Filamentous Structures Associated with Epstein-Barr Virus-Infected Cells

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After the onset of Epstein-Barr virus DNA and protein synthesis 10 h after superinfection of Raji cells (a cell line containing Epstein-Barr virus DNA but not producing virus), filamentous structures 25 nm in diameter and 0.2 to 1.4 μ m in length could be detected in the cell cytoplasm by electron microscopy. These structures banded in metrizamide gradients with viral DNA and proteins, but at a density different from that of virions or nucleocapsids. These filaments, enriched in a 155,000-dalton protein similar in size to a major nucleocapsid protein of Epstein-Barr virus, may represent intermediates in viral nucleocapsid assembly.

The interaction of Epstein-Barr virus (EBV) with B-lymphoblastoid cell lines has been studied in detail and exhibits several strikingly different patterns of virus development. In cell lines such as P3HR-1 (14), a lytic cycle occurs with the production of virus containing a linear form of double-stranded DNA (28). Other established cell lines, such as Raji, contain multiple copies of the EBV genome (25), but normally do not produce virus particles. In Raji cells, all of the viral genomes are present as circular, superhelical molecules (1, 20) and exhibit a regular micrococcal nuclease digestion pattern typical of the host cell chromatin (32). Superinfection of Raji cells with EBV results in an abortive process involving the synthesis of several thousand copies of viral DNA per cell (33) and the induction of virus-specific proteins (10), but the release of only a few mature virions (31, 36). As in the virus-producing cell lines, the viral nucleoproteins produced during this superinfection are not organized into nucleosome-like structures when examined by micrococcal nuclease digestion (32).

In an effort to search for viral nucleoprotein complexes under these different conditions of infection, we used electron microscopy and metrizamide density banding. With these techniques, we identified filamentous structures (FS) that are unique to EBV-infected cells actively synthesizing viral components, such as superinfected Raji cells and the P3HR-1 producer cells. These FS were not found in the virus-nonproducing Raji line.

MATERIALS AND METHODS

Cell lines and virus. The B-lymphoblastoid cell lines, Raji (29) and P3HR-1 (14), were maintained at 2×10^5 to 1×10^6 cells per ml by dilution in RPMI 1640

medium containing 10% fetal calf serum (33). [3 H]thymidine and [35 S]methionine, both at 10 μ Ci/ml, were added to the cell media to label nucleic acids and proteins, respectively. For specific labeling of viral-induced products, label was added at 10 h postinfection (2, 33). For inhibition of viral DNA replication, acyclovir (ACV) (100 μ M) was added to the cells at 1 h postinfection (7).

Extracellular virus was prepared from P3HR-1 cells induced with 12-O-tetradecanoylphorbol-13-acetate (19) and was used for superinfection as described previously (33). Before analysis by metrizamide gradients, virus pelleted from the supernatant of P3HR-1 cells was further purified by banding in sucrose. Nucleocapsids were prepared by treatment of partially purified virus with detergent (9).

Preparation of cell lysates. Cells (1×10^6 to 5×10^6) were pelleted at 2,000 rpm for 5 min and suspended at 0°C in 0.1 to 0.5 ml of 0.1 mM potassium borate buffer (pH 9). The cell suspension was diluted 10-fold with a solution of 0.25% Joy detergent (Procter and Gamble) containing 5 mM EDTA and 0.1 mM borate (pH 8.6), and the cells were lysed by swirling them gently on ice for 5 to 10 min. For electron microscopy, the solution was again diluted 5- to 10-fold with 0.1 mM borate buffer (pH 8.6) containing 5 mM EDTA. Samples were further processed by the chromatin spreading technique of Miller and Bakken (22). For metrizamide gradient analysis, the cell lysate was sonicated at 0°C for 3 s; brought to 0.15 M NaCl, 10 mM MgCl_2 , 10 mM Tris-hydrochloride (pH 7.8), and 3% (wt/vol) metrizamide; and layered directly onto the metrizamide gradient.

Electron microscopy. For examination of cell chromatin, cell lysates were mounted on carbon-coated grids by the procedure of Miller and Bakken (22), and the grids were rotary shadowed with tungsten (13). For electron microscopic analysis of metrizamide gradients, samples from each fraction were fixed with 0.6% glutaraldehyde in 0.02 M sodium phosphate (pH 7.6) for 15 min on ice. Samples were mounted on grids and tungsten shadowed as described previously (13). FS were quantitated by direct counts from these grids.

For negative staining, samples from the metrizamide gradient were adsorbed for 1 min onto charged, carbon-coated grids. The grids were rinsed thoroughly with water, stained with uranyl acetate for 10 s, and blotted dry.

To prepare material for thin sections, cells were pelleted onto Millipore filters, fixed with 2% glutaraldehyde in 0.01 M sodium cacodylate (pH 7.4), and then fixed with 2% osmium tetroxide. The preparation was then stained with 1% uranyl acetate, dehydrated, and embedded in Epon (21).

Metrizamide gradient analysis. Metrizamide gradients (10 to 60% [wt/vol] in 10 mM Tris-hydrochloride [pH 7.5] with 1 mM EDTA) were overlaid with 1 ml of the cell lysate and centrifuged for 3 h at 35,000 rpm in a Beckman SW41 rotor at 4°C. After centrifugation 0.5-ml fractions were collected from the bottom of the tube.

Equilibrium centrifugation of DNA on CsCl. Samples were deproteinized for 1 to 2 h at 37°C by digestion with 0.15% pronase in 1% Sarkosyl-25 mM Tris-hydrochloride (pH 8.6)–10 mM EDTA. After digestion, ³²P-labeled EBV DNA marker was added, and the samples were diluted to 7 ml with 50 mM Tris-hydrochloride (pH 8.0). Solid CsCl (8.75 g) was dissolved in each 7-ml sample, and then the samples were centrifuged in a Beckman T40 rotor at 37,000 rpm for 48 h at 20°C as described previously (33). The gradients were analyzed after centrifugation for the presence of ³H- and ³²P-radionuclides at the buoyant densities of viral (1.718 g/ml) and cellular (1.700 g/ml) DNAs.

Gel electrophoresis. Proteins were analyzed by slab polyacrylamide gel electrophoresis with 7.5% gels by the method of Laemmli (18). Gels were prepared for fluorography (5) and exposed at -70°C to X-ray film.

RESULTS

Visualization of FS in whole-cell lysates. To search for possible differences in chromatin structure, whole cell lysates of P3HR-1 cells, mock-infected Raji cells, and superinfected Raji cells were prepared for electron microscopy by the Miller spreading technique (22). Whole-cell lysates rather than isolated nuclei were used for two reasons. First, this method gives better preservation of chromatin structure (O. L. Miller, personal communication), and second, nuclei of EBV-superinfected cells lyse easily, making clean fractionation of the nuclei and cytoplasm very difficult.

Chromatin from each cell preparation exhibited the beaded nucleosomal arrangement typical of eucaryotic cells (Fig. 1a). In the superinfected Raji cells only, large aggregates of structures resembling rods or filaments were also observed (Fig. 1b). These FS had a constant diameter of 25 nm but varied in length from 0.2 to 1.4 µm. Because total cell lysates were used, it was not possible to determine the intracellular location of the FS by this procedure.

Intracellular location of FS. The intracellular location of FS was determined by thin-section

electron microscopy. FS were found in the superinfected Raji and the P3HR-1 cells, but not in mock-infected Raji cells, and were usually arranged in bundles in the cytoplasm (Fig. 2a). Striations, similar to those observed by Oda and Mori (26) in cells infected with herpes simplex virus, could be seen in some sections of the FS (Fig. 2a, arrows). The FS had an outer diameter of 22 nm and a central core 9 nm in diameter (Fig. 2c). Mature virions were observed in many of the superinfected cells containing FS (Fig. 2b). Unusual double-unit membranes, resembling those reported previously in EBV-infected cells (12) or in cells infected with non-A non-B hepatitis virus (34), were also common in the superinfected cells (Fig. 2b).

Isolation of FS by metrizamide gradient centrifugation. Metrizamide, a nonionic buoyant density medium which allows separation of nucleoprotein complexes without prior fixation, was used to purify FS from other cell components and chromatin. Gradient profiles of radioactive labels incorporated into DNA and protein of Raji cells before and after superinfection are compared in Fig. 3.

FS were observed by electron microscopy in the lower half of both gradients of superinfected cell preparations (arrows, Fig. 3B and C) but were not present in Raji cells before infection (Fig. 3A). The greatest number of FS banded at fraction 9, at a density of 1.22 g/ml. (The relative abundance of FS is indicated by the length of the arrow at each fraction). Labeled host cell chromatin banded near the top of the gradient (fractions 18 to 21) in mock-infected (Fig. 3A) and superinfected (Fig. 3B) cells. Most of the labeled viral DNA also remained in this region of the gradient (Fig. 3C).

Virions and nucleocapsids prepared by detergent treatment were centrifuged in metrizamide under the same conditions as for FS, and their positions in the gradient are indicated in Fig. 3C. Virions banded several fractions higher in the gradient than did the peak fractions of the FS, whereas nucleocapsids banded several fractions lower and at a position corresponding to the small peak of labeled viral DNA in Fig. 3C.

The FS isolated by metrizamide gradient centrifugation (Fig. 1c) were identical in appearance and diameter (25 nm) to those observed with the Miller chromatin spreading technique (Fig. 1b). When purified FS were negatively stained with uranyl acetate (Fig. 1d), the outer and central core diameters were 18 and 3 nm, respectively, and therefore were smaller than those determined from thin-section analysis (Fig. 2c).

FS were not observed in metrizamide gradients of mock-infected Raji cells (Fig. 3A). Metrizamide gradient analysis of lysates of P3HR-1 cells revealed FS like those found in superinfected

FIG. 1. (a) Direct visualization of chromatin from a lysate of mock-infected Raji cells prepared by the Miller spreading technique and tungsten shadowed. Bar = 400 nm. (b) Direct visualization of FS from a lysate of superinfected Raji cells prepared by the Miller spreading technique and tungsten shadowed. Bar = 250 nm. (c) FS recovered from a metrizamide gradient and tungsten shadowed. Bar = 250 nm. (d) Negative stain of FS from a metrizamide gradient. Bar = 100 nm.

FIG. 2. Thin-section electron microscopy of superinfected Raji cells at 48 h after infection. (a) FS in the cytoplasm of superinfected cells. The arrows mark the fine striations seen in some sections. (b) Mature virions (large arrows) and double-unit membranes (small arrow) in superinfected cells. (c) Cross section of a bundle of filamentous structures. Bar = 200 nm.

FIG. 3. Metrizamide gradient profiles of DNA and protein. (A) Mock-infected Raji cells. (B) Superinfected Raji cells, labeled for 24 h before infection and harvested at 36 h postinfection. (C) Superinfected Raji cells, labeled at 10 h postinfection and harvested at 36 h postinfection. ●, [^3H]thymidine; ○, [^{35}S]methionine; arrows, relative amounts of FS in each fraction; N, nucleocapsid; V, virion.

ed Raji cells, although the number of FS in the virus-producing cell line was approximately 10-fold lower than that found in superinfected cultures, when equivalent numbers of cells were compared. These results with the P3HR-1 culture, in which approximately 5% of the population is actively producing virus, demonstrated that the metrizamide gradients could concentrate low quantities of FS, which were not detectable in P3HR-1 cells by the direct visualization technique.

To examine the time course of FS appearance, superinfected Raji cells were harvested at 5, 10, 18, 24, 36, and 48 h after infection. Total cell lysates were sedimented through metrizamide, and the gradient fractions were examined by electron microscopy. A few FS were detectable in superinfected cells at 10 h, but FS were most abundant at 18 h after infection (Table 1). The number of FS declined at later times.

DNA and protein composition of FS recovered from metrizamide gradients. Because FS could be isolated by metrizamide gradient centrifugation, a preliminary characterization of the DNA and proteins of these structures was possible. DNA in superinfected Raji cells was labeled at 10 to 24 h postinfection, and the FS from these cells were banded in metrizamide. An analysis of the labeled DNA from the FS-containing regions of the gradient by isopycnic centrifugation in CsCl showed that it was EBV DNA, since it banded exactly with ^{32}P -labeled EBV marker DNA (Fig. 4) at the characteristic buoyant density of 1.718 g/ml. DNA from mock-infected Raji cells was mixed with ^{32}P -labeled EBV DNA and banded in CsCl on a separate gradient. The ^3H -labeled DNA profiles of both gradients are plotted together in Fig. 4 after alignment of the profiles of ^{32}P -labeled EBV marker DNA. These results demonstrated that the DNA labeled during superinfection and recovered in FS did not band with host DNA.

Superinfection results in the shutdown of host protein synthesis and the subsequent appearance of viral-induced polypeptides (10). Proteins

TABLE 1. Appearance of FS during superinfection of Raji cells

Time postinfection (h)	No. of FS per Grid Square ^a
5	0
10	2
18	420
24	110
36	230
48	70

^a One grid (400 mesh) square is equivalent to 1,500 μm^2 .

FIG. 4. Comparison of the buoyant density of [^3H]thymidine-labeled DNA which bands with FS on metrizamide gradients (●) with that found in mock-infected Raji cells (○). The arrow marks the position of ^{32}P -labeled EBV DNA (1.718 g/ml), which was included in each gradient. FS were obtained from superinfected Raji cells at 24 h postinfection.

labeled at 36 to 48 h after infection were recovered from a metrizamide gradient, such as the one shown in Fig. 5, and analyzed by polyacrylamide gel electrophoresis (Fig. 6). The fractions containing FS (fractions 8 to 10, Fig. 5) were consistently enriched in a 155-kilodalton (155K)

FIG. 5. Metrizamide gradient profile of superinfected Raji cells labeled with [^{35}S]methionine (○) and [^3H]thymidine (●) at 10 h postinfection and harvested at 48 h postinfection. Fractions containing FS are marked with arrows. Nucleocapsids band at fraction 6.

polypeptide (Lane d, Fig. 6). This particular pattern of enrichment was not found in other regions of the gradient such as fraction 6 (Lane e, Fig. 6), which is the region corresponding to the banding position of nucleocapsids (Fig. 3C). Although it is suggested by these results that EBV-specific polypeptides are components of FS, further purification is required to establish a specific association. It is not known whether the components of FS include host cell DNA and proteins which are not labeled significantly 36 to 48 h after superinfection.

FIG. 6. Polyacrylamide gel analysis of proteins recovered from mock-infected and superinfected Raji cells. (a) Total cell lysate of mock-infected Raji cells. (b) Total cell lysate of superinfected Raji cells labeled with [^{35}S]methionine at 10 to 48 h postinfection. (c) Labeled proteins from fraction 20 (see Fig. 5). (d) Labeled proteins from fractions 8 to 10 (see Fig. 5). (e) Labeled proteins from fraction 6 (see Fig. 5). The arrow marks the position of the 155K polypeptide.

Effect of ACV on the appearance of FS. ACV inhibits EBV DNA synthesis in superinfected Raji cells but does not release the virus-induced block of cellular DNA replication (7). When ACV was added at 1 h after superinfection and left throughout the infectious cycle, the incorporation of label into total DNA was reduced by approximately 50% compared with that of a drug-free culture of infected cells. Thus, a significant number of cells in the culture had been infected with virus. The synthesis of the 155K polypeptide was also partially inhibited in the superinfected culture exposed to ACV, in agreement with previous findings (11), and there was a 50% reduction in the number of FS. These results suggest that FS are composed of late virus proteins, since the expression of late virus functions requires the synthesis of viral DNA.

DISCUSSION

After the onset of viral DNA and protein synthesis, FS appear in the cytoplasm of Raji cells superinfected with EBV. An analysis of FS isolated by banding in metrizamide suggests that they contain viral DNA and proteins. We have attempted equilibrium banding of FS as a method of purification, but the purity of the FS by this procedure was unacceptable. Velocity sedimentation through preformed gradients has been the most effective method of obtaining large quantities of FS. Attempts to pellet and reband FS a second time on metrizamide have resulted in a 10-fold or greater loss of these structures.

FS resemble the dense bundles of hollow tubules observed in thin sections of superinfected Raji cells by Seigneurin (31) and may be related to the cytoplasmic crystals which are present in Burkitt somatic cell hybrids expressing late EBV functions (12). Structures similar in morphology and diameter have been found in the nuclei of herpes simplex virus type 2-infected cells, but not in herpes simplex virus type 1-infected cells (8, 30, 37). Tubular aggregates are also present in the nucleus and cytoplasm of guinea pig cells infected with an endogenous herpesvirus and are often found in the region of nucleocapsid formation (3). Tubular structures appearing in herpes simplex virus type 2-infected cells have been isolated by sucrose gradient centrifugation (26), and negative staining and optical diffraction analysis demonstrate that these have a helical assembly of subunits (17).

Although FS are characteristic of some herpesvirus infections, their biological significance is not known. They may function as intermediates in viral replication and assembly. In recent studies with herpes simplex virus type 2, low concentrations of compounds which inhibit viral DNA and late protein synthesis cause an accumulation of FS with a concurrent decrease in

production of virus (15, 27). High concentrations of these antiviral compounds completely inhibit the appearance of FS and virus. When these antimetabolites are removed, the FS decrease in number, and virus particles reappear. In the present study, we showed that an inhibitor of EBV DNA synthesis (ACV) reduces the number of FS in superinfected Raji cells.

Superinfection of Raji cells with EBV results in abundant production of viral DNA and virus-induced proteins. Yet, this is only a semipermissive infection, yielding small amounts of mature virus when high multiplicities of infection are used (31, 36). Most of the EBV DNA produced during superinfection is found in the nucleus but is not organized into nucleosomes (32). It is sensitive to DNase digestion (33) yet is not susceptible to the viral-induced nuclease which fragments cell chromatin (6), and it may be protected by a unique nucleoprotein structure. FS are located primarily in the cytoplasm, and the bulk of the viral DNA in superinfected Raji cells does not cosediment with FS. These findings indicate that FS are probably not the major form of viral nucleoprotein.

In superinfections of Raji cells, the appearance of FS correlates with the onset of viral DNA and viral protein synthesis beginning 10 h after infection. FS and virions are often present in the same cells. A major component of FS preparations is the 155K protein, which is similar in size to one of the major nucleocapsid proteins of EBV (9). This polypeptide first appears at 9 h postinfection, with maximum production occurring at 15 to 17 h postinfection (10, 11), corresponding to a time when most FS are observed. At this time, the synthesis of the 155 K protein is blocked by inhibitors of EBV DNA replication (11). A protein of similar size and sensitivity to inhibitors has also been reported by others to appear at intermediate times (9 to 12 h) after superinfection of Raji cells (4) and NC37 cells (23) and has been observed in *N*-butyrate-treated, but not in iododeoxyuridine-treated, P3HR-1 producer cells (16). By immunoprecipitation studies, it was demonstrated that the virus capsid antigen complex of EBV contains a 150K nucleocapsid protein (24, 35), and this antigen is expressed in superinfected Raji cells (2, 31).

Experiments involving side-by-side comparisons of purified virion and FS polypeptides would be necessary to establish that the major component of FS is indeed a virus capsid protein. If this is the case, FS may represent intermediates in viral nucleocapsid assembly which are normally rapidly processed but accumulate in the semipermissive superinfected cells because of a bottleneck in the maturation sequence. Alternatively, they could be aberrant

structures which condense from saturated pools of nucleocapsid protein because the normal assembly pathway is blocked. Further studies will be necessary to clarify the relationship of these FS to the infectious process.

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LITERATURE CITED

- Adams, A., and T. Lindahl. 1975. Epstein-Barr virus genomes with properties of circular DNA molecules in carrier cells. *Proc. Natl. Acad. Sci. U.S.A.* 72:1477-1481.
- Bayliss, G. J., and M. Nonoyama. 1978. Mechanisms of infection with Epstein-Barr virus. III. The synthesis of proteins in superinfected Raji cells. *Virology* 87:204-207.
- Bia, F. J., W. C. Summers, C. K. Y. Fong, and G. D. Hsiung. 1980. New endogenous herpesvirus of guinea pigs: biological and molecular characterization. *J. Virol.* 36:245-253.
- Bodemer, W. W., W. C. Summers, and J. C. Niederman. 1980. Detection of virus-specific antigens in EBV-(P3HR-1) virus-superinfected Raji cells by immunoprecipitation. *Virology* 103:340-349.
- Bonner, W. M., and R. A. Laskey. 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* 46:83-88.
- Cheng, Y. C., J. Chen, P. Hoffman, and R. Glaser. 1980. Studies on the activity of DNase associated with the replication of the Epstein-Barr virus. *Virology* 100:334-338.
- Colby, B. M., J. E. Shaw, G. B. Elion, and J. S. Pagano. 1980. Effect of acyclovir [9-(2-hydroxyethoxymethyl)guanine] on Epstein-Barr virus DNA replication. *J. Virol.* 34:560-568.
- Couch, E. F., and A. J. Nahmias. 1969. Filamentous structures of type 2 *Herpesvirus hominis* infection of the chorioallantoic membrane. *J. Virol.* 3:228-232.
- Dolyniuk, M., E. Wolff, and E. Kieff. 1976. Proteins of Epstein-Barr virus. II. Electrophoretic analysis of the polypeptides of the nucleocapsid and the glucoasimine- and polysaccharide-containing components of enveloped virus. *J. Virol.* 18:289-297.
- Feighny, R. J., M. P. Farrell, and J. S. Pagano. 1980. Polypeptide synthesis and phosphorylation in Epstein-Barr virus-infected cells. *J. Virol.* 34:455-463.
- Feighny, R. J., B. E. Henry, Jr., and J. S. Pagano. 1981. Epstein-Barr virus polypeptides: effect of inhibition of viral DNA replication on their synthesis. *J. Virol.* 37:61-71.
- Glaser, R., R. Farrugia, and N. Brown. 1976. Effect of the host cell on the maintenance and replication of Epstein-Barr virus. *Virology* 69:132-142.
- Griffith, J. D., and G. Christiansen. 1978. Electron microscope visualization of chromatin and other DNA-protein complexes. *Annu. Rev. Biophys. Bioeng.* 7:19-35.
- Hinuma, Y., M. Konn, J. Yamaguchi, D. J. Wudarski, J. R. Blakeslee, Jr., and J. T. Grace, Jr. 1967. Immunofluorescence and herpes-types particles in the P3HR-1 Burkitt lymphoma cell line. *J. Virol.* 1:1045-1051.
- Iwasaka, T., H. Oda, R. Mori, J. Miyazono, and S. Nagafuchi. 1979. Production of tubular structures in Vero cells infected with herpes simplex virus type 2: effects of ultraviolet light irradiation and antimetabolites. *J. Gen. Virol.* 44:57-67.
- Kawanishi, M., K. Sugawara, and Y. Ito. 1981. Epstein-Barr virus-induced polypeptides: a comparative study with superinfected Raji, IUdR-treated, and *N*-butyrate treated P3HR-1 cells. *Virology* 109:72-81.
- Kitamura, K., J. Miyazono, R. Mori, H. Oda, and Y. Nonomura. 1978. Optical diffraction analysis of electron microscope images of tubular structures found in cells infected with herpes simplex virus type 2. *J. Gen. Virol.* 41:167-170.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680-685.
- Lin, J. C., J. E. Shaw, M. C. Smith, and J. S. Pagano. 1979. Effect of 12-*o*-tetradecanoyl-phorbol-13-acetate on the replication of Epstein-Barr virus. I. Characterization of viral DNA. *Virology* 99:183-187.
- Lindahl, T., A. Adams, G. Bjursell, G. Bornkamm, C. Kashka-Dierich, and U. John. 1976. Covalently closed circular duplex DNA of EBV in a human lymphoid cell line. *J. Mol. Biol.* 102:511-530.
- Miller, M. F., P. T. Allen, and L. Dmochowski. 1973. Quantitative studies of oncornaviruses in thin sections. *J. Gen. Virol.* 21:57-68.
- Miller, O. L., and A. H. Bakken. 1972. Gene transcription in reproductive tissue. *Acta Endocrinol. (Copenhagen)* Suppl. 168:155-172.
- Mueller-Lantzsch, N., B. Georg, N. Yamamoto, and H. Zur Hausen. 1980. Epstein-Barr virus-induced proteins. III. Analysis of polypeptides from P3HR-1 EBV-superinfected NC37 cells by immunoprecipitation. *Virology* 102:231-233.
- Mueller-Lantzsch, N., N. Yamamoto, and H. Zur Hausen. 1979. Analysis of early and late EBV associated polypeptides by immunoprecipitation. *Virology* 97:378-387.
- Nonoyama, M., and J. S. Pagano. 1972. Separation of Epstein-Barr virus DNA from large chromosomal DNA in nonvirus producing cells. *Nature (London) New Biol.* 238:169-171.
- Oda, H., and R. Mori. 1976. Electron microscope observations on tubular structures in cells infected with herpes simplex virus type 2. *Arch. Virol.* 50:159-168.
- Oda, H., R. Mori, J. Miyazono, and T. Iwasaka. 1979. Effects of antimetabolites on the production of tubular structures in Vero cells infected with HSV type 2. *Arch. Virol.* 62:175-187.
- Pritchett, R. F., S. D. Hayward, and E. D. Kieff. 1975. DNA of Epstein-Barr virus. I. Comparative studies of the DNA of Epstein-Barr virus from HR-1 and B95-8 cells: size, structure, and relatedness. *J. Virol.* 15:556-569.
- Pulvertaft, R. J. V. 1965. A study of malignant tumors in Nigeria by short term tissue culture. *J. Clin. Pathol.* 18:261-273.
- Schwartz, J., and B. Roizman. 1969. Similarities and differences in the development of laboratory strains and freshly isolated strains of herpes simplex virus in Hep-2 cells: electron microscopy. *J. Virol.* 4:879-889.
- Seigneurin, J. M., M. Guillaume, B. Lenoir, and G. De-Thé. 1977. Replication of Epstein-Barr virus: ultrastructural and immunofluorescent studies of P3HR1-superinfected Raji cells. *J. Virol.* 24:836-845.
- Shaw, J. E., L. F. Levinger, and C. W. Carter, Jr. 1979. Nucleosomal structure of Epstein-Barr virus DNA in transformed cell lines. *J. Virol.* 29:657-665.
- Shaw, J. E., T. Seebeck, J.-L. H. Li, and J. S. Pagano. 1977. The Epstein-Barr virus DNA synthesized in superinfected Raji cells. *Virology* 77:762-771.
- Shimizu, Y. K., S. M. Feinstone, R. H. Purcell, H. J. Allen, and W. T. London. 1979. Non-A, non-B hepatitis: ultrastructural evidence for two agents in experimentally infected chimpanzees. *Science* 205:197-200.
- Thorley-Lawson, D. A. 1979. Characterization of cross-reacting antigens on the Epstein-Barr virus envelope and plasma membranes of producer cells. *Cell* 16:33-42.

36. **Yajima, J., and M. Nonoyama.** 1976. Mechanisms of infection with Epstein-Barr virus. I. Viral DNA replication and formation of noninfectious virus particles in superinfected Raji cells. *J. Virol.* **19**:187-194.
37. **Young, S. K., N. H. Rowe, and K. C. Sanderlin.** 1977. Herpes simplex types 1 and 2 in clinical infections: differences observed by electron microscopy. *J. Infect. Dis.* **135**:486-489.