Brief Communication

Infection of Human Embryonic Cell Cultures with the Rauscher Murine Leukemia Virus¹

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Although infection of mouse cell lines with murine leukemia and sarcoma viruses can be achieved with relative ease (4, 6, 12), similar infection of human cell cultures has not been reported until very recently. Boiron et al. (2) have described the successful infection of a diploid human embryonic lung culture, WI-38, with the murine sarcoma virus Moloney strain (MSV-M). Infection and continuous replication of animal oncogenic RNA viruses in human cells is of considerable interest as the phenomenon may be a step towards proving the viral etiology of human leukemia. This report describes the infection of human embryonic cell cultures with the Rauscher murine leukemia virus (RLV) and some of the physical and biologic properties of the virus synthesized.

Monolayer cultures from the 4th transfer of human embryonic muscle cells, G8-6, growing in RPMI medium supplemented with 20% fetal calf serum were washed with fresh medium. One ml of mouse plasma virus, HL-67-4 (Rauscher mouse plasma virus supplied by Hazleton Laboratories) diluted 1:4 in growth medium was added to each monolayer in 30-ml plastic tissue culture flasks, and the cultures were incubated at 37°C for 30 minutes. After virus adsorption, the monolayers were washed once and refed with the growth medium. Cytopathic effects were not observed, and after one week the cultures were transferred to 250-ml Falcon tissue culture flasks. Electron microscopic studies of the infected cells did not reveal the presence of virus at that time; however, after four weeks, during the 17th passage, virus particles were seen budding from the plasma membranes (Fig. 1A) as well as into cytoplasmic vacuoles (Fig. 1B). Virus particles found in the extracellular spaces (Fig. 1C) had the morphology of the “C-type” particles found in the murine leukemias (1). In subsequent passages, many virus particles were seen by the negative stain technic (3) in concentrates of the infected tissue culture fluids.

Extracellular virus in medium from a 5-day-old culture in its 25th transfer since infection was concentrated by ultracentrifugation in a Sharples continuous flow centrifuge at the rate of one liter per hour (approx. 55,000 X g). The virus was resuspended in either fresh medium or 0.05 M sodium citrate, pH 6.8, to give a 100-fold concentrate.

These preparations, which were estimated by electron microscopic studies to have approximately 5 X 10⁹ virus particles per ml, were used for mouse inoculation, serologic tests, and infection of other cell cultures in an effort to characterize the virus. Sixty newborn and ten weanling BALB/c mice were inoculated intraperitoneally with 0.1 ml of this virus preparation. At the present time, three months postinoculation, none of the mice has shown any of the typical Rauscher virus disease symptoms (8). Previous experience with equivalent doses of Rauscher virus derived from infected mouse cell cultures has shown that leukemia should develop within one to two months (5, 10). After six months these mice will be challenged with a Rauscher mouse plasma virus to determine their immune status.

Complement fixation tests were conducted using 100-fold concentrates from control and virus-infected cultures as antigens. The antiserum was prepared in rats against mouse-derived virus according to the procedure described by Hartley et al. (5). A titer of 1:16 was obtained against four units of antibody with the positive control RLV that was produced by infected JLS-V9 cells (11). In contrast, the human cell-derived virus concentrate, which had the same virus count, gave a 1:4 titer. The uninfected human cell culture concentrate was negative in this test. The four-fold difference in titers between the two antigens, which was confirmed by a repeat test, would seem to indicate a modified nature of the virus produced in human cells.

A potent rabbit anti-RLV serum prepared against mouse plasma virus was used for the immunodiffusion tests. While ether-treated virus from the infected human cells produced precipitin lines, the intact virus showed a negative reaction. The Rauscher leukemia virus produced in mouse cells (JLS-V9), whether ether-treated or intact, was always positive in these tests. The electron microscopic agglutination test (7) was also negative for the intact human cell-derived virus.

Further characterization of this virus was attempted by density gradient centrifugation. Two ml of a 50-fold virus concentrate were layered on top of a preformed sucrose gradient. This gradient, along with a similar one containing the

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mouse plasma virus, was centrifuged in a Spinco Model L-2 ultracentrifuge for 5 hours at 25,000 rpm in an SW-25.1 swinging bucket rotor. Each tube contained a plastic bead of a known 1.16 gm/ml density (plastic beads obtained from Sondell Scientific Instruments, Inc., Palo Alto, California). After centrifugation both tubes had a diffuse band, with the heaviest concentration just above the bead. Equal volumes of the bands were collected and found to contain similar amounts of virus. Both samples contained virus that was greater than $2 \times 10^{10}$ particles per ml when examined by the negative stain technic. When rebanded with a RLV from infected JLS-V9 cells, all particles per ml when examined by the negative stain technic. 

When rebanded with a RLV from infected JLS-V9 cells, all particles per ml were collected and found to contain similar amounts of virus. Both samples contained virus that was greater than $2 \times 10^{10}$ particles per ml when examined by the negative stain technic. When rebanded with a RLV from infected JLS-V9 cells, all three viruses banded at the same density 1.16 gm/ml.

The human cell-derived G8-6 virus was found to be infectious when passaged at least once to control G8-6 cells and to several other human embryonic cultures. It was also found to be infectious for the JLS-V9 mouse cell line.

Since the G8-6 cells were growing slowly, attempts were made to infect other primary human embryonic cell cultures with either tissue culture or mouse plasma RLV. A total of ten different human embryonic tissues have been infected, and two showed new virus in as little as two to three weeks.

Infection of the embryonic human tissues was accomplished both with unfiltered and filtered mouse plasma virus. The latter was passed through 0.45-µ Millipore filters, and infection was accomplished whether the cells were in suspension or treated as monolayers.

In order to confirm the human origin of the infected cell lines, three such cultures, G8-6, G8-2, and HE 15-1, were submitted for cytogenetic analysis. Preliminary chromosome counts showed all three to be definitely of human origin.

The ability of murine viruses to infect cell cultures from species other than the mouse is becoming more evident. Recently, Thomas et al. (9) described the replication of the MSV-M in bovine embryo skin cells; while Boiron et al. (2) reported on the multiplication of the MSV-M in human embryonic lung cells. In the present study, we not only report the successful infection of a human embryonic muscle cell culture with the RLV but other embryonic cultures as well. It is interesting to note that in each case embryonic tissues had been infected successfully.

The virus produced in G8-6 cell culture infected with RLV is similar to the mouse plasma virus in two physical characteristics, namely, morphology and density. The former virus during its budding process acquires an envelope or coat from the plasma membrane of a human cell. Such a coat may thus explain the observed differences in the leukemogenicity and immunologic characteristics of the human cell-derived virus and RLV. Our preliminary results thus indicate that the former virus has the nucleoid or core of the murine leukemia virus and a protein coat acquired from a human cell. However, one cannot exclude the possibility of the RLV acting as a helper for an inactive human leukemia virus. This seems unlikely, unless the human virus has a nucleoprotein core that is similar, if not identical, to the RLV and is found in normal embryonic tissues. Since human cells apparently are capable of replicating murine “C-type” viruses, it raises the question of whether such viruses, after serial passage in human cells, might also cause leukemia in humans. In vivo studies to examine this possibility in subhuman primates are in progress.

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Fig. 1A-C. C-type virus particles budding from the plasma membrane (Fig. 1A) or into cytoplasmic vacuoles (Fig. 1B) and mature (Fig. 1C) extracellular virus found in a human embryonic muscle culture, G8-6, infected with the Rauscher murine leukemia virus. The bar indicates 0.1μ.
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