Effect of Cyclophosphamide on Friend Virus Leukemogenesis in Virus-sensitive and Virus-resistant Mice

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ABSTRACT

The influence of cyclophosphamide (CY) on Friend virus leukemogenesis was studied in SJL/J, C57BL/10J, and C57BL/10J x SJL/J F1 (hereafter called B10SJF1) mice. All three differ in their susceptibility to the virus oncogenic effect. Immunosuppressive doses of CY, which by themselves produced no cancer, were followed 2 days later by injection of Friend leukemia virus. The virus doses were the same as used previously. Although in other experiments preinjection of various chemical carcinogens augmented leukemogenesis by Friend leukemia virus in SJL/J mice, in the present study, pretreatment by CY had no such effect. In contrast, CY increased Friend erythroleukemia incidence from 15 to 100% in B10SJF1 mice, and from 0 to 85% in C57BL/10J mice. The disease in C57BL/10J mice had a 190-day incubation period, which is approximately 5 times that in the SJL/J and B10SJF1 mice. During this latent period, the C57BL/10J mice harbored infectious Friend leukemia virus in their plasma.

INTRODUCTION

The sensitivity of various inbred strains of mice to Friend viral leukemogenesis varies and is under complex genetic control (10, 11). Some genetic loci that influence virus sensitivity interfere with virus replication (7, 9, 24), while others may influence the ability of the host to inactivate virus and/or virally transformed cells by immune mechanisms (3, 7, 27). The Friend virus used in most laboratories appears to be a complex of at least 2 elements (5), and some of the virus resistance genes are concerned with the functioning of a helper virus (9, 24).

Chemical carcinogens and irradiation both potentiate transformation by oncogenic viruses of several types of cells in vitro (2, 13, 21) and in vivo (8, 15–17, 19). MMS, BP, and DMBA appear to potentiate Friend viral leukemogenesis in vivo by direct effect on virus target cells (15–17, 19).

In the present study, the influences of immunosuppressive doses of CY (a chemotherapeutic drug) on Friend virus leukemogenesis were compared in 3 genetically different mouse strains. These were the highly resistant C57BL/10J mice, the highly sensitive SJL/J mice, and C57BL/10J x SJL/J F1 (hereafter called B10SJF1) mice, their moderately virus-resistant hybrid. CY produced significant changes in FLV leukemogenesis in the 2 virus-resistant strains only. This difference in the reactions of SJL/J and B10SJF1 mice to chemical pretreatment with CY contrasts with results of previous studies (15–17, 19), in which carcinogenic chemicals potentiated FLV leukemogenesis in both SJL/J and B10SJF1 mice.

MATERIALS AND METHODS

Animals. Female SJL/J and C57BL/10J mice were purchased from The Jackson Laboratory when they were 7 weeks old and were held in quarantine until use at 10 to 12 weeks of age. The B10SJF1 mice were bred in our facilities from C57BL/10J females and SJL/J males. Both sexes were used when they were 10 to 14 weeks old. All animals were housed 6 or fewer per cage in plastic cages with wood chip bedding and filter tops. They were given autoclavable Purina laboratory chow and slightly acidified water ad libitum. All animal supplies were autoclaved before use, and the animal room was environmentally regulated.

Virus. FLV stock was obtained from the National Cancer Institute in 1969 and has been stored at -60°C. It was passaged once in SJL/J mice, and a plasma preparation was obtained for use in these studies. The FLV-rich plasma was titered by the spleen enlargement assay (4). The virus-containing plasma was diluted immediately before use with cold phosphate-buffered saline to give the appropriate SED of virus and injected i.p. The doses used were 0.1 SED for SJL/J mice and 100 SED for C57BL/10 and B10SJF1 mice. Our virus preparation produced polycythemia. Also, it is NB-tropic, since we have found it to be potently leukemogenic in BALB/c mice, which are Fv-1<sup>−</sup>, as well as in SJL/J mice, which are Fv-1<sup>+</sup>.

CY. CY (Mead Johnson) was dissolved in 0.9% NaCl solution (saline) immediately before use and injected i.p. at 150 or 200 mg/kg of body weight 2 days before the injection of FLV.

Monitoring for Leukemia Development. Experimental animals were followed for 300 days after virus or control (saline) injections. Their peripheral WBC counts were measured on Days 14, 30, 45, 72, 100, 150, and 200. Blood samples with elevated WBC counts were also spot checked for morphology of the cells involved on Wright-stained blood smears. Dead animals were picked up daily and necropsied. Any abnormalities were noted, and the spleens were weighed. Representative enlarged spleens were examined with hematoxylin-eosin staining. Plasma of C57BL/10 mice that received FLV was also checked for the presence of FLV. For this purpose, 3 C57BL/10 mice from each of the 2 groups that received FLV were sacrificed on Days 41 and 90 after virus injection. Blood was collected from the inferior vena cava of anesthetized mice. One-half ml of plasma so obtained was injected i.p. to test SJL/J mice, and these were monitored for leukemia development.

RESULTS

The survival data of mice given injections of CY alone, FLV alone, or CY 2 days before FLV are shown in Chart 1, A to C, for SJL/J, B10SJF1, and C57BL/10J mice, respectively. The doses of CY and FLV used were those that produced minimal mortality in each strain when injected alone. Thus, while CY, 200 mg/kg of body weight, was used in SJL/J and B10SJF1 mice, only 150 mg/kg was used in C57BL/10J mice, because the latter were more sensitive to the drug. Also, 100 SED doses of FLV were used in B10SJF1 and C57BL/10J mice, while only 0.1 SED was used in SJL/J. The latter represents a minimum workable dose, which nevertheless caused 90% mortality in SJL/J mice.
by Day 240.

SJL/J Mice. Only one of the 10 mice given CY alone died of undetermined cause, whereas 9 of the 10 mice given FLV alone died with signs of either erythroleukemia and/or lymphoma, as was expected for this virus dose (Ref. 16; Chart 1A). The 12 mice given CY 2 days before FLV died in a pattern indistinguishable from those that received FLV alone, although splenomegaly and WBC counts were lower in the group that received both as compared to the group that received virus only (Table 1). This difference was not significant by the Student t test.

B10SJF1 Mice. Neither CY nor FLV alone had a significant effect on the survival of B10SJF1 mice (Chart 1B). However, the 4 of 47 mice in the FLV group that did die had signs of erythroleukemia, i.e., splenomegaly due to cells that appeared malignant in hematoxylin-eosin-stained slide preparations and elevated peripheral WBC counts. All of the 23 mice that received CY plus FLV died with these signs (Table 1).

C57BL/10J Mice. FLV alone had no effect on the survival of the 14 mice tested, and only one of the 10 mice given CY alone died of undetermined cause (Chart 1C). The survival of 12 mice given injections of CY 2 days before FLV was followed for 300 days. Ten of these (83%) died between Days 190 and 300 after FLV injection with signs of erythroleukemia (Table 1). The WBC elevation, although moderate, was due mainly to typical Friend cells (12) and to erythroblasts. The onset of erythroleukemia in C57BL/10J mice was delayed by approximately 150 days as compared to SJL/J or B10SJF1 mice. Twice during the latent period, i.e., on Days 41 and 90 after virus injection, sample mice were sacrificed to obtain plasma, which was then tested for its leukemia-producing potential in SJL/J mice. The plasma of C57BL/10J mice that received FLV only did not cause erythroleukemia in test SJL/J mice, while 100% of the SJL/J test mice given injections of plasma from C57BL/10J mice that received both CY and FLV died with erythroleukemia (Table 2).

DISCUSSION

The present results show that similar pretreatments by CY potentiate FLV-caused leukemogenesis in B10SJF1 and C57BL/10J mice but not in SJL/J mice. On the contrary, in SJL/J mice,

### Table 1

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Treatment</th>
<th>Spleen wt (g)</th>
<th>WBC ($n \times 10^9$/ml blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SJL/J</td>
<td>CY only</td>
<td>0.3880 ± 0.0980</td>
<td>14.4 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>FLV only</td>
<td>1.5896 ± 0.2259</td>
<td>7.9 ± 14.07</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>0.6637 ± 0.1352</td>
<td>18.8 ± 2.88</td>
</tr>
<tr>
<td>B10SJF1</td>
<td>CY only</td>
<td>0.1622 ± 0.3340</td>
<td>9.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>FLV only</td>
<td>0.3822 ± 0.1034</td>
<td>21.7 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>2.0751 ± 0.2272</td>
<td>39.9 ± 8.2</td>
</tr>
<tr>
<td>C57BL/10J</td>
<td>CY only</td>
<td>0.1001 ± 0.0100</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FLV only</td>
<td>0.1262 ± 0.1026</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>0.6637 ± 0.1352</td>
<td>17.8 ± 5.6</td>
</tr>
</tbody>
</table>

* Average ± S.E. of spleen weight at death.
* Average ± S.E. of peripheral WBC count.
* WBC counts not done in these groups. WBC counts in normal C57BL/10J mice do not exceed 15 x 10^9.

### Table 2

<table>
<thead>
<tr>
<th>Day after FLV injection on which plasma was collected</th>
<th>CY 2 days before FLV</th>
<th>FLV only</th>
</tr>
</thead>
<tbody>
<tr>
<td>41</td>
<td>5/5</td>
<td>0/4</td>
</tr>
<tr>
<td>90</td>
<td>3/3</td>
<td>0/4</td>
</tr>
</tbody>
</table>
CY had a slight protective effect (Table 1), which was more pronounced with higher FLV doses (data not shown), indicating that the lack of a potentiating effect on FLV leukemogenesis in SJL/J mice is not due to the lack of CY activation in these mice. Moreover, the absence of leukemogenesis potentiation is not an inherent characteristic of this experimental system because, as reported previously, FLV potentiation in SJL/J mice can be demonstrated with MMS, BP, or DMBA (15, 16, 19). Therefore, CY apparently does not produce virus-potentiating lesions of the type produced by MMS, BP, and DMBA.

Two principal modes of action by which chemical pretreatment can potentiate in vivo viral oncogenesis are: (a) by directly acting on the virus target cell to be transformed and (b) by indirectly affecting the survival of transformed cells via immunosuppression.

Analyses of the in vivo effects of MMS and BP in correlation with FLV potentiation (15, 19) as well as other studies of the in vitro action of these chemical carcinogens (2, 13, 20, 21) indicate that they use primarily the direct mode of action. CY, an alkylating agent and a suspected carcinogen (25), which produces severe immunosuppression largely due to effects on T-cells (6, 22), may act mainly via the second, indirect route. Characteristics of the mouse strains used in the present study support this conclusion.

Leukemia virus infection in B10SJF, mice, but not in SJL/J mice, increases immune activity (14). Thus, immune elimination of virus and/or virally transformed cells appears to be an important part of the viral resistance of B10SJF, mice. Other F, hybrid of virus and/or virally transformed cells appears to be an important part of the viral resistance of B10SJF, mice. Therefore, not expected to play a role in the resistance of B10SJF, hybrids. Moreover the Fv-1 locus cannot be involved in this resistance because our FLV preparation is NB-tropic.

Immune functions have also been implicated in the resistance of C57BL/10 mice, since FLV can produce leukemia in these mice when injected to neonates (3) or after bone marrow is destroyed in C57BL/10 mice with strontium-89 treatment (7). Leukemia virus resistance of C57BL/10 mice based on their Fv-2* genotype that limits intratarget cell replication of one of the viral components (1) may help explain why even with severe immunosuppression leukemia develops in C57BL mice only after an extremely long lag period. During this, the few leukemic cells, established while the mice are immunosuppressed by CY, must expand to eventually become clinically evident. This extended lag period also explains why previous experiments of shorter duration failed to demonstrate the viral potentiation effect of CY in this mouse strain (23).

The present measurements of the fate of the virus inoculum in the plasma of CY-treated and untreated C57BL/10 mice agree with a previous paper (18), that the number of virus particles assayable by the XC-plaque test increased in FLV-inoculated athymic (nude) or CY-treated C57BL/10 mice but not in normal C57BL/10 mice given injections of either FLV or CY alone. The present results indicate that sufficient spleen focus-forming virus particles (which are not measured by the XC-plaques test, but which are required for erythroleukemia production) are also present in the plasma of immunosuppressed C57BL mice during a long latent period.

It is concluded that, in SJL/J mice, where cell transformation by leukemia virus is extremely efficient, immunosuppression does not affect leukemia progression. In other mouse strains, like B10SJF, and C57BL/10J where the number of cells transformed by the virus is low, even transient immunosuppression may be important for prognosis.

REFERENCES

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