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Cancer Research Laboratories
320 East North Avenue
Pittsburgh, Pennsylvania 15212

CONFERENCE REPORT

EFFECTS OF CHEMICAL CARCINOGENS ON THE SUSCEPTIBILITY OF C57BL/10 AND (SJL/J X C57BL/10) F_1 HYBRIDS TO FRIEND LEUKEMIA VIRUS

R.B. Raikow
J.P. OKunewick
K.C. Magliere
B.J. Brozovich
P.R. Seeman

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TABLE OF CONTENTS

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ABSTRACT

Under normal circumstances cells of C57BL/10 mice are resistant to infection by Friend leukemia virus (FLV). Pre-treatment by chemical carcinogens does not affect the susceptibility of C57BL/10 mice to FLV leukemogenesis. However, immunosuppression by cyclophosphamide or a congenitally athymic condition allows the replication of the LLV component of FLV to take place in these mice. F1 hybrids between C57BL/10 and SJL/J mice are also resistant to virus, although about twenty percent of these hybrids develop leukemia after massive doses of FLV. Unexpectedly, the F1 hybrid with the virus-sensitive SJL/J mother was more resistant than the F1 hybrid from the reciprocal cross. Pre-treatment of the F1 hybrid or SJL/J mice with chemical carcinogens, such as methyl methane sulfonate and benzo(a)pyrene, but not cyclophosphamide, increased the incidence of leukemia with a peak of increased susceptibility developing at a specific time after treatment. This increase was not related temporally to a suppression of plaque forming response to sheep red blood cells although a blastogenic response to B and T cell mitogens was so related. A chemical carcinogen-caused depression of the viability of hematopoietic cells in the spleen, which cells are the major target for FLV oncogenesis, was also temporally related with the increase in susceptibility to the virus. Our data correlated with information on the alleles known to affect resistance to murine leukemia viruses.

In order to elucidate the nature of cancer etiology, which probably involves a combination of factors, we have investigated whether a single exposure to chemical carcinogens will increase susceptibility to Friend leukemia virus (FLV) in three strains of mice that have different sensitivities to this virus: C57BL/10, SJL/J, and their F_1 hybrid. C57BL/10 mice are highly resistant to FLV leukemogenesis (Stutman and Dupuy, 1972; Odaka, 1969) and their genotype with regard to FLV resistance has been relatively well characterized. SJL/J mice, in contrast, are highly sensitive to FLV and harbor an endogenous lymphoma virus that causes spontaneous Hodgkins-like lymphoma late in life (Siegler and Rich, 1968). The F_1 hybrid between the C57BL/10 and SJL/J mice shows a very low, but demonstrable susceptibility to FLV. We measured the effects of methyl methane sulfonate (MMS) and benzo(a)pyrene (BP) on the incidence of FLV-caused erythroleukemia, on the viability of hematopoietic stem cells in the spleen (which are a major target for FLV leukemogenesis), on the humoral immune response to sheep red blood cells, and on lymphocyte blastogenesis.

Results and Discussion

C57BL/10: Immunosuppression by rabbit anti-lymphocyte serum (Stutman and Dupuy, 1972), by cyclophosphamide (Raikow, et al. 1980), or because of the congenital athymic (nude) condition (Raikow, et al. 1980) does not allow the development of leukemia in C57BL/10 mice even if extremely high doses of the virus are injected. The Fv-2 locus, whose homozygosity is apparently responsible for the resistance of these mice (Odaka, 1969), affects the infectibility of only the SFFV component of FLV, accordingly immunosuppressed C57BL/10 mice have been

shown to support the replication of the LLV component of FLV even though no trace of either component can be found in normal C57BL/10 mice by 40 days after injection of large virus doses (Raikow, et al. 1980). Nevertheless, under such conditions as neonatal infection (Ceglowski and Freedman, 1969) or following bone marrow ablation by ^{89}Strontium (Kumar, et al. 1978) erythroleukemia induction by FLV can occur in these mice, indicating that some C57BL/10 cells must be transformed, perhaps by the SFFV contained in the inoculum itself, and normally these few transformed cells are eliminated by bone marrow dependent elements. We have found that MMS or BP injected at times before FLV that have proven to be effective in increasing the susceptibility of SJL/J or B10SJF$_1$ hybrid mice (Raikow, et al. 1979; Raikow, et al., in press), did not affect the sucepti-bility of C57BL/10 mice (data not shown).

SJL/J: The alleles relevant to FLV sensitivity carried by this strain have not been characterized, however this strain is apparently homozygous for the sensitive allele of the Fv-2 locus, and the virus tropism defined by its Fv-1 locus must be compatible with the FLV virus utilized by us (Raikow, et al. 1979) because extremely low doses of virus (even 1/1000th of that needed to produce erythro-leukemia in our F1 hybrids) are effective in SJL/J mice. We have demonstrated an increase in the incidence of erythroleukemia if SJL/J mice were pre-treated with either MMS (Raikow, et al. 1979; Raikow, et al., in press) or BP (OKunewick, et al. 1980), when these mice were injected with doses of virus that by themselves caused less than 50% of the animals to develop the disease. The erythroleukemia

was characterized by early splenomegaly and polycythemia (Raikow, et al., in press and unpublished). The increased susceptibility peaked at specific times after the chemicals were injected, i.e. at 5 hrs after MMS and 2 days after BP. We next attempted to determine whether suppression of immune functions could be related to these peaks of increased susceptibility. No temporal correlation between any suppression of the induction of humoral immune response to sheep red blood cells could be demonstrated (Figs 1 a and b), since the suppression of this response occurred after the 5 hr and 2 day virus-potentiation peaks for MMS and BP, respectively. On the other hand, the blastogenic response (Fig. 2) was affected by these chemicals at the times required for the optimum virus potentiation. However, to date these measurements have been made only at these specific times and additional study is required to determine whether this correlation is significant to virus susceptibility. In addition, our blastogenesis data (Fig. 2) shows that this response changes dramatically with the age of the mice and that at 24 weeks it is barely demonstrable. Therefore, the virus susceptibility of such older mice should be tested, even without carcinogen treatment.

Studies on the viability of colony forming units (CFU-S) in the spleen after MMS and BP treatment (OKunewick, et al. 1980) have shown a good temporal correlation between this effect and the increase in virus susceptibility. It is therefore possible that the crucial event caused by these chemical carcinogens that leads to increased virus susceptibility takes place at the virus target cell itself at some early stage of transformation rather than at some later stage involving the elimination of virus transformed clones by an immunological

mechanism. In support of this we have also noted that immunosuppression by cyclophosphamide does not increase FLV-caused leukemogenesis in SJL/J mice.

F_1 Hybrids: We have designated these as B10SJF1 if they have a C57BL/10 mother and SJB10F1 if they have an SJL/J mother. Both of these hybrids are quite resistant to FLV-caused leukemogenesis. However, when large doses of 100 spleen enlargement units are injected into B10SJF1 mice around 20% of the animals develop erythroleukemia, provided that they are at least 10 weeks old (Table 1).

Table 1. Susceptibility of B10SJF1 and SJB10F1 mice to FLV infection.

The percent of mice that developed fatal erythroleukemia by 200 days after injection of 100 SED of FLV is shown. Numbers in parenthesis are the total numbers of mice tested.

Age in weeks:		<10	10 - 14	>14
B10SJF1	♀	0%(14)	22.6%(53)	23.5%(17)
	♂	-	27.3%(11)	13%(15)
SJB10F1	♀	0%(3)	0%(8)	-
	♂	25%(4)	0%(8)	-

The SJB10F1 mice, on the other hand, appear to be more resistant to murine leukemia viruses than B10SJF1 mice. (Table 1 and R.F. Meredith, pers. comm.). This is surprising because other reports of maternal effects favor the passage of resistance from mother to offspring (Lilly and Pincus, 1973). Possibly the presence of endogenous lymphoma virus in the SJL/J mothers allows for the induction of anti-virus antibodies and gives some advantage to the SJB10F1 offspring. We have seen a low transient white blood cell elevation in the hybrid mice at around day 20 post infection, indicating that immunological elimination of incip-

ient leukemia may take place. The B10SJF$_1$ mice showed a dramatic increase in virus susceptibility following MMS treatment (Raikow, et al. 1979; Raikow, et al., in press). Furthermore, the timing of the peak of this reaction was the same as that defined by our studies with SJL/J mice.

The genetic basis of the resistance of the hybrids is not clear. The Fv-2 resistance has been defined as recessive (Lilly and Pincus, 1973) and therefore cannot account for the F$_1$ resistance. Moreover resistance based on the Fv-1 locus can usually be overcome with high titres of infecting virus (Pincus, et al. 1975) and this does not appear to happen with these F1 hybrids. Possibly the Fv-3 (Kumar, et al. 1978) or Fv-4 (Yoshikura and Odaka, 1978) or other genes associated with virus resistance (Lilly and Pincus, 1973) may be involved. H-2 related genes have also been implicated in virus resistance with the H-2b allele, carried by C57BL/10 mice being associated with relatively high virus resistance (Lilly, 1967, Dawson and Fieldsteel, 1973). However, data on F1 hybrids between different histotypes indicate that the hybrids are generally intermediate between the parents with regard to resistance, provided other resistance alleles are not epistatic to this function (Lilly and Pincus, 1973).

Conclusions: Methyl methane sulfonate and benzo(a)pyrene increase the incidence of FLV transformation in strains of mice which, when not treated by such carcinogenic chemicals, support some virus transformation. However, such chemicals do not increase leukemogenesis in C57BL/10 mice, which under most circumstances do not show any virus sensitivity. Thus the chemical pre-treatment in no way lessens the effect of the Fv-2 gene, but does affect the genes responsible for the resistant phenotype of our F$_1$ hybrid. The nature of these latter genes is not known, nor

is it clear whether they are directly related to immune functions.

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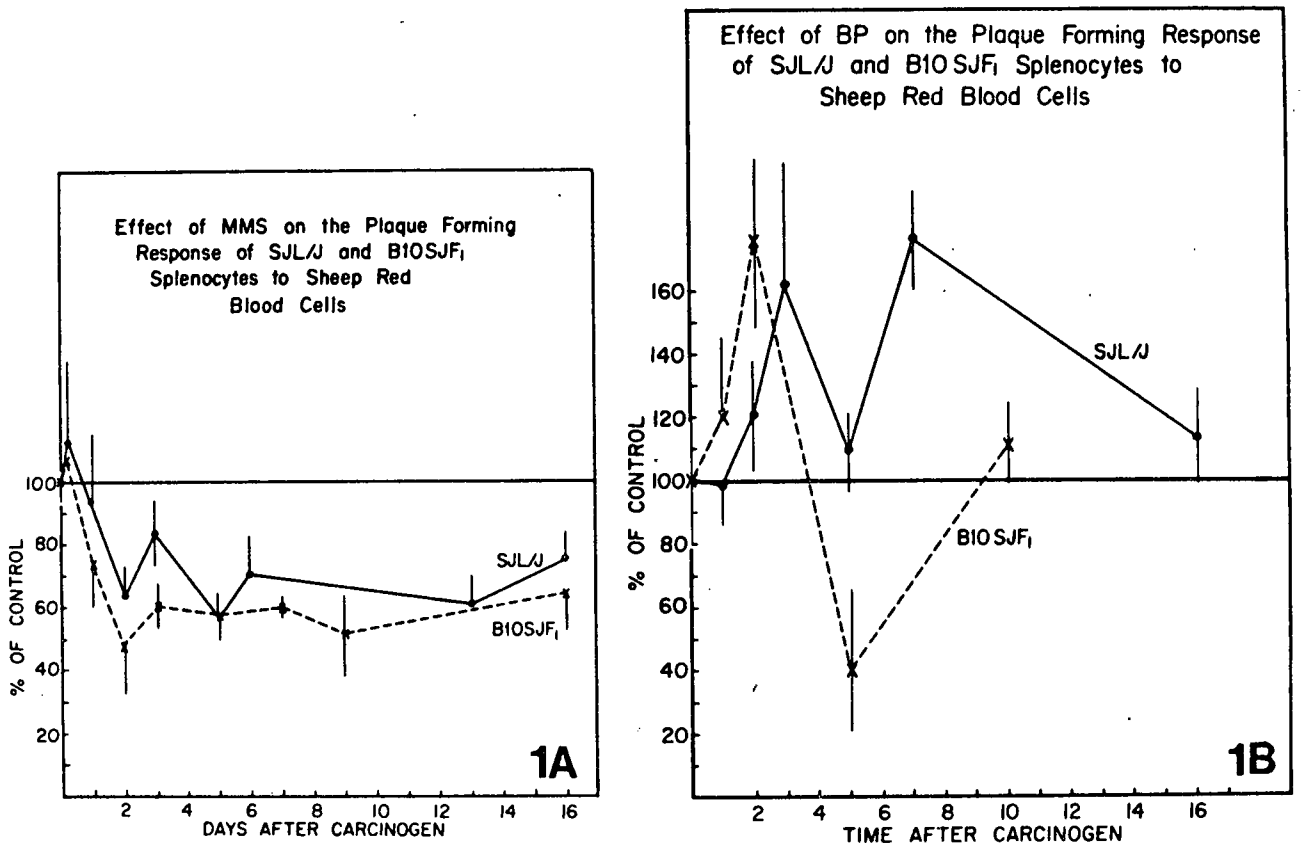

Figure 1 a and b. 2 mg/mouse of MMS in saline and 500 µg/mouse of BP in trioctanoin oil were injected i.p. The plaque forming response was measured as described previously (Raikow, et al. 1978). The data points are mean values of at least 6 mice ± 1 S.E. The mean control values were 1228 and 807 plaques per 10^6 spleen cells for the MMS and the BP experiments, respectively.

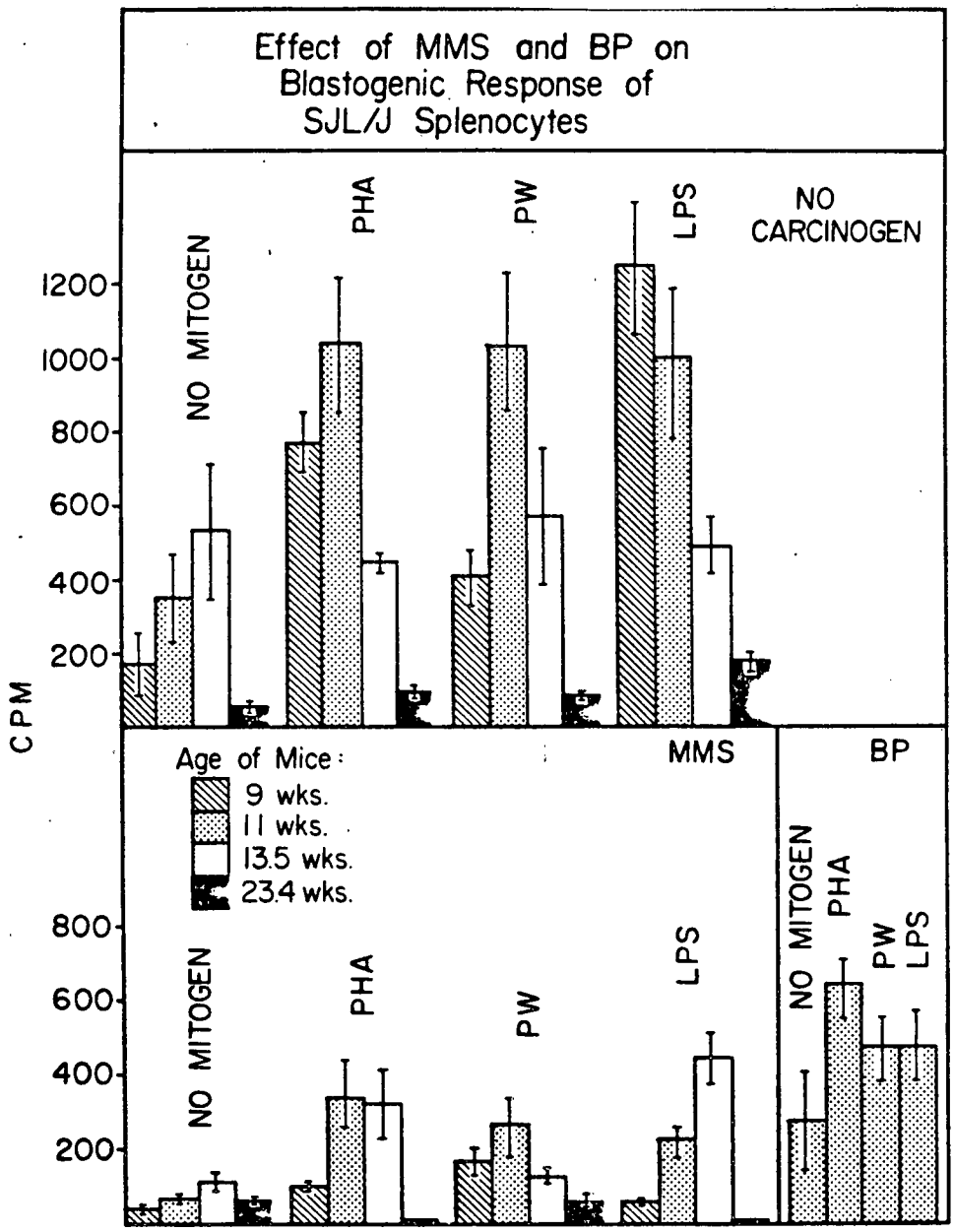

Figure 2: The blastogenic response was measured as described previously (Meredith, et al. 1978), 5 hrs after 2 mg of MMS per mouse and 2 days after 500 µg of BP per mouse was injected i.p.