Rat Virus-mediated Suppression of Leukemia Induction by Moloney Virus in Rats

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SUMMARY

Rats of the Sprague-Dawley and W/FU strain, inoculated intraperitoneally at birth with a mixture of Moloney leukemia virus (MLV) and rat virus (RV), strain RV-13 or 9HV-B, developed fewer leukemias than rats inoculated with the leukemia virus alone. In several experiments the incidence of leukemia was reduced from 100, 95, 62, and 62% in control rats, that received MLV alone, to 67, 60, 23, and 17% in rats inoculated with the same dose of MLV in combination with RV. Serologic data indirectly substantiated the evidence for RV being the suppressive factor. This was indicated by the fact that among rats which had been inoculated with both viruses, generally higher levels of hemagglutination-inhibition antibody to RV were found in nonleukemic animals as compared to leukemic ones in the same group of inoculum. The latter finding also suggests, but does not prove, reciprocal interference between MLV and RV in rats.

INTRODUCTION

The recovery of viruses serologically similar or identical to rat virus (RV) (7) from rat leukemias of different origin has been reported by several investigators (1, 6, 7, 9, 11). Such viruses have also been isolated from other types of tissues derived from the rat as discussed in a recent review by Toolan (12). The association of RV with neoplastic or preneoplastic tissues has led to speculations concerning its possible role as an oncogenic agent of the rat (4). In view of the lack of evidence for a leukemia-inducing ability of RV, despite its frequent association with this disease in rats, we have considered RV as a potential inhibitor of leukemogenic viruses, aside from any other biologic effects that RV may be able to produce. This consideration was borne out during studies in which the 9H virus (9HV), serologically related to Kilham’s RV, was isolated from leukemias induced by extracts derived from chemically induced rat mammary tumors (1). In further studies (3) it was found that the 9HV consists of two infectious hemagglutinating components which differ in heat sensitivity and size and were termed 9HV-A and 9HV-B respectively, the 9HV-B being similar to RV.

The present communication describes the results of experiments, conducted with the Moloney leukemia virus (MLV) in combination with RV-13 or 9HV-B, which lend support to the hypothesis cited above.

MATERIALS AND METHODS

Moloney leukemia virus, Lot No. 3062-59, was received from Flow Laboratories through the courtesy of Dr. John B. Moloney of the National Cancer Institute. The virus, which had been prepared from BALB/c mouse plasma, was diluted 1:10 with 0.05 M potassium citrate and inoculated intraperitoneally (0.1 ml) into newborn BALB/c mice purchased from the Jackson Laboratory, Bar Harbor, Maine. Stock virus was prepared from pooled livers and spleens of leukemic mice by a modification of the extraction procedure described by Moloney (10). Twelve grams of the tissue were homogenized as a 20% suspension by weight with 0.15 M potassium citrate containing Alundum. The Alundum and coarse tissue debris were removed by centrifugation at 500 rpm; the extract was sonicated for 1 minute and then centrifuged at 1,600 rpm for 20 minutes. The supernatant was centrifuged in a Model L Spinco centrifuge at 73,000 X g for 1 hr, and the resulting pellet was suspended in 12 ml of 0.05 M potassium citrate and homogenized. The preparation was placed in ampules and stored at -70°C until used. The rat-infectivity titer of this MLV stock was 10^2.0 ID_{50}/0.1 ml as determined by assay in Sprague-Dawley rats; this consisted of inoculating groups of 6 newborn rats each with serial 10-fold dilutions of the virus. The number of animals that became leukemic in each inoculum group by the end of 6 months was recorded and the ID_{50} calculated.

Rat virus strain RV-13, was kindly supplied by Dr. H. J. Spencer of the Mary Imogene Bassett Hospital, Cooperstown, N. Y. The virus had been passed 4 times in hamsters, and the preparation received represented a 10% extract of hamster liver-spleen-kidney tissue. The 4th passage in rat embryo cell cultures of the virus was used for experiments. This stock virus was prepared as follows. The cells and supernatants of infected cultures were pooled 10 days after infection and sonicated for 60 seconds. Cell debris was removed by centrifugation at 1,600 rpm and the virus sedimented at 105,000 X g for 1 hr.

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1This investigation was supported by Office of Naval Research Contract N00014-67-A-0201-0001, by USPHS Research Grant CA-08708 from the National Cancer Institute, and by an equipment grant from the Florida Division of the American Cancer Society.

Received May 20, 1968; accepted May 12, 1969.
The pellet was resuspended in Hanks' balanced salt solution containing 1% newborn calf serum (HBSS-NCS) and homogenized. This virus preparation had an infectivity titer of $10^{5.0} \text{TCID}_{50}/0.1 \text{ml}$, as determined by titration in secondary W/FU rat embryo cell cultures, and a hemagglutinating (HA) titer of 512 (with a 0.5% suspension of guinea pig erythrocytes).

The 9H virus (9HV) had been passed exclusively in the REL line of rat embryo cells (1) since its original isolation from leukemic rat tissues. The B component of the 9HV or 9HV-B (3), which cross-reacts with RV-13 in serologic tests and appears to be a new strain of RV, was used in this study. It was obtained by filtration of the 9HV through Gradocel membranes of 30-mu porosity. It was then passed 5 times in rat embryo cell cultures and the 5th-passage virus was prepared as described above for RV-13. This stock had an infectivity titer of $10^{5.0} \text{TCID}_{50}/0.1 \text{ml}$ and an HA titer of 128.

In order to determine the effect of rat virus on the incidence of leukemia upon simultaneous inoculation with MLV, RV-13 or 9HV-B was mixed with MLV in a ratio of 1:1 or 2:1 at about 4°C. The mixture was inoculated intraperitoneally (0.1 ml) into newborn W/FU or Sprague-Dawley rats. Control rats were inoculated with MLV mixed in the same ratio with the diluent (HBSS-NCS) in which RV-13 or 9HV-B was suspended. The pregnant mothers were purchased from commercial breeders, and 1–2 ml of blood were obtained by cardiac puncture a few hours before inoculation of their offspring with virus. The inoculated animals were inspected three times a week, and any nonspecific deaths occurring during the first 3 days following inoculation were disregarded. The animals were supplied with water and food brisquets ad libitum and weaned at 3 weeks of age. The time which elapsed before clinical symptoms appeared was recorded as the latent period. The leukemic state, manifested by enlarged thymus, spleen, and/or liver, was ascertained by autopsies of the dead or moribund animals. At the termination of the experiments, the remaining healthy-appearing animals were sacrificed and autopsied to verify the absence of macroscopic leukemic symptoms.

Blood samples were obtained from a number of moribund leukemic animals, as well as the healthy survivors at 6 months, in all experimental groups. Serums prepared from these as well as of the mothers' blood samples were tested for the presence of hemagglutination-inhibition (HI) antibody against RV-13 as follows. A given serum sample was diluted 1:10 and then serially in 2-fold steps with phosphate-buffered saline (PBS), pH 7.2. Two-tenths ml of each serum dilution was mixed with 0.2 ml of virus antigen (8–16 hemagglutinating units) and allowed to stand at room temperature for 1 hr. Subsequently, 0.2 ml of a 1% suspension of guinea pig erythrocytes in PBS were added to each tube and the test read after 2 hr at 4°C. The reciprocal of the highest serum dilution that inhibited agglutination of the erythrocytes was recorded as the HI titer of the serum.

**RESULTS**

Reduction by 9HV-B and RV-13 of the Incidence of MLV-induced Leukemia in Rats

In three separate experiments MLV alone and mixed with either 9HV-B or RV-13 was inoculated intraperitoneally into newborn rats of the random-bred Sprague-Dawley strain and the inbred W/FU strain. The results of these experiments are presented in Table 1. It can be seen that consistently a 33% to 45% reduction in the incidence of leukemia occurred when MLV and 9HV-B or RV-13 were inoculated simultaneously as a mixture into newborn rats. Early (2 months) and late (5–6 months) leukemias developed in rats inoculated with the mixture or with MLV alone; however, the incidence at any given time in groups of animals inoculated with the former was 30–50% below those inoculated with the latter. At 6 months, when 100% of the MLV-inoculated animals had developed leukemia in Experiment #1 and 95% in Experiment #2, only 67% and 60% respectively of the rats inoculated with the mixture had developed the disease. The remaining ones were apparent-

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Type of virus inoculum</th>
<th>Strain of recipient rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sprague-Dawley</td>
</tr>
<tr>
<td></td>
<td>Number inoculated</td>
<td>Number with leukemia</td>
</tr>
<tr>
<td>1</td>
<td>MLV + Diluent</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>MLV + 9HV-B</td>
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<tr>
<td>2</td>
<td>MLV + Diluent</td>
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</tr>
<tr>
<td></td>
<td>MLV + 9HV-B</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>MLV + Diluent</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>MLV + 9HV-B</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>MLV + RV-13</td>
<td>23</td>
</tr>
</tbody>
</table>

Inhibition by rat virus, strain 9HV-B and RV-13, of the induction of leukemia by Moloney leukemia virus (MLV) in rats. Observed for 6 months after inoculation.

- $10^{2.0} \text{TCID}_{50}/0.1 \text{ml}$ of MLV.
- $10^{5.0} \text{TCID}_{50}/0.1 \text{ml}$ of 9HV-B or RV-13.
- $10^{5.0} \text{TCID}_{50}/0.1 \text{ml}$ of MLV.
leukemia had considerable serum HI titers; in fact, half of the
ated low HI titers, with the exception of 2 animals whose
ulated control rats which, like the groups of rats inoculated
ated by appreciable HI antibody titers to this virus in the ma-
serums tested had titers of 160-640. Interestingly, the uninoc-
mixture of MLV and rat virus and became leukemic also exhib-
tained before initiation of the experiments. It should be noted
data on the HI antibody titers of their mothers' serums ob-
Table 1, it failed to inhibit leukemia induction when used as
diluent for MLV.

Relationship of HI Antibody Titer:s in Maternal Serums
and Serums of Experimental Rats to the Occurrence
of Leukemia

The immune status in regard to rat virus was determined for
inoculated rats as well as their mothers. The mothers were
bled a few hours before inoculation of their offspring. The
inoculated offspring that developed clinical symptoms of leu-
emia were bled at that time and then sacrificed to ascertain the
diagnosis. The blood from healthy nonleukemic animals
was obtained at the end of 6 months postinoculation. The titer
of HI antibody against RV-13 was determined in each serum
thus obtained.

Table 2 shows the titers of leukemic and nonleukemic
Sprague-Dawley rats at the time of sacrifice. It also includes
data on the HI antibody titers of their mothers' serums ob-
tained before initiation of the experiments. It should be noted
that all the animals inoculated with MLV plus diluent had very
low or insignificant levels of serum HI antibody to RV, irre-
pective of whether they had developed leukemia and despite
the fact that their mothers had been exposed to RV, as reflected
by appreciable HI antibody titers to this virus in the mat-
ernal serums. Similarly, rats that had been inoculated with a
mixture of MLV and rat virus and became leukemic also exhib-
ited low HI titers, with the exception of 2 animals whose
serums had titers of 1:80. On the other hand, most rats which
had been inoculated with the mixture and did not develop
leukemia had considerable serum HI titers; in fact, half of the
serums tested had titers of 160-640. Interestingly, the uninoc-
ulated control rats which, like the groups of rats inoculated
with MLV plus diluent, were kept in close proximity to the
animals inoculated with the mixture, also exhibited uniformly
high serum HI titers. This latter finding appears to represent an
eexample of natural transmission of rat virus.

**DISCUSSION**

A consistent reduction in the incidence of rat leukemia, pro-
duced by a given dose of Moloney virus, was demonstrated
when the same dose of virus was deliberately mixed with a
constant dose of rat virus and administered intraperitoneally
to newborn rats. The possibility that the suspending medium
used for the rat virus inhibited or inactivated MLV when the
two viruses were mixed in vitro was ruled out by the fact that
the same medium failed to inhibit per se the induction of
leukemia by MLV. The protective effect is therefore attribut-
able to the rat virus and appears to represent a case of viral
interference. Interference between certain leukemogenic and
nonleukemogenic viruses in mice has also been reported from
other laboratories (14, 15). It is conceivable that, by varying
the dose pairing of rat virus and leukemia virus, one may ob-
tain a low or zero incidence of leukemia upon inoculation into
rats. Such a situation may have prevailed in the previously
mentioned natural host system where attempts were made at
qualitative serial cell-free transmission of a rat mammary
tumor-derived leukemia containing an unknown and presum-
ably small quantity of either virus (1).

The exact mechanism responsible for the suppressive action
of rat virus on leukemia induction by MLV in rats is not
known at the present time and needs elucidation. It is possible
that rat virus-induced circulating interferon (5) inhibited MLV
and its leukemogenic effect; however, one has to see whether
the strains of rat virus used in the present study were capable
of inducing the production of interferon. An alternative mech-
nism could involve a stimulating interaction between the two
viruses. Such an interaction between Adenovirus 12 and rat
virus in vitro was reported by Chany and Brailovsky (2). If
MLV, like Adenovirus 12, initially stimulates the production
of rat virus, then the latter may subsequently interfere with

<table>
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<tr>
<th>Type of inoculum</th>
<th>Type of serum</th>
<th>No. of serums tested</th>
<th>No. with HI titer of:</th>
<th>HI titer of mothers, serums</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLV + Diluent</td>
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<td>&lt; 10</td>
<td>80-320</td>
</tr>
<tr>
<td>MLV + Diluent</td>
<td>Nonleukemic</td>
<td>10</td>
<td>10</td>
<td>80-320</td>
</tr>
<tr>
<td>MLV + RV-13b or 9HV-B</td>
<td>Leukemic</td>
<td>10</td>
<td>20</td>
<td>10-80</td>
</tr>
<tr>
<td>MLV + RV-13 or 9HV-B</td>
<td>Nonleukemic</td>
<td>28</td>
<td>40</td>
<td>10-80</td>
</tr>
<tr>
<td>None</td>
<td>Nonleukemic</td>
<td>10</td>
<td>80</td>
<td>10-80</td>
</tr>
</tbody>
</table>

Hemagglutination-inhibition (HI) antibody titers to rat virus in serums of leukemic and nonleukemic Sprague-Dawley rats and
serums of their mothers. Obtained at various times within 6 months postinoculation from leukemic animals and at 6 months
from nonleukemic animals as ascertained by autopsies. Obtained a few hours before inoculation of respective offspring.

*MLV = Moloney leukemia virus.
*9HV-B = Strain of rat virus.
the multiplication and leukemia induction by MLV. In preliminary experiments in vitro, we have not found any appreciable stimulating effect of MLV upon rat virus (unpublished data). On the contrary, MLV, under certain conditions, exhibited a transient inhibitory effect, suggesting the possibility of reciprocal interference between the two viruses. This may also be the case in vivo as suggested indirectly by serologic data presented in this communication: (a) HI antibody titers against rat virus in the group of rats that received both viruses were generally lower in those animals which developed leukemia, and (b) appreciable HI antibody was absent in rats inoculated with MLV alone, despite the fact that uninoculated control rats had high HI antibody titers, presumably resulting from natural transmission of rat virus through close proximity to rats inoculated with the latter. The possibility cannot be excluded, however, that the multiplication of MLV causes a general suppression of the host's immunologic mechanism which could be reflected in a lowered production of antibody to rat virus. Small to moderate levels of HI antibody to rat virus in the maternal sera of Sprague-Dawley rats did not seem to significantly affect the outcome of the experiments because the results obtained with W/FU rats, whose mothers' HI titers were uniformly less than 1:10, were essentially the same. Similarly, about 60% of the Sprague-Dawley mothers whose offspring were used in the experiments had appreciable amounts of HI antibody to the H-1 virus.

The findings reported in this communication raise the question as to the role of rat virus in inhibiting oncogenesis in its natural host in which it is latent and widespread under natural conditions (4). They also provide another example of inhibition of viral oncogenesis by the Picodna viruses as previously reported for other members of this group of small DNA viruses (8, 13).

ACKNOWLEDGMENTS

The author is indebted to Dr. M. M. Sigel for providing an opportunity to carry out this study. The technical assistance of Mrs. R. Miller is gratefully acknowledged.

REFERENCES

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