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TECHNICAL REPORT

LEUKEMIA INDUCTION BY FRIEND VIRUS IN NORMALLY LEUKEMIA VIRUS RESISTANT MICE AFTER TREATMENT WITH METHYL METHANE SULFONATE

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ABSTRACT

F1 offspring of virus-resistant C57Bl/10 females and virus-sensitive SJL/J males are normally resistant to Friend leukemia virus. However, after treatment of these hybrids with methyl methane sulfonate (MMS), Friend leukemia virus given within 5 hrs of the treatment caused many individuals to succumb to erythro-leukemia with characteristic elevated white counts, hepatometaly, splenomegaly, and high hematocrit. Although MMS was found immuno-suppressive in these mice when given alone, it was found to enhance humoral immune function measured by a plaque forming assay against sheep red blood cells, when given in combination with the virus. These results suggest that MMS enhances viral leukemogenesis by some mechanism other than immunosuppression.

INTRODUCTION

Sensitivity to leukemia induction by Friend leukemia virus varies in different mouse strains (1,2). While the molecular mechanisms involved in the resistance to this virus are only partially understood, the resistance is known to be inheritable (3).

C57B1/10 mice are one of the types that have been designated as absolutely resistant by Stutman and Dupuy (1), meaning that they could not be made leukemic even by relatively high virus doses. In the present study we have used hybrid mice, which we have designated as BD10SJF1, that result from crossing of C57B1/10 females and virus-sensitive SJL/J males. This hybrid is intermediate to the two parents in its response to leukemia virus and seems to develop a transient leukemia after injection of Rauscher or Friend leukemia virus (4, and unpublished data). The intent of the present study was to evaluate the response of this mouse which contains genes for both leukemia resistance and leukemia sensitivity, to the combined exposure to the leukemia virus and a chemical carcinogen.

Based on previous reports concerning its mechanism of action (5,6), our choice for a chemical carcinogen was the alkylating agent, methyl methane sulfonate (MMS). In previous studies, Casto et al (6) have shown in vitro that MMS potentiates adenovirus-dependent transformation of hamster cells in culture. From this it could be inferred that MMS facilitates viral integration into the host genome. In the present study we have asked the question as to whether a 5 hour pretreatment with MMS also might facilitate in vivo the leukemogenic action of FLV in B10SJF1 mice. We have also measured the effect of MMS on the humoral immune response of these mice, both alone and in combination with FLV, to determine whether immunosuppression is important in MMS action.

MATERIALS AND METHODS

Animals - C57B1/10 female and SJL/J male mice were purchased from Jackson Laboratories. Their F1 hybrids were bred in our facilities. Ten to fourteen week old female F1 hybrids were used. All animals were maintained in temperature and humidity controlled rooms with a 12 hour light cycle. They were given Purina lab chow and acidified water ad libitum.

Virus - Friend leukemia virus (FLV) preparations were made by passaging Friend virus stock obtained from NCI through mice of the SJL/J parental strain, obtaining the virus rich plasma and diluting it with saline. The virus dose to be administered was measured in spleen enlargement units (SED), which are defined as that amount of virus which causes spleen enlargement in 50% of injected SJL/J assay mice within two weeks. The B10SJF1 mice were given the virus by intraperitoneal injection.

MMS - Methyl methane sulfonate (MMS) (Sigma) was diluted for injection with isotonic saline. Two mg were injected intraperitoneally per mouse. Preliminary experiments indicated that this dose is close to the highest tolerated by the mice without any obvious toxic affects. MMS was given 3.5-5 hrs before FLV, because our experiments with SJL/J mice (7) indicated that pretreatment within 24 hours of virus injection is most effective in augmenting FLV leukemogenesis.

Plaque forming assay - The ability of splenocytes to lyse sheep red blood cells (Sacks Farms) was measured by the Jerne plaque forming technique as described previously (4).

RESULTS

Table I illustrates the effect of exposure of B10SJF1 mice to MMS only, virus only, or virus plus MMS, as compared to untreated controls and as a function of time after virus injection. Mice that received 50 SED units of virus 5 hrs after a dose of 2 mg of MMS had increased white blood cell counts (WBC) when compared to mice that received FLV only. The mice that received both carcinogens had a greater elevation in WBC at 30 days than those receiving either agent alone. Mice with both virus and MMS also had significantly higher hematocrits: 77 and 80 at the forty-fourth day after virus injection, as compared to 54 in the one mouse with elevated WBC in the virus only group and 45 for normal mice. Coupled with these changes in the peripheral blood, survival in the group receiving combined exposure to MMS and FLV was poorer than any other group, and those mice which died had gross hepato- and splenomagely. On day 169 the experiment was terminated and all animals were autopsied. Two of the three remaining mice in the MMS and FLV exposed group had enlarged spleens of 2.6170 and 0.6210 g, while all of the mice in the other groups, including those which had received virus only, had normal spleens of less than 0.3 g in size.

Figure 1 shows the results of a second experiment in which B10SJF1 mice received 2 mg MMS 3.5-4 hrs before 100 SED FLV, and illustrates that survival was greatly shortened when compared to mice receiving virus only. There were no deaths in the group receiving MMS only. All of the mice that died had leukemia, as judged by elevated WBC and hepato- and splenomegaly at death.

Table I

Effect of MMS Pre-treatment on the Leukemogenic Action of FLV in B10SJF1 Mice.

2 mg MMS was given IP to B10SJF1 mice on day 0 5 hrs before IP injection of 50 SED FLV. Average white blood cell counts, their range and the number of mice with elevated white blood cell counts per total number of mice per group are given.

Day	Control	Elevated Total	MMS Only	Elevated Total	Virus Only	Elevated Total	Both	Elevated Total
16	10,550 (6,763-14,210)	0/5	10,814 (6,454-13,719)	0/5	12,646 (10,184-17,305)	0/5	16,630 (9,154-38,335)	1/5
30	9,000 (8,361-9,646)	0/5	44,294 (9,846-183,278)	1/5	12,833 (8,897-17,234)	0/5	24,285 (7,419-48,537)	2/5
44	8,565 (6,397-11,362)	0/5	7,378 (6,234-9,002)	0/5	39,214 (6,806-156,258)	1/5	43,734 (8,241-138,827)	2/5
70	8,779 (6,419-12,913)	0/5	16,907 (4,004-62,062)	1/5	7,900 (7,554-8,433)	0/5	19,362 (8,820-57,177)	1/4
100	9,634 (5,922-13,086)	0/5	5,924 (4,411-9,344)	0/5	8,968 (7,922-11,705)	0/5	10,782 (8,933-12,630)	0/3
123	13,014 (11,012-14,948)	0/5	12,338 (6,259-14,891)	0/5	12,667 (10,616-15,107)	0/5	16,015 (14,270-17,090)	0/3
Survival at day 169	100%		100%		100%		60%	

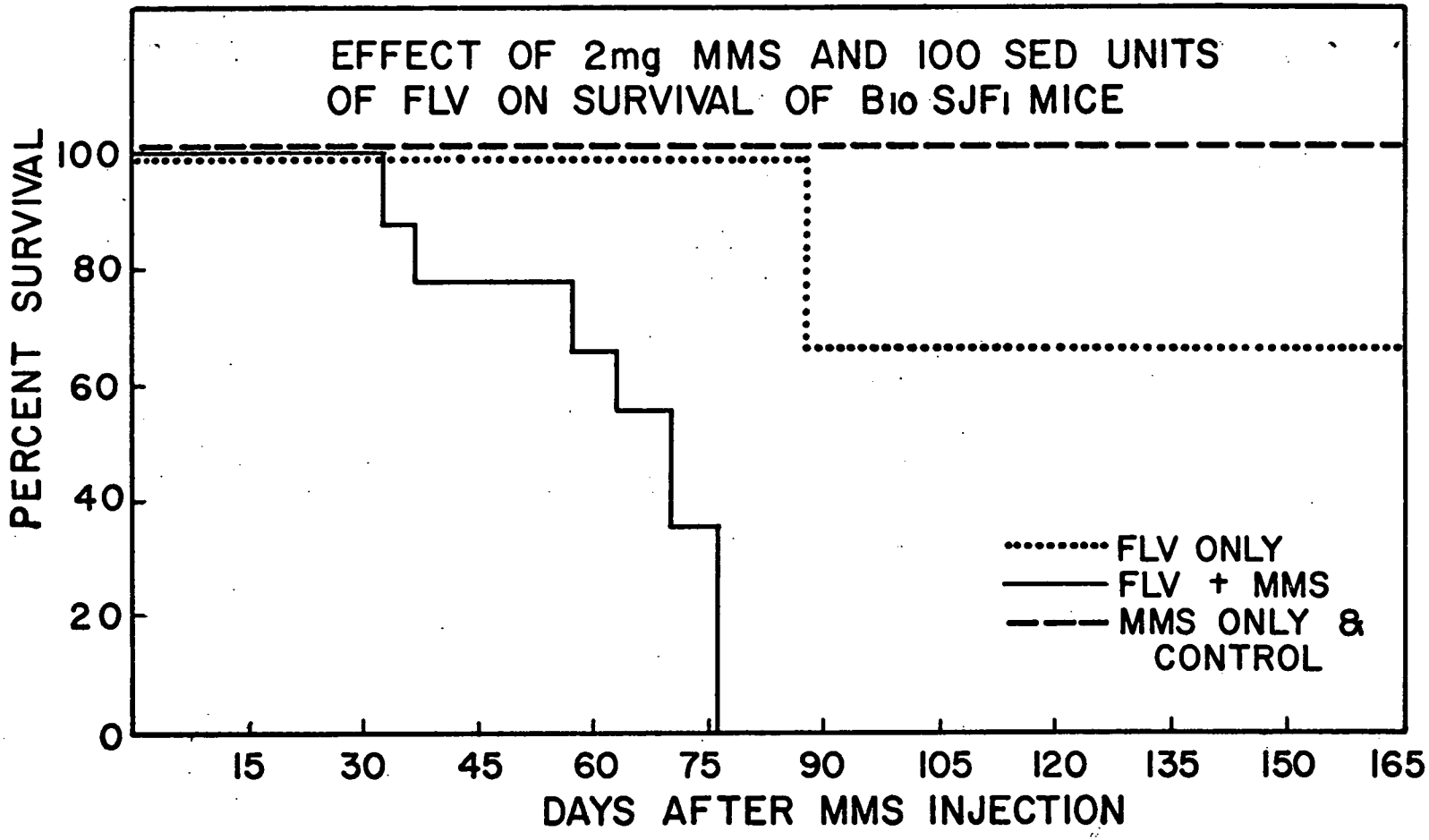

Fig. 1. 10-14 week old B10SJF1 female mice were given MMS intraperitoneally 3.5-4 hrs before 100 SED FLV. Other groups were given one or none of these carcinogens. There were 4 mice in each group, except in the group that received both carcinogens which consisted of 10 mice.

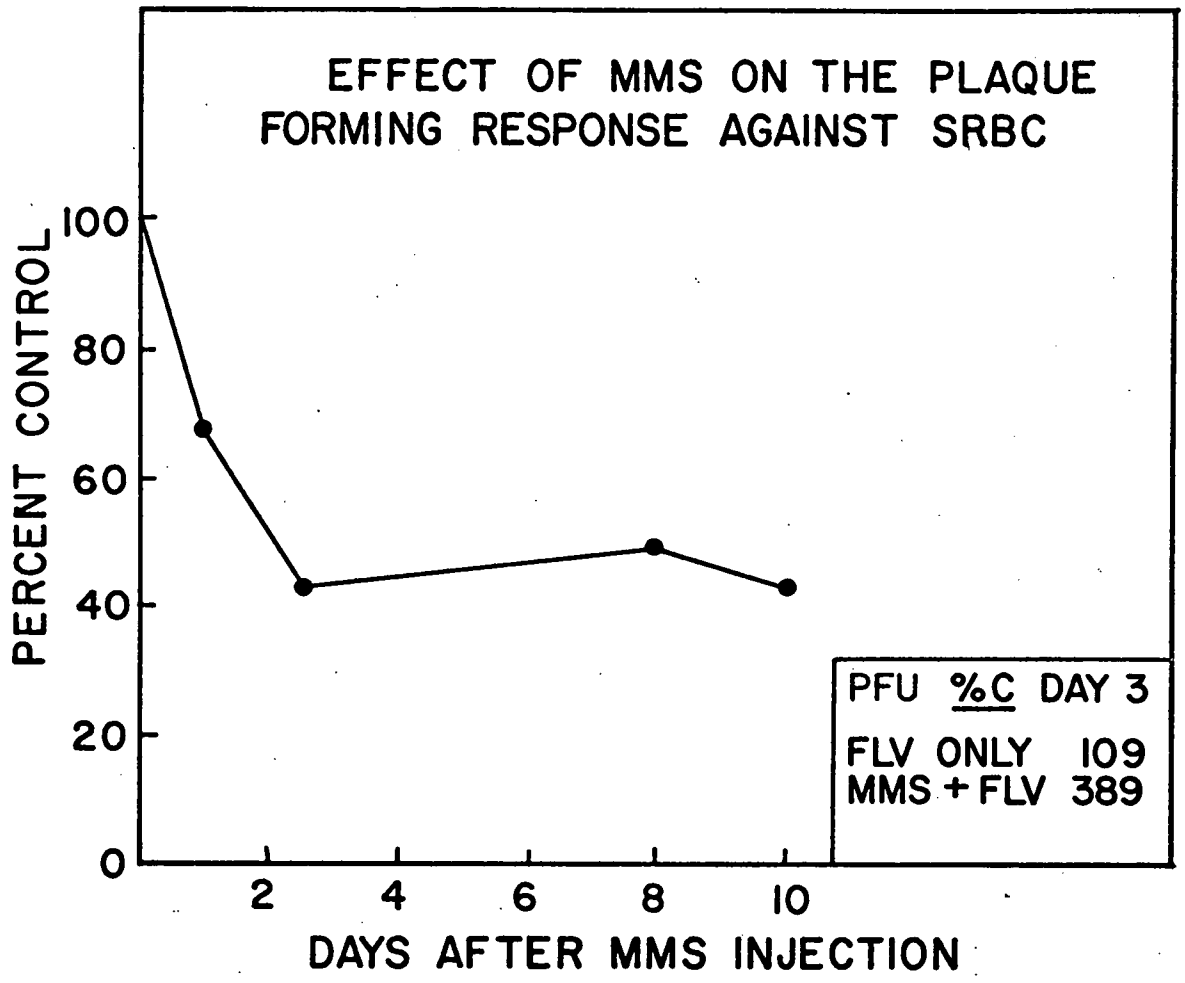

Fig. 2. B10SJF1 mice were injected with 2 mg MMS IP. Then on the days indicated they were injected with SRBC. Five days later their splenocytes were isolated and tested for ability to lyse SRBC in vitro. The insert shows the same function measured in B10SJF1 mice that were injected with 100 SED FLV alone or with 2 mg MMS plus 100 SED FLV 3 days before SRBC sensitization. The values represent the average of 3 mice.

Figure 2 shows the effect of MMS on the ability of B10SJF1 mice to mount a humoral immune response to sheep red blood cells (SRBC) as determined by the plaque forming technique (4). Plaque forming cell (PFC) response decreased as a function of time after MMS given alone, while when virus was given in conjunction with MMS there was an apparent increase (box, fig. 2).

DISCUSSION

Our results show that MMS pretreatment increases the sensitivity of normally FLV-resistant B10SJF1 mice to the leukemogenic action of this virus. This implies that the juxtaposition of these two different carcinogens can have a synergistic effect. Normal B10SJF1 hybrid mice seem to be able to control leukemia virus action, as is evident by a lower mortality and the transient nature of leukemia symptoms such as WBC elevation. Since exposure of the F1 to leukemia virus actually results in an increased humoral immune response, as measured by the plaque forming response to SRBC (4), it was of interest to measure the effect of MMS on this immune response and in relation to the observed effect of MMS on leukemogenesis. Although MMS did decrease the level of plaque forming response when given singly, when FLV was given following MMS the PFC response was elevated above control levels by the third day, and above the level of FLV exposure alone. In the previous study with this hybrid (4), elevated PFC response was not seen until the eighth day after virus infection. Thus the data from this study suggest that not only is the PFC response enhanced when the two agents are given together, but that this enhancement occurs earlier. Additional studies will have to be performed to confirm this.

However, the preliminary results do suggest that this observed increase in leukemogenesis following combined administration of MMS and FLV may not be related to humoral immunosuppression.

Results of in vitro studies of adenovirus in cultured hamster cells suggest that MMS may act directly on the virus target cells. The fact that our data show an effect as early as 3.5 to 5 hours would be in agreement with that. It has also been postulated (8,9) that virus enhancement by radiation and chemicals that mimic radiation damage to DNA might be accomplished during post replication repair of DNA damage. Whether the same is true in our system can be tested by the use of inhibitors of such repair, for example caffeine (9). Such experiments are now in progress.

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