Late-Passage SC-1 Cells Resistant to N- and B-Tropic Viruses but Sensitive to NB-Tropic Viruses

THEODORE PINCUS
The Wistar Institute, Philadelphia, Pennsylvania 19104

A late-passage derivative of SC-1 cells with stable relative resistance to N- and B-tropic viruses but with retained sensitivity to NB-tropic viruses has been identified. Dual resistance was seen with the XC plaque assay, as well as reverse transcriptase assay and radioimmunoassay for viral p30. Evidence was found for resistance in clonal derivatives. These findings suggest a possible instability in determinants of cellular resistance to endogenous retroviruses and may be useful in further analysis of resistance to retrovirus infection.

Cells of inbred mouse strains are resistant to either N- or B-ecotropic endogenous murine retroviruses (6, 10), with cellular resistance determined in large part by the murine genetic locus Fv-1 (8, 10). Two cell lines, which are sensitive to both N- and B-ecotropic viruses, have been isolated in the laboratory: (i) the 3T3/FL line, which had originally shown an Fv-1b phenotype, but was found on passage to show dual sensitivity (2); and (ii) clonal derivatives from a feral mouse embryo, entitled SC-1, SC-2, etc., in which certain clones had shown Fv-1a sensitivity (5). The SC-1 cell line has been widely used for the isolation and titration of ecotropic viruses.

I have recently observed late-passage SC-1 cells which show stable dual resistance to both N- and B-ecotropic viruses but remain sensitive to NB-tropic viruses. This phenomenon appears of pragmatic concern to workers using SC-1 cells and of possible interest in the analysis of cellular resistance to endogenous retroviruses.

MATERIALS AND METHODS

Cell cultures. SC-1 cells (5) were initially provided by J. W. Hartley and W. P. Rowe and maintained in this laboratory in Autopow Eagle minimal essential medium (Microbiological Associates, Walkersville, Md.) supplemented with 2× vitamins-glutamine-penicillin-streptomycin-10% fetal calf serum. Primary embryo cultures were prepared from NFS and BALB/c mice on days 14 to 16 of gestation (4) and stored in liquid nitrogen in Eagle minimal essential medium supplemented with 20% fetal calf serum-10% dimethyl sulfoxide-penicillin-streptomycin. Other continuous cell lines, including NIH-3T3, BALB-3T3, normal rat kidney (NRK), mink lung, and rabbit cornea (SIRC), were also initially provided by J. W. Hartley and W. P. Rowe and maintained in this laboratory.

Viruses. Endogenous N- and B-ecotropic viruses, isolated from AKR, BALB/c, and C57BL/6 mice, as well as tissue culture-passage NB-tropic Moloney leukemia virus, were initially obtained from J. W. Hartley and W. P. Rowe (6). Tissue culture-passage NB-tropic Gross virus was isolated in this laboratory from thymocyte cultures of BALB.K mice rendered leukemic after neonatal infection with NB-tropic Gross virus (K. Blank and T. Pincus, unpublished data).

Virus assays. Virus titrations were performed with the standard XC plaque assay (13) in secondary cultures of sensitive and resistant SC-1 cells, secondary cultures of NFS and BALB/c mouse embryo fibroblasts, and NIH-3T3 and BALB-3T3 cells. Virus titrations performed with SC-1 cells were modified by plating cells on a clonal cell line at a density of 10⁶ cells per 60-mm plastic petri dish or 10⁷ cells per six 35-mm cluster dishes (Linbro Division, Flow Laboratories, Inc., Hamden, Conn.) and performing the UV-XC procedure 3 days after infection. Infectious center titrations were performed as previously described (9, 11). Cells were trypsinized at 12 h or 3 days after infection and plated as infectious centers on susceptible and resistant cells as described in the text.

Reverse transcriptase assays were performed by the procedure of Tsuchida et al. (15), with results expressed as the counts-per-minute incorporation of [3H]deoxythymidine triphosphate into acid precipitable counts. Competition radioimmunoasays for viral p30 were performed as described by Stephenson et al. (14), measuring the interspecies reaction with goat anti-feline antiserum and 125I-labeled p30, with results expressed as microgram equivalents of p30 competing with binding of the antiserum at a dilution binding 40% of the reference antigen. The viral proteins and antiserum were generously supplied through the Viral Resources Branch of the National Cancer Institute.

Cloning of resistant SC-1 cells. Cloning of resistant SC-1 cells was performed by plating 0.1 ml of a cell suspension at 1 cell per ml in every other well of two 96-well microtiter plates (Falcon Plastics, Oxnard, Calif.). All wells were checked, and single cells were identified in seven wells, and none were identified in other wells. The seven clones were then passed in 24- and 6-well plates and in 75-cm² flasks before testing in routine XC plaque assays.

RESULTS

SC-1 cells with dual resistance to infection by N- and B-tropic viruses. SC-1 cells were received from J. W. Hartley and W. P.
Rowe during 1974 in passage 72. These cells have been maintained for 5 years in this laboratory and have provided highly reproducible XC plaque assay titration patterns of ecotropic viruses, although late-passage cells have shown a tendency toward "peeling off" from the tissue culture plasticware. The SC-1 cell line used in routine XC plaque titrations was noted in passage 103 to show a markedly reduced plaquing efficiency of the N-tropic viruses WN1802N and AKR-L1, as well as of the B-tropic virus WN1802B, without significant change in the plaquing efficiency of the NB-tropic Moloney virus (Table 1). The possibility that a laboratory error had resulted in the mislabeling of SC-1 cells was excluded by a testing of all cell lines maintained in this laboratory, including NRK, mink lung, and SIRC, which were dissimilar in the XC plaque assay from relatively resistant late-passage cells, as well as by chromosome analysis (kindly performed by C. Croce, The Wistar Institute), which indicated a pattern identical to earlier-passage sensitive murine SC-1 cells. The pattern of resistance has remained stable through subsequent passages.

Similar patterns in late-passage cells, termed C-177, were found in studies of all viruses tested, with relative resistance to N- and B-tropic viruses and sensitivity to NB-tropic viruses (Table 2). Dually sensitive SC-1 cells showed titration patterns similar to those of either the sensitive prototype N-type NFS embryo or NIH-3T3 cells with N-tropic viruses or the B-type BALB embryo and BALB-3T3 cells with B-tropic viruses, whereas dually resistant C-177 cells showed titration patterns similar to those of the reciprocal resistant prototype.

Cellular resistance not resulting from anomalous function in the XC assay. Endogenous murine ecotropic retroviruses do not necessarily register as plaques in the XC plaque assay (12), and the possibility that viruses could replicate efficiently in C-177 cells but not induce XC plaques was considered. Inoculation of the WN1802N and WN1802B viruses on C-177 cells yielded minimally detectable p30 and no reverse transcriptase activity, in contrast to sensitive SC-1 cells, which showed high levels of viral reverse transcriptase and p30 protein under similar conditions (Table 3). Therefore, resistance of C-177 cells in the XC plaque assay does not appear to result from anomalous behavior in this assay, but rather from a general cellular resistance to infection.

Cellular resistance found in all C-177 cells. Reduced titers of N- and B-tropic viruses in C-177 cells imply that the great majority of cells are resistant, but the possibility that a few cells could confer resistance to a majority of sensitive cells required consideration. Resistant C-177 and sensitive SC-1 cells were mixed in equal numbers, plated, and inoculated with WN1802N and WN1802B viruses. The cell mixture showed approximately half the number of plaques as the sensitive cells (Table 4), suggesting that half the cells in the culture were sensitive to virus and half the cells were insensitive, i.e., that all C-177 cells are resistant and do not secrete factors which cause generalized resistance in sensitive cells.

Cellular resistance was also studied by the titration of cells inoculated with WN1802N and WN1802B viruses as infectious centers on sensitive and resistant cells (Table 5). The proportion of infected C-177 and SC-1 cells was similar to the multiplicity of infection in the direct XC plaque assay, i.e., approximately 1 to 2 for SC-1 and 1/250 to 1/3,000 for C-177, indicating that only a small fraction of C-177 cells is infected, as determined in the XC plaque assay. Plaques in C-177 cells were considerably smaller, with an approximately twofold reduction, although SC-1 cells can register effectively as infectious centers in insensitive C-177 cells. These results suggest that there is restricted virus spreading in cultures of C-177 cells infected with N- and B-tropic viruses.

Dually resistant C-177 and dually sensitive SC-1 cells were trypsinized 12 h after initial infection and plated as infectious centers on sensitive cells to analyze dose-response relations of resistant cells (1, 9, 11). Several experiments indicated one-hit dose-response relations in both SC-1 and C-177 cells, with approximately three log10-lower titers in C-177 cells at various dilutions, similar to the results shown in Table 6.

C-177 cells were cloned in microtiter dishes, with the isolation of seven clonal derivatives for testing virus sensitivity. All seven clones showed at least one log10 reduction in titer of both the N- and B-tropic viruses, with similar or greater sensitivity to NB-tropic viruses, when compared with reference earlier-passage sensitive SC-1 cells. The results of these experiments are given in Table 7. The clones are designated C-177-1 to C-177-7.

### Table 1. XC plaque titer estimates in the SC-1 cell line with acquired resistance to N- and B-ecotropic viruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Host range</th>
<th>Titer estimate at the following cell passage no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>101 103 112</td>
</tr>
<tr>
<td>WN1802N</td>
<td>N</td>
<td>5.7 1.0 2.2</td>
</tr>
<tr>
<td>WN1802B</td>
<td>B</td>
<td>5.0 2.3 1.6</td>
</tr>
<tr>
<td>AKR-L1</td>
<td>N</td>
<td>6.2 3.0 2.8</td>
</tr>
<tr>
<td>Moloney</td>
<td>NB</td>
<td>5.6 5.3 4.8</td>
</tr>
</tbody>
</table>

* Results of routine XC titrations at various passage levels, expressed as log10.
for the routine p30, Rauscher 10-3 as expressed dilution. 

Virus infection, with goat anti-feline antigen, with antiserum and antigen, as described in the text, is done. Infection, retrovirus cells. Cells all described deoxythymidine triphosphate as NWN1802N VN1802B and WN1802B viruses in SC-1 and C-177 cells.

**TABLE 2. XC plaque titer estimates of ecotropic viruses in various cell lines**

<table>
<thead>
<tr>
<th>Virus</th>
<th>NFS-ME</th>
<th>NIH-3T3</th>
<th>BALB-ME</th>
<th>BALB-3T3</th>
<th>SC-1</th>
<th>C-177</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-tropic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AKR-L1</td>
<td>5.4</td>
<td>5.3</td>
<td>1.6</td>
<td>3.1</td>
<td>5.6</td>
<td>2.9</td>
</tr>
<tr>
<td>WN1802N</td>
<td>5.2</td>
<td>3.3</td>
<td>1.5</td>
<td>2.4</td>
<td>5.6</td>
<td>1.7</td>
</tr>
<tr>
<td>C57BL/6-N</td>
<td>4.6</td>
<td>3.3</td>
<td>1.7</td>
<td>2.2</td>
<td>4.5</td>
<td>2.2</td>
</tr>
<tr>
<td>B-tropic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WN1802B</td>
<td>1.3</td>
<td>0.7</td>
<td>4.3</td>
<td>4.7</td>
<td>4.6</td>
<td>1.6</td>
</tr>
<tr>
<td>C57BL/6-B</td>
<td>2.5</td>
<td>2.0</td>
<td>5.6</td>
<td>6.1</td>
<td>6.1</td>
<td>2.5</td>
</tr>
<tr>
<td>NB-tropic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gross (NB-tropic variant)</td>
<td>5.3</td>
<td>3.3</td>
<td>4.6</td>
<td>5.0</td>
<td>4.7</td>
<td>5.2</td>
</tr>
<tr>
<td>Moloney</td>
<td>5.3</td>
<td>3.9</td>
<td>4.3</td>
<td>4.4</td>
<td>4.9</td>
<td>4.8</td>
</tr>
</tbody>
</table>

* Results of routine XC titrations, expressed as log_{10}.

**TABLE 3. Virus growth in SC-1 and C-177 cells**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Host range</th>
<th>Reverse transcriptase activity*</th>
<th>p30 presence*</th>
<th>p30 presence*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SC-1</td>
<td>C-177</td>
<td>SC-1</td>
<td>C-177</td>
</tr>
<tr>
<td>WN1802N</td>
<td>N</td>
<td>5,698</td>
<td>303</td>
<td>80</td>
</tr>
<tr>
<td>WN1802B</td>
<td>B</td>
<td>13,188</td>
<td>149</td>
<td>60</td>
</tr>
<tr>
<td>Moloney</td>
<td>NB</td>
<td>17,802</td>
<td>30,960</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Reverse transcriptase was measured, as described in the text, 4 days after the inoculation of cells. Levels of enzyme are expressed as the counts per minute incorporation of [3H]-deoxycytidine triphosphate into acid-precipitable counts.

**TABLE 4. XC PFU in mixtures of SC-1 and C-177 cells**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Dilution</th>
<th>PFU</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SC-1*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SC-1 + C-177*</td>
</tr>
<tr>
<td>WN1802N</td>
<td>10^-3</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>10^-3.4</td>
<td>23</td>
</tr>
<tr>
<td>WN1802B</td>
<td>10^-3</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>10^-3.8</td>
<td>19</td>
</tr>
</tbody>
</table>

* Results expressed as XC PFU at a particular virus dilution.

**TABLE 5. Direct and infectious-center XC plaque titer estimates of WN1802N and WN1802B viruses in SC-1 and C-177 cells**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Virus</th>
<th>Direct titer*</th>
<th>Calculated Infectious-center titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MOI*</td>
</tr>
<tr>
<td>SC-1</td>
<td>WN1802N</td>
<td>4.3</td>
<td>-0.40</td>
</tr>
<tr>
<td></td>
<td>WN1802B</td>
<td>4.8</td>
<td>0.20</td>
</tr>
<tr>
<td>C-177</td>
<td>WN1802N</td>
<td>1.0</td>
<td>-3.7</td>
</tr>
<tr>
<td></td>
<td>WN1802B</td>
<td>1.5</td>
<td>-3.5</td>
</tr>
</tbody>
</table>

* Replicate cultures were inoculated with WN1802N and WN1802B viruses before the routine XC assay. One set of cultures was trypsinized and plated as infectious centers on SC-1 and C-177 indicator cells as described in the text. Results are expressed as log_{10} Routine XC plaque titration.

**DISCUSSION**

These studies indicate that a stable change has taken place in late-passage SC-1 cells main-
Table 6. XC plaque titer estimates of various viruses in seven clonal isolates derived from dually resistant C-177 cells*  

<table>
<thead>
<tr>
<th>Virus</th>
<th>Host range</th>
<th>SC-1 (84b)</th>
<th>SC-1 (80b)</th>
<th>C-177 (112b)</th>
<th>4D7⁺</th>
<th>4D3⁺</th>
<th>4H9⁺</th>
<th>1G11⁺</th>
<th>4D11⁺</th>
<th>4B3⁺</th>
<th>4E12⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>WN1802N</td>
<td>N</td>
<td>4.7</td>
<td>4.4</td>
<td>2.9</td>
<td>2.7</td>
<td>3.0</td>
<td>2.4</td>
<td>2.8</td>
<td>3.1</td>
<td>3.1</td>
<td>3.4</td>
</tr>
<tr>
<td>WN1802B</td>
<td>B</td>
<td>4.8</td>
<td>4.9</td>
<td>2.1</td>
<td>2.6</td>
<td>2.9</td>
<td>2.0</td>
<td>2.7</td>
<td>2.7</td>
<td>2.5</td>
<td>2.7</td>
</tr>
<tr>
<td>Gross (NB-tropic variant)</td>
<td>NB</td>
<td>3.4</td>
<td>3.3</td>
<td>4.5</td>
<td>4.9</td>
<td>4.5</td>
<td>4.1</td>
<td>5.2</td>
<td>4.9</td>
<td>4.8</td>
<td>4.5</td>
</tr>
<tr>
<td>Moloney</td>
<td>NB</td>
<td>4.0</td>
<td>3.8</td>
<td>4.5</td>
<td>4.5</td>
<td>4.7</td>
<td>4.0</td>
<td>4.3</td>
<td>4.7</td>
<td>4.7</td>
<td>4.5</td>
</tr>
</tbody>
</table>

* Results of routine XC titrations, expressed as log_{10}.

† Numbers in parentheses represent the passage number.

Clonal derivative of C-177.

Fig. 1. Cell growth kinetics of SC-1 and C-177 cells and two clonal derivatives of C-177, 4D7 and 4E12. Cells were plated in triplicate at a standard concentration of 10⁴ cells per 60-mm Linbro well, trypsinized each day, and counted. Results are expressed as total cells recovered at each point.

In this laboratory, C-177 cells, with dual resistance to both N- and B-tropic viruses and residual (even augmented) sensitivity to NB-tropic viruses. Cellular resistance was seen with the XC plaque assay and also with the reverse transcriptase assays and radioimmunoassays for the p30 viral protein. Evidence was found that all cells in C-177 cultures were resistant to N- and B-tropic virus infection, and mixtures of resistant and sensitive cells showed intermediate resistance. Late-passage dually resistant C-177 cells showed shorter doubling times than earlier-passage dually sensitive SC-1 cells, which might explain the rapid emergence of resistance in cell cultures. C-177 cells differ from cells of the G strain, which are resistant to murine retroviruses (7), as C-177 cells are derived from previously sensitive cells and show selective resistance to N- and B-tropic viruses but not to NB-tropic viruses.

The SC-1 cell line was isolated from a single fetus of wild mouse embryo WM1511, initially given by M. Gardner and the late J. E. Officer to A. Friedman, who established a continuous cell line (5). The cells were then cloned by M. Lander, J. W. Hartley, and W. P. Rowe to select for a cell line highly sensitive to both N- and B-tropic virus infection. The WM1511 line had initially been observed to show variable sensitivity to the B-tropic virus, although sensitivity to the N-tropic virus was always seen. Cells were selected at passage 21 for three serial clonings. In the first cloning, one of six lines was sensitive to both N- and B-tropic viruses, indicating the absence of genetic uniformity among WM1511 cells. In the second cloning, 8 of 12 clones were sensitive to B-tropic viruses, as well as to N-tropic viruses. After the third cloning, three cell lines remained sensitive through 30 subsequent passages to both N- and B-tropic viruses, including the SC-1 (I16A) cell line (5). The early passage history of SC-1 cells indicates instability in the expression of resistance to B-tropic viruses. It is not known whether variants resistant to both N- and B-tropic viruses were present in earlier cell passages leading to the establishment of SC-1 cells.

The resistance of C-177 cells may involve the Fv-1 locus, as resistance is relative and is found with N- and B-tropic viruses, but not with NB-tropic viruses. Three components have been identified in Fv-1 resistance affecting virus replication: (i) two-hit dose-response relations; (ii)
“refractoriness,” i.e., only a fraction of resistant cells becomes a virus producer; and (iii) “reduced expression,” i.e., relative inefficiency of subsequent infection as determined in the plaque assay (11). The finding of one-hit dose-response relations in C-177 cells suggests that mechanisms other than Fv-1 may be involved, although resistance could involve the refractoriness component of Fv-1, and reduced expression is seen in the infectious center titrations. Earlier studies of Fv-1 resistance were not performed with congenic mouse strains, so effects may be due to variables other than Fv-1. SC-1 and C-177 cells may be congenic with respect to sensitivity to N- and B-tropic viruses at Fv-1 or at another gene.

These studies, along with a retrospective analysis of SC-1 cells in the initial studies, may reflect the possible instability in the expression of cellular resistance to endogenous retroviruses. Such resistance, e.g., Fv-1 resistance, has been regarded as a static phenomenon. However, there exists evidence that cellular interactions with retroviruses may vary with the age of the mouse, e.g., the early observation that a high-frequency lymphoma in vivo can be induced by the inoculation of an endogenous ecotropic virus only during the first week of life (3), and recent evidence of changes in the complexity of virus-specific nuclear RNA in C57BL/6 mouse tissue at different ages (D. L. Florine, T. Ono, R. G. Cutler, and M. J. Getz, Cancer Res., in press). It is not known whether changes seen in SC-1 cells are rare, sporadic, or even regular events at late passage. Further studies are in progress to characterize the occurrence and mechanism of the change from dual sensitivity to dual resistance in these SC-1 cells.

ACKNOWLEDGMENTS

I thank Ann Dannenberg and Leigh Sasser for excellent technical assistance; J. W. Hartley and W. P. Rowe for the provision of SC-1 and other cells, as well as virus stocks; J. Cole, Viral Resources Branch, National Cancer Institute, for cooperation in the provision of viral proteins and antisera; C. Croce for chromosome analysis of C-177 cells; and R. W. Tennant and M. Halpern for helpful discussions.

This work was supported by Public Health Service grants CA 10815, CA 24744, and CA 25315 from the National Institutes of Health.

LITERATURE CITED


