Relationship of Infecting Dose to Recovery of Rauscher Murine Leukemia Virus (RMLV) in Random Bred Swiss Mice

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SUMMARY

Random bred Swiss mice were infected with graded decimal doses of Rauscher murine leukemia virus via the intravenous or intraperitoneal routes. Samples of spleen and plasma were then collected at various times after infection. Bioassay of these samples in recipient mice revealed a definite relationship between the amount of virus used to initiate the infection and the amount of virus recovered as measured by a spleen focus assay. Highest infective titers were obtained when the most concentrated inocula were used and the relative titers of virus in the pooled plasmas and spleens decreased as successively less concentrated inocula were used to initiate the infection. Plasma and spleen samples contained more virus when the inocula were injected intravenously than when injected intraperitoneally. In addition, virus was detectable earlier and persisted at higher levels when the initiating virus was injected intravenously rather than intraperitoneally.

INTRODUCTION

Bryan et al. (1) showed that the amount of infective virus demonstrable in individual chicken sarcomas was directly related to the infecting dose of Rous sarcoma virus. Later, studies in this laboratory (3, 9) showed that recovery of virus from chick brain also was directly related to the infecting dose, i.e., the virus content of brains from infected chicks decreased as successively smaller amounts of virus were contained in the inocula. This direct relationship between infecting dose and recovery of virus does not occur with many nononcogenic viruses such as influenza (4) or pneumonia virus of mice (2). The data presented here show that the relationship of infecting dose to recovery of virus also obtains with a mammalian oncogenic virus, namely, the Rauscher murine leukemia virus (RMLV) (9).

MATERIALS AND METHODS

Virus

RMLV, sample P-881, was used as the seed virus in this series of experiments, and was kindly supplied through the courtesy of Dr. F. J. Rauscher of the National Cancer Institute. It was a frozen, 10 percent cell-free extract prepared from infected BALB/c mouse spleens and was stored at −70°C in glass-sealed ampules. For use, the frozen aliquot samples were thawed and diluted in diluent consisting of saline containing 2 percent inactivated horse serum and 100 units of penicillin and 100 μg of streptomycin per ml. Virus suspensions were inoculated intravenously via a tail vein or intraperitoneally into mice in 0.2-ml amounts.

Mice

Random bred, female, barrier-raised Swiss mice were purchased from Taconic Farms. They were housed in standard stainless steel cages and fed food pellets and water ad libitum. All mice weighed 15 to 18 gm when inoculated.

Spleen Focus Assay

The virus content of plasma and spleen samples was determined by means of the spleen focus assay described by Pluznik and Sachs (5). Serial 10-fold dilutions of spleen or plasma samples, to be assayed for virus content, were made, and at each dilution 0.2 ml was injected intravenously into each of seven mice. Seven days later, which was optimum for these mice, the mice were killed by cervical dislocation. Their spleens were then removed and immediately immersed in fixative consisting of 91 parts 70 percent ethanol, 4.5 parts glacial acetic acid, and 4.5 parts formalin. Foci appeared rapidly on the surface of the spleens and were counted within one hour after fixation. Virus titers of the inocula that induced the formation of the foci were calculated and expressed as the log focus-forming units (FFU) per ml of plasma or log FFU per gm of spleen. In each assay experiment groups of mice inoculated with diluent alone or serial decimal dilutions of standard RMLV (P-881) served as controls. Approximately 3000 mice were required to assay all samples described in this series of experiments.

RESULTS

Titration of the Standard Virus

In order to determine its potency, 0.2-ml amounts of serial 10-fold dilutions of the seed virus pool (P-881) were inoculated intravenously or intraperitoneally into groups of 10 mice each. The mice were palpated at regular biweekly intervals to detect splenomegaly, and the titer of the inoculum pool was calculated according to the method of Reed and Muench (10). These data are summarized in Table 1. The titer of the inoculum was 10^4 or 10^5.

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TABLE 1
Titration of Rauscher Murine Leukemia Virus (RMLV) in Swiss Mice

<table>
<thead>
<tr>
<th>Virus dilution</th>
<th>Intravenous route</th>
<th>Intrapertoneal route</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spleno-megaly/Total</td>
<td>Av. time to response (days)</td>
</tr>
<tr>
<td>2 x 10⁻¹</td>
<td>7/7</td>
<td>8.1</td>
</tr>
<tr>
<td>10⁻²</td>
<td>10/10</td>
<td>10.6</td>
</tr>
<tr>
<td>10⁻³</td>
<td>9/10</td>
<td>25.0</td>
</tr>
<tr>
<td>10⁻⁴</td>
<td>6/10</td>
<td>26.5</td>
</tr>
<tr>
<td>10⁻⁵</td>
<td>2/9</td>
<td>33.0</td>
</tr>
<tr>
<td>10⁻⁶</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>ID₅₀</td>
<td>10⁻⁵.⁹</td>
<td>10⁻⁵.⁹</td>
</tr>
</tbody>
</table>

* RMLV (P-881), 0.2 ml per mouse.

ID₅₀/0.2 ml when it was injected intravenously and 10⁻⁹ ID₅₀/0.2 ml when the intraperitoneal route was used. This 10-fold difference in response depending upon the route of injection employed is consistent with earlier observations of others (5).

Effects of Infecting Dose on the Recovery of Virus

Serial 10-fold dilutions of seed virus, from 10⁻¹ through 10⁻⁶, were made and 0.2-ml amounts of each dilution were injected intravenously into groups of 25 donor mice each. The same dilutions of seed virus were also injected intraperitoneally into similar groups of 25 mice each. At 1, 2, 4, 8, and 12 weeks, respectively, after injection, 3 mice from each dilution group were selected at random, bled from the brachial artery and killed. The presence or absence of palpable spleens in these donor mice are summarized in Table 2. At the time of bleeding, blood samples were collected in equal volumes of saline containing 5 units of heparin per ml. The samples were centrifuged at 2000 rpm for 15 minutes in a refrigerated International PR-2 centrifuge after which the supernatant plasma was collected. In addition, spleens from these mice were also collected for assay. All samples were stored in a Dry Ice chest until assayed in recipient mice for virus content.

Immediately prior to use the plasma and spleen samples were thawed. A 10 percent weight/volume suspension of the spleens was then made by grinding in diluent with Alundum and the acellular supernatant fluid obtained after centrifugation at 2000 rpm for 20 minutes was used for assay. Serial 10-fold dilutions of the spleen extracts and plasma samples were made and at each dilution 0.2-ml amounts were injected intravenously into each of seven mice. Seven days later, the mice were killed and the spleen foci were counted.

When infecting virus was injected intraperitoneally into donor mice, their plasmas were found to contain highest titers of virus when the most concentrated inocula were used to initiate the

CHART 1. Relationship of infecting dose to recovery of virus from plasmas of mice injected intraperitoneally with serially diluted Rauscher leukemia virus. FFU, focus-forming units.

CHART 2. Palpable Spleens in Donor Mice Whose Spleens and Plasmas Were Assayed for Virus Content

<table>
<thead>
<tr>
<th>Infected dose³</th>
<th>Intravenous route</th>
<th>Intrapertoneal route</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mice killed at week:</td>
<td>Mice killed at week:</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2 x 10⁻¹</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10⁻²</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10⁻³</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10⁻⁴</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10⁻⁵</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

³ Rauscher virus pool P-881, 0.2 ml per mouse.

* No survivors at 12 weeks.
infection. These data are illustrated in Chart 1. Here, plasma samples were collected from donor mice at 1, 2, 4, 8, and 12 weeks, respectively, after infection. Upon assay in recipient mice, virus was first detectable at two weeks when large amounts of virus were injected. The relative potencies obtained were directly related to the initiating dose employed. Decreasing amounts of virus were recoverable as successively less concentrated inocula were used and little or no virus was recoverable from the plasma when the infecting dose of virus approached the LD50. Maximum amounts of virus were obtained from plasma samples taken at 4 weeks after infection. At the higher (10^{-1} and 10^{-2}) dose levels no donor mice survived to 12 weeks. The spleens from these same donor mice infected intraperitoneally with graded doses of virus were also assayed in recipient mice. The results of this experiment are shown in Chart 2. Here too, as expected, a dose response to the initiating virus was evident. The curves are essentially the same as those obtained from the plasma samples in that virus was first detected by 1 or 2 weeks and reached maximal titers in the spleen by 4 weeks. At low infecting doses (10^{-4} and 10^{-5} dilutions) little or no virus could be detected at 8 or 12 weeks after infection.

Plasma and spleens from donor mice initially infected with virus via the intravenous route were similarly assayed for virus content. Charts 3 and 4 show that virus was detectable in the

**Chart 2.** Relationship of infecting dose to recovery of virus from spleens of mice injected intraperitoneally with serially diluted Rauscher leukemia virus. FFU, focus-forming units.

**Chart 3.** Relationship of infecting dose to recovery of virus from plasmas of mice injected intravenously with serially diluted Rauscher leukemia virus. FFU, focus-forming units.
Infecting Dose and Recovery of RMLV in Mice

VIRUS INJECTED I.V. SPLEENS ASSAYED

CHART 4. Relationship of infecting dose to recovery of virus from spleens of mice injected intravenously with serially diluted Rauscher leukemia virus. FFU, focus-forming units.

DISCUSSION

Groupé and Rauscher (3) observed that the growth curve of Rous sarcoma virus in chick brain paralleled that of many non-neoplastic animal viruses. Their data showed that virus disappeared rapidly from the brain immediately after injection. Following an eclipse period, virus reappeared and increased logarithmically until maximal titers were reached in moribund birds. As anticipated from the previous studies of Bryan et al. (1) on the recovery of virus from sarcomas, they also noted that the amount of virus extractable from infected brains was found to be directly related to the dose of virus used to initiate the infection. In later studies Rauscher and Groupé (9) found that the duration of the eclipse period and the rate of viral synthesis in chick brain were also directly related to the infecting dose of Rous sarcoma virus. More recently, Rauscher and Allen (8) observed that the growth curve of RMLV in the plasma or spleen of infected mice also paralleled that of other viruses.

Prior to the development of a spleen focus assay for the titration of RMLV (5), it was necessary to titrate the virus by dilution to extinction of leukemic response as determined by splenomegaly or death. This required several months of observation when dilute inocula were employed. On the other hand, a titration can be completed in approximately seven days when the spleen focus assay is employed. However, it should be emphasized that this latter method is less sensitive. Pluznik and Sachs (5) have shown that titers based on mortality or splenomegaly were higher than that obtained using the spleen focus technic by a factor of 6 to 71 depending upon the strain and age of the mouse employed and the source of virus being titered. Nevertheless, because of the short period of observation required, the spleen focus assay offers many advantages and was used in this study.

The data presented here show that the amount of virus recoverable from the plasma and spleens of mice infected with RMLV is indeed correlated with the amount of virus used to initiate the infection. Typical growth curves for virus in the plasma and spleens were obtained. However, in general, at each point on the growth curves the relative virus content of the plasma or spleen extracts correlated with the infecting dose. In view of the enormous number of recipient mice that would have been required to assay samples taken from individual donor mice sacrificed at various times after infection, samples for bioassay consisted of pooled plasma or spleen extracts from 3 animals. Since pooled samples were assayed, each point on the curves reflects only the virus titer of the individual tissue containing the most virus in that pool. Nevertheless, the curves obtained clearly show a definite correlation of infecting dose to recovery of virus.

When the intravenous route was used for initiating the infection, an apparent 10-fold increase in titer of the inoculum virus was obtained than when the intraperitoneal route was used. This increase in susceptibility is also reflected in the virus content of the samples collected. Charts 3 and 4 show that virus was detected earlier in the spleens and plasmas when donor mice were injected intravenously. In addition, viremia persisted at higher levels than when virus was injected intraperitoneally into donor mice. Other responses to RMLV shown to be dose dependent, in addition to the recovery of virus from infected tissues, include
the number of foci produced on the spleen (5), the ratio of mice responding with erythropoiesis alone to those developing both erythropoiesis and leukemia, the percent incidence of disease, and the latent period to splenomegaly or death (6, 7).

The data presented here re-emphasize some of the difficulties to be anticipated in attempting to isolate oncogenic viruses from tumor tissues where the infecting dose of the unknown agent may be presumed to be low and, in addition, demonstrate the usefulness of RMLV as a model leukemogenic agent in random bred Swiss mice.

REFERENCES

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