

The B95-8 Isolate of Epstein-Barr Virus Arose from an Isolate with a Standard Genome

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Blot hybridization studies revealed that the deletion which characterizes the DNA from the B95-8 strain of Epstein-Barr virus was not present in the virus from which the B95-8 strain was derived (883L). The deletion event must have occurred during establishment of the B95-8 cell line or very soon afterward, since the deletion was present in Epstein-Barr virus DNA from a cell line established with B95-8 virus soon after it became available. The presence of the deletion correlates with decreased expression of the gp220 viral envelope glycoprotein.

The most extensively studied strain of Epstein-Barr virus (EBV) is the B95-8 prototype, because for many years the B95-8 cell line produced more virus than any other cell line. The B95-8 cell line (a marmoset line which secretes the B95-8 strain of EBV) was originally immortalized by infection with virus (883L strain) from the 883L cell line (8). The 883L cell line was obtained by culture of lymphocytes from an elderly person with transfusion-induced infectious mononucleosis. The B95-8 virus has biological properties indistinguishable from other strains (except for the nontransforming P3HR-1 strain). However, it is known to have unique physical properties. First, the viral genome is different in that it has a 7.6-megadalton deletion (1, 2, 4, 5, 9). Second, it has a disproportionately low amount of a viral envelope glycoprotein, gp220 (3).

A study of the EBV envelope proteins synthesized in the 883L cell line showed normal expression of the gp220, unlike the progeny cell line B95-8 (3). This result prompted us to examine the EBV DNA in the 883L cell line to determine whether the deletion observed in the B95-8 genome was present. We found that the deletion is not present in the EBV DNA in 883L cells and that the deletion in the B95-8 genome occurred during or soon after the establishment of the B95-8 cell line. Thus, we conclude that the B95-8 strain of EBV is not a naturally occurring isolate, but rather is a laboratory-derived variant.

These conclusions came from blot hybridization studies with DNA from B95-8 cells, 883L cells, and JY cells (a human cell line which was made by infection of peripheral blood lymphocytes with the B95-8 strain of EBV soon after B95-8 virus became available). The cellular DNA was extracted with phenol after Sarkosyl-

pronase incubation and was then digested with *SalI*, *HindIII*, or *BamHI* restriction endonuclease. Electrophoresis on 0.7% agarose gels was followed by denaturation and transfer of the DNA from the gel to nitrocellulose sheets. Later, either EBV virion DNA or cloned *BamHI* fragments were radiolabeled with ³²P and hybridized with EBV fragments on the blots. The EBV fragments were detected after autoradiography.

Figure 1 shows the results of the blot hybridizations. The *SalI*-digested DNA (Fig. 1d, e, and f) shows fragments G' and G'' in 883L that are not present in B95-8 and JY EBV DNA. The *HindIII*-digested DNA (Fig. 1g, h, and i) has D', D'', and K' fragments in 883L which are not present in B95-8 or JY. The differences in *BamHI* fragments (Fig. 1a, b, and c) are less easily discerned, but 883L has B', I', and W' fragments instead of the I fragment present in B95-8 and JY. In all cases, the 883L DNA has a structure similar to standard isolates (1, 2, 4, 5, 9). Figure 2 shows how these differences are reflected in the restriction maps. Therefore, the major conclusion to be drawn from Fig. 1 is that the B95-8 strain of EBV is a laboratory variant rather than a clinical isolate.

There are minor unexpected findings in Fig. 1. For example, *SalI* D is a different size in each of the cell lines. This difference can be understood as arising from different numbers of terminal redundancies (6, 7) in each genome. This would not be detected in *HindIII* fragments because the fragments are so large that the 0.35-megadalton differences would make no difference in their rate of migration.

In the *BamHI* digest, the differences at the termini of the linearized genome are confined to the N and J fragments. In the B95-8 cells, there is so much heterogeneity in the size of the N and

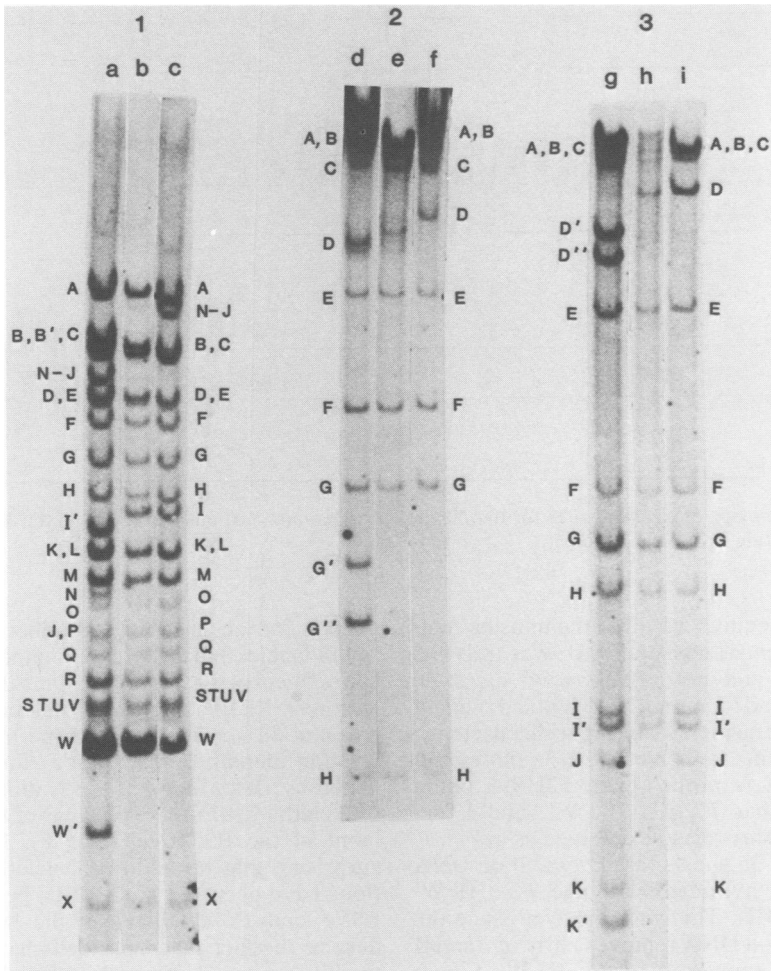


FIG. 1. Autoradiography of EBV restriction fragments. DNA from the 883L, B95-8, and JY cell lines was digested with either *Bam*HI, *Sal*I, or *Hind*III, and the fragments were electrophoresed in a 0.7% agarose slab gel. The DNA in the gel was denatured in situ with alkali and then transferred to a nitrocellulose filter. The filter was incubated with 32 P-labeled EBV DNA (purified from the P3HR-1 strain) under conditions which favor hybridization of the labeled DNA to the complementary EBV DNA in the nitrocellulose filter (10). DNA from the 883L, B95-8, and JY cell lines was digested with *Bam*HI (lanes a, b, and c), *Sal*I (lanes d, e, and f), and *Hind*III (lanes g, h, and i). (The larger bands in the *Sal*I digests are distorted because the gel in that vicinity was overloaded with DNA. The *Sal*I fragments from cellular DNA are particularly large because many of the *Sal*I recognition sites contain methylcytosine and are therefore resistant to cleavage.)

J fragments that they do not migrate as single bands. However, the predominant species are of 2.8 and 3.5 megadaltons. Their sum, if the genome were a circle (as it is in nonproducing cells), would be 6.3 megadaltons. The observed molecular size for the N-J fragment is 5.3 megadaltons for 883L and 7.0 megadaltons for JY. (Note that there is little heterogeneity in the sizes of these fragments.) The B95-8 genome has, on the average, about seven terminal redundancies. Based on the sizes of the N-J frag-

ments, we conclude that the 883L genome has only four terminal redundancies per genome as opposed to nine for the genome in the JY cell line. (In 883L, a minor *Bam*HI fragment which comigrates with the N fragment of B95-8 is seen. This may be an N or a J fragment which has lost two of its 500-base pair terminal redundancies. The 883L cell line produces small quantities of virus, so some of the viral genomes are linear and therefore have separate *Bam*HI N and J fragments.)

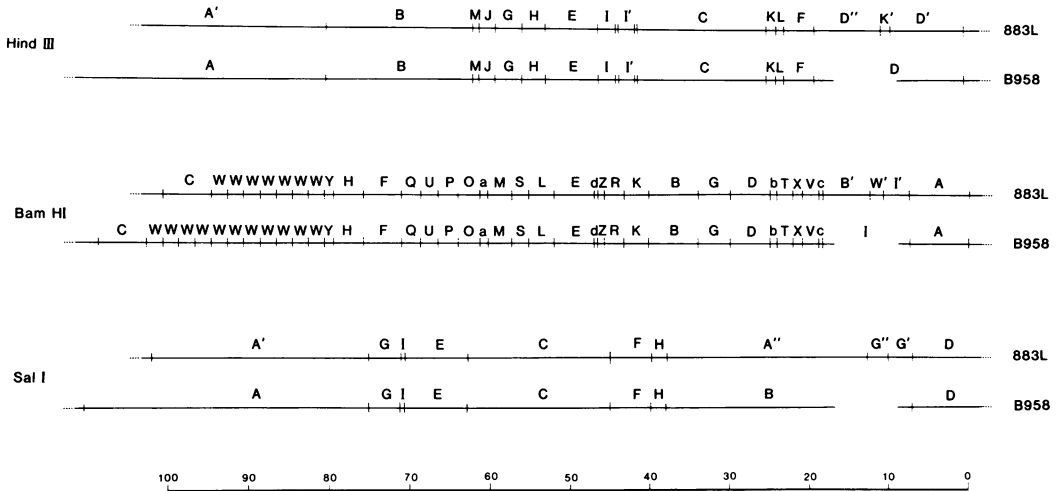


FIG. 2. Scale maps of cleavage sites for the *Hind*III, *Bam*HI, and *Sal*I endonucleases in the 883L and B95-8 genomes. The scale is in megadaltons.

To prove unequivocally that the unusual fragment in the *Bam*HI digest of 883L was truly the N-J fragment and not some partial digestion product, we made a number of identical blots of 883L *Bam*HI fragments and hybridized cloned *Bam*HI fragments to them. Figure 3a shows that when the blot is probed with P3HR-1 virion DNA (which has *Bam*HI B', W', and I' sequences) the same result is obtained as in Fig. 1. However, Fig. 3b shows that B95-8 virion DNA probe fails to hybridize with the *Bam*HI W' fragment of 883L. This was expected since the deletion in B95-8 DNA removes parts of *Bam*HI B' and I' and removes all of *Bam*HI W'. Figure 3c shows the results of hybridization with pooled cloned *Bam*HI fragments (10). This collection of cloned fragments lacks the terminal fragments, and it does not hybridize to the unusual 5.3-megadalton fragment. This proves that the unusual fragment in 883L is indeed the N-J fusion product and by analogy that the 7.0-megadalton fragment in strain JY is also N-J.

Also in Fig. 3 are hybridizations with cloned DNAs which could be constituents of partial digestion products which had sizes near 5.3 megadaltons. None of these probes hybridized to the N-J fragments, but as expected, the B95-8 *Bam*HI I hybridizes to the B' and I' of 883L (Fig. 3e). Also, as is the case in all other isolates of EBV, part of the *Bam*HI H fragment has homology with part of *Bam*HI B' (Fig. 3d). The minor bands visible are the *Bam*HI fragments of mitochondrial DNA, which have some homology with the *Escherichia coli* DNA that is also in the probe.

These data show that the EBV genome in JY cells is identical to that of the B95-8 strain

except for an increased number of terminal redundancies and a decreased number of *Bam*HI W fragments (Fig. 1). This means that the B95-8 strain of EBV acquired its deletion when the parental 883L virus infected the marmoset cell or soon thereafter. The JY cell line was established by Dean Mann at the National Institutes of Health in 1972, within a year of the establishment of the B95-8 cell line (8). It is perhaps surprising that no additional deletions or additions have occurred in the EBV genomes in the B95-8 and JY cell lines in the last 10 years, despite the fact that these cells have been continually passed during this time. If such disregard for passage number were practiced in the passage of herpes simplex virus, for example, the viral DNA population would become heterogeneous. This striking stability upon culture in the laboratory may be characteristic of episomal EBV DNA (with the possible exception of the nontransforming P3HR-1 strain). Certainly the kind of deletion event that gave rise to B95-8 DNA must be rare, since the W91, M-ABA, and FF41 strains have no such deletion, despite the fact that they are also produced by marmoset cells (1, 4, 5).

As mentioned earlier, B95-8 cells, unlike 883L cells, express a disproportionately low level of the gp220 viral envelope component. JY cells have never been induced to a productive cycle, so their potential for expression of gp220 is unknown. However, other cell lines transformed by B95-8 virus share the characteristic low-level gp220 expression (3). The fact that this change is concomitant with the loss of about 7.6 megadaltons of viral DNA suggests that the deletion which is characteristic of B95-8 is responsible

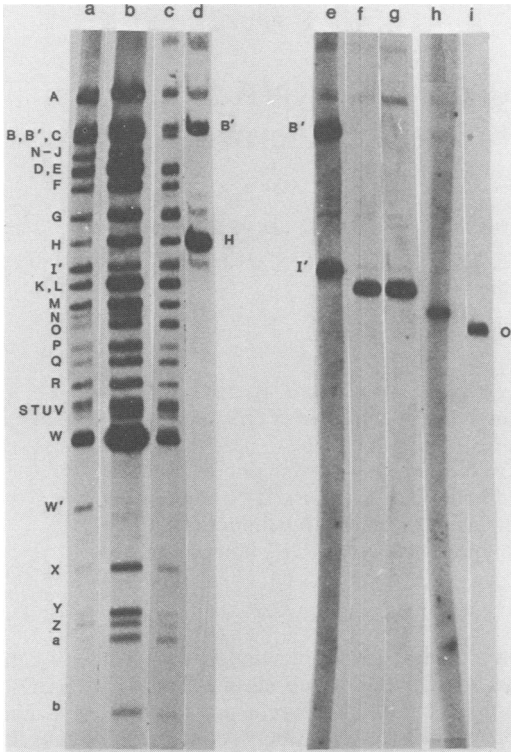


FIG. 3. DNA from the 883L cell line digested with *Bam*HI and applied to a single trough of a 0.7% agarose gel. The DNA in the gel was transferred to nitrocellulose, and the nitrocellulose was cut into strips (10). Each of the strips was hybridized with a different probe as follows: a, P3HR-1 virion DNA; b, B95-8 virion DNA; c, a mixture of all the cloned *Bam*HI fragments of B95-8 DNA (except for the termini); d, *Bam*HI H; e, *Bam*HI I; f, *Bam*HI K; g, *Bam*HI L; h, *Bam*HI M; i, *Bam*HI O.

for its depressed gp220 level. How could this deletion decrease gp220 expression? Any feasible explanation would require the postulate that the gp350 gene would be regulated independently of the gp220 gene, despite the fact that both are late membrane antigens and cross-react with the same monoclonal antibodies. One possibility is that the gp220 (but not gp350) is regulated by a gene product specified in the deleted sequence. Prokaryotic analogs of such regulation might be the catabolite repression system of *E. coli* or the

N gene of bacteriophage lambda. Another possibility is that the deletion actually removes some sequence at the 3' end of the gp220 mRNA, either preventing the mRNA from reaching the cytoplasm or conceivably resulting in an altered carboxyl terminus on the gp220. An altered mRNA or an altered protein could be short-lived. A third possibility is that the deletion removes promoter or enhancer sequences upstream from the gp220 structural gene. By using the technique of hybrid-selected translation, it should be possible to map the gp220 gene precisely, and this should lead to an understanding of its depressed expression in the B95-8 strain.

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