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Effects of Benzo(a)Pyrene on Friend Viral Leukemogenesis in B10SJF$_1$ Mice[1] (41524)

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Abstract. A single intraperitoneal injection of benzo(*a*)pyrene (BP) given 1, 2, or 3 days before an ip injection of Friend leukemia virus (FLV) significantly increased the leukemogenic effect of the virus in B10SJF$_1$ mice. These hybrids are the offspring of C57BL/10 females and SJL/J males and are highly resistant to FLV leukemogenesis when the virus is injected alone.

The possibility that viral carcinogenesis can be potentiated by chemical carcinogens or by radiation has been suggested by results obtained in several systems: some of these involved the activation of endogenous virus (1–3) and others involved increasing effectiveness of externally applied virus (4–8). *In vitro* studies demonstrated that the chemical agents can affect viral target cells directly (4, 5, 9) and some results suggested that the critical virus-potentiating lesions are correlated with DNA strand breaks (4, 10).

We have previously reported that methyl methane sulfonate (MMS) potentiates FLV leukemogenesis in SJL/J and B10SJF$_1$ mice (6). The cocarcinogenic effect of this low-molecular-weight alkylating agent was evident when MMS was injected 5 hr before virus but not when MMS was injected 24 hr before or 5 hr after virus to SJL/J mice, suggesting that the MMS-caused effect is quickly repaired. Similarly, Casto (4) showed that quickly repaired, MMS-caused lesions in DNA are potentiating to adenovirus transformation of hamster embryo cells in tissue culture. We have also shown that BP causes significant potentiation of Friend viral leukemogenesis in SJL/J mice when the chemical is injected 2 days before the virus, but that this potentiation is not seen when the interval between BP and virus injections is 1 or 3 days (7).

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B10SJF$_1$ mice are produced by crossing SJL/J males and C57BL/10J females. They are relatively resistant to FLV. In the present experiments a high dose of this virus (spleen enlargement dose, SED = 100), when given alone, produced fatal erythroleukemia in 20 to 25% of these mice. This should be compared with the total resistance of C57BL/10J mice (13) and the extreme sensitivity of SJL/J mice to this virus. In C57BL/10J mice 100 SED of FLV given to adult mice produced no leukemia or lymphoma within a 300-day observation period (our data in preparation). However, in SJL/J mice a 1000-fold lower virus dose (0.1 SED) caused 80% or more deaths due to erythroleukemia and/or lymphoma within our 300-day observation period (12).

This report presents results that show potentiation of viral leukemogenesis in B10SJF$_1$ hybrids when BP is injected at 1, 2, or 3 days before the virus. The fact that BP was potentiating after all of these time periods contrasts with our experience with SJL/J mice where significant BP potentiation was seen at Day 2 only and not at 1 or 3 days.

Materials and Methods. *Animals.* B10SJF$_1$ mice were bred in our own facilities from SJL/J males and C57BL/10 females purchased from Jackson Laboratories, Bar Harbor, Maine. The animals were maintained in plastic cages with wood chip bedding, filter tops, autoclavable Purina Lab Chow, and slightly acidified drinking water. All of these materials were autoclaved before use. Both female and male mice ranging in age from

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10 to 19 weeks were used. Data from all mice that received the same treatment were pooled since no significant differences between the responses of male and female mice could be demonstrated.

Benzo(a)pyrene (BP). BP (Sigma, St. Louis, Missouri) was dissolved in trioactanoin oil at 5 mg/ml and filter sterilized. One-tenth of 1 ml of this solution was injected ip per mouse. Control mice received 0.1 ml of trioactanoin oil only.

Friend leukemia virus (FLV). Our stock of FLV was obtained from NCI in 1968. It was passaged once through SJL/J mice: the virus-rich plasma of these mice, sacrificed 2 weeks after virus injection, was diluted 1:1 with 9% Na-citrate buffer, titered by a spleen enlargement assay (14), and stored at −60° in small aliquots. Appropriate dilutions with cold physiological saline were made immediately before use.

Monitoring of the experimental animals. Cages were checked daily and dead removed for necropsy. The weight of the spleen and the state of the thymus gland and lymph nodes were noted. The mice were monitored for 300 days, with the day virus was injected taken as Day 0. White cells in the peripheral blood were counted on Days 14, 30, 45, 72, 100, 150, 200, and 250 after the injections of virus or the virus vehicle. Spot checks of the cell profile of peripheral blood showed characteristics of erythroleukemia (15) whenever the white blood cell counts were elevated. All of the surviving animals were sacrificed on Day 300 and necropsied.

Statistics. The significance of the differences between survival curves was tested using life table statistics as described by Colton (16).

Results. Figure 1 shows the survival of B10SJF$_1$ mice that were given BP alone, FLV alone, or BP 1, 2, or 3 days before FLV. BP alone had no significant effect on the survival of the 10 mice tested since only one of these mice died (on Day 280). FLV injected alone produced 20–25% fatal erythroleukemia by Day 300. BP injected at 1, 2, or 3 days increased the leukemogenic effect of FLV. For the BP -1 and -2 days experiments statistically significant potentiation of FLV leukemogenesis began around Day 100 and continued to the end of the monitoring period.

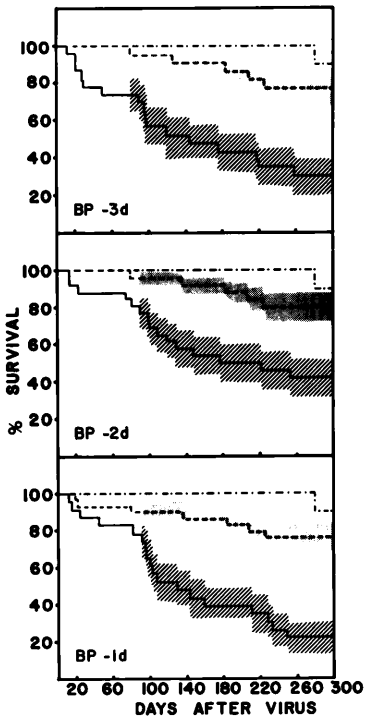

FIG. 1. Survival of B10SJF$_1$ mice injected intraperitoneally with BP alone (500 μg/mouse) (-·-·-) with FLV alone (100 SED/mouse) (- - -) or with BP at 1, 2, or 3 days before FLV as indicated (——). Day 0 equals the day of FLV or FLV vehicle injection. Data shown represent five independent experiments all of which produced consistent results. There were 10 mice in the BP only group and all of the other groups had 20 or more mice. The shaded areas represent the extent of the standard error.

For the BP -3 days experiment significant potentiation started on Day 50 and also continued to the end of the experiment.

All of the mice that died within the 300-day observation period displayed splenomegaly (spleen weight at least three times normal) without any thymic or lymph node enlargement. Splenomegaly is the most characteristic sign of Friend erythroleukemia (15, 17) and indicates that the decreased survival was probably due to FLV-caused leukemia.

The white blood cell count data was consistent with the spleen enlargements. White blood cell count elevations, i.e., at least 2× normal, were nearly always seen in the mice that subsequently died with enlarged spleens. A few exceptions to this correlation between

white blood cell count elevations and death with splenomegaly were found in mice that died just before scheduled white blood cell counts. The white blood cell elevations ranged from two to five times normal level. The peak elevations were not always found at the last sampling before death, but in approximately 50% of the mice there was a decrease to nearly normal levels before death. The cause of this decrease is not known but was associated with the general debilitation of the animal. Spot checks of peripheral cell morphology smears of blood with elevated white blood cell counts indicated that the elevations were mostly due to proerythrocytes, Friend cells (described by Metcalf *et al.* (15) as cells with foamy nuclei) and polymorphonuclear leukocyte (also described by Metcalf *et al.* (15) in blood of mice infected with Friend virus). Moreover, at least a few of these cell types, characteristic of Friend leukemia, were found in mice dying with enlarged spleens but with blood counts that had returned to normal levels. Thus the peripheral blood cell profiles corroborated the splenomegaly in evidence that the mice were dying of leukemia caused by the Friend leukemia virus.

Discussion. The demonstration of potentiation of viral leukemogenesis in F_1 hybrid mice which are relatively resistant to viral leukemia when virus is injected alone, may be more relevant to natural situations than such demonstration in inbred mouse strains that are highly sensitive to virus alone, since extremely sensitive individuals are rarely found in nonlaboratory situations. The genetic basis of the resistance of the $B10SJF_1$ hybrid is not known.

Resistance to Friend virus is known to be influenced by several genes (18), the most characterized of which being the Fv-1 and Fv-2 loci. The Fv-1 locus decreases susceptibility to the virus when its allele is not matched with the tropism of the infecting virus (19). Since the C57BL/10 parent carries an Fv-1bb genotype (20) and resistance is dominant (19), the Fv-1 gene could be a factor in the resistance of our $B10SJF_1$ hybrid if our virus were N-tropic. However, our virus is either B or NB tropic since we have found it potently leukemogenic in Balb/c mice that carry the Fv-1bb genotype (19, 20). Therefore, the Fv-1 gene is probably not a factor in the resistance of the $B10SJF_1$ mice to our FLV stain.

The C57BL/10 parent is extremely resistant to FLV primarily because of the resistance alleles it carries at the Fv-2 locus. This was shown by Odaka (21), who tested a congenic strain to C57BL/10 mice that differed from this strain only in that it carried the sensitivity allele of the Fv-2 locus, and found this congenic strain to be highly sensitive to FLV leukemogenesis. However, the resistance allele of the Fv-2 locus is recessive in crosses with several virus-sensitive mice other than SJL/J (22). Therefore, unless the Fv-2 sensitive allele of SJL mice is unusual or gene complementation occurs, other genes beside Fv-2 are involved in the resistance of the $B10SJF_1$ hybrid (23, 24). Tests of humoral function of this hybrid indicate that leukemia virus stimulates the ability of sheep red blood cells to induce antibody synthesis (25). Therefore, immunological factors may be important in the virus resistance of $B10SJF_1$ mice. The importance of immunological factors in the resistance of C57BL/10 mice has also been suggested by the observation that marrow ablation with ^{89}Sr makes C57BL mice virus sensitive (26).

All of the $B10SJF_1$ mice that died in the experiments reported here did so with gross splenomegaly and without significant lymph node enlargements. Furthermore, elevated white blood cell counts due to cells resembling proerythrocytes and "Friend cells" (15) were seen in most of the mice that died. Thus, their deaths were most likely caused by the FLV inoculum (15, 17). We have previously reported that BP also enhances FLV leukemogenesis in virus-sensitive SJL/J mice. However, in contrast to the situation in $B10SJF_1$ mice, in SJL/J mice we often saw lymph node enlargement after virus injection (7, 11). This difference between the signs caused by FLV in $B10SJF_1$ and SJL/J mice may be due to the nature of our virus inoculum. FLV is a complex of at least two elements, the more numerous of which is a virus that by itself can induce a lymphoma with a late time of onset (LLV) and which appears to be a necessary helper for the erythroleukemia inducing virus (SFFV) (27). In order to be able to demonstrate virus poten-

tiation by BP in SJL/J and B10SJF$_1$ mice, we needed to use a 1000× lower virus dose in the former than in the latter. Thus, it is possible that certain individual SJL/J mice received only the LLV component of the FLV complex. Indeed the original demonstration of the LLV element was accomplished by just such virus dilution (28). Because of the relatively large doses of FLV used, such a separation is unlikely to occur in B10SJF$_1$ mice.

Another difference in the responses of SJL/J and B10SJF$_1$ mice to BP and FLV involved the optimal timing between the injection of chemical and the virus. In our previous experiments with SJL/J mice, we have seen that significant FLV potentiation by BP occurred only when the chemical was injected 2 days before virus and not when it was injected 1 or 3 days before virus (7, 11). On the other hand, our present data with B10SJF$_1$ mice show that BP pretreatments at 1, 2, or 3 days before virus are equally effective. The reason why the BP potentiation of FLV seems to be shorter lived in SJL/J mice than in B10SJF$_1$ mice is not known. Our results with SJL/J mice indicate that the presently used dose of BP causes potentially lethal lesions in virus target cells, CFU-S (11) with a peak effect at 2 days after BP injection. On the other hand, a depression of the ability to mount a humoral immune response was not found to be linked to the BP caused virus potentiation (7). The observation that BP potentiation of FLV is shorter lived in SJL mice than in the hybrid suggests the possibility that different mechanisms of BP-FLV cocarcinogenesis may be operative in these two mouse strains.

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