Effect of benzo[a]pyrene on Friend virus leukemogenesis, CFU-S viability, and induction of humoral immunity[1]

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Summary. Benzo[a]pyrene (BP) potentiated the induction of leukemia by low doses of Friend virus in SJL/J mice if injected 2 days before the virus. BP also reduced the viability of hematopoietic stem cells (CFU-S) within this time interval but had little effect on the induction of humoral immunity (the PFC response).

We have previously reported that benzo[a]pyrene (BP) injected i.p. to SJL/J mice at 25 mg/kg potentiates viral leukemogenesis in vivo and that caffeine injected 4 h before the virus further increases this effect[2]. This action of caffeine suggested that repairable damage to target cell DNA may be involved, but this interpretation was not the only possible explanation[3]. Since many carcinogens are immunosuppressive[4] it was also possible that the expression of leukemia in vivo might be enhanced by effects on the immune response.

We now report further data on the timing of the BP-caused virus potentiation and compare it with the timing and magnitude of BP effects on the viability of hematopoietic stem cells, CFU-S[5] and on the induction of humoral immunity or plaque forming cell (PFC) response. CFU-S or hematopoietic stem cells are major targets for Friend leukemia virus[6]. Therefore BP effects on CFU-S viability could indicate the possibility of a potentiation of viral leukemogenesis by BP at the target cell level. Among the progeny cells of the CFU-S are mature lymphocytes that are active in the PFC response[6]. Because the PFC response technique measures a function that reflects the interaction of both T and B lymphocytes[7], evaluation of this response may provide some information on the immunosuppression effect of chemical carcinogens and on the role of immunosuppression in the potentiation of viral leukemogenesis by carcinogenic chemicals.

Materials and methods. Animals. 8-week-old female SJL/J mice were purchased from Jackson Laboratories and held for 2–4 weeks before use. The animals were housed in plastic cages with wood chip bedding in an environmentally controlled room and fed Purina Lab Chow and acidified water ad libitum. All the supplies and food were sterilized before use.

Injections. BP (Sigma) was dissolved in trioctanoin oil at 5 mg/ml. This was then filter-sterilized and stored in a dark container under refrigeration for no longer than 30 days. The Friend leukemia virus (FLV) (stock previously described[2,8]) was diluted with cold saline to 0.1 SED 50/14 (spleen enlargement dose[8]) immediately before use. Both the chemical and viral carcinogen were injected i.p. at the times indicated.

Survival studies. Mice injected with BP and/or FLV were monitored for 300 days after virus injection. Their peripheral white blood cell counts were checked periodically to monitor the development of leukemia. The cause of death was confirmed by autopsies. Splenomegaly is one of the principal indications of Friend leukemia. Accordingly the weight and condition of the spleen as well as the condition of thymus glands and lymph nodes were noted.

CFU-S assay. The measurement of CFU-S followed the Till and McCulloch method[9]. This involves the injection of spleen cell suspensions via the tail vein into lethally irradiated SJL/J mice. After 8 days the recipient mice are sacrificed and foci of lightly colored cell clones are counted on the surface of Picric acid-fixed spleens.

PFC assay. The measurement of PFC followed the Kennedy Axelrad modification[10] of the Jerne plaque assay technique[11]. This involves the sensitization of mice with an i.p. injection of washed sheep red blood cells followed by the assay of antibody-producing spleen cells 5 days later. For the in vitro visualization of these cells a monolayer of sheep red cells is fixed to plastic petri dishes with poly-L-lysine. Spleen cells are then layered on top of this monolayer and allowed to incubate at 37 °C for 1 h. Plaques formed by red cell lysis are visible with approximately magnification × 10.

Results. Mortality due to Friend leukemia. The figure shows survival data indicating BP potentiation of Friend viral leukemogenesis in SJL/J mice. All of the mice that died before day 180 of the period monitored had signs of Friend leukemia, i.e. grossly enlarged spleens and 3–5-fold elevations of peripheral white blood counts due at least partially to proerythroblasts[2,8]. The mice that died after day 200 did not have these signs of Friend leukemia but had enlarged lymph nodes. Therefore the cause of death in these mice could not be unequivocally attributed to the FLV inoculum. These deaths are not included here because the plot ends at day 200. BP increased the number of mice dying of erythroleukemia when injected 2 days before the virus. This effect was statistically significant after day 135 by life table analysis[12]. The slight potentiation evident in the figure after 3 days was not statistically significant.

CFU-S. Column 1 of the table gives data on the number of colonies formed by the injection of spleen cells from mice that were treated with BP 1, 2, 3 or 5 days before sacrifice.

Effect of BP on CFU-S viability and PFC number

Administration of BP (days before assay)	CFU-S colonies/10^5 spleen cells*	% of control group	PFC/10^6 spleen cells*	% of control group
No BP (control)	12.64(±0.41)	–	835(±64)	–
1 day	7.06(±0.50)**	55	808(±210)	97
2 days	4.48(±0.28)**	35	926(±81)	111
3 days	5.28(±0.42)**	42	789(±144)	94
5 days	12.84(±0.65)	102	827(±11)	99
7 days	–	–	532(±63)**	63
10 days	–	–	766(±178)	92

* The values of column 1 and 3 are means (±SE) of values obtained from individual mice. ** Indicates values that were found to differ significantly from the control values by the Student t-test.

The means of these counts from individual recipient mice ± 1 SE are shown. The numbers in column 2 show the ratios of CFU-S numbers of BP treated mice to those of control mice. Similarly to the survival data of the figure, the maximum effect was observed at 2 days after BP, and was partially reversed by day 3. By 5 days the BP effect was no longer measurable.

PFC. Column 3 of the table gives the number of plaque forming units measured in spleen cells that received BP at various times indicated. The only significant variation from the control was seen at 7 days after BP and this effect was smaller than the BP-mediated changes in virus potentiation and CFU-S viability seen at day 2.

Discussion. We measured the effect of single injections of BP on 3 parameters: 1. FLV leukemogenesis, 2. CFU-S number and 3. PFC number. BP was significantly potentiating for FLV leukemogenesis and also significantly reduced CFU-S number but its effect on PFC number was marginal and seen at day 7 only.

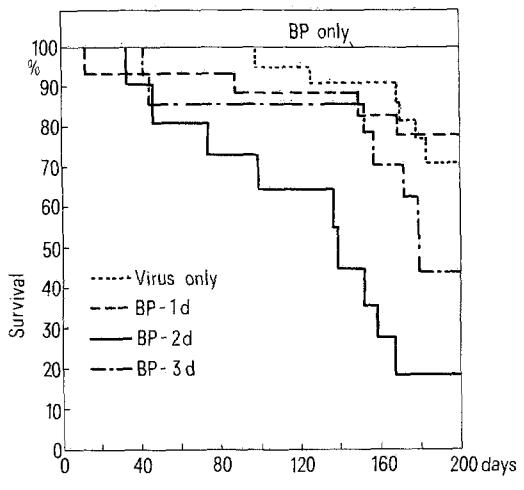
Since BP had very little effect on PFC response and the small BP-related reduction of PFC number occurred after virus potentiation was no longer demonstrable, immunosuppression as measured by this technique was probably not a factor in the BP-caused virus potentiation. However, since our data bear on the induction of humoral immunity, the possibility remains that T-cell mediated immunity against the virus or against virally-transformed cells may be inhibited. The hypothesis that suppression of the PFC-response is not significantly potentiating to Friend viral leukemogenesis is supported by our previous results with methyl methane sulfonate (MMS). Our data with this chemical[8] indicated that suppression of the PFC response was not related to a MMS-mediated potentiation. In that study[8] we observed a MMS-caused potentiation which had an extremely short duration, being maximal 5 h after MMS injection and fully recovered at 24 h after MMS injection. Meanwhile the immunosuppressive effect of PFC function produced by the same MMS dose lasted from day 1 to day 14 after MMS injection, but could not be demonstrated at 5 h.

Significant potentiation of FLV leukemogenesis was observed only if the interval between the BP and the subsequent virus injections was 2 days. Similarly the maximal effect of BP of CFU-S number occurred at 2 days. Although we cannot be certain that the BP effect on CFU-S number is related to the in vivo potentiation of viral leukemogenesis, the temporal correlation of the 2 maxima and the fact that the hematopoetic stem cells, assayed by the CFU-S technique, are major targets for Friend leukemia virus[6] suggests this possibility.

In the assay of CFU-S viability by the Till and McCulloch spleen focus method[9] the CFU-S cells from BP-treated and control animals are enumerated only if they form visible clones. This rapid cell proliferation in the assay could result in a lethal expression of stem cell damage that would otherwise be repaired if the cells were left in situ and remained in a more slowly proliferating population[13]. On the other hand, if BP did cause lethal cell damage in situ, a compensatory cell proliferation would probably follow this effect[14]. Either situation could be relevant to viral potentiation since both damaged[15,16] and replicating[17] DNA's have been shown to be more susceptible to viral transformation than intact, non-replicating DNA.

Other investigators have used in vitro systems to demonstrate the potentiation of viral transformation by chemical carcinogens thus proving that the chemicals work directly on the virus target cells. For example, Casto et al.[15] showed that various carcinogens, including BP and MMS, potentiate adenovirus transformation of Syrian hamster embryo cells, and Lavi[18] recently reported amplification of SV-40 virus and potentiation of SV-40 transformation efficiency by a variety of chemical carcinogens in Chinese hamster cells.

Although it is such in vitro systems that hold the greatest promise for the definition of the molecular mechanisms of such viral potentiations, our in vivo demonstration of this effect lends credibility to the relevance of such mechanisms in physiological cancer induction.



Survival of SJL/J mice injected with BP (25 mg/kg) and/or Friend leukemia virus (0.1 SED). Day 0 is the day on which virus or saline was injected. A single injection of BP was giben 1, 2 or 3 days before the virus, as indicated.

1 This work was supported by Department of Energy Contract No. EP-78-S-02-4800.

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