Studies with the Murine Leukemogenic Rauscher Virus

I. Chemotherapy Studies with in Vivo and in Vitro Assay Systems*

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SUMMARY

Assay systems are described for the chemotherapeutic testing of drugs against animals given inoculations of Rauscher virus. The parameters of drug testing employed were survival time of the animals, extent of splenomegaly and transmissibility of the Rauscher disease. Treatment with triethylene melamine (TEM), 6-mercaptopurine, and 2-chloro-4',4''-2-imidazolyl-2-ylterephthalanilide (NSC-60339) increased the survival time of mice given inoculations of Rauscher virus when five daily treatments were administered starting 3 days or 17 days following viral inoculation. Methotrexate had no effect when treatment was initiated early, but was effective when treatment was delayed. Treatment with Cytoxan, TEM, and 5-fluorouracil resulted in transient suppression of splenomegaly, and TEM had an inhibitory effect on the transmissibility of Rauscher disease.

Of the drugs tested in an assay system with a transplantable line of Rauscher leukemia, NSC-60339 produced the most extensive increase in survival time.

The Rauscher virus and blood cells of animals with primary Rauscher leukemia were exposed to TEM and Melphalan in vitro and bioassayed in vivo. The drugs appeared to exert more extensive antileukemic than antiviral activity.

The increasing number of known oncogenic viruses and some understanding of their biology and of the neoplasms they induce (4, 11) is providing the investigator with test systems with which to conduct chemotherapeutic studies. With such test systems a drug may be evaluated not only for antiviral activity, but also for its capacity to influence neoplasia and the growth of virus-induced neoplasms. Groupé et al. (5, 6), Johnson and his co-workers (7, 8) and Bather (1) reported in vivo studies with Rous sarcoma virus. Sugiura (13, 14) and Mirand et al. (10) have studied the effect of a variety of compounds on the Friend virus-induced leukemia. Chirigos et al. (2) reported an in vivo assay system for the chemotherapeutic testing of drugs against the Moloney virus-induced leukemia.


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Recently, Rauscher (12) described the recovery of a new, potent, rapidly acting virus which induces erythrocytopenosis and lymphocytic leukemia in many strains of mice and rats. The Rauscher disease is characterized by a very rapid and extensive proliferation of predominantly erythrocytic and leukocytic elements as early as 7 days following parenteral inoculation. In general, the mice develop palpable spleens and have intense viremia as early as 7 days following virus inoculation.1 Mice may succumb early (25–35 days) after inoculation, with spleens weighing 30–50 times those of normal mice. In mice that survive the acute phase of the disease, erythrocytopenosis is followed by the development of lymphocytic leukemia (30–45 days after inoculation). The latter mice generally succumb in 60 or more days, usually with grossly evident leukemia. Recently, more potent virus preparations have been obtained

1 F. J. Rauscher and B. Allen, Growth Curve of a Murine Leukemia Virus in Mice (to be published).
which induce lymphocytic leukemia as early as 17 days after parenteral inoculation in adult BALB/c mice.

The successful use of primary Moloney virus-induced and transplantable leukemia in mice for testing candidate chemotherapeutic agents prompted a similar investigation employing Rauscher virus. The present report presents the results of studies in which procedures were explored, including: (a) treatment of the Rauscher disease in mice in which treatment was initiated shortly after inoculation of virus or after the development of splenomegaly, (b) treatment of a transplantable line of Rauscher leukemia initially induced by the virus, (c) in vitro exposure of Rauscher virus and leukemic cells to drug, and subsequent bioassay.

MATERIALS AND METHODS

Preparation of standard virus.—A procedure involving differential centrifugation and citrate salts was used for the preparation of cell-free virus. Splenic tissue from mice of the second to tenth intraperitoneal virus passage was the source of virus for all the experiments described here. The spleens were removed from mice showing marked splenomegaly, usually within the 25th–30th day after inoculation. A 10 per cent homogenate of pooled spleens was prepared in a chilled Waring Blendor, with 0.153 M potassium citrate containing 1 mg per cent of hyaluronidase as diluent. The homogenate was allowed to digest for 1 hour at room temperature and was then centrifuged at 1800 × g for 20 minutes. The middle two-thirds of the supernatant was recentrifuged at 2300 × g for 20 minutes to insure a more complete separation of the heavier cell fractions. A final clarification of the supernatant was carried out as follows: A frozen aliquot of the virus concentrate was diluted with an equal amount of sterile 0.9 per cent saline, and 0.1 or 0.2 ml. of the diluted virus preparation was inoculated intraperitoneally into 6- to 8-week-old BALB/c or 6- to 10-week-old CDBA3 mice.

Leukemic cell suspension.—The transplantable lymphoid leukemia line MCDV-12 was isolated from a virus-inoculated BALB/c male mouse. It had been carried in the ascites form for over 50 transplant generations in BALB/c mice prior to use in the current study. Although the tumor grew slowly when first isolated, repeated passage of tumor cells resulted in more rapid development of generalized leukemia and early death. The current study on drug effect against the transplantable leukemia was conducted in 6- to 8-week-old BALB/c mice given subcutaneous inoculations in the right inguinal region of 0.2 ml. of a 1:10 dilution of ascitic fluid.

Randomization of test animals.—In all experiments mice were randomized shortly after inoculation and distributed into appropriate groups.

Drugs and treatment.—Triethylenemelamine (TEM), 5-fluorouracil (5-FU), and cyclophosphamide, were dissolved in 0.9 per cent NaCl. Methotrexate (MTX) was dissolved in 2 per cent NaHCO3. Terephthalanilide (NSC-60339) was suspended in 0.1 per cent methyl cellulose. 6-Mercaptopurine (6-MP) was dissolved in dilute alkali. The schedule of treatment for each experiment is noted in “Results.” Melphalan employed in the in vitro study, was dissolved in calcium-free Krebs-Ringer phosphate buffer (S).

Blood bioassay.—For retransplant experiments, individual virus-inoculated BALB/c male mice were anesthetized with ether, a midline incision was made, and the diaphragm was exposed and cut transversely. Blood was collected in a heparinized syringe by cardiac puncture and 0.2 ml. inoculated intraperitoneally into recipient BALB/c mice.

In vitro exposure of Rauscher virus and blood cell suspension from mice infected with Rauscher virus.—A 1 to 100 dilution (10–1) of standard virus was prepared in calcium-free Krebs-Ringer phosphate buffer.

Blood was collected and pooled from leukemic BALB/c male mice 105 days after inoculation with virus. The cells were washed 4 times in 0.9 per cent NaCl and suspended in the original volume after the final wash. Two ml. of the virus or 2 ml. of the blood suspension were incubated for:

* The compounds in this study were obtained through the Cancer Chemotherapy National Service Center from the following sources: Methotrexate and triethylene melamine, Lederle Laboratories Division of the American Cyanamid Co.; 5-fluorouracil, Hoffman-LaRoche, Inc.; Cyclophosphamide (Cytoxan) (2H-1,3,2-Oxazaphosphorine,2-[bis(2-chloroethyl)amino]tetrahydro-2-oxide), Mead Johnson and Co.; 6-mercaptopurine, Burroughs Wellcome Company; Terephthalanilide (2-chloro-4,4'-di-2-imidazolin-2-ylterephthalanilide) (NSC-60339), Wander Co.; Melphalan (p-di-(2-chloroethyl)aminolphenylalanine), Chester Beatty Research Institute.
with 2 ml. of buffer containing TEM or Melphalan. Control blood cell suspension and virus were similarly prepared. All materials were incubated for 30 minutes at 37° C., after which the cell and virus suspensions were washed free of drug with 0.9 per cent NaCl and suspended in the original volume prior to inoculation of BALB/c male mice with 0.2 ml. of each mixture. The virus suspensions were washed by sedimenting the virus at 30,000 × g in a Spinco ultracentrifuge for 30 minutes.

RESULTS

CHEMOTHERAPY OF VIRUS-INOCULATED MICE

Effect of drug therapy on splenomegaly and transmissibility of Rauscher disease.—The results of the

No marked decrease in the incidence of deaths was apparent, although one of seven mice treated with 5-FU, as compared with four of ten untreated control animals, succumbed to the disease. Extensive daily treatment with 12.5 mg/kg of Cytoxan was not sufficient to completely suppress the development of splenomegaly and eventually became toxic for the host.

Chart 1 illustrates the results obtained in a second experiment in which a detailed dose-response study with TEM was conducted. In this experiment, treatment was initiated 15 days after virus inoculation and continued daily to the end of the experiment. Groups of mice were sacrificed at regular intervals after therapy was started, and

TABLE 1

<table>
<thead>
<tr>
<th>GROUP NO.</th>
<th>DRUG</th>
<th>DAILY DOSE (mg/kg)</th>
<th>NO. OF TREATMENTS FROM DAY 15</th>
<th>SPLEEN PALPATIONS (DAYS)</th>
<th>DEAD/TOTAL (DAY 181)</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>0.5</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Cytoxan</td>
<td>0.5</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>TEM</td>
<td>0.5</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>TEM</td>
<td>0.5</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>0.5</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5-FU</td>
<td>0.5</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>None</td>
<td>0.5</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>TEM</td>
<td>0.5</td>
<td>45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mice were given inoculations intraperitoneally of 0.1 ml. of virus preparation.
† Group Nos. 1–4: 6-week-old male CDBA mice.
‡ Group Nos. 5–6: 10-week-old male CDBA mice.
§ Group Nos. 7–8: 10-week-old female CDBA mice.

first experiment on the chemotherapy of the virus-induced disease are presented in Table 1. The hybrid CDBA mice, ranging in age from 6 to 10 weeks were given inoculations of virus, and therapy was initiated 15 days later. Spleen size, the criterion of response for this experiment, was arbitrarily graded from 1+ to 4+. A 4+ spleen was one which extended across the peritoneal cavity and weighed approximately 3 gm. This represented a twentyfold increase in weight over the normal.

A limited course of therapy with Cytoxan, TEM, or 5-FU resulted in some degree of inhibition of spleen enlargement. However, after cessation of therapy, spleen size increased in every case, becoming similar to that of untreated controls. spleen weights were recorded. From day 15 to 48, each point represents the average spleen weight of five mice; from day 50 to 78, each point represents the average spleen weight of three mice.

The results show that TEM was effective in diminishing splenomegaly and that the degree to which spleen weights were suppressed was directly related to the dose of TEM employed.

It may be noted that a decrease in spleen weight in the untreated control animals occurred between 30 and 50 days following virus inoculation (top curve, Chart 1). The most probable explanation of this phenomenon, which has been seen repeatedly in other studies,1 is that at about day 30 the predominantly erythropoietic response terminates with the development of organized hemorrhage and

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fibrosis. This is accompanied by a transient decrease in spleen weight, with a concomitant infiltration of splenic parenchyma by immature lymphoid cells. As the latter process becomes more intense, a second progressive increase in spleen weight occurs.

During the course of this experiment, from each animal that was sacrificed for the determination of spleen weight, heart blood was removed and inoculated into recipient mice. The data in Table 2 show the effect TEM therapy had on the capacity of blood from donor mice to transmit the disease to recipient mice. Blood from donor mice treated with the highest level of TEM tested failed to transmit the disease to recipients to the same extent as did blood from donor mice treated with lower levels of the drug. Since treatment was withheld in this experiment until splenomegaly was evident (15 days after inoculation of virus), the above result may be attributable to either an antiviral or an antitumor effect of TEM.

**Effect of drug therapy on survival time.**—Charts 2A and 2B show the results of treatment on the survival time of BALB/c mice given inoculations of virus. Treatment was initiated either on the 3rd day (Chart 2A) or on the 17th day (Chart 2B) following virus inoculation and continued daily for 5 days. The range of individual deaths in the untreated controls (Chart 2A), from the time of virus inoculation, was from 31 to greater than 140 days. The median survival time (MST) for the group was 72 days. Treatment from the 3rd to the 7th day with 100 mg/kg 6-MP, 0.5 mg/kg TEM, and 50 mg/kg NSC-60339 (Chart 2A) resulted in increases in the MST of the mice. Although treatment with MTX failed to increase the MST of the mice, there was a moderate increase in the percentage of mice surviving to 140 days.

When treatment was initiated 17 days following virus inoculation (Chart 2B), significant increases in survival times were obtained with all of the four drugs tested. An approximately twofold or greater increase in the MST over untreated controls was obtained with 0.36 mg/kg MTX, 36 mg/kg 6-MP, 0.25 and 1.0 mg/kg TEM, and 50 and 100 mg/kg NSC-60339. Delayed therapy resulted in a somewhat greater number of mice surviving for more than 140 days (46 mice, Chart 2B) than did when treatment was initiated early (27 mice, Chart 2A). In general, with the delayed therapy the animals appeared to show greater tolerance to drug toxicity. This was particularly evident for TEM, where a 1.0 mg/kg dose was toxic for the younger mice and increased the survival time of the older mice. All the mice given inoculations of virus which survived to day 140, including both the untreated control group and the drug-treated groups, had enlarged spleens.

**CHART 1.—Influence of treatment with TEM on splenomegaly in 6- to 8-week-old female CDBA female mice given intraperitoneal inoculations of 0.1 ml. of virus preparation. Subcutaneous daily treatment with drug initiated 15 days following virus inoculation. Last treatment with drug 24 hours prior to sacrifice.**

**TABLE 2**

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>PROPORTION OF RECIPIENT MICE EXHIBITING SPLENOMEGALY</th>
<th>DAY OF SACRIFICE OF DONOR†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22</td>
<td>29</td>
</tr>
<tr>
<td>Controls</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>0.1 mg/kg TEM</td>
<td>4/4</td>
<td>4/5</td>
</tr>
<tr>
<td>0.2 mg/kg TEM</td>
<td>2/3</td>
<td>5/5</td>
</tr>
<tr>
<td>0.4 mg/kg TEM</td>
<td>3/3</td>
<td>4/3</td>
</tr>
</tbody>
</table>

* 6- to 8-week-old CDBA female mice given intraperitoneal inoculations of 0.1 ml. of virus preparation. Subcutaneous daily treatment with drug initiated 15 days following virus inoculation. Last treatment with drug 24 hours prior to sacrifice.

† One virus-inoculated donor mouse was sacrificed for inoculation of each recipient; 0.3 ml. of donor blood inoculated intraperitoneally into recipient. Inoculated recipient mice were observed for 41⁄2 months.
During the course of this experiment, virus-inoculated mice were bled at different times, and the heart blood was transferred to recipient mice to determine whether the Rauscher disease could be transmitted. The data presented in Table 3 show that, as early as 3 days following virus inoculation, whole blood was capable of transmitting the disease to recipient mice.

Also, as part of this experiment, untreated control mice were sacrificed 3, 7, 17, and 21 days after inoculation with virus, and complete autopsies were performed. The tissues were fixed in Zenker-formol solution, and the sections were stained with hematoxylin and eosin. In agreement with previous observations (12), histological examination of the tissues revealed pathological changes progressing from hyperplasia and erythrocytopenosis to generalized lymphatic leukemia as follows:

a) On day 3 the only noticeable change was generalized hyperplasia of the lymphoid follicles in lymph nodes and of the malpighian bodies in the spleen.

b) On day 7, examination of spleens showed hyperplasia of malpighian bodies with severe congestion. Large immature lymphoid cells with vesiculated nuclei, many of which were in mitosis, were seen along the trabeculae, immediately beneath the capsule, and scattered throughout the parenchyma. The lymph nodes and the thymus were characterized mainly by hyperplasia of lymphoid elements and dilation of sinusoids.

c) By day 17, the normal splenic architecture was almost completely absent. Areas of hemorrhage, in various stages of organization, were numerous and were composed of nucleated red cells interspersed with mature erythrocytes. Non-hemorrhagic areas of the spleen consisted predominantly of the large immature lymphoid cells. With the exception of marked erythrocytopenosis in liver and continued hyperplasia of lymph nodes, the other tissues were not unusual.

d) By day 21, the spleen was characterized by massive areas of hemorrhage and fibrosis. Numer-

![Table 3](chart.png)

**Table 3**

Transmission of Disease from Virus-Inoculated Donor Mice to Recipient Mice

<table>
<thead>
<tr>
<th>DAY VIRUS-INOCULATED DONOR MICE</th>
<th>MICE RECEIVING BLOOD FROM VIRUS-INOCULATED DONORS†</th>
</tr>
</thead>
<tbody>
<tr>
<td>WERE SACRIFICED*</td>
<td>Splenomegaly/total</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2/3</td>
</tr>
<tr>
<td>7</td>
<td>3/3</td>
</tr>
<tr>
<td>10</td>
<td>3/3</td>
</tr>
<tr>
<td>14</td>
<td>3/3</td>
</tr>
<tr>
<td>17</td>
<td>3/3</td>
</tr>
<tr>
<td>21</td>
<td>3/3</td>
</tr>
<tr>
<td>28</td>
<td>3/3</td>
</tr>
</tbody>
</table>

* 6-week-old BALB/c male mice given intraperitoneal inoculations of 0.2 ml. of virus preparation.
† One virus-inoculated donor mouse was sacrificed for each individual recipient; 0.2 ml. of heart blood from each donor mouse inoculated intraperitoneally into one recipient mouse.
‡ No further deaths occurred by day 140.
ous foci of lymphoid leukemic cells frequently interspersed with immature erythrocytes were prevalent.

A more complete pathogenesis study is in progress in which the disease-inducing capacity of blood and tissues is being related to pathological alterations found at autopsy.

Chemotherapy of transplanted virus-induced leukemia.—Studies also were undertaken on the chemotherapy of a virus-induced transplantable leukemia (line MCDV-12). Histological examination of tissues from mice 10 days after subcutaneous inoculation of leukemia MCDV-12 showed widespread infiltration and destruction of spleen, liver, and regional lymph nodes. The tumor arising at the site of implantation rapidly invaded adjacent muscle and infiltrated the subcutaneous fat. The primary tumor and the infiltration invariably seen in the tissues of the host were composed predominantly of large lymphoid cells, the nuclei of which were vesicular and pleomorphic. The cells were also characterized by prominent nucleoli and by a very narrow rim of intensely basophilic cytoplasm. Mitotic figures were numerous.

The chemotherapeutic response of mice implanted with this leukemia is summarized in Chart 3. Time to death of the untreated controls was more rapid and less variable than for mice given inoculations of virus alone. The MST of the control group (Chart 3, 1st panel) was 19.0 days. In the treated groups, drug therapy was initiated 3 days after implantation of cells and continued daily for 5 days. Of the four drugs tested, NSC-60339 exerted the most extensive therapeutic effect, 100 mg/kg producing a 7-day increase in MST over the untreated controls. Of the three levels of MTX tested, 0.36, 0.5, and 1.0 mg/kg produced a 2.5-, 2-, and 5-day increase in MST, respectively. There were minimal increases in MST following treatment with TEM and a 1-day increase in MST with the highest dose of 6-MP (100 mg/kg daily X 5).

In vitro effect of drug on Rauscher virus and on blood cells of Rauscher virus-infected mice.—In the in vitro system a known concentration of a standard virus preparation and a suspension of washed blood cells harvested from virus-inoculated mice were incubated with various concentrations of drug. Following incubation, the virus and cells were washed free of drug and inoculated into test mice.

Melphalan appeared to exert some antiviral effect when incubated in vitro with a cell-free virus preparation (Table 4). When mice were given inoculations of a virus suspension previously incubated with Melphalan (0.5 mg/ml), an increase in survival time was noted, and five out of ten mice did not develop splenomegaly. The lower concentration of Melphalan was essentially ineffective. TEM also appeared to have some effect at the lower concentration of the drug (0.25 mg/ml).

Melphalan, at the concentrations tested, had an inhibitory effect on the disease-inducing capacity of the blood cell suspension. More than a twofold increase in survival time over controls was obtained, and eighteen out of twenty mice did not develop splenomegaly. Similarly, mice given inoculations of TEM-treated cells showed a longer survival time than did control mice. In contrast to Melphalan, however, only five out of twenty mice did not develop splenomegaly.

Histological examination of tissues of mice sacrificed 190 days post-inoculation revealed characteristic erythropoiesis and infiltration with leukemic cells in the mice with splenomegaly. On the other hand, mice without splenomegaly appeared free of the disease.

DISCUSSION

Inoculation of the Rauscher virus into mice results in rapid virus multiplication and initiates a progression of responses in the host culminating in a systemic leukemia.

The current studies illustrate the use of mice given inoculations of the Rauscher virus for the assay of antitumor agents. Animals may be treated shortly after inoculation of the virus, prior to the appearance of any appreciable number of leu-
chemic cells, to study the influence of drugs on the virus and on the virus-host relationship. In the current experiments increases in the survival time of the mice were observed following treatment with 6-MP, TEM, and NSC-60339 from the 3d to the 7th day after inoculation of the Rauscher virus. The above may be supplemented by in vitro studies in which the virus is exposed directly to drug and subsequently bioassayed in vivo. Melphalan and TEM were tested for activity in vitro and appeared to exert limited antiviral effect. A more pronounced effect was noted when the drugs were tested in a similar manner against a suspen-

It is of interest to note that MTX, 6-MP, and TEM were also effective in limiting spleen weight with the Friend mouse leukemia (13, 14). MTX and 6-MP were effective in decreasing spleen size and spleen uptake of Fe59, and 6-MP prolonged the life span of mice with Friend virus (10). Similarly, MTX and 6-MP retarded the increase in weight of thymus, liver, and spleen of mice given inoculations of the Moloney virus (2).

The influence of drugs may also be tested for inhibition of splenomegaly and for their capacity to interfere with the transmissibility of the disease from treated donor to recipient animals. TEM,

| Incubation additions | Bioassay results (day 190) | Average spleen weight (gm.) of surviving mice sacrificed 190 days post-inoculation+
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Drug</td>
<td>Concentration (mg/ml)</td>
<td>Median survival time (days)</td>
</tr>
<tr>
<td>----------------------</td>
<td>---------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Virus 10⁴+ Cells:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melphalan</td>
<td>0.5</td>
<td>&gt;190</td>
</tr>
<tr>
<td>Melphalan</td>
<td>0.25</td>
<td>&gt;190</td>
</tr>
<tr>
<td>TEM</td>
<td>0.5</td>
<td>&gt;190</td>
</tr>
<tr>
<td>TEM</td>
<td>0.25</td>
<td>&gt;190</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal environment controls</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses represent the number of mice sacrificed for determining the average spleen weight. BALB/c mice were used throughout this experiment.

sion of blood cells from an animal with Rauscher leukemia. In the latter case a definite inhibitory effect on the disease-inducing capacity of the tumor cells was observed.

Alternatively, treatment of mice given inoculations of the virus may be withheld until the animals show definite evidence of leukemia or become grossly leukemic, for purposes of investigating the action of drugs on the leukemic cells and the virus-leukemia-host relationship. In the experiments where treatment was initiated at 24 weeks following inoculation of virus, treatment with methotrexate, 6-mercaptopurine, TEM, and NSC-60339 elicited significant increases in the lifespan of the animals.

Cytoxan, and 5-FU were shown to have some inhibitory effect on spleen enlargement in the primary host when treatment was given 15 days following virus inoculation. Under similar circumstances treatment with a relatively high dose of TEM appeared to interfere with the capacity of whole blood to transmit Rauscher disease to donor mice. If the drugs are administered shortly after viral inoculation, this type of experiment may be employed to determine the influence of drugs on viral multiplication in vivo. Such a system is providing the basis for a rapid in vivo antiviral screen in our laboratory (Chirigos).5

Collateral investigations of the influence of

| * M. A. Chirigos et al. (to be published).
drugs on transplantable lines of virus-induced Rauscher leukemia may provide additional information on the tumor-virus-host relationship. With the one line of leukemia employed in this study, NSC-60339 was effective in increasing survival time. MTX and TEM produced minimal increases in survival time and 6-MP was ineffective. It will be of particular interest to survey a number of transplantable lines of Rauscher leukemia in view of the findings of Glynn et al. that different sublines of transplantable Moloney leukemia show a spectrum of sensitivity to drugs.

Two leukemogenic viruses, the Moloney (2) and the Rauscher, are currently being employed in this laboratory in detailed studies on the influence of drugs on the host-tumor-virus-drug relationship. With the advent of transmissible viruses as etiological agents in the induction of murine leukemia, the importance of the host-parasite-drug triad stressed by Marshall (9) must now be considered as a tetrad, to include the virus too.

REFERENCES


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Cancer Research

Studies with the Murine Leukemogenic Rauscher Virus: I. Chemotherapy Studies with in Vivo and in Vitro Assay Systems


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