The Formation and Nature of Foci Induced by a Modified Sarcoma Virus in Human Cells

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Murine sarcoma virus (MSV), which in its natural state is enveloped in a murine leukaemia virus coat, can, under select conditions of aggregation with feline leukaemia virus (FeLV), be made to infect and transform cat embryo cells (Fischinger & O'Connor, 1969). The product of such an infection is a new FeLV pseudotype of sarcoma-MSV (FeLV) which replicates to a high titre in cat cells, possesses a new host range, and is neutralized by FeLV antisera (Fischinger & O'Connor, 1969; Sibal et al., 1970). The infectious capacity of MSV (FeLV) is not limited to cat cells but is also manifest by a morphological transformation of cultured human, dog and pig cells (O'Connor & Fischinger, 1970; Chapman, Fischinger & O'Connor, 1970). The MSV infection of mouse cells is defective in the sense that both focus formation and progeny virus replication are only initiated by an infection of a cell with both MSV and murine leukaemia virus (Hartley & Rowe, 1966; Bassinet al., 1968; O'Connor & Fischinger, 1968). In other cell systems MSV seemed to remain defective because it retained the necessity for FeLV as a helper virus. Because transformation of human cells by MSV (FeLV) was also dependent on an exogenous leukaemia virus, a quantitative focus assay could also detect potential homologous human helper virus(es) (Fischinger & O'Connor, 1970). Up to now, transformation of human embryonic cells by a large inoculum of the MSV (FeLV)–FeLV virus complex was manifest by typical hyper-refractile changes and production of new MSV (FeLV) and FeLV. But as the MSV (FeLV) inoculum became progressively more dilute, morphological changes became more sparse, and discrete foci were rare. At terminal sarcoma virus dilutions, single hyper-refractile cells were scattered in an apparently normal layer of cells. We now report the necessary conditions for efficient MSV (FeLV) visualization in human cells by a direct focus essay.

The origin of the cat cells, human cells, murine and feline leukaemia viruses and the production of MSV (FeLV) were previously detailed (Fischinger & O'Connor, 1969; 1970). The focus assays for sarcoma and the helper assays for leukaemia viruses followed standard procedures (Fischinger & O'Connor, 1968; 1969). The human cell strain chosen for MSV (FeLV) assay consisted of normal human embryo muscle-skin cells (HEMS) which seemed very susceptible to transformation by large doses of MSV (FeLV) (Fischinger & O'Connor, 1970). Initial experiments defined the optimal cell plating concentration (9 x 10⁴ HEMS cells/20 cm² plastic dish) and suitable media (McCoy's 5A with 15% foetal calf serum for growth and 7.5% foetal calf serum for maintenance). Medium was changed on infected cell cultures every 3 to 4 days. Because murine leukaemia pseudotypes of MSV could be significantly enhanced in rodent cells by pre-treatment of cells by DEAE-dextran we determined the optimal amounts for both cat and human cells (Duc-Nguyen, 1968; Ting & Valentine-Bader, 1969). In both cases a treatment with 25 μg. DEAE-dextran/ml. in growth medium for 30 min. was optimal for cellular integrity and for MSV (FeLV) focus potentiation. In cat cells, DEAE-dextran augmented the number of foci by a factor of 2 in the presence of optimal helper FeLV. In the presence of DEAE-dextran, a defective virus titration pattern remained
defective, but the increase in foci at identical dilutions was increased by a factor of four (Table 1). In HEMS cells, pretreatment with several concentrations of DEAE-dextran (1 to 75 μg./ml.) did not produce discrete foci at terminal MSV (FelLV) dilutions. An addition of FelLV (2 × 10⁵ to 4 × 10⁶ leukaemia virus helper units LVHU) did not help focus formation with or without DEAE-dextran treatment of cells. The diffuse transforming effect of MSV (FelLV) could have resulted from poor FelLV growth in HEMS cells. An inoculum of 1 × 10⁶ LVHU of FelLV was used to infect 9 × 10⁴ HEMS cells and 1 × 10⁶ cat embryo cells and growth of new FelLV was followed. In both cell types virus eclipsed (< 10⁴ LVHU) during the first 6 hr. In cat cells a rapid burst of growth appeared at 12 hr after infection, and FelLV production reached a high value of 2 × 10⁶ LVHU/ml. within 36 hr. In contrast, less than 10⁵ LVHU were detected in human cells during the first few days, and even after 5 days of infection, only about 10⁵ LVHU/ml. were present. The severely reduced output of FelLV from HEMS cells could have been responsible for poor visualization of discrete foci, if foci arise in HEMS cells from successive cycles of virus spread to contiguous cells. Accordingly, FelLV was added to MSV (FelLV) infected HEMS cells at recurring intervals in various quantities. Under those conditions detailed in Table 1, distinct single focal areas of infection were readily quantitated on the 8th day. The sequential 2-day addition of FelLV in both DEAE-dextran-treated and untreated human cells produced the highest numbers of discrete foci. In all cases the enhancement of foci was significant and generally averaged about fivefold. Because an adaptation of FelLV to human cells might be required for HEMS cells to express MSV (FelLV), we also attempted to see whether human cell-adapted FelLV was a more efficient helper virus. A culture of human embryonic lung cells, which was initially infected with FelLV 10 months previously, and yielded ample (> 10⁶ LVHU/ml.) FelLV continually, was used as the source of helper virus in quantities comparable to cat cell-passaged FelLV. FelLV released from human cells was an adequate but not a more advantageous helper in both cat cells and in HEMS cells.

Table 1. Effect of DEAE-dextran pretreatment and sequential feline leukaemia virus addition on the production of foci at terminal MSV (FelLV) dilutions in HEMS cells

<table>
<thead>
<tr>
<th>Virus</th>
<th>MSV (FelLV)</th>
<th>MSV (FelLV), filtered P 36, DEAE-dextran, 25 μg./ml. pre-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat embryo cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Per se</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FelLV (1 × 10⁵ LVHU)</td>
<td>Diffuse</td>
<td>Diffuse</td>
</tr>
<tr>
<td>Human embryo muscle-skin cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Per se</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FelLV (1 to 20 × 10⁶ LVHU once)</td>
<td>2.8 × 10⁴</td>
<td>1.2 × 10⁶ (4:3)</td>
</tr>
<tr>
<td>FelLV (1 × 10⁴ LVHU every 2 days)</td>
<td>1.5 × 10⁴</td>
<td>7.5 × 10⁴ (5:0)</td>
</tr>
<tr>
<td>FelLV (1 × 10⁴ LVHU every 2 days)</td>
<td>1.1 × 10⁴</td>
<td>6.6 × 10⁴ (6:0)</td>
</tr>
<tr>
<td>FelLV (3 × 10⁴ LVHU every 2 days)</td>
<td>5.5 × 10³</td>
<td>2.7 × 10⁶ (5:0)</td>
</tr>
</tbody>
</table>

* Enhancement factor of the virus titre obtained with DEAE-dextran pre-treatment relative to virus titre in untreated cells.
† The presence of a DEAE-dextran required a fourfold smaller amount of FelLV as helper (2.5 × 10⁴ LVHU/dish) for optimal focus visualization in cat cells. No reduction of FelLV concentration was necessary for HEMS cells.
‡ Titre expressed in f.f.u./ml.
Terminal foci induced in HEMS cells by MSV (FelLV) and FelLV were morphologically similar to localized lesions induced by sarcoma virus in other species (Fig. 1). Both hyper-refractile fusiform and round cells were observed against a background of normal cells. The necessity for continual FelLV supplementation suggested that foci in HEMS cells were derived by localized virus spread. Thus, new MSV (FelLV) would be required, and the final lesion should yield both sarcoma and leukaemia viruses. Eleven foci derived either from DEAE-dextran-treated or normal HEMS cells were tested for virus production on cat embryo cells. Each single focus was obtained from dishes which contained only one terminal focus. Every one of the eleven foci produced 10 to 1000 new focus-forming units of defective MSV (FelLV). This progeny virus had no apparent enhanced potential for discrete focus formation in HEMS cells but required the above-mentioned procedure.

The infection and transformation of human cells by both DNA and RNA oncogenic viruses, including the murine leukaemia pseudotypes of MSV, have been described (Koprowski et al. 1962; Todaro, Green & Swift, 1966; Jensen et al. 1964; Aaronson & Todaro, 1970). Transformation of human cells with viruses other than MSV (FelLV) or feline sarcoma virus required about 1000 times as much infectious virus as was needed for cell transformation in the natural host (Sarma et al. 1970). In HEMS cells the efficiency of registering a virus-transforming event was only tenfold lower than transformation of cat embryo cells under ideal conditions. Of all known oncogenic viruses, a sarcoma genome wrapped in a cat leukaemia envelope seems to be the most efficient transforming agent for human cells.

Fig. 1. Focus of transformed human embryonic muscle-skin cells 8 days after infection with MSV (FelLV) and FelLV. Additional FelLV was added to the infected cells every 2 days.

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Sarcoma virus derived from passage in HEMS cells had no overtly superior infective capacity for fresh human cells. This is in contrast to Rous sarcoma virus derived from rat cells, which infected new rat cells with greater efficiency (Altaner & Temin, 1970). None of the sarcoma virus which emerged from HEMS cells was infectious for mouse cells. Recent data suggested that focus formation by MSV required a leukaemia virus only in mouse cells but not in other cell types (Parkman, Levy & Ting, 1970). We reiterate that MSV can remain defective and require a helper virus for focus formation in several other species unrelated to the murine host of origin (Fischinger & O’Connor, 1970; O’Connor & Fischinger, 1970). Efficient and discrete transformation of human cells with terminal dilutions of a defective sarcoma virus, which so far needed a heterologous helper virus, now allows a search for human helper viruses.

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