Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 7772–7776, August 1992
Medical Sciences

Marked variation in the size of genomic plasmids among members of a family of related Epstein–Barr viruses

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Communicated by Dorothy M. Horstmann, April 30, 1992

ABSTRACT **Epstein–Barr virus (EBV) genomes in the P3J-HR-1 (HR-1) Burkitt lymphoma cell line rearrange at a high rate. Previously described deletions and rearrangements in HR-1 cells have been found at sites of EBV replication *in vivo*, suggesting that DNA rearrangement may be an integral aspect of EBV biology and pathogenesis. We examined the structure of linear EBV genomes in subcultures of HR-1 cells using contour-clamped homogenous electric field gel electrophoresis. We developed a second pulsed electrophoretic technique to separate intracellular circular EBV plasmids. The standard, linear HR-1 EBV genome was ≈155 kilobases in length. Linear molecules of less than unit length, presumably defective genomes, were seen in numerous subcultures. Linear intracellular genomes >155 kilobases were also detected, but only linear genomes of 155 kilobases or less were packaged into virions. The size of circular EBV plasmids also varied greatly among HR-1 subcultures, some of which contained two plasmids of different size. The progeny of the unusual circular plasmids could be either standard or nonstandard linear genomes. No aberrant linear or circular form was detected in a subculture carrying the previously described het fragments. Pulsed-gel electrophoresis has provided two additional characteristics of mutant EBVs: abnormal linear and circular genome configurations.**

The Epstein–Barr virus (EBV) genome exists in two different forms. Circular genomes are maintained in latently infected, immortalized cells, and linear molecules are packaged into virions during lytic infection (1). EBV genome structure and replication have been extensively examined in the P3J-HR-1 (HR-1) Burkitt lymphoma cell line (2–4). The HR-1 virus is immortalization incompetent due to deletion of nuclear antigen coding sequences (EBNA2 and LP) (5–7). It is maintained predominantly in the latent state but exhibits a low level of lytic viral DNA replication. The cell line responds strongly to chemical agents that induce lytic replication.

Several naturally occurring mutants found in the HR-1 cell line or its progeny (8–10) were first identified as aberrant viral DNA restriction fragments, termed het fragments. The majority of HR-1 subclones are het-free [e.g., clone 16 (Cl16)], whereas rare subclones are enriched for het fragments (e.g., Cl5). Standard, unrearranged EBV DNA restriction fragments are maintained in the parental HR-1 and Cl5 cell lines, suggesting that het DNA is part of a defective genome that requires helper functions provided by the standard genome. het DNA in Cl5 cells is maintained as an independent replicon, is spread from cell to cell in EB virions (11), and is easily lost (12). The complete structure of the defective genome is not known, primarily because the defective and standard genomes have not been separated.

Defective genomes correlate with spontaneous lytic viral DNA replication in cell clones of the HR-1 family (9, 12). Furthermore, gene transfer of the WZhet fragment, comprised of sequences from the standard *Bam*HI-W and -Z regions, disrupts the latent viral life cycle of a standard EBV genome (13). WZhet encodes a single intact viral gene, whose product is termed ZEBRA (14, 15). Altered expression of ZEBRA is responsible for induction of the lytic life cycle by defective genomes (16, 17).

Viruses with deletions and rearrangements similar to those of the HR-1 family have now been found in sites of EBV replication *in vivo* (18, 19). Therefore, study of the structure, function, and generation of defective HR-1 EBV DNA populations should continue to provide insights into EBV biology and pathogenesis.

Our study of HR-1 viral DNA addresses the following issues. Are the defective EBV genomes in parental and Cl5 cells distinct in size from the standard genome? Do HR-1 cell lines, with or without defective molecules, generate new defectives? Are there EBV genome rearrangements, other than WZhet, that can activate lytic replication?

We addressed these questions by analyzing EBV genome size and configuration in subcultures of HR-1 cells. Linear EBV genome size was determined by the contour-clamped homogenous electric field [CHEF (see *Materials and Methods*)] gel technique. The number and size of EBV circular plasmids were determined using a further technique, the pulsed Gardella gel (20), which separates high molecular weight circular DNA. The presence of het DNA, determined by Southern analysis, was correlated with genome size, spontaneous expression of ZEBRA, and spontaneous lytic viral replication.

MATERIALS AND METHODS

Cells. The P3H3 subculture, obtained from W. Henle (12), was used as a source of parental HR-1. Two HR-1 single cell clones, HH543-5 (Cl5) and HH514-16 (Cl16), have been described (9). Individual subcultures had been handled independently for 16 or more weekly passages and were designated by a letter suffix (e.g., P3H3A). Chemically induced cells were harvested after 3 days of exposure to 1.5 mM sodium butyrate and 10 ng of phorbol 12-myristate 13-acetate per liter. Aged cultures were harvested 10 days after a 1:2 dilution.

Preparation of Cell Plugs for CHEF Gels. Agarose plugs of total cellular DNA were prepared using a modification of a published method (21). Cells were resuspended in molten low-melting temperature agarose (Sea-Plaque; FMC) in 0.25 M EDTA at 60°C to a final concentration of 3×10^5 cells per 50 μl. Solidified agarose plugs were incubated overnight at 60°C in lysis buffer [10 mM Tris, pH 8.5/425 mM EDTA/1 mg of Pronase per ml (Boehringer Mannheim)/1% SDS], dialyzed against 50 mM EDTA/10 mM Tris, pH 7.5, for 2 hr at 22°C with nutation, and then stored in dialysis buffer at 4°C.

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Abbreviations: CHEF, contour-clamped homogeneous electric field; EBV, Epstein–Barr virus; Cl, clone.

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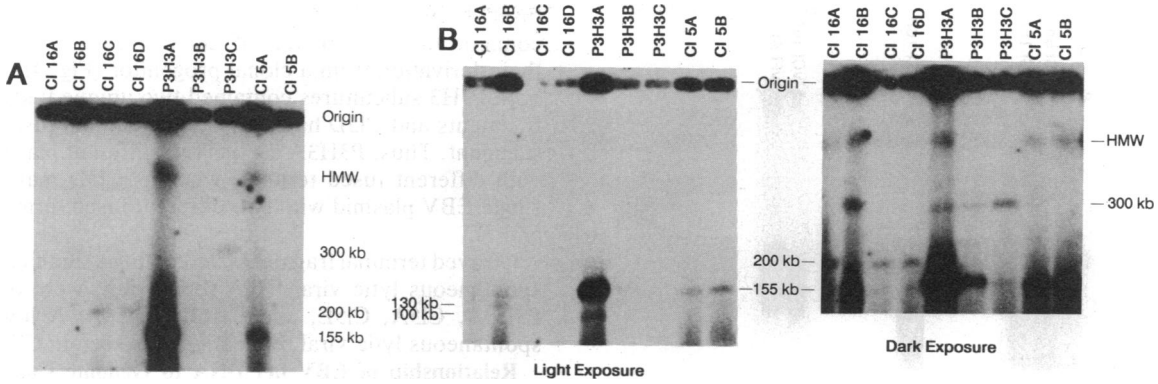

FIG. 1. CHEF gel analysis of linear EBV genomes in HR-1 cells with and without induction of the viral lytic cycle. Viral sequences were detected with an IR-1 probe. In each panel, the origin of the gel, location of high molecular weight hybridization (HMW), and mobilities of linear EBV bands are indicated. (*A*) Uninduced subcultures. (*B*) Analysis of the same cultures after chemical induction. Light and dark exposures of the same gel are shown to portray all of the relevant bands. Defectives of 130 and 100 kb in cultures Cl16B and P3H3A, respectively, are indicated on the light exposure (left). Molecules of 200 and 300 kb are marked on the dark exposure (right). Hybridization at the origin represented unlysed cells. The HMW signal comigrated with chromosomal DNA and was proportional to the amount of EBV DNA in the sample. This signal may represent circular EBV plasmids, linear concatemers of EBV genomes, or EBV sequences integrated into host cell DNA. Most samples also contained low molecular weight background hybridization (0–50 kb). This probably represents small, single-strand DNA fragments released from the highly nicked EBV genome in the course of sample preparation (28).

Preparation of Viral DNA. Cells were induced with sodium butyrate and phorbol 12-myristate 13-acetate for 5 days. Virus was then harvested as per Adams (22).

CHEF Gel Analysis. A custom-built apparatus was used for CHEF gel analysis (21, 23, 24). Ten × 10 cm, 1% agarose CHEF gels were run in 0.5× TBE (10× TBE = 890 mM Tris, pH 7.9/890 mM boric acid/20 mM EDTA) at 10°C for 19–24 hr. A potential of 250 V was alternated every 25 sec between electrodes separated by 29.8 cm. This empirically derived pulse profile resolved molecules between 100 and 300 kilobases (kb). Phage λ multimers were included on every gel as size standards.

Pulsed Gardella Gels. Horizontal 0.75% Gardella gels were prepared in 0.5× TBE and loaded with whole B cells (20). Phage λ multimers were included as linear size markers. Standard electrophoresis was performed in 0.5× TBE/0.1% SDS at 22°C and 40 V for 1–3 hr. A pulsed electric field was then applied using a PPI-100 pulse controller (MJ Research, Watertown, MA) at 22°C and 150 V for 16–20 hr. Pulse program numbers four and seven (switcher chip version: 100.3) were used. These pulse profiles separated molecules in the range of circular plasmids; consequently, linear molecules were poorly separated.

Genomic Southern Analysis for het DNA. Five micrograms of total cellular DNA (12) was digested with *Bam*HI and separated on 0.8% agarose gels in 1× TBE.

DNA Hybridization. Gels were transferred to nitrocellulose and hybridized using standard methods (25).

DNA Probes. EBV DNA probes were as follows (26): *Bam*HI-W (IR-1) [14,877–16,287 base pairs (bp)], *Bam*HI-H (52,381–53,817 bp), which cross-reacts with *Bam*HI-B′, *Bam*HI-R (103,816–105,118 bp), *Bam*HI-Z (101,947–103,741 bp), *Bam*HI-C (4345–5393 bp), and fused terminus (TR) (167,037–168,976 bp) (27). DNA probes were radiolabeled as described (17).

ZEBRA Expression. Immunoblots were done using the polyclonal rabbit anti-BZLF1 antiserum (ZACK) as described (17).

RESULTS

Size of Linear EBV DNA Molecules in the HR-1 Family of Cells. The CHEF gel technique was used to study linear EBV genome size in subcultures of the HR-1 family that were untreated or induced into lytic replication by addition of sodium butyrate and PMA (Fig. 1; see Table 1). A molecule of 155 kb, representing the standard linear HR-1 genome, was identified in two cultures (P3H3A and Cl5A) that spontaneously produced virus (Fig. 1*A*). The 155-kb form appeared in several batches of cells (Cl16A, -B, -C, and -D, P3H3B and -C, and Cl5B) after chemical induction (Fig. 1*B*) and in three cultures (Cl5F, Cl16A and -B) in which replication was activated by ageing (Fig. 2). The 155-kb genome was found in virions prepared from supernatant fluids of chemically induced P3H3A, Cl5A, Cl16A and -B cultures (Fig. 3). Virion DNA of the HR-1 family was ≈35 kb smaller than that of FF41 (29).

An EBV linear genome of 300 kb was detected in one uninduced cell line (P3H3C, Fig. 1*A*). A similar sized band was seen following chemical induction of P3H3A, -B, and -C and Cl16B cells (Fig. 1*B*), and ageing of Cl5E (Fig. 2). Cl5D exhibited a 250-kb linear molecule after ageing (Fig. 2). Neither of the larger forms in P3H3A or Cl16B was identified in virion DNA (Fig. 3).

A linear genome of 200 kb was unique to several Cl16 subcultures (Cl16C and -D, Fig. 1). It was evident in Cl16A only after chemical induction or ageing (Figs. 1*B* and 2) but was not packaged into virions (Fig. 3). The 200-kb form was never detected in Cl16B.

P3H3A and Cl16B harbored additional linear genomes of 100 kb and 130 kb, respectively (Fig. 1*B*). Both forms were

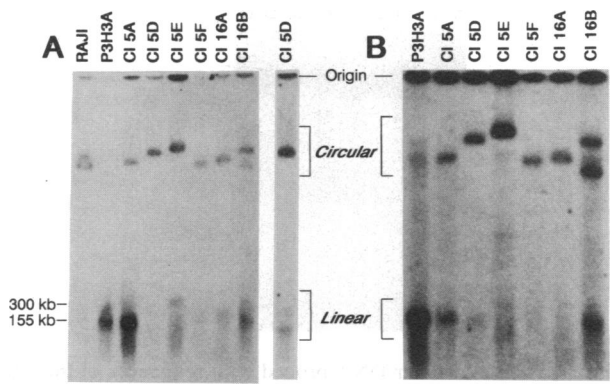

FIG. 2. Pulsed Gardella gel analysis of intracellular EBV genomes in aged cells. Viral sequences were detected with a *Bam*HI-Z probe. Raji cells were included as a size standard for a unit length EBV circular plasmid. *A* and *B* represent two analyses separated by 2 months. A darker exposure of the Cl5D lane is provided in *A*.

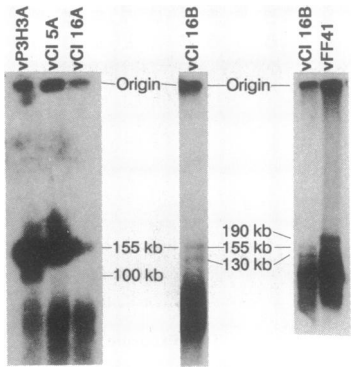

FIG. 3. CHEF gel analysis of DNA extracted from virions. Virion DNA was separated and probed as in Fig. 1. The mobilities of linear EBV bands are indicated.

packaged into virions (Fig. 3). A linear genome of 130 kb was evident in aged Cl5D cells (Fig. 2). Aged Cl5E cells carried two indistinct linear molecules, possibly 120 and 30 kb (Fig. 2).

Circular Plasmids in Subcultures of HR-1 Cells. Conventional pulsed gels require considerable sample preparation and often damage circular DNA molecules. The Gardella technique lyses cells in the well of the gel, preserving circular DNA, and separates circles from linears but does not efficiently separate circular molecules of different size (20). The pulsed Gardella gel was developed as a reliable method to separate large intracellular circular DNA molecules.

The size and number of circular EBV plasmids varied greatly among the related HR-1 subcultures (Fig. 2). Single plasmids of similar size were found in Cl5A and Cl5F. Larger plasmids were seen in Cl5D, Cl5E, and Cl16A. Two plasmids of different size were detected in P3H3A and Cl16B cells. The EBV plasmids detected by this analysis were stable for 2 months, or >20 cell divisions (Fig. 2*B*).

Analysis of the EBV Fused Terminus. Fused terminus analysis has been used to assess the clonality of virus in a population of EBV-infected cells (27). A unique restriction fragment encompassing the fused terminus indicates a single EBV plasmid resulting from infection by a single virus. A population of cells infected with several EBVs often carries several fused terminal fragments of different length. The size of the fused terminus depends upon the number of terminal repeats used to circularize the viral genome. We used the technique to determine if the plasmids we had detected by pulsed Gardella gel could be distinguished by the size of their fused termini (Fig. 4). A single dominant fused terminus common to the Cl16 and Cl5 derivatives is in keeping with their derivation from a clonal progenitor (Fig. 4). However, both P3H3 subcultures contained two unique fused terminal fragments and Cl5D had a second prominent fused terminal fragment. Thus, P3H3A carries two different plasmids, each with different fused termini, whereas Cl5D may contain a single EBV plasmid with two different fused termini (Figs. 2 and 5).

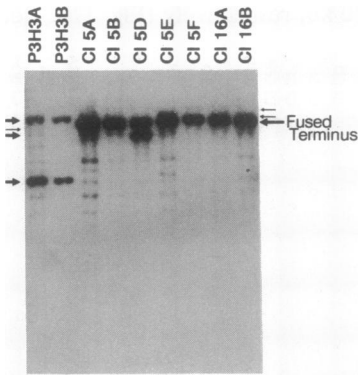

FIG. 4. Total cellular DNA probed with sequences of the EBV genome terminus. Uninduced Cl5 and Cl16 cells exhibited a common high molecular weight fused terminus (Fused Terminus). Additional fused termini in P3H3A and Cl15D are indicated by heavy arrows. Hypomolar fused termini in Cl5A (slightly smaller than the standard terminus) and Cl5E and -F (slightly larger than the standard terminus) are indicated by light arrows.

Cleaved terminal fragments, indicating a significant level of spontaneous lytic viral DNA replication, were detected in P3H3A, Cl5A, Cl5E, and Cl16B (Fig. 4). A low level of spontaneous lytic viral replication was seen in Cl5B and -D.

Relationship of EBV het DNA to Genome Configuration, ZEBRA Expression, and Spontaneous Lytic Viral DNA Replication. Uninduced subcultures of the HR-1 family were examined for het DNA and ZEBRA expression (Table 1). The P3H3 cultures did not contain the defective fragments described by Cho *et al.* (10). Instead, P3H3A carried three novel het fragments: IR-1het 5.3, Rhet 2.1, and Zhet 8.1; P3H3B had the same IR-1 and Zhets as P3H3A but lacked Rhet 2.1 (Table 1). The presence of Rhet 2.1 in P3H3A

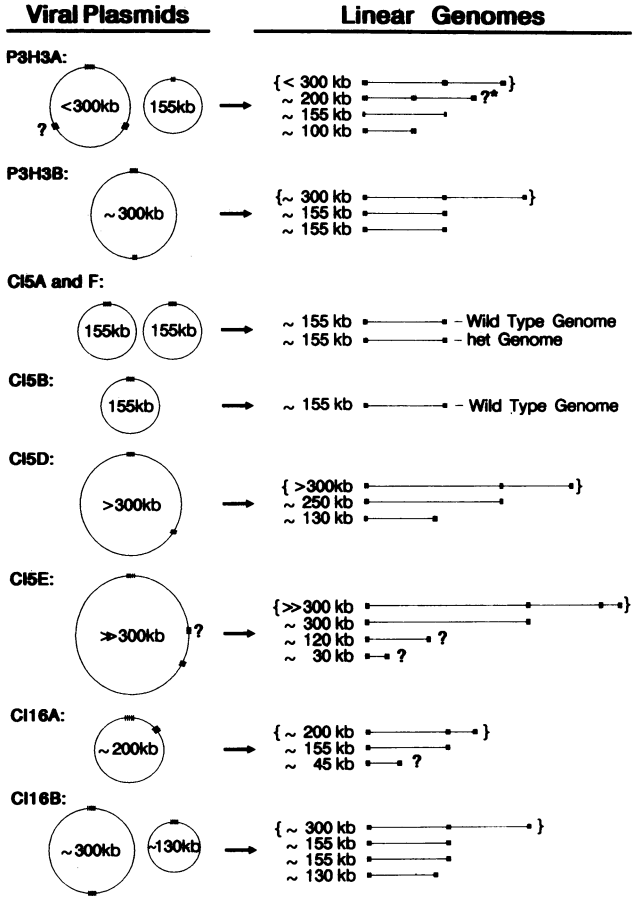

FIG. 5. Potential relationship between circular and linear EBV molecules in HR-1 subcultures. (Left) Deduced sizes and configurations of the stable circular plasmids present in each cell line. (Right) Possible linear genomes that would result from lytic replication and cleavage of the plasmids. In each case, the terminal repeats are depicted as a series of small perpendicular lines. The number of repeats is uniform except for P3H3A and Cl5D, which have two different fused termini (see Fig. 4). Bracketed linear genomes may be the result of a single cleavage at terminal repeat sequences or nonspecific double-strand breaks in circular plasmids. The 200-kb linear genome predicted for P3H3A (marked with an *) was never detected. Genomes of <155 kb may be defective. Genomes <100 kb were generally not detected due to low molecular weight background hybridization.

Table 1. *Bam*HI het DNA fragments and spontaneous ZEBRA expression in the HR-1 family of cells

Culture	het DNA,* kb	ZEBRA, kDa
P3H3A	IR-1, 5.3; Z, 8.0; R, 2.1	36
P3H3B	IR-1, 5.3; Z, 8.0	None
Cl5A	IR-1, **1.28**; Z, **2.8**; MB', **7.2**; C, **7.2**	39,† 36
Cl5B	None	None
Cl5D	IR-1, **1.28**, 9.05; Z, **2.8**; MB', 7.55; C, **7.2**	39, 36
Cl5E	IR-1, **1.28**, 2.5, 5.7, 9.85; Z, **2.8**; MB', 7.8; C, 4.8, 4.9, **7.2**	40, 36
Cl5F	IR-1, 10.25; Z, **2.8**; C, 6.9, **7.2**	None
Cl16A	IR-1, 5.0	None
Cl16B	IR-1, 10.0	None

Fragments listed in bold type were observed in Cl5 cells previously (12).
*het DNA is designated viral DNA homology, size in kb.
†A larger "het" ZEBRA was detected in addition to the standard sized ZEBRA.

correlated with spontaneous expression of ZEBRA, spontaneous lytic viral DNA replication as measured by the generation of 155-kb linear genomes in untreated cells (Fig. 1*A*), and the presence of cleaved terminal repeats (Fig. 4).

Cl5A contained the het fragments previously reported (ref. 12 and Table 1), although it lacked an anomalous linear or circular defective genome molecule (Figs. 1 and 2). het DNA was no longer present in Cl5B, whereas it continued to evolve in other subcultures of Cl5 cells (Table 1). These DNA rearrangements correlated with anomalous genome configurations in Cl5D and -E. However, only standard size EBV genomes were detected in Cl5F (Fig. 2).

Spontaneous expression of ZEBRA in Cl5 cells was detected in subcultures that contained the original Cl5 het DNA fragments (Cl5A, -D, and -E, Table 1). These same cultures exhibited cleaved terminal repeats indicative of spontaneous lytic viral DNA replication (Fig. 4). Cl5F lacked IR-1het 1.28 and an MB'het (Table 1). It did not express ZEBRA (Table 1) or exhibit cleaved terminal fragments (Fig. 4). Cl5B was the exception. It lacked het DNA but exhibited a low level of cleaved terminal repeats.

Cl16 cells, which originally lacked het DNA (9), had acquired a het fragment homologous to IR-1. In Cl16A (and two other subcultures, not shown) this IR-1 het fragment was 5.0 kb (Table 1). An IR-1het of 10.0 kb was detected in Cl16B cells that bore a 300-kb circular genome (Table 1 and Fig. 1*B*). However, none of the Cl16 subcultures spontaneously generated 155-kb linear genomes (Fig. 1*A*), nor did they express ZEBRA or carry a Zhet (Table 1). Oddly, cleaved terminal repeats were seen in Cl16B cells, suggesting that spontaneous lytic viral DNA replication in these cells could be initiated by low levels of ZEBRA, undetected by immunoblot, or by a mechanism entirely independent of ZEBRA.

Relationship of EBV Plasmids to Linear Genomes in HR-1 Cells. Fig. 5 shows how the specific linear EBV genomes generated during lytic viral DNA replication could be derived from the EBV circular plasmids detected by pulsed Gardella gel (Figs. 1–4). Lytic viral DNA replication occurs via the rolling-circle mechanism: unit length genomes are released for packaging into virions from a large linear concatemer of viral DNA by cleavage at successive viral terminal sequences (1). EBV sequences have been found integrated into cellular DNA (30); however, it is not known if these genomes are competent for lytic replication. Thus, we have not considered the possibility that extrachromosomal linear viral genomes may have arisen from an integrated virus.

Subclones Cl5A and -F carried plasmids with mobility similar to that of RAJI (Fig. 2) and produced linear genomes of 155 kb (Figs. 1*B* and 3). Therefore, the plasmids were unit length and contained a single dominant viral fused terminus [Fig. 5 (20)]. However, both clones contained het DNA (Table 1), indicating that there were two species of unit length circles in these cells: standard and defective.

Cl5D and -E maintained the largest plasmids (Fig. 2). Linear molecules of 250 and 130 kb were detected in Cl5D upon ageing (Fig. 2), consistent with the complete cleavage of its large circular plasmid at two sets of terminal repeat sequences into two linear components. Therefore, Cl5D cells carried a heterodimeric plasmid composed of two nonstandard genomes, of which the smaller may be packaged (Fig. 5). Cl5E, on the other hand, produced a 300-kb linear molecule and one or two poorly defined smaller molecules after ageing (Fig. 2). The large Cl5E plasmid may have contained two sets of terminal repeats that permitted it to be preferentially cleaved once per 300 kb. Other terminal repeat sequences elsewhere on the plasmid may have accounted for the less abundant smaller linear forms (Fig. 5).

The circular plasmid in Cl16A was slightly larger than unit length (Fig. 2) and is consistent with a size of 200 kb, similar to the linear molecule of 200 kb observed by CHEF gel analysis (Fig. 1*B*). The 200-kb linear molecule detected in these cells may have been the result of lytic replication and cleavage at one viral terminal sequence or a single nonspecific double-strand break incurred by the 200-kb plasmid during sample preparation. Cl16A also exhibited intracellular and virion linear molecules of 155 kb (Figs. 1*B* and 3), indicating that the 200-kb plasmid contained two sets of terminal repeats separated by 155 kb (Fig. 5).

The Cl16B and P3H3A subcultures each contained two plasmids (Fig. 2) and three linear species (Fig. 1*B*). We believe that the 300-kb linear genomes seen on CHEF gels arose from circular plasmids in the course of lytic replication or as a result of a double strand break (Fig. 1*B*). Thus, the slower migrating plasmid bands in P3H3A and Cl16B were circles of ≈300 kb in length (Fig. 2). The second circular molecule observed in Cl16B cells was slightly smaller than unit length (Fig. 2). This plasmid probably generated the 130-kb linear genomes that were seen during lytic replication (Fig. 1*B* and 3). Thus, the 300-kb circular plasmid in Cl16B encoded at least one copy of the 155-kb linear molecule found in these cells after induction (Figs. 1*B*, 3, and 5).

The smaller plasmid in the P3H3A subculture comigrated with a 155-kb species (Fig. 2*B*, compare P3H3A with Cl5A) and probably generated the 155-kb linear seen in these cells (Fig. 1*B*). Thus, the 300-kb P3H3A plasmid is the likely source of the 100-kb linear genome (Fig. 1*B*). The balance of the large plasmid would have encoded a 200-kb linear genome; however, a molecule of this mobility was never detected in P3H3A cells with probes against the IR-1 and *Bam*HI-Z viral sequences. It may be that the 200-kb molecule contained no IR-1 or *Bam*HI-Z sequences and, therefore, was not detected. Alternatively, the 300-kb circle may have encoded three copies of the 100-kb genome (Fig. 5).

DISCUSSION

A Modified Pulsed-Gel Technique for Resolving Large Eukaryotic Plasmids. This technique is the combination of two recently developed tools for the study of very large DNA molecules: (*i*) pulsed gel electrophoresis, which can efficiently separate DNA molecules of 100 kb to >1 Mb (21, 23), and (*ii*) Gardella gel electrophoresis, which preserves circular DNA molecules extracted from eukaryotic cells (20). The pulsed Gardella gel preserves molecular conformation, easily separates circles of different size, and is amenable for routine use.

The pulsed Gardella gel was used in this study to examine EBV genome configuration, spontaneous mutagenesis, and replication in cultured cells. Although EBV DNA plasmids of

non-standard length have been seen by electron microscopy in some Burkitt lymphoma cell lines, the molecules were not positively identified as EBV (31, 32). The pulsed Gardella gel technique may also be useful in the study of other large circular DNA molecules—for example, latent herpes simplex virus (33) and extrachromosomal DNA elements generated during gene amplification in tumor cells [e.g., episomes and double minute chromosomes (34)].

Variation in EBV Plasmid Size. EBV subcultures derived from a single cloned cell line have diverged over time. We detected circular EBV genomes of unit length, some of which were dimeric and others that were intermediate in size. The linear progeny of the aberrant plasmids could be unit length or non-unit length; however, only linear molecules unit length or less were detected in virions. Unlike encapsidated linear genomes, it appears that the size of the intracellular viral plasmid is not restricted. This spontaneous variation in EBV genome size and configuration must be carefully monitored in genetic studies that rely upon the HR-1 virus (6, 7).

The exact genetic composition of the aberrant plasmids is not known. However, some EB viral elements (e.g., for plasmid maintenance and lytic viral DNA replication) must be present since the plasmids were stable for numerous generations and could be induced to replicate (Figs. 1 and 2). Furthermore, many of the aberrant plasmids carried defective DNA that influenced the biology of the virus. For example, only Cl5 subcultures that carried the previously detected rearrangements spontaneously entered lytic viral DNA replication (Cl5A, -D, and -E). A Cl5 subculture that had lost IR-1het 1.28 and an MB'het (Cl5F, Table 1) was no longer productive. This is consistent with the observations of Rooney *et al.* (16), which indicated that sequences upstream (IR-1het 1.28) and downstream (MB'het) of WZhet in the Cl5 defective genome were necessary for maximal expression of ZEBRA.

Similarly, the defective genome in the P3H3 subcultures expressed ZEBRA only when Rhet 2.1 was present. This rearrangement may activate ZEBRA expression by altering the bicistronic ZEBRA promoter in *Bam*HI-R (35). The P3H3B defective lacked the *Bam*HI-R rearrangement and remained quiescent. The hypothesis that defective genomes with Rhet 2.1 spontaneously activate the viral lytic cycle in P3H3 cultures remains to be tested.

A third anomaly revealed by this analysis is an extra 45 kb of DNA carried by most Cl16 viruses. This derivative of HR-1 was first characterized as het negative but a strong responder to chemical induction, as opposed to >100 het negative sibs that were weakly responsive to chemicals (9). It is possible that the extra 45 kb of DNA present in this genome is responsible for the altered response to chemical stimuli.

Significance of the Findings. EBV DNA in HR-1 cells rearranges at a high rate. This may be dependent upon the preexistence of het DNA. The continuous low-level induction of replication by ZEBRA, which itself is activated by het DNA, might cause DNA rearrangements as the result of replication errors. EBNA2 and LP are also deleted from the HR-1 genome. Perhaps these proteins are involved in control or fidelity of latent DNA replication. Alternatively, the site-specific recombinases needed for immunoglobulin expression in B cells may inadvertently alter the viral genome.

Pulsed-gel analysis offers additional means of characterizing EBV mutants. Previously, EBV mutants were identified by het fragments. Now, their genomes may also be typed as standard or non-standard linears or circles. However, not all mutant EBV genomes assume a non-standard linear or circular form. Cl5A carries all of the het fragments described previously and yet maintains circular and linear genomes of unit length.

The mutants we have detected are not likely to be artifacts of cells in culture. The EBNA2 deletion and rearrangements similar to WZhet have now been detected at sites of EBV replication *in vivo* (18, 19). This suggests that DNA rearrangement is common in EBV biology and may be involved in the pathogenesis of the virus as well.

We thank Mike Snyder, John Sedivy, and Greg Howe for pulsed-gel advice and N. Raab-Traub for the TR clone (Xho-1.9). Special thanks go to Sithideth Thonkham for technical assistance and for acting as a liaison. This work was supported by American Cancer Society Grant MV-548L and National Institutes of Health Grants CA12055, CA52228, CA16038, and AI22959.

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