Different Cell Culture Characteristics of Two Strains of Murine Sarcoma Virus* (33900)

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A number of strains of virus which rapidly induce sarcomas when inoculated into newborn rodents have been described (1–3). These agents induce foci of altered cells in cultures of secondary mouse embryo cells and some continuous mouse cell lines (4, 5). During titrations of murine sarcoma virus (MSV) *in vitro*, it was found that the two strains of MSV being used gave different morphological types of foci.

Materials and Methods. Viruses. The source of MSV (Harvey) has been previously described (5). MSV(Moloney) was obtained as a tumor extract from Dr. R. J. Huebner, U. S. National Institutes of Health. Fluids from infected cultures of Prince Henry mouse (6) embryo cells showing extensive transformation were filtered through 0.45 μ membrane filters (Millipore) and stored at —65° for use as working virus stocks.

Cell culture techniques. Cultures of Prince Henry embryonic mouse cells (PHEM) were prepared and maintained as previously described (5). A line (CL-1) of BALB/C mouse embryo cells (4) was obtained from Dr. R. J. Huebner in its thirty-fifth passage, and was used after three passages in this laboratory. Eagle's minimum essential medium (Grand Island Biological Co., Powder Medium F15) supplemented with 10% tryptose phosphate broth and 10% unheated calf serum was used for all of the cells. Cell cultures were kept at 37° in domestic polythene "vacuum seal" containers, a suitable carbon dioxide atmosphere being provided by addition of a known amount of ENO fruit salt (Beecham Pty. Ltd.) to a water reservoir in the container.

The MSV focus assays were carried out by adding 5 ml of cell suspension (1.25×10^5 cells/ml) to 0.2 ml of virus dilution in each 60-mm plastic petri dish. The cultures were incubated for 7 days, the medium being changed on the third or fourth day. Where necessary, cultures were stained with May–Grünwald–Giemsa for microscopic examination.

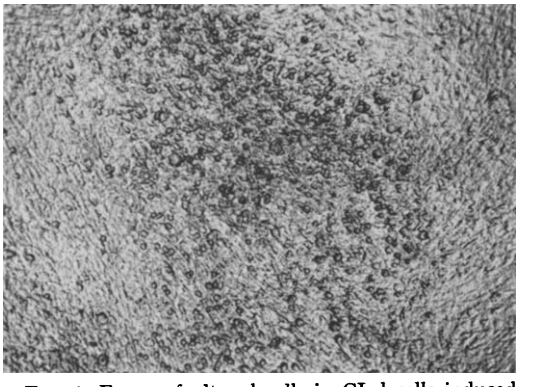

FIG. 1. Focus of altered cells in CL-1 cells induced by MSV(Moloney); unstained; 40×.

Results. Morphology of altered cell foci in CL-1 cells. Uninfected CL-1 cell cultures consisted of a uniform layer of epithelioid cells. Scattered throughout the sheet were large cells possessing two or three nuclei.

Cultures infected with MSV(Moloney) showed foci of altered cells consisting of a mixture of fusiform and round cells. These two types of cells had more distinct outlines than normal cells, making the foci easily visible by low power microscopy in unstained plates (Fig. 1). Staining the cultures made it more difficult to see the foci against the background of normal cells.

The MSV(Harvey)-induced foci had a completely different appearance, due to the presence of what appeared to be large clear areas in their centers (Fig. 2). Scattered throughout each focus were distinct fusiform and round cells. This "fenestrated" effect made the foci easily visible against the uniform background of normal CL-1 cells. Stain-

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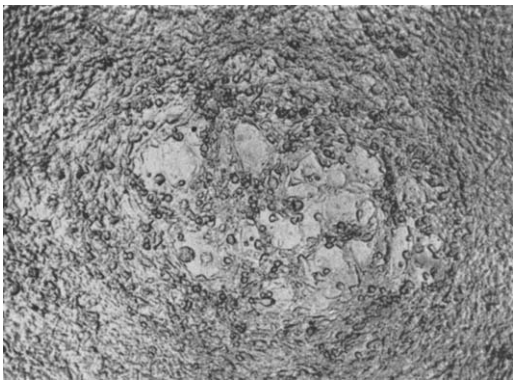

FIG. 2. Focus of altered cells in CL-1 cells induced by MSV(Harvey); unstained; 40×.

ing MSV(Harvey)-infected cultures accentuated the alterations, and the foci on stained plates could be counted very easily using a low-powered microscope. Staining also showed that the "clear areas" were in fact cells with large nonstaining cytoplasmic areas (Fig. 3). Some of the large clear cells contained several nuclei, others with a single large nucleus often possessed one or more micronuclei.

Subculture of altered CL-1 cultures. Assay plates of CL-1 cells which had developed 20–30 MSV(Moloney) foci were trypsinized and split into new dishes. After 2- or 3-days incubation it was difficult to distinguish the transformed cells. Even after 4- or 5-days further incubation it was difficult to distinguish between the infected and control cultures. On the other hand when similar MSV(Harvey) cultures were split, the alterations spread rapidly to involve the whole cell sheet. Stained preparations showed that the secondary cultures contained fibroblastic cells, densely staining fusiform cells, and giant multinucleated cells. Many of the giant cells possessed large bizarre nuclei (Fig. 4).

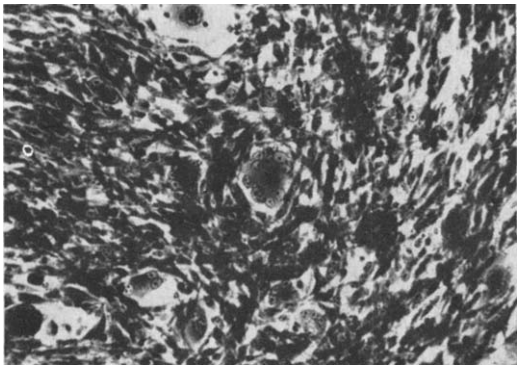

FIG. 3. The center of a MSV(Harvey) focus stained with May–Grünwald–Giemsa, and showing the large clear cells; 100×.

Morphology of altered cell foci in PHEM cells. The results in early passage embryonic mouse fibroblasts were essentially the same as for the CL-1 cells. The MSV(Harvey) foci contained the large clear cells which were not seen in the MSV(Moloney) foci. The foci were more diffuse than in CL-1 cells, the large clear cells not being so prominent.

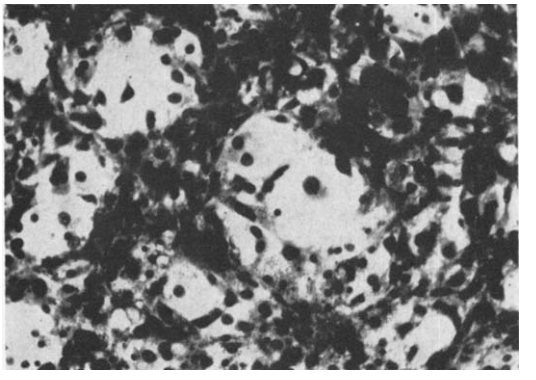

FIG. 4. Passaged MSV(Harvey)-infected CL-1-cells, stained with May–Grünwals–Giemsa; 100×.

Discussion. The morphology of the MSV (Moloney)-induced foci agrees with previous descriptions (4, 7). MSV(Harvey) presented a different picture due to the presence of large, often multinucleated, clear cells in the middle of the foci. The clear cells gave the foci a plaque-like appearance. The CL-1 cultures consisted essentially of uniform epithelioid cells giving a dense background against which the foci could easily be seen. The early passage PHEM cultures contained many cell types giving a less uniform background. Photographs of MSV(Harvey) foci showing the large clear cells have been published (5), but because heterogeneous, early passage mouse embryo cells were being used, they were not thought to have special significance.

Passage of infected cultures indicated that there were further qualitative differences between the MSV(Moloney) and MSV(Harvey)transformed cells. Subcultured MSV

(Moloney)-infected cultures did not show extensive alterations and the transformed cells were overgrown by apparently normal cells. Passage of MSV(Harvey) infected cells led to extensive alterations in the cultures and overgrowth of normal cells by transformed cells. Bather *et al.* (8) showed that MSV(Moloney)-infected cells are incapable of the unrestrained division necessary to form foci and that virus release and reinfection of neighboring cells are necessary for focus formation. Presumably under the conditions of the assay the accompanying helper murine leukemia virus rapidly spreads throughout the culture rendering cells resistant to MSV(Moloney) infection (9). Our experience with MSV(Harvey)-infected cells indicates that they are not subject to the same restriction and divide readily on subculture. The giant cells also persist and cells with a number of nuclei or with a large lobulated nucleus appear. Such cells form an appreciable proportion of the total cells in the cultures. The presence of multinucleated giant cells has been reported in tumors in mice induced by both MSV(Harvey) and MSV(Moloney). Tumors induced by the Moloney strain are more clearly rhabdomyosarcomas and the giant cells are said to resemble myoblasts (10). The MSV(Harvey)-induced tumors in mice are less clearly rhabdomyosarcomas (11). The multinucleated giant cells look like cells of the foreign body type (11), and resemble the cells found in passaged MSV(Harvey)-infected CL-1 cultures. These findings indicate that MSV(Harvey) is able to induce the formation of giant cells *in vitro*, and this gives rise to foci with a characteristic appearance. In the same system, MSV (Moloney) apparently cannot induce giant cell formation. This provides further evidence that the two strains of MSV possess differing biological properties. However, it is important to emphasise that the MSV(Harvey) used in these studies has been through two cycles of "focus purification" (5) and may represent a variant from the parent stock. Detailed studies on the origin of the giant cells *in vitro* are being undertaken.

Summary. The production of different morphological types of foci by two strains of MSV is described. MSV(Harvey) foci contain large clear cells which are absent from the MSV(Moloney) foci. Cells transformed by MSV(Moloney) are apparently restricted in their ability to divide. MSV(Harvey) transformed cells are not subject to the same restriction.

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