Oncogenicity of Non-transforming Mutants of Avian Sarcoma Viruses

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For practical purposes avian RNA tumour viruses have been divided into sarcoma and leukosis viruses. Sarcoma viruses have the ability to transform chick embryo fibroblasts in vitro and to induce fibro-sarcomas in vivo after a latent period of about 7 to 21 days. Leukosis viruses do not transform chick embryo fibroblasts, although they replicate in them, and in vivo most wild strains induce principally lymphoid leukosis after a latent period of over 90 days. They also frequently induce erythroid leukosis (erythroblastosis) and osteopetrosis, and occasionally other tumours.

Non-transforming mutants of sarcoma viruses, which have all the in vitro properties of leukosis viruses, have been produced experimentally by exposure to ultraviolet light (Toyoshima, Friis & Vogt, 1970), by γ-irradiation (Goldé, 1970) and by treatment with chemicals (Graf et al. 1971). These results suggest that leukosis viruses may originate from sarcoma viruses, perhaps by loss of genetic material either by mutation or by segregation of subgenomic components (Toyoshima et al. 1970; Graf et al. 1971; Martin & Duesberg, 1972). It has been suggested that non-transforming mutants arise spontaneously when cloned strains of Rous sarcoma viruses are grown (Vogt, 1971). However, regardless of whether such non-transforming viruses arise spontaneously or are produced experimentally, it is necessary to know whether they are oncogenic in vivo and behave like wild-type leukosis viruses.

Two non-transforming mutants were chosen for study—the non-transforming, or non-converting derivatives of SCHMIDT-RUPPIN strain 1 Rous sarcoma virus (NC-SRV-I) and of SRV-H (NC-SRV-H) (Graf et al. 1971) which belong to subgroups A and D, respectively. These virus mutants were chosen because there was a detectable loss of RNA in one (NC-SRV-I) but not in the other (NC-SRV-H), which suggested that the former originated by deletion and the latter by point mutation (Bolognesi & Graf, 1971).

The chickens chosen as hosts were HPRS-Brown leghorns (HPRS-Br.L.) and HPRS Line 151 White Leghorns (HPRS-L 151). The former were used in this Laboratory (HPRS) for the production of sarcomas with subgroups A, B, C and D sarcoma viruses; the latter are highly susceptible to the induction of tumours by leukosis viruses (Burmester et al. 1960).

Both HPRS-Br.L. and HPRS-L 151 have been kept in isolation at Houghton Poultry Research Station since 1961 and are considered free of leukosis sarcoma viruses of subgroups A, B, C and D because tests on each generation have not detected antibodies to representative sarcoma viruses of these subgroups. No spontaneous cases of lymphoid leukosis were detected during this period.

Groups of 1-day-old HPRS-L 151 and HPRS-Br.L. chicks were placed in each of three isolation rooms maintained under positive pressure with filtered air (Kenzy & Biggs, 1967). Chicks in one room were kept as controls and were inoculated with phosphate buffered saline (pH 7.2) by the same routes and in the same volume as the experimental chicks. Chicks in the second room were inoculated with NC-SRV-I and chicks in the third room with NC-SRV-H. Although it is well recognized that leukosis viruses spread horizontally
with difficulty (Purchase & Burmester, 1972), these rooms were chosen because in 7 years use there has been no evidence of cross-infection with leukosis viruses.

Each HPRS-L 15I chick was inoculated at 1-day-old with 1000 tissue culture infective doses (TCID)/chick, 250 TCID into the wing web and 750 TCID intraperitoneally. The birds were examined weekly for 5 weeks for tumours in the wing web. The surviving birds were killed at 272 days old.

HPRS-Br.L. chicks were inoculated at 1-day-old into the wing web. For NC-SRV-I a dose of 10000 TCID/chick was used, but because of low infectivity only 1600 TCID/chick of NC-SRV-H were used. The birds were examined for tumours in the wing web weekly for 3 weeks. The surviving birds were killed at 259 days old.

All birds were examined post mortem and the liver, spleen, gonad, bursa of Fabricius and other tissues which had lesions suggestive of neoplasia were studied microscopically. For analysis of the results, the number of birds dying of causes other than neoplasia or Marek's disease were subtracted from the number inoculated to give the effective experimental number.

A summary of the incidence of tumours and Marek’s disease is given in Table 1. Inadvertent infection with Marek’s disease virus occurred in the control chickens and the chickens inoculated with NC-SRV-1. However, only the lymphoid tumours were likely to be misdiagnosed, and histological and cytological examination of these and the bursa of Fabricius left little doubt of the correctness of each diagnosis (Biggs & Payne, 1964; Siccardi & Burmester, 1970). The absence of lesions of, and precipitating antibodies to, Marek’s disease in chickens inoculated with NC-SRV-H, confirmed this group’s freedom from infection with Marek’s disease virus.

No sarcomas were seen in the wing webs of either the HPRS-Br.L. or the HPRS-L 15I chickens. The only tumour that occurred in the HPRS-Br.L. chickens, other than those due to Marek’s disease, was a fibrosarcoma at a site other than the wing web in a chicken 212 days after inoculation with NC-SRV-H.

For chickens of strain L 15I the incidence of tumours other than those of Marek’s disease was 35.6% (65.2% if chickens with Marek’s disease are omitted) following inoculation with NC-SRV-1 and 53.5% following inoculation with NC-SRV-H. In the former group these consisted of erythroid leukosis (erythroblastosis) and lymphoid leukemia only, whereas in the latter group there were also two cases of osteopetrosis and one of an osteochondrosar-
Table 2. Relative plating efficiencies of subgroup A, B, C, and D sarcoma viruses on chick embryo fibroblasts pre-infected with viruses isolated from tumours induced in chickens by NC-SRV-1 and NC-SRV-H mutants of SCHMIDT-RUPPIN Rous sarcoma virus

<table>
<thead>
<tr>
<th>Interfering virus</th>
<th>A (BH-RSV)</th>
<th>B (BH-RSV (RAV 2))</th>
<th>C (BH-RSV (RAV 49))</th>
<th>D (BH-RSV (RAV 50))</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>1, from tumour induced by NC-SRV-1, subgroup A</td>
<td>&lt; 0.004</td>
<td>0.9</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>2, from tumour induced by NC-SRV-H, subgroup D</td>
<td>0.9</td>
<td>0.3</td>
<td>0.9</td>
<td>0.004</td>
</tr>
<tr>
<td>3, from tumour induced by NC-SRV-H, subgroup D</td>
<td>1.0</td>
<td>0.4</td>
<td>1.0</td>
<td>&lt; 0.004</td>
</tr>
</tbody>
</table>

Sarcoma. In the group inoculated with NC-SRV-1, the first cases of erythroid leukemia and lymphoid leukemia occurred 118 and 168 days after inoculation, respectively, and in the group inoculated with NC-SRV-H both first occurred 164 days after inoculation.

Viruses were isolated from two lymphoid tumours induced by NC-SRV-H and one by NC-SRV-1. All three isolates were found to be non-transforming viruses in cell culture, and, based on interference tests, each belonged to the same subgroup as the mutant which produced the respective tumour (Table 2).

The parent viruses (SRV-1) and SRV-H) produce sarcomas in 1-day-old chicks within 1 week. These results show that both non-transforming mutants of SCHMIDT-RUPPIN RSV lost their ability to produce sarcomas characteristic of their parent viruses. The loss of this property, together with the loss of the ability to transform chick embryo fibroblasts in cell culture, supports the view that these two properties are associated. The production in vivo of other tumours agrees with the findings of Kurth & Bauer (1972) that these non-transforming mutants induce in chickens a strong anti-tumour-specific surface antigen (TSSA).

There are three possible interpretations for the occurrence of tumours characteristic of leukemia viruses in the chickens inoculated with the non-transforming mutants: (1) there was inadvertent infection with leukemia viruses in the groups inoculated with the mutants; (2) there was recombination or complementation with, or induction of, a subgroup E leukemia virus (Weiss et al. 1971) in the cells in which the non-transforming mutants were isolated, or present in the cells of at least all chickens which developed these tumours; and (3) the mutants possess genetic information derived from the sarcoma virus responsible for the production of tumours characteristic of leukemia viruses.

The first alternative can be discarded because all other chickens used in the same animal accommodation building at the time of these experiments were free of leukemia viruses belonging to subgroups A, B, C and D. Inadvertent infection with leukemia viruses is also unlikely to occur twice, and it is unlikely that in each case the virus would belong to the same subgroup as the inoculated mutant virus, and that the control group would remain free of leukemia virus. In addition, subgroup D leukemia viruses have not been isolated from natural infections of chickens.

It would be difficult to choose between the second and third alternatives. However, the mutants belong to the same subgroup as the sarcoma viruses from which they were derived...
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(Graf et al. 1971) and each virus isolated from tumours belonged to the same subgroup as the inducing mutant.

Whichever alternative is correct, these results provide evidence for more than one gene in the genome of leukosis/sarcoma viruses responsible for neoplasia. If the third alternative is correct these genes may be present in the genome of some or all sarcoma virus particles. Because of the clonal origin of the sarcoma viruses from which the non-transforming mutants were derived, these results also suggest that leukosis viruses can be multipotential with respect of their oncogenicity. Whether different parts of the virus genome are responsible for each kind of tumour remains to be determined.

The two non-transforming mutants were equally oncogenic despite one originating as a deletion and the other as a point mutation. It would be interesting to know if the RNA of each mutant lacked the $a$ subunit found for other non-transforming mutants and variants (Duesberg & Vogt, 1970; Martin & Duesberg, 1972).

Whether or not the tumours produced in chickens by the inoculation of non-transforming mutants are the result of recombination or complementation with, or induction of, endogenous subgroup E virus, their production by such mutants supports the hypothesis that leukosis viruses can originate from sarcoma viruses through loss of genetic material.

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