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Identification of a rare Epstein–Barr virus variant that enhances early antigen expression in Raji cells

(latency)

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ABSTRACT **Early antigens (EAs) are made when the P3J-HR-1 strain of Epstein–Barr virus (EBV) infects cells that already harbor latent EB viral genomes. We wished to identify EBV genes that might participate in induction of EAs. We have recently isolated from the HR-1 line EB viral variants that are unable to induce EAs. We have now isolated a clone of HR-1 cells that releases virus with the capacity to induce EA. We compared the genome of the EB variant that possesses the capacity to induce EA with that of a variant that is unable to induce EA and with parental stock that was the source of both different biotypes of EBV. The variant that is able to induce EA contains, in molar or greater quantities, additional fragments of EBV DNA not found in the variant that lacks that capacity. These same DNA fragments are present in submolar quantities in the parental DNA, indicating that they represent a subpopulation in the parental viral DNA mixture. We thus provide evidence that EA induction is brought about by unusual forms of EBV DNA that are likely to act by regulating expression of the genome.**

Viruses released from the P3J-HR-1 (HR-1) cell clone of the Jijoye Burkitt lymphoma line (1) are unique among Epstein–Barr viruses (EBVs). They fail to immortalize normal B lymphocytes and they do not cause lymphomas in marmosets (2–4). HR-1 viruses induce an abortive replicative cycle with the synthesis of early antigens (EAs) in Raji cells (5), which already contain latent EBV genomes. If a high multiplicity of HR-1 viruses is added, some virions are produced which are able to immortalize lymphocytes (6, 7).

Biological and biochemical evidence indicates that HR-1 viruses are heterogeneous (8, 9). By partial denaturation mapping of HR-1 DNA, it has been shown that there are at least two different populations of molecules (10). Furthermore, when HR-1 DNA is digested with restriction endonucleases, a variety of submolar fragments, designated heterogeneous, are found, in addition to the expected molar fragments (11, 12). These heterogeneous DNA fragments are homologous to a number of different regions of the genome and are not to be confused with heterogeneity at the terminus of EBV DNA (13).

The HR-1 line is a mixture of cells that carry distinct EBV variants (14). The DNA of four clonal HR-1 EBVs studied lack submolar heterogeneous restriction fragments found in parental DNA. All subclones spontaneously shed lower amounts of virus than does the parental HR-1 line. None of our first set of 29 clonal HR-1 EBVs is able to induce EA in Raji cells. Yano *et al.* (15) have also isolated a subclone of HR-1 cells that releases EBV that lacks EA induction ability.

We now report the identification, among 171 additional subclones, of one clone that resembles parental HR-1 virus in high spontaneous production of virus having the ability to induce EA. We find that the genome of this HR-1 subclone contains, in molar quantity or greater, at least three heterogeneous DNA fragments that are present in submolar quantities in parental HR-1 DNA. Thus, we provide evidence that unusual forms of EBV DNA released from a rare subpopulation of HR-1 cells are likely to account for the ability of the HR-1 strain to induce EAs.

MATERIALS AND METHODS

Cell Cloning. Two batches of P3J-HR-1 cells were cloned by either limiting dilution or plating in semisolid agarose medium (16). Both systems used unirradiated feeder layers of fibroblastic cell strains from human placenta.

Estimates of Amounts of Viral DNA. The content of viral DNA was estimated in supernatant fluids by nucleic acid spot hybridization (17). Four milliliters of fluid was centrifuged for 15 min in a microcentrifuge and then suspended in 20 μl of Hanks' buffered saline. Five-microliter aliquots were spotted on nitrocellulose and processed as described (17). Probes were chimeric plasmids containing EBV DNA fragments radiolabeled with ^{32}P by nick-translation. Standard curves were constructed from serial dilutions of Raji cells known to contain about 100 copies of EBV DNA per cell. The curves were log–log plots of number of Raji cells vs. cpm hybridized. The amount of EBV DNA was estimated by extrapolation to these standard curves.

Detection of EA Induction. Equal volumes of the fluid under test and Raji cells at 2×10^6/ml were incubated for 3 days at 37°C. Cell spreads were fixed in acetone and examined by indirect immunofluorescence using either a polyvalent human serum from a patient with nasopharyngeal carcinoma or murine monoclonal antibodies (gifts of G. Pearson, G. Hoffman, and B. Sugden).

Analysis of Viral DNA Sequences. Intracellular DNA was prepared from the parental P3H3 line and from two cell clones (clone HH514-16 and clone HH543-5; see Table 1) according to the procedure of Wahl *et al.* (18). Total cellular DNA was digested with *Bam*HI, electrophoresed through 0.5% agarose gels, and transferred to nitrocellulose. EBV DNA sequences were detected by probing with ^{32}P-labeled plasmids containing cloned EBV DNA *Eco*RI and *Bam*HI fragments from strain FF41 (19). Conditions for hybridization have been described (19).

RESULTS

Properties of Virus from 200 HR-1 Clones. Cellular subclones of the HR-1 line were obtained with high efficiency, ranging from 15% to 58% (Table 1). The clones were isolated from two batches of cells, one (HR-1) that had been passaged

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Abbreviations: EBV, Epstein–Barr virus; EA, early antigen; EAI, EA-inducing; PMA, phorbol 12-myristate 13-acetate; VCA, viral capsid antigen.

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Table 1. A rare clone of HR-1 cells stably releases EBV that is competent to induce EA in Raji cells

Exp.	HR-1 cells*	Cloning method	% cloning	Clones tested, no.	Clones able to induce EA, no.
FF452	A	Agarose	33	5	0
GG68	A	Agarose	37	24	0
HH198	A	Dilution	35	43	0†
HH492	A	Dilution	58	14	0†
HH514	B	Agarose	15	102	0
HH543	B	Dilution	25	12	1
Total				200	1

* A, our laboratory stock of HR-1 cells; B, W. Henle laboratory stock of P3H3 cells.
† In exp. HH198, one clone initially released EAI^+ virus but, on further testing, released EAI^- virus. In exp. HH492, four clones were initially EAI^+ and later became EAI^-.

in our laboratory for 12 years and another (P3H3) obtained recently from W. Henle. Only one cellular subclone, designated HH543-5, derived in a limiting-dilution experiment from the P3H3 parental stock released virus having the ability to induce EA in Raji cells. This clone will be called EAI^+ (EA-inducing positive) and the remainder of the clones, EAI^-.

Lack of EA Induction by Clonal HR-1 Virus Is Not Due to Lack of Virus. Because 199 of 200 HR-1 subclones were EAI^-, we asked whether this was simply a matter of the quantity of virus shed into the culture medium. That this is not the case is shown by representative data in Tables 2 and 3. Virus was raised from the parental line and five EAI^- HR-1 subclones in the presence or absence of phorbol 12-myristate 13-acetate (PMA; 20 ng/ml), which induces the EB viral replicative cycle (20). The amount of viral DNA present in the supernatant was estimated by spot hybridization and the fluids were also tested for their biologic activity in Raji cells. Only the parental HR-1 stock induced EA. Although supernatants from all five of these clones contained low amounts of viral DNA in the absence of PMA, in the presence of PMA, three of five clones shed levels of viral DNA that were equivalent to the amount spontaneously shed by the parental line. In further studies, stocks prepared from ("superinducible") clones that, after PMA treatment produced more viral DNA than the parental line, were still unable to induce EA.

Although there were considerable amounts of EBV DNA in the supernatants of PMA-induced EAI^- HR-1 clones, we had not proven that this DNA was in virions. Therefore, the supernatant fluids obtained from parental HR-1 and a superinducible clone, GG68-5, after PMA treatment were exposed to DNase. The amount of viral DNA in such fluids was compared with that in fluids that had not been so treated. As a control, we exposed purified plasmid DNA containing the EBV *Eco*RI fragment B to DNase. Plasmid DNA was destroyed by DNase whereas EBV DNA in the supernatant was protected from DNase, as would occur if the DNA were encapsidated. We also metabolically labeled, with [^{3}H]thymidine, cultures of two clones, GG68-5 and GG68-13, that are induced to synthesize large amounts of viral DNA and capsid antigen after treatment with PMA. When supernatant fluids were concentrated by centrifugation and the pellets were sedimented on sucrose gradients, we found a peak of radioactivity at 34–36% sucrose, where virions are generally found (data not shown).

Table 2. Clonal variants of HR-1 cells release EBV that fails to induce EA in Raji cells

Virus	EBV DNA,* ng/ml		EA positive-Raji cells†	
	Without PMA	With PMA	Without PMA	With PMA
HR-1 parent stock A	0.63	2.9	20%	20%
Clone FF452-1	0.02	0.5	Negative	Negative
Clone FF452-2	0.01	0.2	Negative	Negative
Clone FF452-3	0.09	1.2	Negative	Negative
Clone FF452-4	<0.01	0.2	Negative	Negative
Clone FF452-5	0.12	0.6	Negative	Negative

Virus was prepared by incubation of parental HR-1 line or clones for 14 days at 33°C; half of each group received PMA at 20 ng/ml on day 1.
* Estimated by spot hybridization. Raji cells served as a positive standard; the probe was HR-1 viral DNA (1 ng/ml = 6×10^6 genomes per ml).
† Detected with a human EBV antibody.

Table 3. Pooling of fluids or cells of clones does not recover EA-inducing capacity of parental HR-1 cells

Pooled fluids added to Raji cells	Conc., x-fold	EBV DNA,* ng/ml	EA-positive Raji cells†
From all GG68 clones	1	0.3	Negative
	35	10.5	Negative
From selected GG68 clones	1	0.4	Negative
	35	14.0	Negative
Parental HR-1 stock A	1	3.3	30%

Fluids from all GG68 clones were obtained 14 days after subculture in the absence of PMA; those from selected clones were prepared from five superinducible clones (16). They were concentrated by centrifugation at 10,000 rpm for 15 min and resuspended in Hanks' buffered saline.
* Estimated by spot hybridization.
† Detected with a human serum.

Pools of Supernatant Fluids or Cell Clones Also Fail to Induce EA. To ask whether EAI^- clones might complement each other, a mixture of fluids was prepared from 24 clones in experiment GG68 (Table 1) and tested for early antigen induction (Table 2). We also concentrated supernatant fluids and created pools of culture fluids from clones GG68-5, -11, -13, -17, and -19, which are known to be superresponsive to PMA (Table 3). All the pooled supernatants failed to induce EA, even though the concentrated cloned virus stocks contained more viral DNA than the parental virus.

In an additional experiment, we pooled cells from all clones isolated in experiments FF452, GG68, and HH198 (Table 1) and prepared virus stocks after culturing these cellular pools together for 27 days. Once again, the pools were unable to induce EA.

Estimation of the Fraction of HR-1 Cells Containing Virus that Can Induce EA in Raji Cells. On the basis of the frequency of obtaining cell clones that release EAI^+ virus, the cell subpopulation having virus with this biologic ability is infrequent, about 1 in 200. In another approach (Table 4), the HR-1 cells were plated at concentrations from 10^5 cells per microwell to 1 cell per 10 microwells. Supernatant virus from lines that grew up were tested for their EA-inducing ability. All sublines established from 1,000 cells or more released EA-inducing virus. Four of eight lines established from 100 cells per well released EA-inducing virus. No lines derived from 10 cells or fewer gave rise to lines having the stable ability to cause EA expression in Raji cells. Thus, by limiting dilution or by direct cloning, we estimate that 1 in 200 HR-1 cells has the capacity to shed EAI^+ virus.

Ability to Induce EA Correlates with Spontaneous Virus Production. The parental HR-1 line spontaneously enters the viral replicative cycle. There are usually 5–10% viral capsid antigen (VCA)-positive cells; approximately 0.5–4.0 ng of viral DNA

Table 4. Estimation of the fraction of parental HR-1 cells that are capable of causing EA induction in Raji cells

Cells plated	Wells with growth	Stable EA-inducing sublines
10^5	8/8	8/8
10^4	8/8	8/8
10^3	8/8	8/8
10^2	8/8	4/8
10^1	16/16	0/16
10^0	14/24	0/14
10^{-1}	1/24	0/1

Cells plated are given as number per well; wells with growth are number per number of wells plated; stable sublines are number per number tested. Calculated values were plating efficiency, 59%; fraction of cells that induce EA, 0.5% (4/800).

is found per ml of supernatant fluid (see, e.g., data of Tables 2 and 3). By contrast among 60 HR-1 cellular subclones studied for spontaneous viral synthesis, only clone HH543-5 entered the viral replicative cycle to an appreciable extent; this is the clone that releases EA-inducing virus. This clone is about 30% VCA positive and, in the absence of induction by phorbol ester, releases about 1.5 ng of EBV DNA per ml (Fig. 1). All other clones spontaneously shed lower amounts of viral DNA (Fig. 1) (<0.1 ng/ml) and produce low levels of viral capsid antigen (<1% VCA+). Thus, the single clone that resembles the parental line in the rate in which the cells enter the viral replicative cycle also sheds EAI^+ virus.

Biological Properties of EAI^+ Virus. Within 72 hr after infection with supernatant fluid from clone HH543-5, 25–30% of Raji cells contain antigens detectable with a polyvalent human antiserum. The antigens that are induced in Raji cells appear to be EAs. The antigens are detectable by immunofluorescence with two murine monoclonal antibodies (R3.3 and K8.1) that contain antibody to the diffuse and restricted components of the EA complex (21). No antigens have been detected with murine monoclonal antibodies directed against the viral membrane antigens, viral capsid antigen, or B-cell surface antigens (22, 23). The EAI^+ virus from clone HH543-5 also induces EA in 10–15% of cells in two lines of immortalized human umbilical cord lymphocytes. No antigens were found in primary lymphocytes or in the EBV genome-negative BJAB Burkitt lymphoma line after exposure to clone HH543-5 virus.

Preliminary Analysis of the DNA of the Viral Variant that Induces EA. We postulated previously that the heterogeneous fragments in parental HR-1 DNA might be responsible for EA induction, because clonal HR-1 virus that lacked ability to induce EA also lacked heterogeneous fragments (14). Therefore, we wished to learn whether the HR-1 viral clone that possessed EA-inducing ability also contained heterogeneous fragments. The viral genome of the EAI^+ variant contains at least three heterogeneous DNA *Bam*HI restriction fragments not present in the clones that fail to induce EA (Figs. 2 and 3). These DNA fragments are not present in submolar quantities, as they are in the parental HR-1 virus, but are present in molar or greater quantities.

In the first experiment (Fig. 2), in which the *Eco*RI fragment B was used as the probe, a *Bam*HI fragment (M_r, ≈1.67 million) was found in HH543-5 DNA that was not present in the EAI^- virus (clone HH514-16). In parental P3H3 DNA, this fragment hybridized more faintly, indicating that it was present in only a subpopulation of HR-1 DNA molecules. All the other *Bam*HI fragments expected to be located in the *Eco*RI fragment B of HR-1 DNA were detected and were of similar molarity in the two clones and in the parental virus (24). When the *Bam*HI fragment W was used as the probe, a fragment of M_r 1.67 million was again detected in molar or greater quantities in HH543-5 DNA but was absent from HH514-16 and present as a heterogeneous fragment in the parental DNA (data not shown). It is not yet known whether the M_r 1.67 million fragments detected by *Eco*RI fragment B and *Bam*HI fragment W are the same fragment.

A second *Bam*HI fragment (M_r, approximately 4.6 million) was identified in the EBV DNA of HR-1 clone HH543-5 by probing with *Eco*RI fragments C, G2, and F (Fig. 3), as well as with *Bam*HI fragment H (data not shown). This fragment contains sequences that are not contiguous on the HR-1 physical map (24). The M_r 4.6 million *Bam*HI fragment is not found in

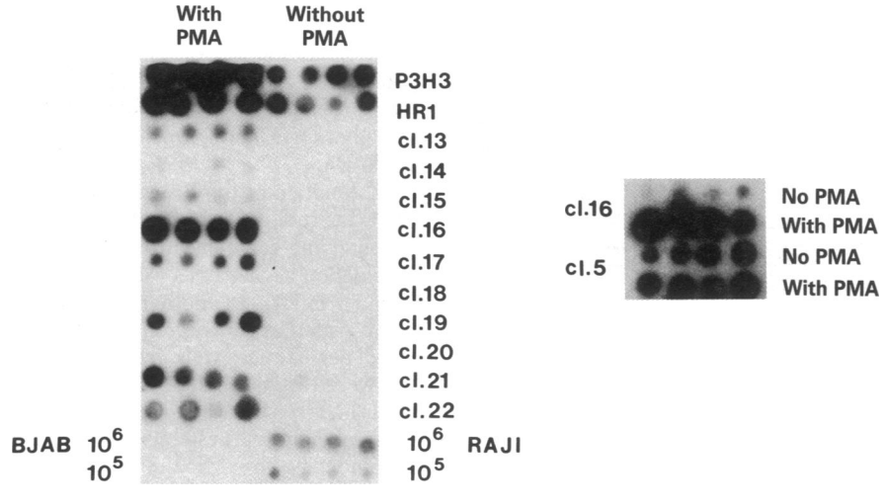

FIG. 1. Parental HR-1 cells and clone HH543-5 spontaneously release EBV. (*Left*) Parental lines and clones 13–22 from experiment HH514 (Table 1) were incubated at 33°C for 2 wk in the presence or absence of PMA. Four-milliliter aliquots were centrifuged, the pellets were suspended in 20 μl of Hanks' buffered saline, and this suspension was spotted in four replicate 5-μl aliquots. The spots were hybridized with a ^{32}P-labeled probe prepared from a mixture of the *Eco*RI B, *Eco*RI C, and *Bam*HI W DNA fragments. Note that both parental lines spontaneously release EBV but none of the clones do so. Clone 16 shows the greatest response to treatment with PMA. (*Right*) Clones HH514-16 and HH543-5 were incubated for 7 days at 33°C with and without PMA; spots were prepared as described above and hybridized with a ^{32}P-labeled probe of the *Bam*HI fragment K of EBV DNA. In this experiment, clone HH514-16 released <0.2 ng of EBV DNA per ml in the absence of PMA and ≈7.4 ng/ml in its presence. Clone HH543-5 released 1.6 ng of EBV DNA per ml in the absence of PMA and 2.5 ng/ml in its presence.

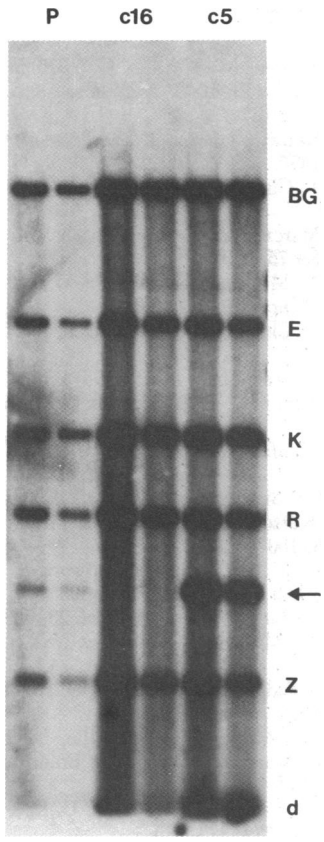

FIG. 2. Digestion of DNA from clone HH543-5 (EAI$^+$) with *Bam*HI gives a DNA fragment (M_r, 1.7 million) that is absent in viral DNA from clone HH514-16 (EAI$^-$). Intracellular DNA was prepared from P3H3 cells (lane P), clone HH514-16 cells (lane c16), and clone HH543-5 cells (lane c5). Approximately 10 or 5 μg of the DNA was digested with *Bam*HI and electrophoresed in a 0.5% agarose gel. The DNA was transferred to nitrocellulose and hybridized with a ^{32}P-labeled probe of *Eco*RI fragment B contained on a chimeric pACYC 184 plasmid (pHH11-29). Note that the EBV DNA fragment indicated by the arrow is present as a submolar fragment in parental P3H3 EBV DNA and appears to be present in greater than molar quantities in clone HH543-5 viral DNA. BG, E, K, R, Z, and d, additional *Bam*HI fragments found in the *Eco*RI fragment B of HR-1 DNA (24).

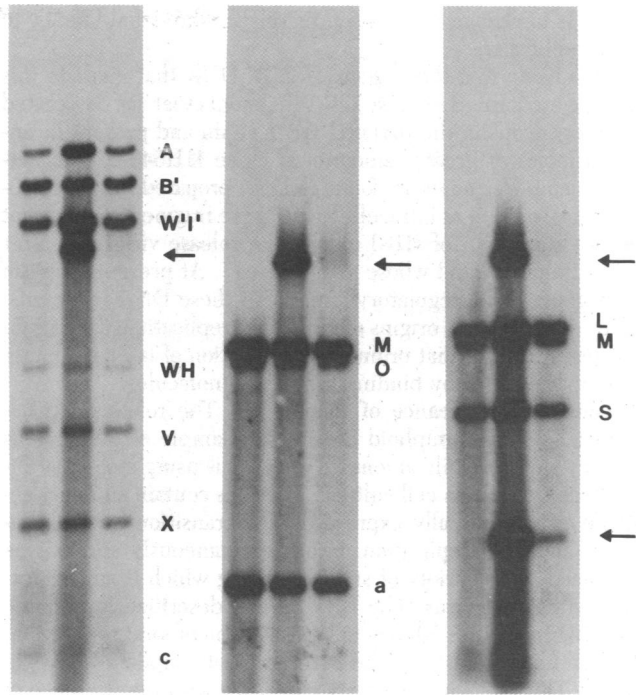

FIG. 3. Two additional *Bam*HI fragments of EBV DNA are present in clone HH543-5 (EAI$^+$) and absent in clone HH514-16 (EAI$^-$). A fragment of M_r 4.7 million was identified by probing with plasmids pHH11-14, -7, and -8 containing the *Eco*RI C (*Left*), G2 (*Center*), and F (*Right*) fragments. A fragment of M_r approximately 1.2 million (lower arrow in *Right*) was also identified with *Eco*RI fragment F. This is present as a hypermolar fragment in clone HH543-5 DNA and as a submolar fragment in parental DNA. Lanes: c16, clone HH514-16; C5, clone HH543-5; P, parental DNA. Letters to the right of the gels identify *Bam*HI fragments known to be contained in the *Eco*RI fragments used as probes (24).

viral DNA of a clone of virus (HH514-16) that is EAI$^-$ and is present as a submolar fragment in the parental P3H3 line. *Eco*RI fragment F (Fig. 3) detects a third *Bam*HI fragment (M_r, $\approx$1.2 million) present in apparent hypermolar quantities in clone HH543-5, as heterogeneous in the parental line, and absent from clone 16.

DISCUSSION

EA induction results from an EB variant that has an unusual genome structure. Virus that is capable of inducing EB is released from a subpopulation of HR-1 cells that is present in low abundance in the cell line. Three types of evidence support this conclusion. Only one clone of 200 shed virus with this biological property (Table 1). In a limiting-dilution assay (Table 4), the ability to establish HR-1 sublines that released EAI$^+$ virus was lost once the cultures were started with 100 cells or less. DNA fragments present in molar or greater quantities in the HR-1 variant having the ability to induce EA were found as submolar restriction fragments of EBV DNA in the parental DNA mixture.

The latter is the strongest argument that EA-inducing virus represents a minor population in the parental stock. The biological evidence based on cloning or limiting dilution of cells is open to other interpretation. For example, if cloning selected against those cells that spontaneously released EAI$^+$ virus, this variant would be isolated in low frequency. Those cells that produced this virus type might lyse before they grow out as clones. Cell cloning might select against the normal partition of certain forms of viral DNA to daughter cells.

EA Induction and Spontaneous Virus Production Are Linked. The single clone that released EAI$^+$ EBV also spontaneously underwent the viral replicative cycle in a high fraction of cells and spontaneously released viral DNA (Fig. 1). In this respect, this clone resembles the parental line and differs from all other clonal HR-1 variants studied. However, it is unlikely that all the spontaneous viral synthesis found in the parental P3H3 line is due to the rare EAI$^+$ cell variant we have cloned. About 10% of the parental line undergoes spontaneous viral production, whereas the EAI$^+$ clone is only 0.5% of the population. This numerical problem could be resolved in one of several ways. (*i*) The fraction of EAI$^+$ virus producer clones may be higher than 0.5% but the virus producer phenotype may not be stable on cell cloning. EAI$^-$ clonal variants might arise by loss of the "defective" DNA sequences. (*ii*) EAI$^+$ virus might superinfect EAI$^-$ nonproducer cell clones in mixed culture. (*iii*) EAI$^+$ virus might interact with the cell membrane of EAI$^-$ clones and induce them to undergo viral replication.

It is tempting to hypothesize a relationship between the ability of EAI$^+$ virus to replicate spontaneously in its own host lymphoid cell and to induce the replicative cycle in Raji and other cells that already carry an EBV genome. In both instances, the

effect is to change the host–virus relationship from latency to viral replication.

It is likely that the regions of EBV DNA that mediate this change are located in those DNA fragments that are designated heterogeneous in the parental HR-1 strain and present in unusual molar or greater amounts in clone HH543-5. The heterogeneous fragments are found in DNA prepared from HH543-5 virions, as well as intracellularly. These fragments are absent from virion DNA of HR-1 clones that release virus only after PMA induction and whose virus is EAI^-. At present, one can only guess at the regulatory function of these DNA fragments. They might contain origins of viral DNA replication; they might express functions that promote transcription of late viral genes either directly or by binding inhibitory molecules.

General Significance of this Result. The relationship between EBV and lymphoid cells is an example of herpesvirus latency and is readily amenable to analysis using molecular biological methods in cell culture. The cells contain an intact genome that is partially expressed. The transition between latency and viral replication occurs spontaneously and can be "induced" by a variety of stimuli, among which is superinfection with HR-1 virus. The EBV variant described here is the one that modulates latency in the direction of viral replication, both in its own host cell and in others that contain a latent genome. This biological property correlates with the presence of certain fragments of EBV DNA that have properties of herpesvirus "defective" DNA (25). It should ultimately be possible by DNA transfer experiments to learn whether these new DNA fragments themselves induce EA or promote viral replication. Further study of the structure of these genes, their products, and their function should provide insights into the molecular mechanisms of herpesvirus latency.

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1. Hinuma, Y., Konn, M., Yamaguchi, J., Wudarski, D. L., Blakeslee, J. R., Jr., & Grace, J. T., Jr. (1967) *J. Virol.* **1**, 1045–1051.
2. Miller, G., Robinson, J., Heston, L. & Lipman, M. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4006–4010.
3. Menezes, J., Leibold, W. & Klein, G. (1975) *Exp. Cell Res.* **92**, 478–484.
4. Frank, A., Andiman, W. & Miller, G. (1976) in *Advances in Cancer Research*, eds. Klein, G. & Weinhouse, S. (Academic, New York), pp. 171–210.
5. Henle, W., Henle, G., Zajac, B. A., Pearson, G., Waubke, R. & Scriba, M. (1970) *Science* **169**, 188–190.
6. Fresen, K. O., Cho, M. S. & zur Hausen, H. (1978) *Int. J. Cancer* **22**, 378–383.
7. Yajima, Y., Marczynska, B. & Nonoyama, M. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2008–2010.
8. Fresen, K. O., Merkt, B., Bornkamm, G. W. & zur Hausen, H. (1977) *Int. J. Cancer* **19**, 317–323.
9. Fresen, K. O., Cho, M. S. & zur Hausen, H. (1978) *Int. J. Cancer* **22**, 160–165.
10. Delius, H. & Bornkamm, G. W. (1978) *J. Virol.* **27**, 81–89.
11. Sugden, B., Summers, W. C. & Klein, G. (1976) *J. Virol.* **18**, 765–775.
12. Heller, M., Dambaugh, T. & Kieff, E. (1981) *J. Virol.* **38**, 632–648.
13. Kinter, C. R. & Sugden, B. (1979) *Cell* **17**, 661–672.
14. Heston, L., Rabson, M., Brown, N. & Miller, G. (1982) *Nature (London)* **295**, 160–163.
15. Yano, S., Tanaka, A., Takada, K., Fujiwara, S., Osato, T. & Nonoyama, M. (1982) *Virology* **119**, 392–398.
16. Sugden, B. & Mark, W. (1977) *J. Virol.* **23**, 503–508.
17. Brandsma, J. & Miller, G. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6851–6855.
18. Wahl, G. M., Padgett, R. A. & Stark, G. R. (1979) *J. Biol. Chem.* **254**, 8679–8689.
19. Fischer, D. K., Miller, G., Gradoville, L., Heston, L., Weststrate, M. W., Maris, W., Wright, J., Brandsma, J. & Summers, W. C. (1981) *Cell* **24**, 543–553.
20. zur Hausen, H., O'Neill, F. J., Freese, U. K. & Hecher, E. (1978) *Nature (London)* **272**, 373–375.
21. Pearson, G. (1983) *J. Virol.*, in press.
22. Hoffman, G. J., Lazarowitz, S. G. & Hayward, S. D. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2979–2983.
23. Kinter, C. R. & Sugden, B. (1981) *Nature (London)* **294**, 458–460.
24. Rabson, M., Gradoville, L., Heston, L. & Miller, G. (1982) *J. Virol.* **44**, 834–844.
25. Frenkel, N., Jacob, R. J., Honess, R. W., Hayward, G. S., Locker, H. & Roizman, B. (1975) *J. Virol.* **16**, 153–167.