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Replication of Viral Deoxyribonucleic Acid and Breakdown of Cellular Deoxyribonucleic Acid in Epstein-Barr Virus Infection

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Infection of Raji cells with Epstein-Barr virus (EBV) causes suppression of cellular deoxyribonucleic acid (DNA) synthesis and fragmentation of the cellular DNA. About 1,000 copies of EBV DNA of normal size (about 5×10^7 daltons in a single strand, as shown in an alkaline gradient) are synthesized per cell.

Infection of human lymphocytes with the Epstein-Barr virus (EBV) leads to proliferation of lymphoblastoid cells (2) and the induction of cellular deoxyribonucleic acid (DNA) synthesis (1). However, the replication of viral DNA has been difficult to demonstrate because of the lack of a cell line that can be productively infected with EBV and the inability to distinguish the small amount of viral DNA from the massive amount of cellular DNA. Raji cells, a nonproductive line of Burkitt's lymphoma, when infected with EBV show formation of "early" antigen (EA), little if any viral capsid antigen (VCA) (3), but also apparently an increase in EBV genome content (5).

We show here the replication of viral DNA, as followed by hybridization with EBV-specific complementary ribonucleic acid (c-RNA) (5), and the fate of the cellular DNA in Raji cells infected with EBV.

Infectious EBV was obtained by centrifuging at 30,000 $\times$ *g* for 1 hr the supernatant fluid of HR1K cells (a productive line originating from Burkitt's lymphoma) that had been kept at 32 C for 11 days in RPM1-1640 medium with 10% fetal calf serum, suspending the pellet in phosphate-buffered saline solution, and passing it through a membrane filter (0.45-nm pore size; Millipore Corp.).

Raji cells, used for infection, lack evidence of virus-specified antigens by immunofluorescence tests, although they contain 65 EBV DNA equivalents per cell (5). Cells free of EBV genome and susceptible to productive infection are not available so far; the established human lymphocyte cell lines contain EBV DNA according to c-RNA hybridization tests (reference 5; zur Hausen, *personal communication*).

Raji cells were grown to a concentration of 7×10^5/ml, concentrated 10-fold, and mixed with a preparation of EBV. After 1 hr of adsorption at room temperature, the infected cells were diluted to 7×10^5/ml in RPM1 medium with 2% heat-inactivated fetal calf serum. This was the zero time of infection.

To determine the multiplicity of infection, each dilution of the EBV preparation was applied as above to Raji cells; those cells showing EA in an immunofluorescence (IF) test were counted (3). Since we used undiluted virus preparations, the extrapolated value indicates the multiplicity of infection (Fig. 1). The antiserum for EA and VCA was a gift from W. Henle. As many as 70% of the Raji cells became IF-positive, but only 1 to 2% of the cells became positive for VCA. All

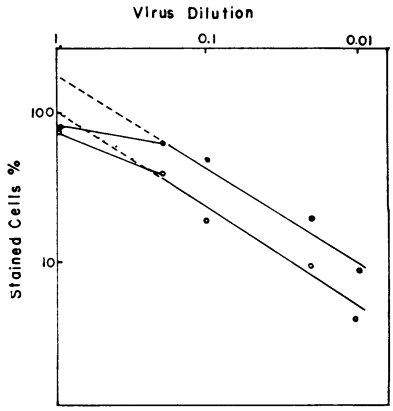

FIG. 1. *Determination of multiplicity of infection. Raji cells were infected as described, kept at 37 C for 30 hr at a concentration of* 7×10^5 *cells/ml, and stained with serum specific for early antigen. About 1,000 cells were counted under a fluorescent microscope. Two different virus preparations (open and closed circles) were titrated.*

other infections were done at a multiplicity of infection of 1 to 2.

Figure 2a shows the incorporation of ^{3}H-thymidine into acid-insoluble material during 6-hr periods postinfection with EBV. The suppression of incorporation of thymidine was quite obvious after 12 to 18 hr of the infection. DNA was extracted from the infected cells at each interval, and the number of EBV DNA equivalents was calculated as described previously (5). Viral DNA replication started around 12 hr and was most active between 18 and 30 hr (Fig. 2b). The quantity of EBV DNA formed reached 1,000 genome equivalents per cell.

Since, despite this substantial replication of EBV DNA, little if any virus is formed (VCA-positive cells are only 1 to 2%), we examined the size of the replicated EBV DNA and also the effect of the infection on the size of cellular DNA. The cells were labeled with ^{14}C-thymidine (0.02 μCi/ml, 50 mCi/mmole) for 24 hr and rinsed. Twelve hours after infection, the cells were labeled with ^{3}H-thymidine (1 μCi/ml, 15 mCi/mmole) for 24 hr. The control cells were treated in the same way, but without EBV infection. In both cases, 7×10^5 cells were gently lysed with 0.5 N NaOH, 0.05 M ethylenediaminetetraacetic acid and 4% Sarkosyl −97 (Geigy) for 20 hr at 4 C on top of an alkaline sucrose gradient (10 to 30% in 0.1 N NaOH, 0.9 M NaCl). After centrifugation for 5 hr at 25,000 rev/min in the SW27 rotor at 4 C, the gradient was fractionated from the top of the tube and assayed for radioactivity.

Figure 3a shows that the DNA of the infected cells, both that labeled with ^{14}C (preexisting) and ^{3}H (newly synthesized after the infection) was degraded to small pieces (~20S to 30S). About 20% of the cellular DNA remained unaffected (~150S). This is in good agreement with the IF result which showed about 70 to 80% of cells positive for EA. This result also indicates suppression of cellular DNA synthesis by EBV infec-

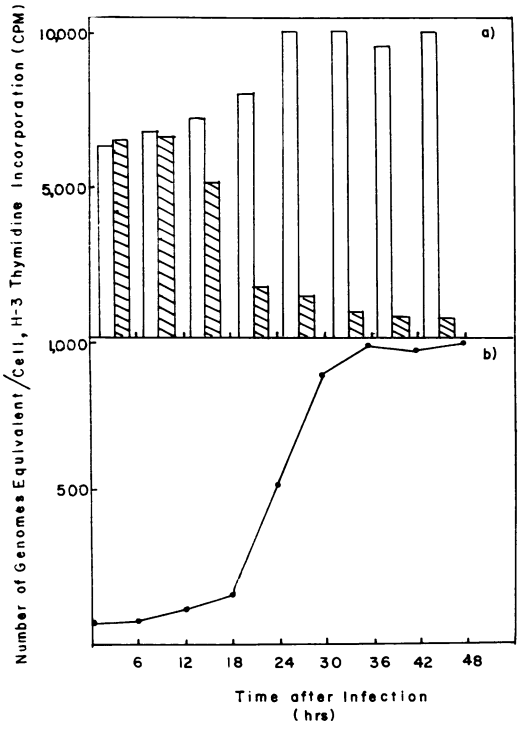

FIG. 2. *Suppression of cellular DNA synthesis and induction of viral DNA replication. (a) After infection with Epstein-Barr virus (EBV), 1 ml of cells was removed at 6-hr intervals from the original bottle and put into a 20-ml vial containing ^{3}H-thymidine (1 μCi/ml, 15 Ci/mmole). After 6 hr, the incorporation was measured by trichloroacetic acid precipitation. Open column, control cells; shadowed column, infected cells. (b) DNA was extracted from the infected cells at each interval and tested for hybridization with EBV-specific complementary ribonucleic acid (c-RNA) (5); 30,000 counts/min of c-RNA and 50 μg of DNA were used. Hybridization of 8,000 counts/min corresponded to 1,000 genome equivalents/cell.*

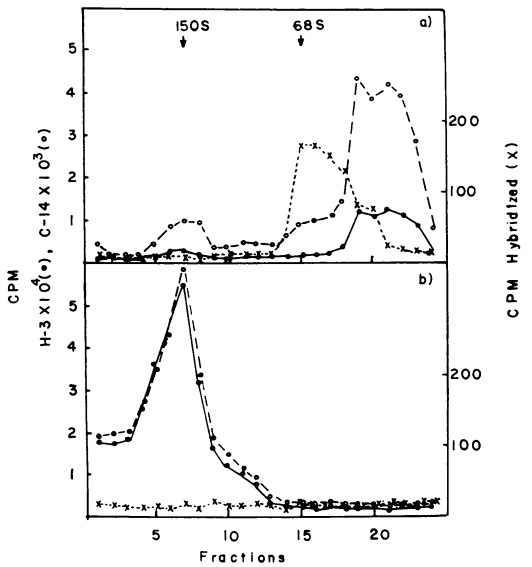

FIG. 3. *Fragmentation of cellular DNA by Epstein-Barr virus (EBV) infection and sedimentation properties of EBV DNA synthesized in infected cells analyzed by alkaline sucrose centrifugation. Conditions for the experiment are described in the text. (a) EBV infection, (b) uninfected control cells. ^{14}C pre-labeled DNA (○); ^{3}H-DNA labeled after infection (●); counts hybridized by EBV complementary ribonucleic acid (×). The 150S value is an estimate. Arrow superimposed for the 68S marker is from the most rapidly sedimenting component of herpes simplex virus DNA and corresponds also to intact single strands of EBV DNA.*

tion when the ratio of ^{3}H to ^{14}C of the infected cells is compared with that of the uninfected control cells.

We centrifuged (in a separate gradient) cold DNA from infected cells and detected EBV DNA by the c-RNA-DNA hybridization technique (5). The replicated virus DNA was in rather broad peaks, the fastest of which was around 68*S* (Fig. 3a). ^{3}H-EBV DNA and ^{3}H-herpes simplex (HSV) DNA were centrifuged in separate tubes. Both DNA species sedimented similarly in heterogeneous peaks as described for HSV (4); the fastest sedimenting components were in the identical position. We took these as 68*S* markers (4). There was no appreciable hybridization above the background level with the same amounts of EBV c-RNA and DNA from uninfected cells.

EBV infection under the conditions used here severely affects cellular DNA, in contrast to the result that EBV infection causes induction of cellular DNA synthesis and lymphoblastic transformation in freshly isolated leukocytes that are ordinarily unable to grow without this stimulus (1, 2). Also, the EBV-Raji system may be a good source of EBV DNA; all cells so far tested yield a paucity of EBV, and this deficit has hindered basic studies. Despite its abundance, most of the replicated EBV DNA is probably not encapsidated, as there are only small numbers of cells that disclose viral capsid antigen, and we could isolate little virus from the infected cells.

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ADDENDUM IN PROOF

L. Gergely, G. Klein, and I. Ernberg (Virology **45**:22–29, 1971) have also shown suppression of DNA synthesis in Raji cells infected with EBV.

LITERATURE CITED

1. Gerber, P., and B. H. Hoyer. 1971. Induction of cellular DNA synthesis in human leucocytes by Epstein-Barr virus. Nature (London) **231**:46–47.
2. Gerber, P., J. Whang-Peng, and J. H. Monroe. 1969. Transformation and chromosome changes induced by Epstein-Barr virus in normal human leucocyte cultures. Proc. Nat. Acad. Sci. U.S.A. **63**:740–747.
3. Henle, W., G. Henle, B. A. Zajac, G. Pearson, R. Waubke, and M. Scriba. 1970. Differential reactivity of human serums with early antigens induced by Epstein-Barr virus. Science **169**:188–190.
4. Kieff, E. D., S. L. Bachenheimer, and B. Roizman. 1971. Size, composition, and structure of the deoxyribonucleic acid of herpes simplex virus subtypes 1 and 2. J. Virol. **8**:125–132.
5. Nonoyama, M., and J. S. Pagano. 1971. Detection of Epstein-Barr viral genome in non-productive cells. Nature N. Biol **233**:103–106.