Rescue of Murine Sarcoma Virus Genome in Mixed Cultures of ‘Non-producer’ Hamster Tumour Cells and Helper Virus Carrier Cell Lines

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The defective genome of murine sarcoma virus (MSV)* (MOLONEY isolate) may be rescued from non-producer hamster tumour cells (HT-1) by co-cultivation of the latter and mouse embryo cells with added helper murine leukaemia viruses (MuLV) (Huebner et al. 1966; Huebner, 1967). Rescue of MSV from HT-1 cells by co-cultivation with MuLV-carrier cells has not, however, been described. This report describes the retrieval of MSV from mixed cultures of HT-1 and various MuLV-carrier cells of mouse and hamster origin. Moreover, it was found that the frequency of success and titres of MSV recovered by co-cultivation of HT-1 and F4, one of the MuLV-carrier mouse cell lines, were much higher than those obtained by the conventional method. We have analysed the factors responsible for such high efficiency of genome rescue.

Among the MuLV-carrier cell lines used, F4 and F5 were derived from two of nine Rauscher pseudotype MSV (MSV(RLV)) transformed foci of NIH Swiss mouse embryo cells isolated by trypsinization within a cloning ring. During the first 2 to 3 passages of these focus-derived cells, most of the cells showed degeneration and failed to grow on further passage. Only F4 and F5 cells survived this critical period and grew well thereafter. During the last 2 years, these two cell lines have been passaged more than 70 times in Eagle’s minimum essential medium (MEM) with 10% foetal bovine serum. The F4 cells have more cytoplasmic projections than mouse embryo fibroblasts, while F5 cells are more refractile, larger in size, and pleomorphic. Both grow into monolayers, although the latter tends to show slightly random orientation. No MSV has been detected in these cells when tested by co-cultivation with mouse, rat, or hamster embryo cells and they are also resistant to super-infection by MSV (MOLONEY) and (HARVEY). Electron microscopic examination showed the presence of typical C-type virus particles. Subcutaneous inoculation of newborn NIH Swiss mice with these cells resulted in splenic enlargement typical of Rauscher virus disease, but no sarcomas have been observed over an 8 month observation period. Infectivity of extracts of these cells was neutralized by anti-RLV serum, but not by anti-AKR (Gross) serum, as tested by in vitro induction of complement-fixing antigen production in mouse embryo cells.

HT-1 and F4 cells were co-cultivated by inoculating $8 \times 10^4$ and $8 \times 10^5$ cells respectively into a Petri dish (50 mm. diameter, Falcon Plastic) containing 4 ml. Eagle’s basal medium (BME) with 10% foetal bovine serum. After incubation for 1 week at 37°, MSV was invariably recovered from the disrupted cells (cell suspension in 0·6 ml. supernatant fluid, freeze-thawed once and subjected to sonic vibration) even though no extraneous helper MuLV was added. MSV was titrated by focus assay (Hartley & Rowe, 1966) on NIH Swiss mouse embryo cells which had been plated 3 to 4 hr previously ($3 \times 10^5$ cells/culture).

Other MuLV carrier cell lines tested were F5 cells (described above), the Walkersville cell line (WH-1) derived from a mouse tumour carrying a wild (Gross) type MuLV, and the RHT cell line derived from hamster embryo cells transformed by RLV (Rhim, Huebner &

* Since MOLONEY strain of murine sarcoma virus is the subject of the present study, the abbreviation MSV will be used in this article to denote this virus unless otherwise specified.
Ting, 1969). Co-cultivation of HT-I and F 4 invariably resulted in MSV recovery in more than 20 attempts, but mixed cultures of HT-I and F 5, WH-I or RHT cells yielded MSV only once or twice in 3 to 5 trials. When compared at optimal cell numbers for co-cultivation, F 4 gave yields of rescued MSV (2.5 × 10⁴ focus-forming units (f.f.u.) culture) approximately 100- to 1000-fold greater than the other cell lines (14 to 130 f.f.u./culture) (Table 1).

The four MuLV-carrier cultures contained the MuLV group-specific antigen at approximately equivalent titre (Table 1), and all synthesized and released large amounts of complete virus particles as measured by incorporation of tritiated uridine into particles with a density of 1.16 g./ml. in sucrose (Fig. 1). While some differences in the latter characteristics were noted, these differences were of low magnitude. The differences between these cultures in rescue efficiency was clearly related to the infectivity of the helper viruses for mouse-embryo cells (Table 1), with the exception of F 5, which exhibited an intermediate infectivity and lowest MSV-rescuing efficiency. Therefore, ability of cells to produce helper virus with a high infectivity is probably a necessary but not sufficient condition for efficient MSV genome rescue by co-cultivation.

The infectivity difference between F 4 and F 5 viruses is surprising, since both were derived from MSV-induced foci on NIH mouse embryo cells. However, alterations of infectivity with murine C-type viruses in continuous culture are well known (Kindig & Kirsten, 1967). The low infectivity of the WH-I virus in NIH mouse embryo cells is as expected for viruses isolated from tumours in Balb/C mice. Such isolates generally grow well in Balb/C cells but not in NIH mouse embryo cells (Hartley, Rowe & Huebner, 1970). However, yields of WH-I and HT-1 co-cultivation did not show foci on Balb/C cells.

In further experiments the minimal numbers of HT-1 or F 4 cells needed to achieve rescue in 2 to 3 weeks after being plated with 8 × 10⁵ F 4, or 8 × 10⁴ HT-1, respectively, were 2 × 10² for both. An approximately 100-fold decrease in recovery of MSV resulted from each tenfold decrease in the number of F 4 cells. Excessive (2 × 10⁵) HT-1 cells in the mixed culture resulted in lower yield of MSV. Excessive numbers of HT-1 in the mixed culture may thus limit cell replication or DNA synthesis probably required for the multiplication of the MSV genome.

### Table 1. CF antigen production and infectivity assay of extracts of leukaemia virus-carrier cell lines compared with their MSV-genome rescue efficiency by co-cultivation procedure

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>CF antigen titre of cell extract after 10 days cultivation*</th>
<th>Infectivity (CF antigen induction) titre on mouse embryo cells †</th>
<th>MSV f.f.u./culture recovered after</th>
</tr>
</thead>
<tbody>
<tr>
<td>F 4</td>
<td>1:4</td>
<td>10⁻⁴</td>
<td>2000</td>
</tr>
<tr>
<td>F 5</td>
<td>1:4</td>
<td>10⁻⁴</td>
<td>0</td>
</tr>
<tr>
<td>WH-I</td>
<td>1:2</td>
<td>10⁻¹</td>
<td>0</td>
</tr>
<tr>
<td>RHT</td>
<td>1:2</td>
<td>10⁻²</td>
<td>0</td>
</tr>
</tbody>
</table>

* Petri dish cultures were started with 10⁵ cells each and incubated for 10 days. The cell sheet was scraped into 0.5 ml. of supernatant fluid, and freeze-thawed three times before titration for group-specific CF antigen.
† Extracts (10%) of each cell line were appropriately diluted and inoculated into cultures of NIH Swiss mouse embryo cells which were harvested 3 weeks later and tested for the presence of group-specific CF antigen.

With optimal numbers of HT-1 (8 × 10⁴) and F 4 (8 × 10⁵) seeded in a culture, MSV could be retrieved as early as 2 days after co-cultivation. In a typical experiment, approximately 2 × 10, 2 × 10², 2 × 10³, 10⁴ and 2·5 × 10⁴ f.f.u./culture could be recovered from co-cultiva-
tion plates harvested at day 2, 4, 7, 10 and 14, respectively. Thus, a reproducible growth curve of MSV retrieval from the mixed culture provides a basis for further study of the roles of cellular functions and mechanisms of MSV genome rescue, which will be reported separately.

![Graph showing incorporation of [3H]uridine into particles isolated in the medium of F4, F5, WH-1 and RHT cell cultures 7 days after plating. [3H]uridine was added to each culture (25 μCi/ml) 5 days after plating of 3 x 10^5 cells/culture. On day 7, 48 hr after [3H]uridine labelling, the fluids were collected from 2 cultures of each line of cells and, after preliminary processing, fractionated in a linear density gradient of 15 to 60% sucrose. (a) O–O, F4; △–△, F5; (b) ●–●, WH-1; ▲–▲, RHT.](image)

At present there is no information about the physical state of the MSV genome in HT-1 cells, its localization, or the mechanism of synthesis of rescued virus. Its availability should provide methods for studying the possible virus aetiology of human cancers. We hoped, for example, that cell-free preparations of HT-1 added to F4 might allow rescue and subsequently the physical state of the MSV genome could be established. Preliminary experiments using sonic extracts of cells or nucleic acid extracts have, however, been fruitless.

The chief difference between the co-cultivation rescue procedure and the conventional method is simply in the use of a culture already replicating infectious C-type virus. This appears to eliminate problems associated with variations in helper virus infectivity and susceptibility of different lots of mouse embryo cells. In the special case of F4, the possibility of complementation of defective MSV genomes is also raised since this cell line was derived originally from a focus induced by MSV. Whatever the basis of the reproducibility and efficiency of the co-cultivation procedure, it should be useful in further studies of the rescue phenomenon. It has been used in a study where the presence of MSV genome could be demonstrated in each of the 50 clones of HT-1 cells (Chang et al. 1969).

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Short communications

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