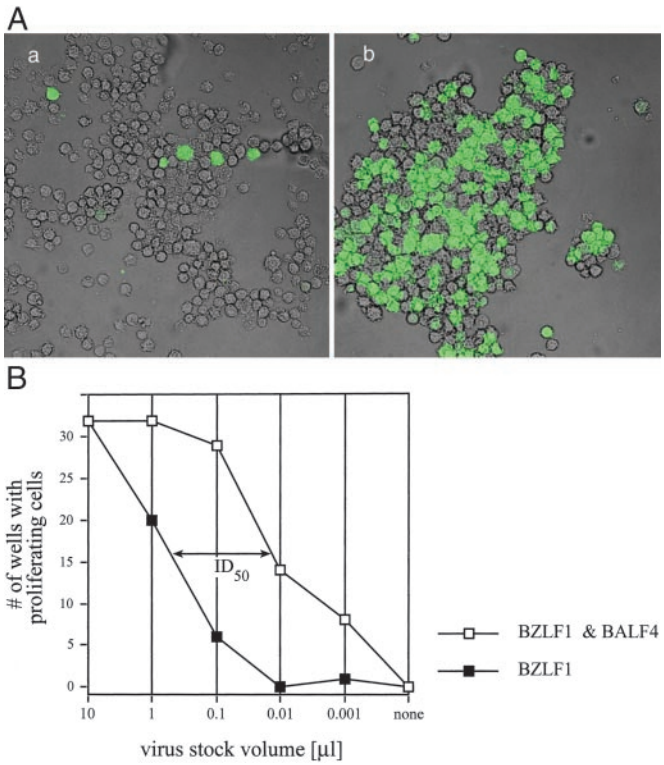

Fig. 1. Transfection of *BALF4* into induced 2089 cells dramatically enhances infectivity of virus stocks. (*A*) Raji cells were infected with gp110^{low} (*Left*) or gp110^{high} (*Right*) viral supernatants. gp110^{high} viruses are more infectious than gp110^{low}, as attested by the number of GFP-positive cells. (*B*) The same experiments were performed with primary human B lymphocytes, and various aliquots or dilutions as shown on the *x* axis were used to infect 10^4 primary B cells per well in a 96-well cluster plate. For each virus stock dilution, 32 wells were plated and the proliferation of B cells was evaluated microscopically 6 wk after infection. As can be seen from the graphs, supernatants containing gp110^{high} viral supernatants contained ≈100-fold more immortalizing units on the basis of the two ID_{50} values. One representative experiment of three is shown.

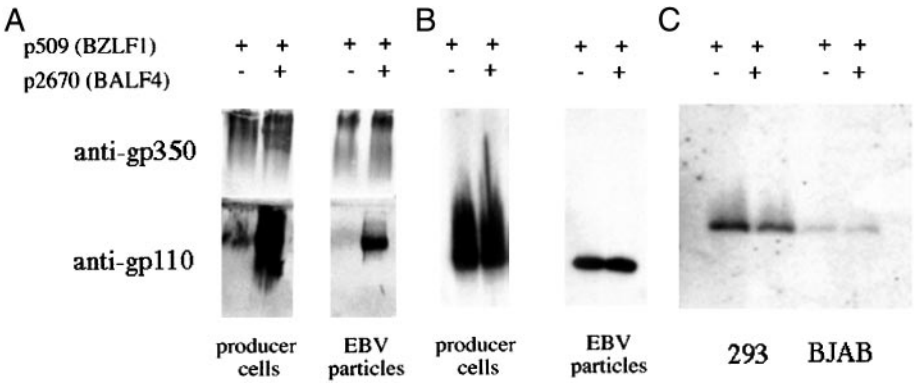

Fig. 2. gp110 does not interfere with virus production or virus binding to target cells. (*A*) The 2089 cells were lytically induced with *BZLF1* alone or in combination with the *BALF4* expression plasmid as indicated and subjected to Western blot analysis using Abs specific for gp350 or gp110. No difference in terms of gp350 expression in the virus-producing cells could be noted, but the expression of gp110 was increased substantially after *BALF4* transfection (*Left*). gp350 and gp110 levels were also analyzed in virus preparations. The amount of gp350 in the virus stocks was unchanged, indicating that *BALF4* does not alter the concentration of virus particles directly. In contrast, virus preparations contained substantially more gp110 protein, after *BALF4* overexpression (*Right*). Protein lysates were separated under nonreducing, nearly naive conditions to prevent precipitation of gp110. (*B*) Gardella gel analysis of unit-length EBV DNA present in 2089 producing cells (*Left*) and virus particles (*Right*). The 2089 cells, transfected with either *BZLF1* or *BZLF1* and *BALF4*, were analyzed by Gardella gel electrophoresis and Southern blot analysis using EBV *Bam*HI W fragment. The signals represent linear forms of the EBV genome generated during lytic viral DNA replication after encapsidation of unit-length genomes. The amount of unit-length DNA is not altered by overexpression of *BALF4* in lytically induced 2089 cells (*Left*) or in PEG-precipitated virus particle preparations (*Right*). (*C*) Overexpression of *BALF4* does not alter virus binding. The 293 and BJAB cells, expressing low and high amounts of the cognate virus receptor CD21, respectively, were incubated with $gp110^{high}$ and $gp110^{low}$ viruses for 3 h at 37°C. After several washes, cells with bound viruses were submitted directly to Gardella gel electrophoresis, followed by Southern blot hybridization using an EBV-specific probe. The signals obtained in the presence or absence of *BALF4* are very similar, indicating that the number of bound viruses is not influenced by gp110 overproduction.

which the GFP gene is expressed (21). Viruses carrying the recombinant EBV DNA can be easily recovered from the 2089 cell line on induction of lytic replication. Cells infected with these recombinant EBV express GFP and are easily identified.

Deletion of *BALF4* has been shown to completely inhibit virus production by impeding viral maturation (11), suggesting that *BALF4* knockouts are unlikely to provide appropriate tools to assess *BALF4* functions. We therefore constructed the *BALF4* expression plasmid p2670 and assessed viral titers by infecting different target cells with supernatants obtained from *BZLF1*-induced 2089 cells cotransfected with p2670 or a control plasmid. Surprisingly, we found that infection of Raji cells was enhanced >10-fold and up to 100-fold after transfection of *BALF4*, as evaluated by counting the number of GFP-positive cells 3 days after infection (Fig. 1*A*).

A similar effect was observed with primary B cells. Primary B cells were plated at 10^4 cells per well in 96-well cluster plates in the presence of virus stocks obtained with *BALF4*- or control-transfected 2089 cells. After 6 wk, the number of wells containing immortalized B cells was counted. As shown in Fig. 1*B*, supernatants obtained by cotransfection of *BALF4* were much more effective in inducing B cell proliferation. Such supernatants contained ≈100 times more immortalizing virions per volume than supernatants from 2089 cells generated by transfection of *BZFL1* only.

Overexpression of gp110 Neither Increases Virus Production Nor Influences Binding to Target Cells. The increased virus titer observed upon heterologous expression of *BALF4* during the lytic phase of 2089 cells might result from an improved production of progeny virus. To test this hypothesis, 2089 cells were cotransfected with *BZLF1* and *BALF4* or *BZLF1* together with a control plasmid and were analyzed in terms of late viral protein expression and viral DNA replication. Similarly, virus stocks were investigated for their content of viral structural proteins and unit-length EBV genomic DNA.

Western blot analysis was used to analyze late viral protein expression in 2089 cells after induction of the lytic cycle. The steady-state level of the EBV late gene product gp350 in the producer cells was found to be the same whether *BALF4* was overexpressed or not, showing that *BALF4* does not influence late gene protein expression (Fig. 2*A*). Similarly, no major difference was found in the amount of gp350 in viruses sedimented by ultracentrifugation from supernatants generated in the presence or absence of exogenous *BALF4* (Fig. 2*A*). gp350 is a major constituent of the virion and abundant in the viral envelope, suggesting that the physical concentration of virus particles in the supernatants was not influenced by *BALF4* overexpression during the lytic cycle of EBV. To confirm these observations, viral DNA content in supernatants and the amount of linear unit-length virion DNA in 2089 cells after induction of the lytic cycle were analyzed by Gardella gel analysis, followed by Southern blotting with the EBV-specific *Bam*HI W probe (Fig. 2*B*). Again, no difference could be found between cells and supernatants with or without transfection of a *BALF4* expression plasmid. These results demonstrated that gp110 overexpression during the lytic cycle of EBV in 2089 cells does not increase the number of viral particles in the virus stocks. Instead, transfection of the *BALF4* expression plasmid led to a clear increase of gp110 incorporated into virions (Fig. 2*A*). Therefore, viruses obtained from *BALF4* or control transfected 2089 cells were termed $gp110^{high}$ and $gp110^{low}$, respectively.

The level of gp110 present in viral particles might influence binding to the target cells of EBV. To test this hypothesis, various cell lines were incubated with $gp110^{high}$ and $gp110^{low}$ viruses for 3 h at 4°C followed by extensive washings. The amount of bound virus was monitored by Gardella gel analysis followed by Southern blot hybridization to determine the amount of genomic virion DNA (Fig. 2*C*) or Western blot detection of gp350 by using protein extracts of the infected cells (not shown). With both methods, we could not detect any differences between $gp110^{high}$ and $gp110^{low}$ viruses in terms of the amount of bound virus, suggesting that gp110 does not influence binding of EBV to its target cells.

gp110 Localizes to the Nuclear and Plasma Cell Membrane in Lytically Induced Cells. Studies performed mainly on herpes simplex virus type 1 (HSV-1) have shown that subcellular location and the function of viral glycoproteins during lytic cycle are related (24).

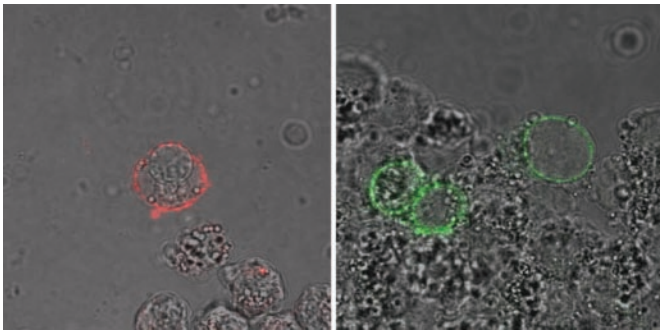

Fig. 3. gp110 is expressed at the cell surface of lytically induced cells. Living (*Left*) or paraformaldehyde-fixed (*Right*) M-ABA cells were stained with an Ab specific to gp110 and a secondary Ab coupled with FITC or Cy5 and counterstained with propidium iodide to exclude dead cells. Positive cells in the two laser scanning microscopy images show a typical surface membrane localization of gp110.

Glycoproteins involved in the first round of envelopment of the viral capsid are found at the nuclear membrane, whereas those expressed at the cellular membrane are eventually incorporated into the mature viral particle just before egress. Immunoelectron microscopy has been used to assess the distribution of gp110 in cells carrying the EBV laboratory strain B95.8 (10). In this work, gp110 was found to be restricted to the nuclear membrane and the near-nuclear endoplasmic reticulum. Prompted by our finding that gp110 can be detected in EBV virion preparations, we reevaluated the subcellular localization of this glycoprotein. To this aim, we stained living or unpermeabilized paraformaldehyde-fixed lytically induced cells with an Ab specific for gp110. In B95.8 cells, which support the lytic cycle of EBV spontaneously, and 2089 cells, which harbor a recombinant EBV genome derived from the B95.8 genome, we were not able to detect gp110 at the plasma membrane. In contrast, lytically induced M-ABA, Akata, P3HR1, or 2089 cells transfected with the expression plasmid encoding *BALF4* were found to unequivocally express gp110 at the plasma membrane (Fig. 3 and data not shown). It therefore appears that gp110 cell surface expression during the lytic phase of EBV is not common to all EBV strains.

The Amount of gp110 Varies Within Various EBV Strains. As already mentioned, expression of glycoproteins at the cell surface is usually followed by their incorporation in mature virions. Because the amount of gp110 expressed at the cell surface during the lytic phase differs in various cell lines, we expected variations in the amounts of gp110 incorporated into the virions of various EBV strains. We analyzed virions from several commonly used laboratory virus strains (P3HR1, B95.8, Akata, and M-ABA) by Western blotting with a gp110-specific Ab (Fig. 4 *Top Left*). Included in the panel were 2089 virus stocks obtained with or without concomitant heterologous expression of *BALF4*. The same samples were also examined for gp350 levels and for DNA content to estimate virus titers (Fig. 4 *Middle Left* and *Bottom Left*). Scanning of the blots indicated that there was a good if not perfect correlation between the amount of gp350 and DNA content of the virions. In contrast to gp350, which is present in similar amounts in different strains, the amount of gp110 varied considerably among different strains. M-ABA was found to carry the highest amount of gp110, even if the DNA content was slightly higher than that of other viral strains. In contrast, gp110 was barely detectable in B95.8 and 2089. Although Akata and P3HR1 viral titers were much lower than those obtained with the other cell lines, Akata viruses were clearly positive for gp110, which suggests that this viral strain is a high gp110 producer. P3HR1 did not show strong gp110 expression in the virions, but

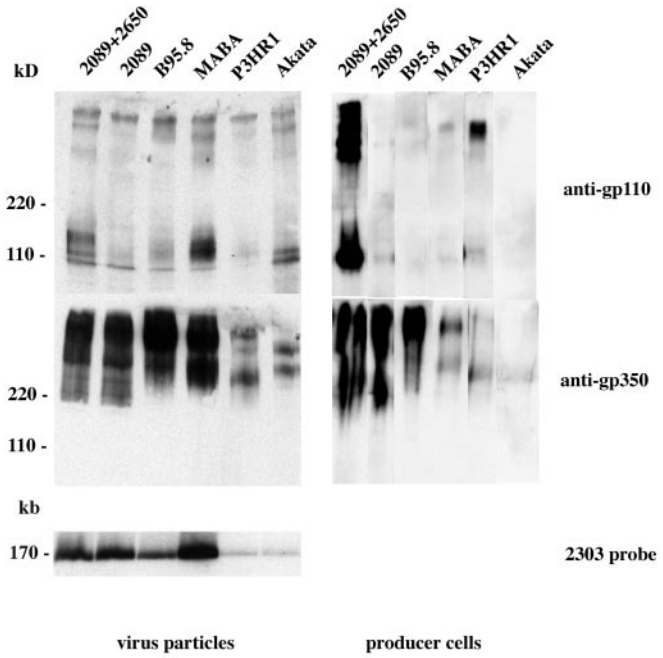

Fig. 4. Variable expression of gp110 in various laboratory EBV strains. (*Top Left* and *Middle Left*) The EBV-infected cell lines M-ABA, P3HR1, Akata, B95.8, 2089, and 2089 cells transfected with *BALF4* were lytically induced, and viruses were collected by ultracentrifugation. After extraction of the viral proteins from the virus pellets, the samples were submitted to Western blot analysis using Abs specific to gp110 and gp350. Intensities of signals obtained with the Ab specific to gp350 were analyzed by using the NIH IMAGE 1.62 software (in percentage, normalized with the signal obtained with 2089: 1, 2089 + 2650: 0.91, B95.8: 0.99, MABA: 1.01, P3HR1: 0.55, Akata: 0.36). (*Top Right* and *Middle Right*) Western blot analysis of the induced cell lines. (*Bottom Left*) Supernatants from the same viral strains were precipitated with PEG and analyzed by Gardella gel electrophoresis. Blots were hybridized with an EBV-specific probe devoid of repeats (p2303). Intensity of obtained signals was estimated by using the NIH IMAGE 1.62 software (in percentage, normalized with the signal obtained with 2089: 1, 2089 + 2650: 0.97, B95.8: 0.75, MABA: 1.21, P3HR1: 0.25, Akata: 0.19).

this is probably related to the low viral titers. Supernatants from lytically induced 2089 cells transfected with the *BALF4* expression plasmid p2650 showed a relatively strong gp110 signal, confirming that overexpression of *BALF4* in the producer cell line during the lytic phase of EBV increased incorporation of the glycoprotein into mature virions. Notably, gp110^{high} 2089 virus still contains less gp110 than some of the laboratory strains such as M-ABA. It therefore appears that the amount of gp110 incorporated in EBV virions after transfection lies within the range observed for other gp110-positive EBV strains. Results gained from experiments performed with gp110^{high} viruses can therefore be extended to wild-type viruses. gp110 and gp350 were positive in all induced cell lines by immunostaining (data not shown), and protein synthesis was further tested by using Western blotting (Fig. 4 *Right*). gp110 was massively produced after transfection into 2089 and appears to be very low in Akata, which is probably related to the low number of induced cells. The remaining cell lines showed comparable expression levels. There was therefore no direct relationship between the amount of gp110 produced in the replicating cell lines and the amount of gp110 present in the mature particle. However, massive overexpression of gp110, even if inefficient, enhanced incorporation.

Increased Incorporation of gp110 Within EBV Virions Extends the Infectious Spectrum of EBV. The tropism of EBV *in vitro* is restricted almost entirely to B cells, with some notable exceptions such as

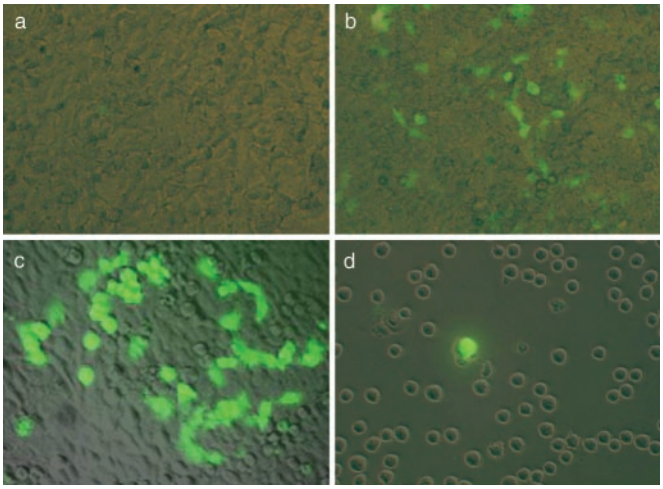

Fig. 5. Infection of 293, HeLa, and MOLT-4 cells with gp110^{high} and gp110^{low} 2089 supernatants. GFP-positive 293 cells after infection with gp110^{low} (*a*) or gp110^{high} (*b*) viral stocks show the influence of *BALF4* on infection efficiency. gp110^{high} supernatants infect HeLa (*c*) and Molt-4 (*d*) cells.

primary gastric epithelial cells. Akata, a viral strain capable of infecting gastric epithelial cells (3), also expressed high amounts of gp110 (Fig. 4), raising the possibility that gp110 might be critically involved in infection of cells other than human B cells. We therefore extended our infection experiments to non-B cell lines. We first infected 293 cells, a cell line reported to express subliminal amounts of CD21 (9, 25), and found that infection with gp110^{high} virus stocks increased the number of infected cells 100-fold as compared with gp110^{low} stocks (Fig. 5 *a* and *b*). HeLa is completely CD21 negative and has been reported to be completely refractory to infection by EBV (8, 9, 26). Incubation of HeLa cells with gp110^{high} supernatants resulted in successful infection of 5–10% of the cells, whereas the control experiment with gp110^{low} supernatants was negative (Fig. 5*c* and data not shown). A low degree of infection (0.1%) was obtained with the T cell lymphoma cell line MOLT-4. This cell line is considered to be completely resistant to EBV infection (27), but a few GFP-positive cells were observed after infection with supernatants obtained from induced 2089 overexpressing gp110 only (Fig. 5*d*).

Interaction between the gH/gL EBV glycoproteins and HLA class II molecules has been shown to be an important modulator of viral infection (28). To assess possible cross-talk between this infection pathway and the enhancing effect of gp110 on infection, we infected RJ2.2.5 cells, an HLA class II-negative mutant of the B cell lymphoma Raji cell line, with both gp110^{high} and gp110^{low} supernatants. Enhanced incorporation of gp110 showed increased infection of the target to the same extent as with the HLA class II-positive Raji parental cell line (data not shown). The effect of gp110 on infection is therefore independent of the gH/gL-HLA class II infection.

Discussion

Herpes viruses possess a large genome coding for at least 80 proteins. Viral protein networks coordinate infection, DNA replication, and in some cases cellular transformation. The HSV-1 envelope contains a dozen viral membrane glycoproteins, four of which (gB, gD, gH, and gL) have been shown to be essential for entry (29, 30). The binding of EBV to its target cells is known to implicate interactions between gp350 and CD21 but also between gH-gL-gp42 trimers and HLA class II molecules (28, 31). In this work, we show that another glycoprotein, gp110, is also present within the mature virus particle. gp110 plays an essential role during virus infection, as gp110^{high} viruses were found to be up to 100-fold more infectious than their gp110^{low} counterparts. This marked increase in infectivity was observed in primary B lymphocytes and in epithelial cells such as 293, suggesting a general effect of gp110 on virus entry. Enhancement of infection by means of gp110 was equally observed in HLA class II-positive and -negative cells (Raji vs. RJ2.2.5) and in both CD21-positive and CD21-negative cells (primary lymphocytes, HeLa cells). gp110 therefore exerts its functions independently of gp350–CD21 or gH/gL–HLA class II interactions.

gp110, sometimes also misleadingly designated VCA because it forms part of the immunologically defined viral capsid antigen complex (32), is distantly related to gB of HSV-1 (10). The finding that gp110 is present in the virion is therefore not entirely unexpected. Interestingly, gp110 also allows access to cell types previously considered to be refractory to EBV infection *in vitro*, indicating that the amount of gp110 present within the mature virus can markedly influence the target cell spectrum of EBV. The precise function of gp110 on virus infection is not clear. Exogenous expression of gp110 neither increased the concentration of infectious particles nor appeared to enhance binding of the virus to its target cell. A *BALF4*-knockout virus would theoretically help defining the function of gp110, but gp110 has been shown to be essential for viral maturation (unpublished data and ref. 11). At this stage of the work, we can only speculate that gp110 might enhance virus penetration or viral transport to the nucleus.

EBV strains are clearly heterogeneous in terms of the amount of gp110 incorporated into the viral particle. M-ABA, a viral strain initially isolated from a nasopharyngeal carcinoma sample before being passaged in lymphoid cells, and Akata, a viral strain isolated from a Japanese Burkitt's lymphoma cell line, both contained large amounts of gp110, whereas B95.8 virions, the prototypic laboratory strain, barely expressed the protein. Although Akata and B95.8 EBV strains have never been compared directly, previous reports showed that the Akata virus efficiently infected gastric epithelial cells, whereas infection of epithelial cells with B95.8 was unsuccessful (4, 8, 26). These results could at least partly be related to the amount of gp110 incorporated into the viral particle. The wider significance of these results is that it is important to use gp110^{high} virions when evaluating EBV target cells. Virions obtained by transfection of *BALF4* in the 2089 cell line that carries the recombinant EBV or virions from the Akata cell line should be suitable for these experiments.

In line with earlier reports, we could not detect significant amounts of gp110 in B95.8 virions (10). It is interesting to note that B95.8 carries a 10-kb deletion upstream of the *BALF4* gene (33). Whether this region contains cis or trans elements important for the optimal incorporation of gp110 into the viral particle is unknown but could be tested by reintroducing the missing sequence into the B95.8 genome. We could not detect significant amounts of gp110 at the cell surface of induced B95.8 cells, reinforcing the view that gp110 is not expressed in a physiological way in B95.8 cells. In contrast, the protein could be easily detected at the cell surface of induced M-ABA cells.

In summary, this work identifies gp110 as an important virulence factor for EBV infection that determines both infectivity and viral tropism. Cellular and humoral responses against gp110 have been reported, showing that gp110 is a potentially important immunogenic determinant (34–36). gp110 therefore is a candidate for peptide vaccination. Vaccination strategies using attenuated live viruses should similarly take into account the variable amounts of gp110 contained in different EBV strains.

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