Simian Virus 40 and Moloney-Murine Sarcoma Virus Infection of 

Bona fide Mouse Epithelium

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SUMMARY

Moloney-murine sarcoma virus (Moloney-MSV) and simian virus 40 (SV40) were found to infect successfully pure cultures of epithelial cells established from mouse liver and mammary tissue. MSV infection resulted in transient morphological foci with persistent production of infectious virus. SV40 infection produced detectable levels of virus-specific T antigen in the cells but morphological transformants were not observed.

Although the majority of human neoplasms are epithelial in origin most of the commonly studied oncogenic viruses induce predominantly sarcomatous tumours (Tooze, 1973). While a great deal is known about the in vitro infection and transformation of fibroblasts by these oncogenic viruses [e.g. murine sarcoma virus (MSV) and simian virus 40 (SV40)], relatively little is known about the interaction of sarcoma-inducing viruses with epithelial cells. Indeed, since these viruses induce few if any carcinomatous tumours in vivo, the possible role of infected epithelial cells in the oncogenic process has been generally ignored. We were interested in whether bona fide mouse epithelium could be infected with MSV or SV40 and, if they were, whether in vitro transformants would result. The transformation of rat epithelium in culture by MSV has been reported (Bomford & Weinstein, 1972; Auersperg et al. 1977; Rhim et al. 1977), but studies demonstrating infection of mouse epithelium have only rarely been published (Ikawa et al. 1970). The infection of epithelial cells in vitro with SV40 also has not been extensively investigated (Black et al. 1966; Diamond et al. 1973; Rubin & Rafferty, 1978), particularly using mouse epithelial cells.

Thus, we have studied the interaction of Moloney-MSV and SV40 with two well characterized epithelial cell lines derived from mouse liver (NMuLi) and mammary gland (NMuMg) (Owens et al. 1974; Anderson & Smith, 1979). In culture both cell lines form even, non-overlapping, cell monolayers ideal for studies of transformation.

Virus infection of NMuLi, NMuMg and a control non-epithelial line (Balb 3T3 A31; Aaronson & Todaro, 1968) with Moloney-MSV resulted in a similar number of morphologically transformed foci 6 days p.i. (Table 1). The formation of virus foci followed single-hit kinetics on all three cell lines. Fig. 1(a) illustrates a typical transformed cell focus consisting of rounded and criss-crossed cells clearly dissimilar from the cobblestone morphology of the surrounding epithelial monolayer. Supernatants collected from infected epithelial monolayers contained infectious MSV and murine leukaemia virus (MLV) (Table 1). The titre of virus produced ranged from $6 \times 10^3$ to $1 \times 10^4$ f.f.u. (MSV) and $4 \times 10^5$ to $8 \times 10^5$ p.f.u. (MLV) per ml supernatant.

A number of MSV-transformed foci of NMuLi and NMuMg cells were removed from the culture monolayers using steel cloning cylinders and saline-trypsin-versene. These cells, when recultured, initially maintained their altered cell morphology with numerous rounded and overlapping cells. However, after several cell passages all of the clones returned to their original monolayer morphology of flat, epithelioid cells even though they continued
### Table 1. Oncogenic virus infection of mouse epithelial cells in vitro

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Infecting virus dilution</th>
<th>Number of morphological foci</th>
<th>Production of</th>
<th>% T antigen-positive cells</th>
<th>Morphological transformants</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMuLi</td>
<td>$10^{-3}$</td>
<td>206</td>
<td>+ +</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$10^{-4}$</td>
<td>19</td>
<td>+ +</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Mock</td>
<td></td>
<td>0</td>
<td>- -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NMuMg</td>
<td>$10^{-3}$</td>
<td>301</td>
<td>- -</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$10^{-4}$</td>
<td>29</td>
<td>+ +</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Mock</td>
<td></td>
<td>0</td>
<td>- -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Balb 3T3 A31</td>
<td>$10^{-3}$</td>
<td>310</td>
<td>- -</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$10^{-4}$</td>
<td>24</td>
<td>ND† ND</td>
<td>70</td>
<td>+</td>
</tr>
<tr>
<td>Mock</td>
<td></td>
<td>0</td>
<td>- -</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Subconfluent cultures (below passage 20) were infected with serial dilutions of Moloney-MSV (containing excess MLV) for 90 min. Fresh medium (MEM plus 10% foetal calf serum plus antibiotics) was then added and the number of morphological transformants counted on day 6 p.i. Supernatants from infected epithelial monolayers were collected (10 ml of medium overnight/75 cm² flask) and assayed for M-MSV and M-MLV on Balb 3T3 A31 cells by standard techniques (Aaronson & Todaro, 1968).

† Subconfluent cultures (below passage 20) were infected with SV40 (approx. m.o.i. 5) or mock suspension for 3 h. Fresh medium (Dulbecco’s MEM plus 10% foetal calf serum plus 10 μg/ml insulin) was added for 2 additional h, then the cells were split 1 to 6 on to coverslips for fluorescent antibody tests for T antigen or split 1 to 50, 100, 1000 or 10000 for detection of transformants. T antigen tests were completed as previously described (Smith et al. 1976) and cultures to detect morphological transformants were followed for at least 2 weeks.

† ND, Not done.

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![Fig. 1. (a) Moloney-MSV focus on epithelial monolayer of NMuMg cells (phase contrast). (b) SV40 T antigen in nuclei of infected NMuLi cells.](image)
to release detectable amounts of MSV (titres ranged from $4 \times 10^1$ to $4 \times 10^3$ f.f.u. per ml supernatant).

Unlike MSV infection, SV40 did not induce morphological transformation in epithelial monolayers (Table 1). However, the mouse epithelial cells were susceptible to SV40 virus infection since immunofluorescence studies for SV40 T antigen showed that a significant number of cells in both epithelial cell lines contained this virus-specific antigen. In vitro cell passage resulted in an even higher level of T antigen expression (up to 90%) in NMuLi cell monolayers (Fig. 1b).

These experiments indicate, as shown by the production of virus or virus-specific antigen, that two of the commonly studied oncogenic viruses are capable of infecting mouse epithelial cells. Thus, the inability of these viruses to induce permanent morphological transformation in mouse epithelial cells must reside in the later stages of the transforming process (i.e. beyond virus infection). The transient nature of the transformation of mouse epithelial cells by MSV in our studies agrees with the earlier findings of Ikawa et al. (1970) using a mouse kidney cell line. Although morphological transformation was temporary, both liver and mammary epithelial cell lines continued to produce infectious MSV and MLV; this finding suggests that epithelial cells may play an important role in virus replication in vivo.

We have also found that SV40 could establish a persistent non-lytic infection of mouse epithelial cells, but did not interact with the cells to induce morphological transformation in vitro. These observations contrast with previous studies reporting morphological transformation of epithelial cells in vitro (Black & Rowe, 1963; Paulson et al. 1967). The contrasting results may stem from the fact that the epithelial cells used by other investigators were derived from different species, or from different organs, than the lines described in this paper.

We recognize that morphological transformation does not necessarily correlate with neoplastic growth, particularly for epithelial cells; however, we have found that in syngeneic mice SV40-infected NMuMg and NMuLi cells do not change in tumorigenic potential (data not shown). Since antigenic changes on the cells may have influenced our results, additional studies using nude mice as well as non-producer MSV-infected cells will be required to demonstrate definitively a lack of neoplastic alteration of our virus-infected epithelial cells in vivo.

Several possibilities exist for the lack of cell transformation in vitro following virus infection. The integration of the virus genome at an improper site for successful transformation in epithelial (cell) DNA is one hypothesis worthy of experimentation. In addition, since embryonal epithelial precursor cells may be more susceptible to transformation by oncogenic viruses (Diamandopoulos & Dalton-Tucker, 1969) than mature epithelium (Diamandopoulos, 1974), the influence of epithelial cell differentiation on virus-cell interaction would be of interest.

Overall, very little is known about the similarities and differences between mesenchymal and epithelial cells in their interaction with known oncogenic viruses and therefore this topic remains an interesting and intriguing area for study.

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REFERENCES


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