The Induction of Myeloid Leukaemias by Rauscher Murine Leukaemia Virus

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SUMMARY

A number of cloned viral preparations isolated from Rauscher virus-producing JLS-V5 cells were compared in their competence to induce different types of leukaemias. All preparations were able to induce myeloid leukaemias, but the induction of lymphatic or erythroid leukaemias was also observed. Serial infection of newborn mice with either cell-free extracts or serum from animals suffering from a myeloid leukaemia did not result in the occurrence of relatively more myeloid leukaemias nor did the infection with virus harvested from ascites fluid of permanent myeloid cell lines. It appears that the mechanism by which myeloid leukaemias are induced is not virus-specific.

Rauscher leukaemia virus causes a rapidly developing erythroblastosis due to the presence of a defective spleen focus-forming virus (SFFV) in combination with an ecotropic lymphatic leukaemia virus (R-MuLV) (Rauscher, 1962; Steeves, 1975). Although a number of other viral components have been described in Rauscher virus preparations (van Griensven & Vogt, 1980), there has been no report of a virus strain that exclusively induces a myeloid leukaemia.

In an earlier study using the culture fluid of Rauscher virus-producing JLS-V5 cells (Wright & Lasfarques, 1965) we obtained both erythroid and lymphatic leukaemias after infection of BALB/c and DBA/2 mice. In addition, a limited number of myeloid leukaemias in BALB/c mice were observed. By serial transplantation of leukaemic blast cells from these animals we could induce tumour formation from which different permanent cell lines were established (de Both et al., 1978, 1981, 1983). Since a specific F-MyLV has been described for the Friend virus complex (McGarry et al., 1974) we report here attempts to isolate a Rauscher virus component (R-MyLV) which exclusively or with high frequency induces a myeloid leukaemia.

As starting material we used Rauscher virus produced by JLS-V5 cells. This virus has been shown to be heterogeneous in RNA composition (Mol et al., 1982). From this viral preparation the ecotropic helper virus was cloned by endpoint dilution and infection of 3T3 cells. The cloned virus was called 3T3-R-MuLV clone 9 and was XC-positive and consisted of only the 35S RNA component. The oligonucleotide pattern was homogeneous and all spots showed a similar labelling density due to a stoichiometric distribution (Mol et al., 1982). Injection of this cloned virus into newborn mice resulted in a lymphatic leukaemia in 75% of the animals. Also, some mixed types of leukaemia were induced which showed, at the same time, features of both erythroid and lymphatic leukaemias. Only 8% of the inoculated animals developed a myeloid leukaemia, which occurred after a latent period of 6 months to 1 year as was the case for lymphatic leukaemias (Table 1).

Passage in vivo of a lymphatic Rauscher leukaemia virus was carried out by sampling the serum of mice suffering from a lymphatic leukaemia and by subsequent infection of 20 newborn mice with this serum. After the mice had become leukaemic the serum was tested for reverse transcriptase activity. If virus was present the serum was used to infect 3T3 cells. The virus was called 3T3-R-MuLV DBA/2. Also, the virus from the serum of an infected C57/Black mouse
Table 1. **Types of leukaemias induced by various Rauscher virus preparations in newborn BALB/c and DBA/2 mice**

<table>
<thead>
<tr>
<th>Virus source</th>
<th>Reverse transcriptase activity</th>
<th>Number of leukaemias induced</th>
<th>Erythroid</th>
<th>Lymphatic</th>
<th>Myeloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>JLS-V5 cells</td>
<td>$322 \times 10^3$</td>
<td>21 (61%)</td>
<td>8 (26%)</td>
<td>2 (6%)</td>
<td></td>
</tr>
<tr>
<td>3T3-R-MuLV clone 9</td>
<td>$155 \times 10^3$</td>
<td>6 (17%)§</td>
<td>27 (75%)</td>
<td>3 (8%)</td>
<td></td>
</tr>
<tr>
<td>3T3-R-MuLV DBA</td>
<td>$101 \times 10^3$</td>
<td>1 (4.8%)§§</td>
<td>15 (71.4%)</td>
<td>5 (23.8%)</td>
<td></td>
</tr>
<tr>
<td>3T3-R-MyLV C57/Black</td>
<td>$283 \times 10^3$</td>
<td>4 (8%)§§</td>
<td>35 (69%)</td>
<td>12 (23%)</td>
<td></td>
</tr>
</tbody>
</table>

* Rauscher virus was obtained from the culture fluid of JLS-V5 cells (Wright & Lasfarques, 1965). From this viral complex the ecotropic lymphatic leukaemia helper virus was cloned by endpoint dilution and infection of BALB/c 3T3 or NIH 3T3 cells (clone 9) (Mol et al., 1982). Other viral preparations originated from sera of infected mice, which showed an overt lymphatic or myeloid leukaemia. 3T3 cells were infected with virus in the presence of 4 μg/ml polybrene for 8 h.

† Virus was measured by reverse transcriptase assay in serum of leukaemic mice or in culture fluids from virus-producing cells according to Verma & Baltimore (1973). Culture fluid of JLS-V5 cells was employed as positive controls and culture fluid of uninfected 3T3 cells was used for negative controls. Reverse transcriptase activity was expressed as c.p.m./h/30 μl serum or culture fluid.

§ The leukaemias were diagnosed on the basis of both pathology and cytological staining. Figures in parentheses indicate the percentage of animals with leukaemias.

§§ Mixed types showed both features of erythroid and lymphatic leukaemias.

which is genetically resistant against SFFV and which suffered from a myeloid leukaemia was transferred to 3T3 cells. The virus produced by these cells was called 3T3-R-MyLV C57/Black. The oligonucleotide pattern of the latter is presented in Fig. 1 and shows a similar stoichiometry to the pattern of 3T3 clone 9 (Mol et al., 1982). It seems likely, therefore, that cloning in vivo has taken place by serial transfer.

Both viral preparations induce predominantly lymphatic leukaemias (Table 1). In only a few cases an erythroid leukaemia could be observed. However, in both series 24% of the cases developed a myeloid leukaemia. Notwithstanding the higher percentage of induced myeloid leukaemias, both viruses are most probably identical to 3T3-R-MuLV clone 9 obtained after cloning in vivo by endpoint dilution as was shown by RNA analysis (Mol et al., 1982). To investigate if permanent myeloid cell lines can produce an R-MyLV, these cells were inoculated into mice intraperitoneally. Cell-free ascitic fluid was used for infection. These infections again resulted in all types of leukaemia. Co-cultivation of serum or extracts of myeloid leukaemic blasts with SC-1 cells, which propagate also dualtropic MCF-like viruses, does not result in the release of virus exclusively inducing myeloid leukaemias.

For a second and third transfer, sera or extracts from myeloid leukaemic tissue were injected directly into newborn mice without the intermediate transfer to 3T3 or SC-1 cells.

The frequency of leukaemias after the first and second transfer was compared. As can be seen from the data of Table 2 the induction of myeloid leukaemias with virus originating from 3T3-R-MyLV C57/Black was higher than with 3T3-R-MuLV. However, within each series of infected animals lymphatic and erythroid leukaemias also occurred. The latter, which have a shorter latent period, increased in frequency in subsequent transfers of virus harvested from myeloid leukaemias.

So far, it can be concluded from these results that passage in vivo of cloned Rauscher virus leads to an initial decrease in the onset of erythroid leukaemias and as a consequence an increase in the number of lymphatic and myeloid leukaemias. Infection of neonatal mice with virus-containing ascitic fluid induced by intraperitoneally growing myeloid cells again induces all types of leukaemia. Serial transfer of virus obtained from myeloid leukaemias results in all series in an increase of erythroid leukaemias but not in that of myeloid leukaemias.

The following explanations for the induction of myeloid leukaemias should be considered.

(i) Cloned viruses are able to recombine in host cells with endogenous sequences. These viruses, which belong to the class of mink cell focus-forming virus (MCFV), are held to be responsible for the onset of lymphatic leukaemias (Chattopadhyay et al., 1982). Cloned MuLVs of Friend and Rauscher virus can induce a limited number of erythroid leukaemias (Troxler &
Fig. 1. Fingerprinting of T1 oligonucleotides. $^{32}$P labelling of Rauscher virus RNA and fingerprinting of RNase T1-digested RNA on two-dimensional polyacrylamide gels was performed according to standard procedures (Mol et al., 1981, 1982). ●, Reference dye positions.

Table 2. Serial infection of newborn mice with sera of mice suffering from a myeloid leukaemia*

<table>
<thead>
<tr>
<th>Virus from sera of myeloid leukaemic mouse (MyLV Black)</th>
<th>BALB/c → BALB/c → BALB/c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythroid</td>
<td>4 → 5 → 13</td>
</tr>
<tr>
<td>Lymphatic</td>
<td>35 → 11 → 1</td>
</tr>
<tr>
<td>Myeloid</td>
<td>12 → 12 → 1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Virus from 3T3 cells infected with cloned helper virus clone 9</th>
<th>BALB/c → BALB/c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythroid</td>
<td>6 → 9</td>
</tr>
<tr>
<td>Lymphatic</td>
<td>27 → 3</td>
</tr>
<tr>
<td>Myeloid</td>
<td>3 → 2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ascitic fluid from myeloid leukaemic cell line (RMB-3)</th>
<th>BALB/c → BALB/c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythroid</td>
<td>9 → 3</td>
</tr>
<tr>
<td>Lymphatic</td>
<td>6 → 2</td>
</tr>
<tr>
<td>Myeloid</td>
<td>2 → 1</td>
</tr>
</tbody>
</table>

* For serial transfer of virus, extracts of leukaemic tissue or serum from leukaemic mice were taken for direct infection of neonatal mice. For this purpose also ascites fluid of mice inoculated previously with a permanent myeloid cell line (RMB-3) was used.

Scolnick, 1978; Vogt, 1982; and present results). MCFV isolated from Rauscher virus is able to induce an erythroid leukaemia (van Griensven & Vogt, 1980) without the synthesis of a deleted envelope protein (Bilello et al., 1980). However, no MCFV could be isolated from Rauscher virus-transformed myeloid cell lines (L. J. L. D. van Griensven, unpublished results). Therefore,
if recombination has taken place it probably only affects the integration of (deleted) proviruses. The fingerprint of virus from C57/Black mice suffering from a myeloid leukaemia shows complete identity with the cloned ecotropic helper virus, which does not favour extensive recombination.

(ii) Recombination of cloned helper virus can take place with cellular oncogenes resulting in defective viruses carrying these oncogenes. For chickens, two acute leukaemia viruses are known which induce myeloblastosis (annr) and myelocytomatosis (mcv) (Graf & Beug, 1978), both of which are related to myeloid leukaemia in mice. However, we were not able to detect any defective viral component when virus inducing myeloid leukaemias was analysed by gel electrophoresis under denaturing conditions.

(iii) Since lymphatic leukaemias have a clonal origin (Reddy & Fialkow, 1983) their induction is dependent on the transformation of one of the available target cells. The lymphoid target cells probably outnumber the myeloid ones. The frequency of lymphatic leukaemias will therefore always be higher than that of the myeloid leukaemias. In view of this, the probability of integration of whole or deleted proviruses in the proximity of cellular onc genes determines the occurrence of a myeloid leukaemia (Neel et al., 1981). Previous unsuccessful attempts to isolate a pure MyLV from cell-free extracts of reticulosarcomas, which frequently induce myeloid leukaemias in mice and rats (Graffi, 1960), are consistent with this explanation. A single report claims the isolation of a pure MyLV from the related Friend virus. This virus was isolated after long-term selection in vivo in C57/Black mice (McGarry et al., 1974). It could induce myeloid leukaemias in a number of different mouse strains and functions as helper virus in the rescue of SFFV. However, its biological properties have not been correlated with its molecular composition.

Our results suggest a close relationship between the myeloid leukaemia-inducing virus preparations and the lymphatic helper viruses both on the basis of RNA composition and inducibility of lymphatic leukaemias, but do not support the existence of a pure Rauscher myeloid leukaemia-inducing virus.

REFERENCES


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