Methyl methane sulfonate induced enhancement of Friend viral leukemogenesis[1]

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Summary. Exposure to the chemical carcinogen, methyl methane sulfonate, enhanced leukemogenesis in mice given threshold doses of Friend leukemia virus, as shown by peripheral white blood cell counts, splenomegaly and survival.

We have previously reported that methyl methane sulfonate[2] (MMS), a weak carcinogen[3,4], given in conjunction with Friend leukemia virus (FLV), will result in a greater incidence of leukemia than that expected on the basis of the effects of either carcinogenic agent given alone. This observation suggests that MMS may act as a potentiator of Friend viral leukemogenesis. In the present paper we report additional data on the potentiating effect of MMS on Friend viral leukemogenesis in 2 mouse strains, one sensitive and the other relatively resistant to FLV, as evidenced by differences in white blood cell counts, splenomegaly, and time of leukemia onset.

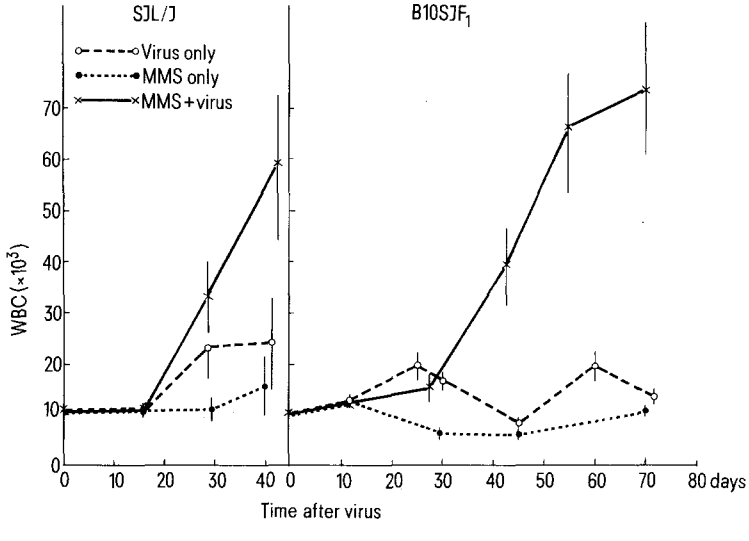
Materials and methods. Female SJL/J mice, the sensitive strain, were purchased from Jackson Laboratories at 6–8 weeks of age and held in quarantine cages until use. Female B10SJF$_1$ mice, the resistant strain, were bred in our facilities by crossing C57B1/10J females with SJL/J males. All mice were used when between 10 and 12 weeks of age. All animals were housed 5 or 6 per cage in autoclaved plastic filter-topped cages in a controlled environment room, and fed autoclaved Purina lab chow and autoclaved acidified water. Prior to MMS injection the animals were randomized into the experimental groups indicated below. MMS at 100 mg/kg b.wt was injected i.p. 5 h before FLV. The dose of virus given to the SJL/J mice was 0.1 $SED_{50/14}$ units per mouse, prepared and titered as described previously[2,5]. The dose of virus given to the B10SJF$_1$ mice was 1000 times higher, 100 $SED_{50/14}$ units per mouse, also injected i.p. The 5-h timing and the doses of FLV and MMS used were previously determined to yield the greatest evidence of a potentiating effect[2]. The animals were observed for up to 300 days after receiving the carcinogens. They were periodically bled from the tail vein and their white counts determined using a Coulter Counter model Fn. Those mice showing elevated WBC were numbered and identified for subsequent close observation. The animals were checked daily for deaths, and the deaths recorded. All dead mice were autopsied and their spleens weighed.

Results. The effect of combined exposure to MMS and FLV on white blood counts is illustrated in the figure. The effects on incidence of leukemia, mean time of leukemia incidence, and splenomegaly at autopsy were illustrated in the table. The data indicate that MMS alone had no significant effect on the parameters monitored. FLV given alone had significantly less leukemogenic effect than when given 5 h after MMS.

When a dose of 0.1 $SED_{50/14}$ units of FLV was given to SJL/J mice there was a significant increase in peripheral white cell counts in about 30% of the animals by day 40. SJL/J mice given the same virus preparation in conjunction with MMS showed white cell counts averaging 2.4 times higher by day 40 than those seen when FLV was given alone. Shortly thereafter the animals with increased white blood cell counts began to die, and upon autopsy showed the splenomegaly characteristic of Friend viral leukemia (table). A 5-h pre-treatment with MMS also substantially enhanced leukemogenesis in the more leukemia-resistant B10SJF$_1$ hybrid. With FLV given alone in a dose 1000 times higher than that given to the SJL/J mice, leukemia was induced in only about 18% of the B10SJF$_1$ mice. However this was increased by a factor of 4 in mice also exposed to MMS. The comparative increase in white blood cells was also greater in the B10SJF$_1$ mouse after exposure to MMS and virus than in the SJL/J mouse.

For both strains of mice the onset and mean times of leukemic deaths was accelerated by exposure to MMS before the virus. Without MMS the mean time of leukemic deaths was about 40 days sooner in the SJL/J mouse than in its histocompatible hybrid. Combined exposure to MMS and FLV also resulted in increased average spleen weight at autopsy which was slightly greater than the value for FLV alone in the case of the SJL/J mouse and significantly greater in the case of the B10SJF$_1$ mouse.

Discussion. The present data support our earlier report[2] that MMS may act as a potentiator of viral leukemogenesis. Both FLV sensitive SJL/J strain and its comparatively FLV resistant hybrid exhibited such a potentiating response in



White blood cell counts in 20 µl samples of blood obtained from the tail vein of SJL/J and B10SJF$_1$ mice. Mice were injected with MMS 5 h before virus. The data represent the results of 3 pooled independent experiments. Each individually yielded similar results. A total of 26 SJL/J and 29 B10SJF$_1$ mice per group were used. The data are presented as the mean ± 1 SE.

Response of mice to combined exposure of Friend leukemia virus (FLV) and methyl methane sulfonate

Mouse strain	Treatment	% of mice that died of leukemia in the course of experiment*	Mean time of leukemia deaths (days after virus)	Mean spleen weight at death**
SJL/J	Sham injected	0 (20)	-	0.3441 (0.2894–0.3988)
	MMS only	0 (22)	-	0.3265 (0.2884–0.3646)
	Virus only	65.4 (26)	104.4 (81.6–127.1)	1.1871 (1.0011–1.3731)
	MMS and virus	93.3 (45)	51.9 (43.6–60.3)	1.3797 (1.2281–1.4713)
$B10SJF_1$	Sham injected	0 (14)	-	Normal Weight 0.3 g
	MMS only	0 (21)	-	Normal Weight 0.3 g
	Virus only	17.9 (28)	143.0 (112.7–173.3)	2.0135 (1.4954–2.5316)
	MMS and virus	80.0 (34)	85.5 (81.1–90.0)	3.2527 (3.0479–3.4575)

Mice were injected with 100 mg MMS/kg b. wt and 5 h later with FLV (0.1 SED). They were followed for 300 days.
* The number in parenthesis equals the total number of mice in group. ** Mice that did not die of leukemia were sacrificed after 300 days.

the data presented in this paper. Because $B10SJF_1$ has a higher degree of FLV resistance than SJL/J, its response to FLV coupled with MMS is especially notable. In our previous studies we found that the time interval between exposure to MMS and FLV is a critical factor in the demonstration of any possible potentiating effect of the chemical carcinogen[2]. MMS given 5 h before FLV had the maximum effect. This time interval agrees closely with the optimum necessary for MMS potentiation of adenovirus transformation of CHO cells as reported earlier by Casto et al.[6]. Although it is not yet certain how MMS produces viral leukemia potentiation in vivo, our previous data demonstrated a rapid appearance and disappearance of the effect in vivo[2], similar to that seen by Casto et al.[5] in vitro, which they suggest may indicate a direct effect on the DNA of the target cell. Relative to this, Regan and Setlow[7] reported that MMS produces direct short strand breaks in DNA similar to those caused by X-rays[4,6,8], and earlier studies with X-rays have shown that radiation is a potentiator of viral leukemias[10,11]. According to Regan and Setlow[7] the DNA damage caused by MMS is quickly repaired. This would be in accord with the rapid disappearance of the potentiating effect of the virus. It is thus possible that potentiation of viral leukemogenesis by MMS may be dependent upon the DNA breaks caused by the chemical and/or their subsequent repair.

1 This work was supported by D.O.E. Contract No. EP-78-S-02-4800.A001.
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A transmission and scanning electron microscopic study of cytoplasmic threads of dividing neuroepithelial cells in early chick embryos[1]

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Summary. Cytoplasmic threads found on the luminal surface of the developing chick neuroepithelium contain a remnant of the spindle complex and an electron-dense midbody. Most threads become long and thin and eventually split at the midbody. This finding suggests that the midbody plays a role in the final separation of daughter cells.

The developing chick neuroepithelium, like many other embryonic epithelia, exhibits interkinetic nuclear migration which results in the accumulation of mitotic figures near its luminal surface[2–5]. Mitotic cells are rounded and remain adjacent to the lumen from late prophase through early telophase. By late telophase, forming daughter cells start to elongate towards the base of the neuroepithelium[6]. During cytokinesis, connections between daughter cells may persist for a relatively long time. As a result, long protoplasmic bridges (=cytoplasmic threads) may be formed. These thread-like structures have been described previously[6–8], but very little is known about the sequence of their formation and function. As part of our interest in the development of the chick neuroepithelium, we have used transmission and scanning electron microscopy to examine dividing neuroepithelial cells with emphasis on their cytoplasmic threads.

Materials and methods. Fertile White Leghorn eggs were incubated at 37.5 °C to obtain embryos at stage 8+ of development[9]. Stage 8+ embryos were chosen because all phases of neurulation are represented in a single embryo[6]. For transmission electron microscopy (TEM), embryos were fixed in 1% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) containing 0.2% tannic acid for