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Potentiation of Friend Viral Leukemogenesis by 9,10-Dimethyl-1,2-benzanthracene in Two Strains of Mice[1] (41619)

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Abstract. The polycyclic aromatic hydrocarbon, 9,10-dimethyl-1,2-benzanthracene (DMBA) produced malignancy involving the spleen in SJL/J and B10SJF1 mice when injected ip at 500 μg per mouse either alone or in combination with threshold doses of Friend leukemia virus (FLV). The mice that received both chemical and virus died significantly sooner than mice that received either chemical or virus alone, and a synergism between DMBA and FLV was demonstrated in both the virus-resistant B10SJF1 hybrids and virus-sensitive SJL/J mice.

Potentiation of viral action by chemical carcinogens has been observed in several *in vitro* (1–3) and *in vivo* (4–8) systems. We reported previously that injection of chemical carcinogens, methyl methane sulfonate (MMS) (6, 7) or benzo(a)pyrene (BP) (8, 9) potentiated viral leukemogenesis in SJL/J and in virus-resistant B10SJF1-hybrid mice. In those experiments the leukemia that developed was characteristic of the Friend leukemia virus (FLV) inoculated. We also demonstrated that a peak of virus potentiation occurred in SJL/J mice when the interval between chemical and virus injections was 5 hr for MMS and 2 days for BP. When virus injection was delayed 24 hr beyond those optimal points the potentiating effects of MMS and BP on viral leukemogenesis in SJL/J mice were no longer significant. Thus, the virus-potentiating lesions produced by MMS and BP were apparently repairable.

The present report deals with the influence of another common carcinogen, 9,10-dimethyl-1,2-benzanthracene (DMBA), on virus leukemogenesis. We will show that this chemical significantly accelerates the incidence of Friend erythroleukemia in SJL/J mice and significantly increases the incidence of typical erythroleukemia in virus-resistant B10SJF1 mice.

Materials and Methods. *Animals.* Female SJL/J mice were purchased from Jackson Laboratories when they were 7 weeks old and were used when they were 10–12 weeks old. B10SJF1 mice were bred in our own facilities from Jackson C57BL/10J males and SJL/J females. Both sexes of B10SJF1 mice were used when they were 10–14 weeks old. The animals were housed six per cage in plastic cages with wood chip bedding and filter tops. They were given autoclavable Purina Lab Chow and slightly acidified water *ad libitum*. All animal supplies were autoclaved before use and the animal rooms were maintained at a constant temperature (21–22°C) and light–dark cycle (12 hr).

DMBA. The 9,10-dimethyl-1,2-benzanthracene (Eastman) was dissolved in trioctanoin oil (Eastman) at 5 mg/ml, filter-sterilized, and refrigerated in dark containers for no more than 30 days before use. Oil alone, or oil containing 500 μg of DMBA were injected ip in mice as indicated.

Virus. The virus stock used was that described previously (6–8). Approximately 0.1 and 100 SED (spleen enlargement dose) was injected ip in SJL/J and B10SJF1 mice, respectively, Virus dilutions were made with cold physiological saline immediately before use. In each experiment all of the groups received the same batch of diluted FLV and the groups receiving virus alone were injected first.

Monitoring malignancy development. Peripheral white blood cell counts were made on Days 14, 30, 45, 72, 100, 150, 200, and 250 after virus or control (saline) injections. Cages were checked daily for dead mice, which were promptly necropsied. The condition and

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weight of the spleen, the condition of the lymph nodes and thymus gland, and any other abnormalities were noted. All remaining animals were sacrificed and necropsied on Day 300 after virus or control (saline) injection. For histologic examination, spleens were fixed in phosphate-buffered Formalin and stained with H&E.

Statistical analysis. The significance of the differences between survival curves was tested by life table statistics (10).

Results. *SJL/J mice.* Figure 1 compares the survival pattern of SJL/J mice given DMBA 8 days before virus (solid line) with the survival of their littermates that were given injections of the same preparations of either DMBA (dashed line) or FLV (dotted line) separately. None of the sham-treated control mice (i.e., mice that were injected with only trioctanoin and saline) died during our 300 day observation period and therefore their survival is not plotted in the figure. Injection of either virus or DMBA alone had a significant effect on survival, reducing the median survival time to 220 and 180 days, respectively. However, mice that received both DMBA and FLV died even earlier than mice that received either of these carcinogens alone. This difference was significant at the level of $P < 0.01$. To determine what extent the increase in deaths seen in the mice receiving both agents might reflect an additive effect of the two agents, the dot–dash line of Fig. 1 was calculated. This was done by combining the data for virus alone and DMBA alone with the assumption that deaths due to either agent were independent of deaths due to the other agent. As the figure indicates, this calculated line lies significantly to the right of the actual observed survival curve for the animals that received both DMBA and then FLV 8 days later. This difference between the calculated and observed curves is significant at $P < 0.05$ for the period between 98 and 190 days after carcinogen exposure. This suggests the possibility of a DMBA induced potentiation of the Friend viral leukemia.

Most of the mice that received virus either alone (23 mice) or in combination with DMBA (25 mice) exhibited signs characteristic of FLV leukemia (12, 13). These signs were peripheral white blood cell counts at least 2× above the average normal level and spleen weights at least 3× above the average normal level. The average normal control values for these were 12,000 WBC/ml and 0.1 g, re-

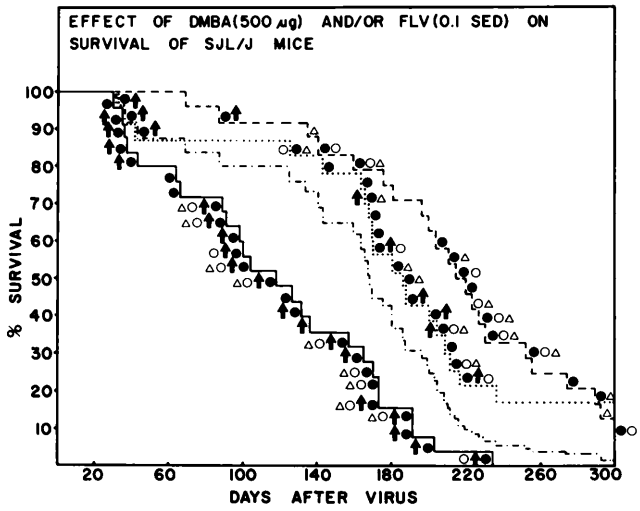

FIG. 1. Survival of SJL/J mice that were injected with either DMBA only, (– – –) FLV only (• • •) or DMBA 8 days before FLV (——). Day 0 is the day of FLV injection. A line representing the theoretical survival curve was calculated by assuming that the effects of DMBA and FLV are additive (–•–). The difference between the calculated (–•–) and actual curves (——) is significant between Days 98 and 190. The symbols represent signs found in the individual mouse that died at each time point. They are as follows: ●, enlarged spleen, △, enlarged thymus; ↑, elevated blood cell counts; ○, enlarged lymph nodes.

spectively. In addition, 52% of the mice that received virus, either alone or in combination with DMBA, had enlarged thymus glands and 23% also had somewhat enlarged lymph nodes. Whether any given mouse had these signs is indicated by various symbols next to the record of its death in Fig. 1. It should be noted that an enlarged spleen was found in all of the mice that received virus except the last to die in the virus alone group, which was cannibalized before it could be autopsied. Moreover, some elevated white blood cell counts may have been missed because the mice died shortly before a scheduled assay.

Twenty-two out of the 24 mice that received only DMBA died by Day 300. Of these, 10 had enlarged spleens (spleen weight > 0.3 g), 8 had enlarged thymus glands, and 8 had enlarged lymph nodes as indicated in Fig. 1. Only one mouse that received DMBA alone had an elevated white blood cell count. This count was 26,100 cells/ml which is only slightly higher than the normal deviation for the control mice. Histological examination of spleens from mice that received DMBA either alone or in combination with virus showed that in each case they were enlarged by proliferation of cells with irregular nuclei, which appeared to be malignant.

Figure 2 shows survival of B10SJF1-hybrid mice that received DMBA and/or 100 SED FLV according to the same schedule as the experiment of Fig. 1. This mouse is a hybrid between the FLV-sensitive SJL/J mouse and the FLV-resistant C57BL/10. Like its C57BL/10 parent, this hybrid is also normally resistant to leukemia induction by Friend virus. The virus inoculum given 5 days after injection with trioctanoin had no effect on survival within the 300-day observation period (12 mice tested, dotted line of Fig. 2) and only one of these mice had an elevated white blood cell count and an enlarged spleen found when it was sacrificed on Day 300. DMBA alone killed 8 out of the 10 mice tested. Five of the mice that died during the study in the group that received DMBA alone had enlarged spleens. However, these spleens looked ulcerated and did not resemble the usual appearance of the enlarged spleens of mice with Friend leukemia. In addition, two of the hybrid mice in the DMBA-alone group had enlarged thymus glands at death. All of the mice that died in the DMBA plus virus group (i.e., 13 out of 15 mice tested) had grossly enlarged spleens. However, only two of these appeared ulcerated, and the others were all characteristic of Friend leukemia. One of the two mice

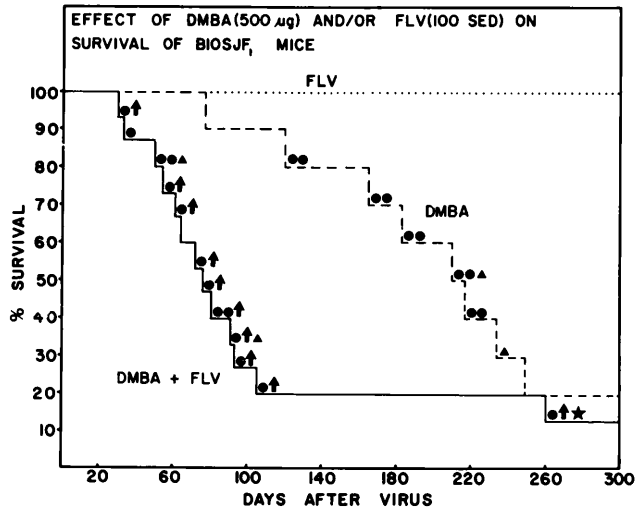

FIG. 2. Survival of B10SJF1 mice that were injected with either DMBA only (– – –), FLV only (· · ·) or DMBA 8 days before FLV (——). Day 0 is the day of FLV injection. The difference between the survival of mice that received DMBA alone (– – –) and 8 days before FLV (——) is significant between Days 60 and 210. The symbols represent signs found in the individual mouse that died at each time point. They are as follows: ●, enlarged spleen; ●●, enlarged and ulcerated spleen; ▲, enlarged thymus; ↑, elevated white blood cell counts; ★, tumor at injection site.

with ulcerated spleen also had a tumor at the DMBA injection site. Eleven of the mice that received DMBA and virus, but none of the mice that received DMBA alone, had elevated white blood cell counts. The occurrence of these various signs is indicated by symbols in Fig. 2. The difference in survival patterns between the mice that received DMBA alone and those that received DMBA and virus was significant between Days 60 and 210 at the level $P < 0.01$.

Discussion. Earlier reports in the literature have indicated that DMBA can induce endogenous leukemogenic virus in C57BL/6 mice (11) and CFW mice to cause malignancy (14). Therefore, our results suggesting a possible interaction between DMBA and FLV are consistent with these earlier findings. In addition to this, SJL/J mice can develop a spontaneous lymphoma after a year or more of life, which may be caused by an endogenous lymphogenic virus (15). Our data for the DMBA only group reflected the possibility that this also may have been accelerated. Other carcinogenic action of the single DMBA exposure used by us was negligible within the 300-day observation period, since only 1 of the 73 mice that received DMBA, either alone or in combination with virus, developed a solid tumor at the site of injection. There were no apparent mammary carcinomas such as are found in Lewis rats fed DMBA (16).

Our FLV stock is a complex of at least two components: the spleen focus forming virus (SFFV) and the lymphatic leukemia virus (LLV) (22). The SFFV component, in the presence of LLV produces erythroleukemia (22). Then after a longer latent period than is required for the action of SFFV, the LLV component produces a lymphoma involving both lymph nodes and thymus (23). Thus, our virus inoculum can cause both erythroleukemia and lymphocytic leukemia. This complication and the possibility that endogenous leukemogenic viruses may become involved (11, 14) often makes classification of the exact subtype of Friend disease (12, 13) found associated with each death difficult. However, despite the difficulty in subtype classification, it is possible to determine if the animal shows the general characteristics of Friend leukemia, and our data do indicate that DMBA can accelerate or potentiate fatal Friend disease. Specifically, the results indicate an acceleration of Friend viral leukemogenesis in the SJL/J mice which are sensitive to the virus and a potentiation of Friend disease by DMBA in B10SJF1 mice which are normally resistant to it. Moreover, in the B10SJF1 mice the disease produced after both DMBA plus FLV was characterized as an erythroleukemia with Friend characteristics. In contrast, although some of the hybrids that received DMBA alone developed a splenic malignancy, none of these malignancies had the characteristics associated with Friend disease.

We have previously demonstrated a potentiating effect of methyl methane sulfonate (MMS) and benzo(a)pyrene (BP) on FLV leukemogenesis (6–9). However, in contrast to the present results where injection of DMBA alone resulted in malignancy of the hemopoietic system, neither MMS nor BP produced a significant incidence of any malignancy when injected alone. DMBA, like BP, is a polycyclic hydrocarbon that is metabolized by microsomal mixed oxidases (17) to yield active carcinogens that form adducts to DNA (17, 18). These can result in breaks in cellular DNA (19) that have been associated with increased transformation of mammalian cells by adenovirus (1) or Rauscher leukemia virus (19). Our previous results (8, 9) indicated that BP potentiation of FLV in SJL/J mice is maximal at 2 days and is reversed by 3 days after virus. In contrast, the present data indicate that the DMBA potentiation of FLV is significant after 8 days. The reason for the difference between the timing of the effectiveness of BP and DMBA is not known. It may possibly relate to the necessity of removing methyl groups that block the bay region of DMBA thought to be active in polycyclic hydrocarbon action (20). Moreover, we have observed that in contrast to BP and MMS, DMBA can cause a prolonged depression of the ability to mount a humoral immune response to sheep red blood cells (21) and this difference between DMBA on one hand and BP and MMS on the other may also play a role in the different timing of their virus-potentiating action and/or their ability to produce malignancy in the hemopoietic system under our conditions.

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