Brief Communication: Relationship Between the Mason–Pfizer Monkey Virus and HeLa Virus: Immunoelectron Microscopy  

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SUMMARY—Envelope antigens of the Mason–Pfizer monkey virus (MPMV) and the morphologically similar HeLa virus, which is continuously produced in some HeLa cell lines, were compared by indirect immunoferritin techniques. Antisera raised against MPMV and HeLa virus revealed cross-reacting antigenic specificities on the surfaces of both agents, and cross-absorption and end-point dilution techniques indicated the presence of identical envelope antigens. The results demonstrated a close relationship, if not an identity, between MPMV and HeLa virus. —J Nati Cancer Inst 56:635–637, 1976.

Several investigators recently reported the detection of a peculiar type of oncornavirus in different, continuous, human cell lines (1–5). Comparative morphologic, serologic, and biochemical studies revealed distinct similarities between several isolates and Mason–Pfizer monkey virus (MPMV), which was originally isolated from a mammary tumor of a rhesus monkey. No relation between the isolates and B- and C-type particles was apparent (2, 5–8).

Consequently, an additional group of oncornaviruses was defined, which until now has been observed only in primates (2, 9). The interrelationship between members of this new group is unknown and requires elucidation. In the present study, we compared the envelope antigens of the HeLa virus and the MPMV, using immunoelectron microscopy (IEM) as a distinctive criterion for their relationships.

MATERIALS AND METHODS

Cells and viruses.—Virus-producing and virus-negative HeLa cells (HeLa-E, HeLa-Sbr; obtained from Dr. E. Kuwert, Essen, and Dr. R. Wigand, Homburg/Saar, FRG, respectively) were grown as monolayers in plastic petri dishes (Greiner and Co., Nürtingen, FRG), in Eagle’s minimum essential medium (Dulbecco modification) supplemented with 5% heat-inactivated normal calf serum and 10% tryptose phosphate broth.

A cell line from a mammary tumor of a rhesus monkey, cocultivated with normal rhesus embryo cells and chronically infected with MPMV (MPMV-M, #3328–28; Pfizer Inc. through the courtesy of Dr. J. Daams, Amsterdam, The Netherlands), was grown as a monolayer under identical conditions.

For IEM, 2,000–6,000 HeLa-E, HeLa-Sbr, or MPMV-M cells per well were seeded in Microtest I #81-3034 plates (Falcon Plastics, Oxnard, Calif.) (10). The cultures were used 24–48 hours later.

For absorption of antisera, virus-negative HeLa-Sbr cells (HeLa-E, HeLa-Sbr; obtained from Dr. E. Kuwert, Essen, and Dr. R. Wigand, Homburg/Saar, FRG, respectively) were grown as monolayers in plastic petri dishes (Greiner and Co., Nürtingen, FRG), in Eagle’s minimum essential medium (Dulbecco modification) supplemented with 5% heat-inactivated normal calf serum and 10% tryptose phosphate broth.

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Antiserum.—Rabbit antiserum to HeLa virus was prepared against purified split virus as described in (6, 7). Respective goat serum directed against MPMV (code #25-612) from Pfizer Inc., was obtained through Dr. J. Gruber, National Cancer Institute (Bethesda, Md.).

Normal rabbit and goat sera were controls. To remove antibodies against normal components of cells, we absorbed all sera twice with equal volumes of packed HeLa-Sbr and n-NC-37 cells. Sera were absorbed for 2 hours at 37°C and subsequently for 10 hours at 4°C with 1:1 mixtures of cells previously fixed with 2.5% glutaraldehyde (GA) to achieve equal conditions for absorption and immunolabeling.

Similarly, cross-absorption was done with fixed, virus-producing HeLa-E or MPMV-M cells. Sera were used in dilutions of 1:5, 1:10, and 1:50.

IEM.—Before they were labeled, the cultures were pretreated with 0.25% ice-cold GA in phosphate-buffered saline (PBS) for 5 minutes. After two washings with PBS supplemented with 0.2% bovine serum albumin and 10 μM ethanolamine, 10 μl antiserum was added to each well. After 30 minutes at 37°C, antiserum was sucked off the wells, and after two washings the appropriate label (i.e., goat IgG–ferritin conjugate with specificity against rabbit IgG, or rabbit IgG–ferritin conjugate with specificity against goat IgG) was reacted under the same conditions. GA-coupled conjugates were prepared in a two-step method (11). After removal of the conjugate, the plates were washed three times in PBS, fixed by 2.5% GA for 15 minutes, postfixed by 1% osmium, treated with 1% uranyl acetate, dehydrated in graded ethanol, and embedded in situ in Epon. Thin sections were cut with a Reichert OmU3 type ultramicrotome, stained with lead citrate, and examined with a Siemens 101 electron microscope at 80 kV.

RESULTS AND DISCUSSION

Control experiments with normal rabbit or goat sera demonstrated the specificity of the process. Neither cell-surface nor virus tagging by the ferritin label was observed (fig. 1). Likewise, all sera were negative when tested on virus-negative HeLa-Sbr cells. Immune sera from rabbit or goat, however, induced a definite reaction with mature (figs. 3–6) and budding (fig. 2) virus particles and to a lesser extent also with the surfaces of virus-producing cells (figs. 2–6). This cell-surface labeling is common in oncornavirus-producing cells and is due to the appearance of certain virus structural polypeptides on the cell membrane (10).

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When reacted with 1:5 dilutions of homologous antiserum, HeLa virus showed a distinct ferritin tagging (fig. 4), whereas the incubation with 1:50 diluted antiserum gave negative results. MPMV, in contrast, was heavily labeled by the 1:50 diluted homologous antiserum (fig. 3). This difference in antibody titers was apparent again in cross-labeling experiments (figs. 5, 6). Remarkably, the labeling activity of each antiserum appeared to be the same for the homologous and the heterologous reaction. Cross-absorption with either MPMV-M or HeLa-E cells completely removed labeling activity from both antisera.

These results, particularly the last finding, mean that the envelope antigens on both the HeLa virus and the MPMV, as revealed by two different antisera, are closely related or identical. The human origin of the HeLa-E is without doubt [(2); Dr. A Espmark, Stockholm, Sweden: Personal communication]. However, despite the fact that the HeLa virus was observed in HeLa cells stored in liquid nitrogen since 1967 (2), an accidental contamination with an MPMV-like virus before that date cannot be ruled out.

ADDENDUM

From nucleic acid hybridization data, Dr. J. Schlom (National Cancer Institute, Bethesda, Md.) has come to the same conclusion as we have. The high degree (>95%) of homology also points to the very close relationship, or identity, of MPMV and HeLa virus. (Data presented at VIIth Symposium on Comparative Leukemia Research, Oct. 15–17, 1975, Copenhagen, Denmark.)

REFERENCES

Figures 1–6.—MPMV-M cells *left* and HeLa-E cells *right* after indirect IEM. × 120,000. Bar represents 1000 Å.

**Figure 1.**—MPMV-M cell incubated with normal goat serum (1:5) and subsequently with ferritin conjugate having specificity against goat IgG.

**Figure 2.**—Ferritin tagging on budding HeLa viruses after incubation with antiserum to MPMV (1:10).

**Figures 3, 4.**—Mature MPMV and HeLa particles, respectively, after incubation with homologous antisera (1:50; 1:5).

**Figures 5, 6.**—Mature MPMV and HeLa particles, respectively, after incubation with heterologous antisera against HeLa (1:5) and MPMV (1:50).