Characterization of Viruses
Obtained after Cell Fusion or Transfection of Chicken Cells
with DNA from Virogenic Mammalian Rous Sarcoma Cells

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SUMMARY
Viruses recovered after transfection of chicken cells with DNA isolated from
virogenic mammalian cells were characterized by serum neutralization, virus
interference and host range tests. The virus obtained after transfection with DNA
from XC cells containing the genome of the PRAGUE strain of Rous sarcoma virus
(PR–RSV) had the properties of subgroup C avian sarcoma viruses. The virus
isolated after transfection with DNA from RSCH cells containing the genome of
the SCHMIDT–RUPPIN strain of Rous sarcoma virus (SR–RSV) exhibited the proper-
ties of subgroup D viruses. The subgroup classification of described viruses corre-
sponds with classes of virus genomes present in virogenic cells.

INTRODUCTION
Hill & Hillová (1971) have isolated the DNA from XC virogenic rat cells transformed
with the PRAGUE strain of Rous sarcoma virus (PR–RSV) (Svoboda, 1960, 1961). After
transfer to chicken embryo fibroblasts this DNA gave rise to transforming virus. Svoboda,
Hložánek & Mach (1972) obtained similar results with XC DNA and also with DNA
prepared from Chinese hamster tumour cells (RSCH) (Hložánek, Donner & Svoboda,
1966), transformed with the SCHMIDT–RUPPIN strain of Rous sarcoma virus (SR–RSV).
The recovered viruses exhibited infectivity and transforming activity in the focus assay and
the CAM test and when inoculated into susceptible chickens they produced progressive
sarcomas. The aim of this paper is to characterize the viruses recovered after transfection
with DNA from virogenic mammalian tumours and to determine to which subgroup of
avian tumour viruses they belong. The criteria described by Vogt & Ishizaki (1966) and
Vogt (1970) were used.

METHODS
Chicken cells. Chick fibroblasts were prepared from embryos from Brown Leghorns (BrL)
(Hložánek & Sovová, 1968) and from the highly inbred Reaseheath C and W lines of
Leghorns (Hašek, Knížetová & Mervartová, 1966; Hála et al. 1966). Embryos from Sykes’s
Line B of Rhode Island Reds (RIR) (Biggs, Thorpe & Payne, 1968), purchased from F. and
G. Sykes Ltd., England, were also used. The lines differ in their susceptibility to the different
subgroups of avian tumour viruses and are designated accordingly by use of phenotype
nomenclature of Vogt & Ishizaki (1965). The cells used throughout the experiments were
of the following phenotypes: Brown Leghorn embryos, C/O; the highly inbred C line, C/A;
and W line, C/B (Payne & Biggs, 1966); the RIR line, C/AB and C/ABC (Payne & Biggs, 1970).

**Hamster and rat cells.** Hamster (HEF) and rat (LWF) fibroblasts were prepared as primary cultures from embryos of Syrian hamsters and Lewis rats obtained from the Institute of Experimental Biology and Genetics, Prague. For the focus assay, secondary cultures grown in Petri dishes were used.

**Viruses.** The virus strains used as controls in individual subgroups were kindly provided by Dr P. M. Biggs and Dr L. N. Payne of the Houghton Poultry Research Station, England. They were propagated in type C/O Brown Leghorn embryos and the stocks were then compared with original viruses. The viruses used in individual subgroups were BS–RSV (F42) and F42, subgroup A; BH–RSV (RAV2) and RAV2, subgroup B; BH–RSV (RAV49) and RAV49, subgroup C; BH–RSV (RAV50) and RAV50, subgroup D.

The following viruses were used for typing:

- xc–RSV, obtained by co-cultivation of cells of XC rat sarcoma induced by pr–RSV (Svoboda, 1960, 1961) with C/O chicken cells;
- sr–RSV-K18, a single focus virus progeny, obtained by co-cultivation of Chinese hamster tumour cells (RSCH) (Hložánek et al. 1966) transformed by sr–RSV with C/O chicken cells;
- V26 obtained after transfection of chicken cells with DNA isolated from XC sarcoma (Svoboda et al. 1972);
- V501 obtained after transfection of chicken cells with DNA isolated from RSCH cells (Svoboda et al. 1972).

**Virus titration.** Viruses were titrated by counting foci formed on cells prepared either from a mixture of Brown Leghorn embryos or from single embryos of the RIR, C, and W lines by the method of Temin & Rubin (1958). The focus counts were read after 10 to 14 days of cultivation.

**Neutralization test.** Equal volumes of an appropriate dilution of the test virus in PBS, with 200 units of penicillin and 200 μg of streptomycin per ml, were mixed with respective antiserum. Sera were tested at a final dilution of 1:10 and incubation was performed at 30 °C for 1 h. Diluted virus mixed with calf serum and incubated for the same period of time, was used as a control. The final virus concentration was estimated to be approximately 100 to 150 focus forming units (f.f.u.) per 60 mm Petri dish. The mixtures were usually assayed at least at two or three plates by focus formation in BrL (C/O) cells to determine the amount of residual virus.

**Antiserum.** Antisera to viruses were produced by inoculating virus into Brown Leghorn chickens (Chubb & Biggs, 1968). After tumour regression the birds were inoculated with virus and the serum collected 1 week later. The antisera against A, B and C subgroups were compared with antisera which were kindly provided by Dr L. N. Payne. All antisera were checked with the control viruses. Antisera were inactivated at 56 °C for 30 min before use.

**Virus interference test.** Interference tests were performed on BrL (C/O) chicken cells infected with the respective helper virus. Test virus was added 7 days after infection with interfering viruses. The amount of virus interference was determined by counting foci of transformed cells 10 to 14 days later.

**Host range.** Cells derived from the stated lines were used to determine the host range of tested viruses. Susceptibility or resistance was estimated according to Vogt (1970), i.e. a plating efficiency ≤ 0·01 was considered significant for resistance; meanwhile a plating efficiency up to 0·1 was considered for partial resistance. The plating efficiency of tested viruses on C/O cells served as a fully susceptible control (1·00).
RESULTS AND DISCUSSION

The results of neutralization tests are summarized in Table 1. Both the virus xc-RSV-obtained by co-cultivation of XC cells with chicken cells and virus v 26 isolated after transfection of chicken cells with DNA from XC cells give comparable virus-neutralizing reactions. Both viruses are neutralized by anti-C serum, and also antiserum against virus v 26 neutralizes the control subgroup C virus BH–RSV(RAV 49). This result is in agreement with the finding that the original PRAGUE strain of RSV, which had been used for the induction of XC sarcoma, contained a subgroup C component (Duff & Vogt, 1969). The same is true of RSCH cells transformed in vitro by sr–RSV, where similarities are found between virus sr–RSV–K 18 isolated after co-cultivation of RSCH cells with chicken cells and virus v 501 obtained after transfection of chicken cells with DNA from RSCH cells. Both viruses are neutralized by anti-B and anti-D sera, and the control subgroup B and D viruses, BH–RSV(RAV 2) and BH–RSV(RAV 50), are neutralized by antiserum against sr–RSV–K 18. This is in accord with the finding that members of both subgroups show immunological cross-reactions in the neutralization test because of shared antigenicity (Duff & Vogt, 1969). In addition, our findings agree with the observation that virus sr–RSV rescued by Bauer & Graf (1969) from virogenic mammalian cells belongs to subgroup D.

Table 1. Virus neutralization tests

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>A subgroup</th>
<th>B subgroup</th>
<th>C subgroup</th>
<th>D subgroup</th>
<th>v 26 subgroup</th>
<th>sr–RSV–K 18</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>BH–RSV (F 42)</td>
<td>0.04</td>
<td>0.81</td>
<td>0.31</td>
<td>0.34</td>
<td>0.51</td>
<td>0.79</td>
<td>1.00</td>
</tr>
<tr>
<td>BH–RSV (RAV 2)</td>
<td>0.94</td>
<td>0.06</td>
<td>0.32</td>
<td>0.34</td>
<td>0.80</td>
<td>0.89</td>
<td>1.00</td>
</tr>
<tr>
<td>BH–RSV (RAV 49)</td>
<td>0.87</td>
<td>NT</td>
<td>0.60</td>
<td>0.34</td>
<td>0.70</td>
<td>0.01</td>
<td>1.00</td>
</tr>
<tr>
<td>BH–RSV (RAV 50)</td>
<td>0.87</td>
<td>0.02</td>
<td>0.60</td>
<td>0.34</td>
<td>0.70</td>
<td>0.01</td>
<td>1.00</td>
</tr>
<tr>
<td>SR–RSV–K 18</td>
<td>0.79</td>
<td>NT</td>
<td>0.65</td>
<td>0.34</td>
<td>0.70</td>
<td>0.01</td>
<td>1.00</td>
</tr>
<tr>
<td>v 501</td>
<td>0.91</td>
<td>0.63</td>
<td>0.04</td>
<td>0.34</td>
<td>0.70</td>
<td>0.01</td>
<td>1.00</td>
</tr>
<tr>
<td>xc–RSV</td>
<td>0.91</td>
<td>0.63</td>
<td>0.04</td>
<td>0.34</td>
<td>0.70</td>
<td>0.01</td>
<td>1.00</td>
</tr>
<tr>
<td>v 26</td>
<td>0.91</td>
<td>0.63</td>
<td>0.04</td>
<td>0.34</td>
<td>0.70</td>
<td>0.01</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Neutralization tests of virus v 26 were kindly checked by Dr L. N. Payne with the same result.
Tests with mixtures of anti A+B, A+C, and B+C sera were set up for the virus v 26 with the results 1.00, 0.08 and 0.04, respectively.
NT = not tested.

The results of virus interference tests summarized in Table 2 reveal that interference occurred between RAV 49 and viruses xc–RSV and v 26. This confirms the assumption that both viruses belong to subgroup C. The interference patterns observed with RAV 50 and viruses sr–RSV–K 18 and v 501 indicated that these viruses may be classified with subgroup D.

The host range was tested in chicken cells of C/O, C/A, C/B, C/AB and C/ABC phenotypes. The results (Table 3) show that virus xc–RSV has, like virus v 26, the same host range in C/O, C/A and C/AB cells. The plating efficiency is reduced (0.001 and 0.01) only on C/ABC cells which can be considered sufficient evidence of resistance of these cells to infection with tested viruses (Vogt, 1970). In the case of viruses sr–RSV–K 18 and v 501, full susceptibility is found on C/O and C/A cells, whereas a reduction in plating efficiency is seen on C/B, C/AB and C/ABC cells. This result supports the finding that viruses sr–RSV–K 18 and v 501 have the same properties as viruses belonging to subgroup D.

None of the viruses tested showed transformation of Syrian hamster embryo fibroblasts and Lewis rat cells in the focus assay.
Table 2. *Virus interference tests*

<table>
<thead>
<tr>
<th>Strain of virus</th>
<th>F 42 (A)</th>
<th>RAV 2 (B)</th>
<th>RAV 49 (C)</th>
<th>RAV 50 (D)</th>
<th>None (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bs–RSV (F 42)</td>
<td>&lt;0.001</td>
<td>0.73</td>
<td>1.03</td>
<td>0.64</td>
<td>1.00</td>
</tr>
<tr>
<td>Bh–RSV (RAV 2)</td>
<td>0.91</td>
<td>&lt;0.01</td>
<td>0.93</td>
<td>&lt;0.01</td>
<td>1.00</td>
</tr>
<tr>
<td>Bh–RSV (RAV 49)</td>
<td>0.98</td>
<td>0.88</td>
<td>&lt;0.001*</td>
<td>0.59</td>
<td>1.00</td>
</tr>
<tr>
<td>Bh–RSV (RAV 50)</td>
<td>1.02</td>
<td>&lt;0.01</td>
<td>1.03</td>
<td>0.01</td>
<td>1.00</td>
</tr>
<tr>
<td>xc–RSV</td>
<td>NT</td>
<td>NT</td>
<td>&lt;0.01*</td>
<td>NT</td>
<td>1.00</td>
</tr>
<tr>
<td>v 26</td>
<td>0.98</td>
<td>0.44</td>
<td>&lt;0.01</td>
<td>NT</td>
<td>0.56</td>
</tr>
<tr>
<td>v 501</td>
<td>0.92</td>
<td>NT</td>
<td>0.68</td>
<td>&lt;0.05</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* Cells infected with helper virus were passaged three times before challenge with sarcoma virus. All interference tests of virus v 26 were kindly checked by Dr L. N. Payne.

NT = not tested.

Table 3. *Relative sensitivity of different cell types to tested viruses*

<table>
<thead>
<tr>
<th>Chicken line and phenotype</th>
<th>Relative sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BrL</td>
</tr>
<tr>
<td></td>
<td>C/O</td>
</tr>
<tr>
<td>Bs–RSV (F 42)</td>
<td>1.00</td>
</tr>
<tr>
<td>Bh–RSV (RAV 2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Bh–RSV (RAV 49)</td>
<td>1.00</td>
</tr>
<tr>
<td>Bh–RSV (RAV 50)</td>
<td>1.00</td>
</tr>
<tr>
<td>xc–RSV</td>
<td>1.00</td>
</tr>
<tr>
<td>v 26</td>
<td>1.00</td>
</tr>
<tr>
<td>v 501</td>
<td>1.00</td>
</tr>
</tbody>
</table>

The results, in all tables, indicate that the virus isolated after transfection of chicken cells with DNA from XC cells belongs to subgroup C, whereas the virus obtained after transfection with DNA from RSCH cells is a member of subgroup D. Also viruses, which have been rescued after fusion of the respective virogenic mammalian cells with chicken cells, exhibit the same properties. Moreover, in agreement with this finding is the fact that virus PR–RSV used for obtaining virogenic XC cell lines contains subgroup C virus responsible for the transformation of mammalian cells, and virus sr–RSV used for obtaining RSCH cells contains subgroup D virus (Duff & Vogt, 1969). The accordance between the subgroup classification of the viruses obtained after transfection and the viruses used for obtaining the respective types of virogenic cells, points to the specificity of transfection in the system used and precludes the possibility that DNA from virogenic cells only activates the pre-existing endogenous virus genomes present in chicken cells. However, the possibility is not excluded that genetic recombination with those genomes occurs when DNA is transferred to chicken cells. This problem cannot be resolved until specific markers of endogenous virus genome, other than virus coat properties, are available.

*Note added in proof.* The tumour inducing capacity of virus, obtained after transfection of CEF with XC-DNA, was tested on 7-day-old Brown-Leghorn chicks. The ID50/ml, according to Reed & Muench (1938), was determined to be 10\(^{24.42}\). The same virus gave a titre of 10\(^{5.47}\) f.f.u./ml when tested in focus assay.
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REFERENCES


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