COO-4800-3

allegheny health, education and research corporation

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TECHNICAL REPORT

LEUKEMIA INDUCTION IN SJL/J MICE TREATED WITH METHYL METHANE SULFONATE AND FRIEND VIRUS

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Prepared for

THE U.S. DEPARTMENT OF ENERGY
UNDER CONTRACT

EP-78-S-02-4800.A000

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ABSTRACT

Methyl methane sulfonate (MMS) given to SJL/J mice in conjunction with Friend leukemia virus (FLV) enhanced leukemogenesis. The time of MMS administration in relation to FLV inoculation was varied. MMS was effective in leukemogenic enhancement only if given within 24 hrs preceding the virus. MMS given 5 hrs after the virus was slightly protective. MMS was also found to have an immunosuppressive effect as measured by a depressed plaque forming response to sheep red blood cells. However, the nadir of this response did not correspond to the peak of leukemia virus enhancement.

INTRODUCTION

Several studies have shown that chemical agents as well as irradiation enhance the effect of carcinogenic viruses in vitro (1-5). One such chemical is the alkylating agent, methyl methane sulfonate (MMS), which has been shown to enhance adenovirus transformation of hamster cells (6). The mechanism by which this occurs seems to depend on a direct effect of this agent on DNA, with the degree of virus co-carcinogenic enhancement being directly correlated with the extent of DNA damage. Possibly the virus becomes more easily integrated at such damaged sites.

In the present study we have asked the question whether MMS was effective in enhancing viral leukemogenesis in vivo; and having found this to be the case, we asked whether this activation involved immunosuppression.

MATERIALS AND METHODS

Animals - SJL/J female mice were purchased from Jackson Laboratories and held until 10-12 weeks of age. They were housed in plastic cages with filter tops in a temperature humidity and 12-hr light cycle controlled room, and were given acidified water and Purina lab chow ad libitum.

Methyl methane sulfonate (MMS) - MMS (Sigma) was diluted with sterile isotonic saline and injected intraperitoneally into 10 to 12 week old mice, at various doses and times in relation to virus, as indicated.

Virus - Friend leukemia virus (FLV) preparations were made by passaging Friend virus stock obtained from NCI through SJL/J mice,

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 the injected SJL/J assay mice within two weeks. The mice were given the virus by intraperitoneal injections.

Plaque forming assay - The ability of splenocytes to lyse sheep red blood cells (Sacks Farms) was measured as described previously (7).

RESULTS

In figure 1 the survival of mice that were injected with 2 mg MMS 5 hrs before or after 0.1 SED FLV is compared to that of mice that were given the same doses of FLV or MMS alone. All of the deaths, with one exception, in this and all experiments described below, were due to erythro-leukemia as indicated by hepato- and splenomegaly at death, elevated white blood cell counts, elevated hematocrits and increased percentages of abnormal red cells in the peripheral blood. The one exception was a single mouse in the MMS only group that died on day 148 which had no splenomegaly but did have enlarged lymph nodes. The cause of death in this instance is unknown.

The experiment illustrated by figure 2 is similar to that of figure 1, except that a lower dose of virus (0.015 SED) and a higher dose of MMS (3 mg) was used. This dose of MMS proved to be toxic, and 44% of the mice receiving it died within 24 hrs of its injection. As these deaths were clearly due to the chemical toxicity of MMS,

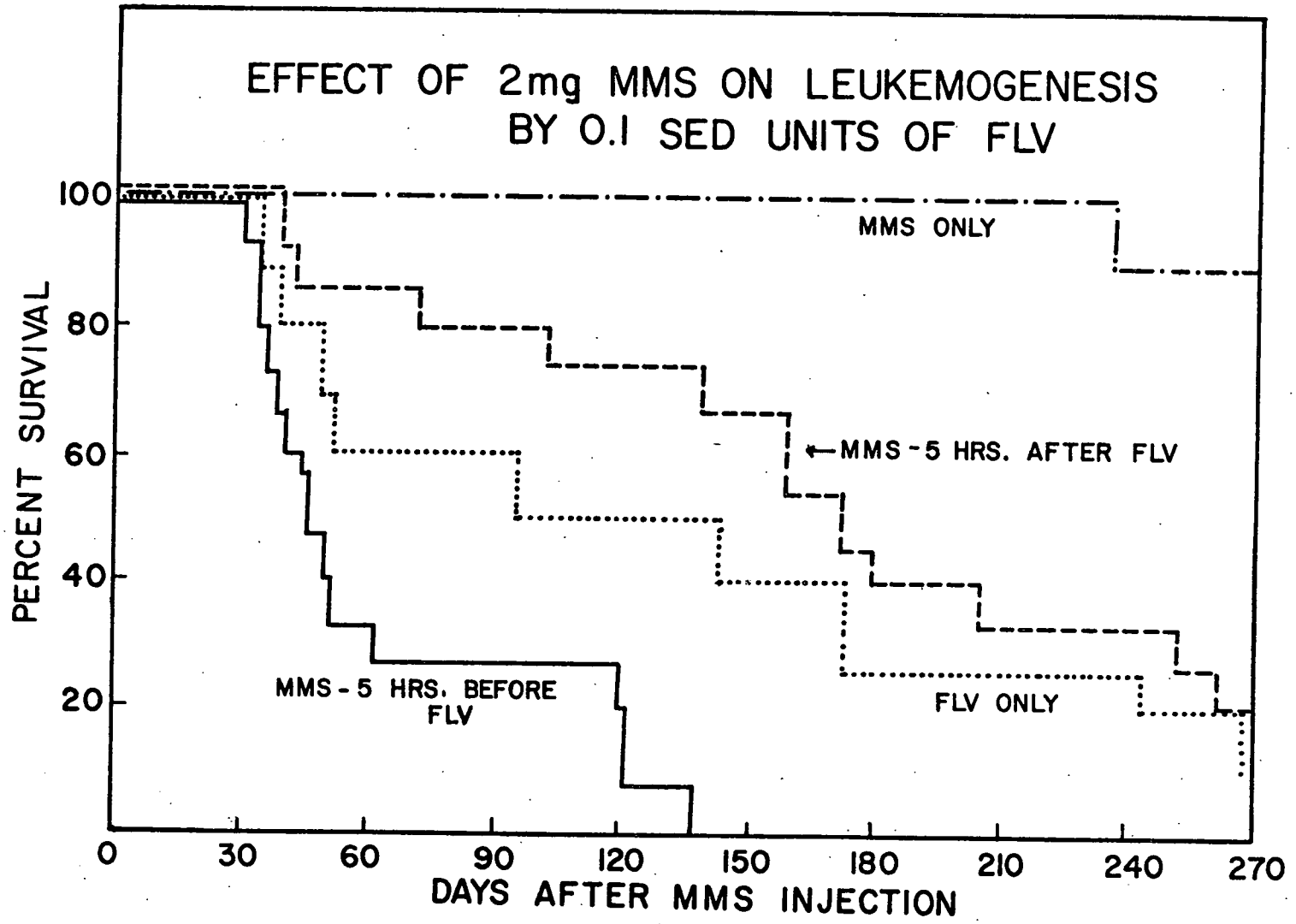

Fig. 1. SJL/J mice were injected IP with 0.10 SED FLV and with 2 mg MMS either 5 hrs before or 5 hrs after the virus. There were 15 mice in the two groups that received both viral and chemical carcinogens. All of the other groups, including a control group that received saline injection only, which is not shown, consisted of 10 mice each. There was 100% survival in the control group.

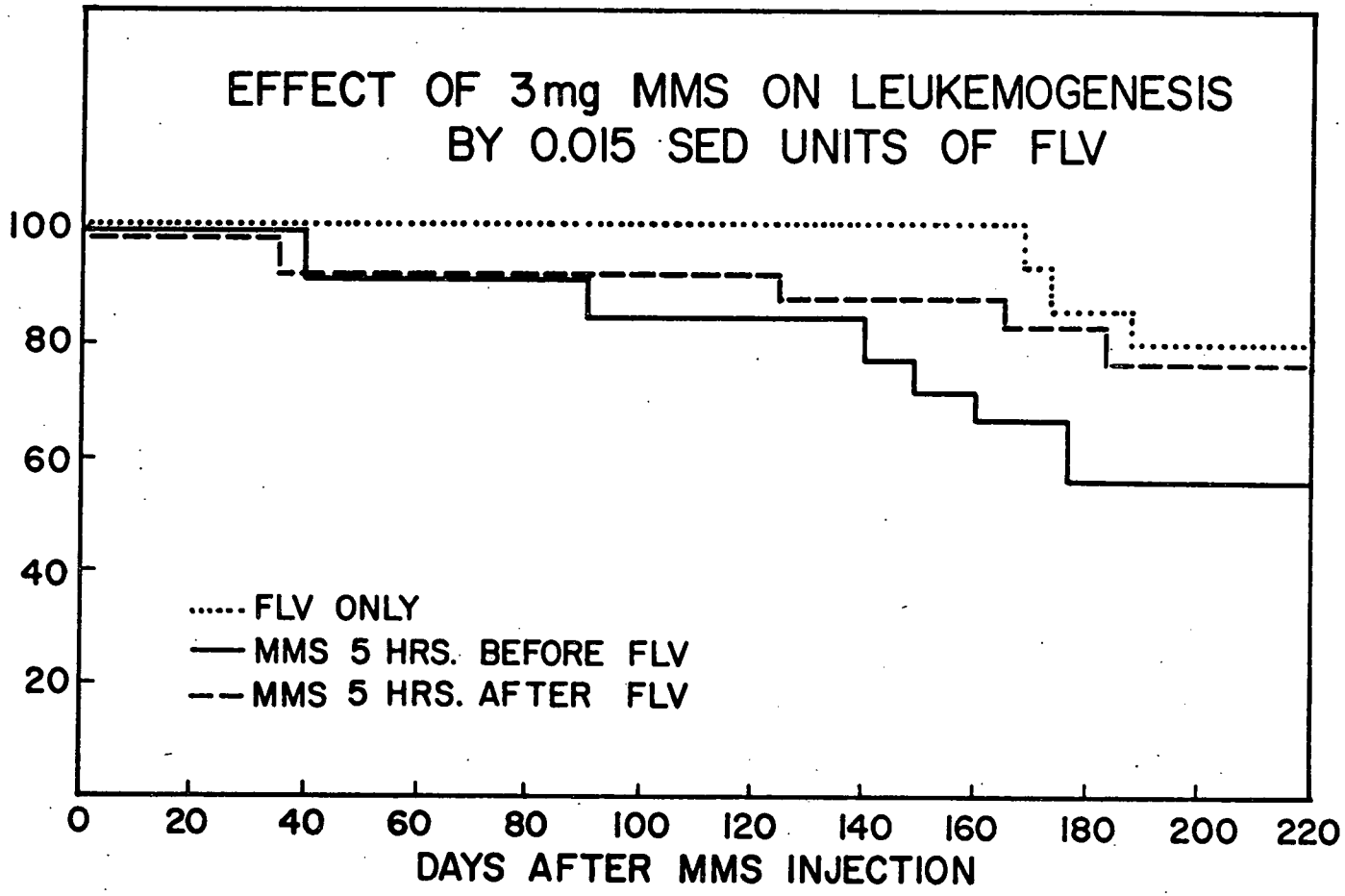

Fig. 2. SJL/J mice were injected intraperitoneally with 0.015 SED FLV and with 3 mg MMS either 5 hrs before or 5 hrs after the virus. There were 5 mice in the virus only group, 7 mice in the group that received MMS before virus and 15 mice in the group that received MMS after virus.

they are not included in the survival data of figure 2. However, in the remaining mice the leukemogenic action of FLV was enhanced by MMS pretreatment in agreement with the data of figure 1. In contrast, MMS given 5 hrs after FLV in this study also enhanced leukemogenesis. In the experiment of figure 3, two mg of MMS were injected at various times, up to 5 days, before 0.1 SED of the virus. The survival data indicate that MMS is equally effective when given 5 or 24 hrs before FLV. However, when 48 hrs or more is allowed to elapse between the two carcinogen treatments, MMS has no enhancing effect and, in fact, appears to be slightly protective. When two 2 mg doses of MMS were given prior to 0.10 SED of FLV (figure 4), a greater enhancement was obtained than when a single dose was given at 24 hrs before FLV.

As shown in figure 5, MMS suppressed the splenic plaque forming response when given to SJL/J mice without virus. The table insert of figure 6 shows plaque forming response was also decreased by FLV injection, as previously reported (8), and when MMS was given in combination with FLV, this immune function was further suppressed.

DISCUSSION

MMS enhancement of viral leukemogenesis - Two different doses of virus were used in this study, both of them substantially below the 100% threshold of 50 SED units previously established by this laboratory to be the lowest dose to cause 100% leukemic deaths in SJL/J mice by day 40. These were 0.1 SED units and 0.015 SED units. The FLV dose of 0.1 SED was sufficient to kill 90% of the mice by

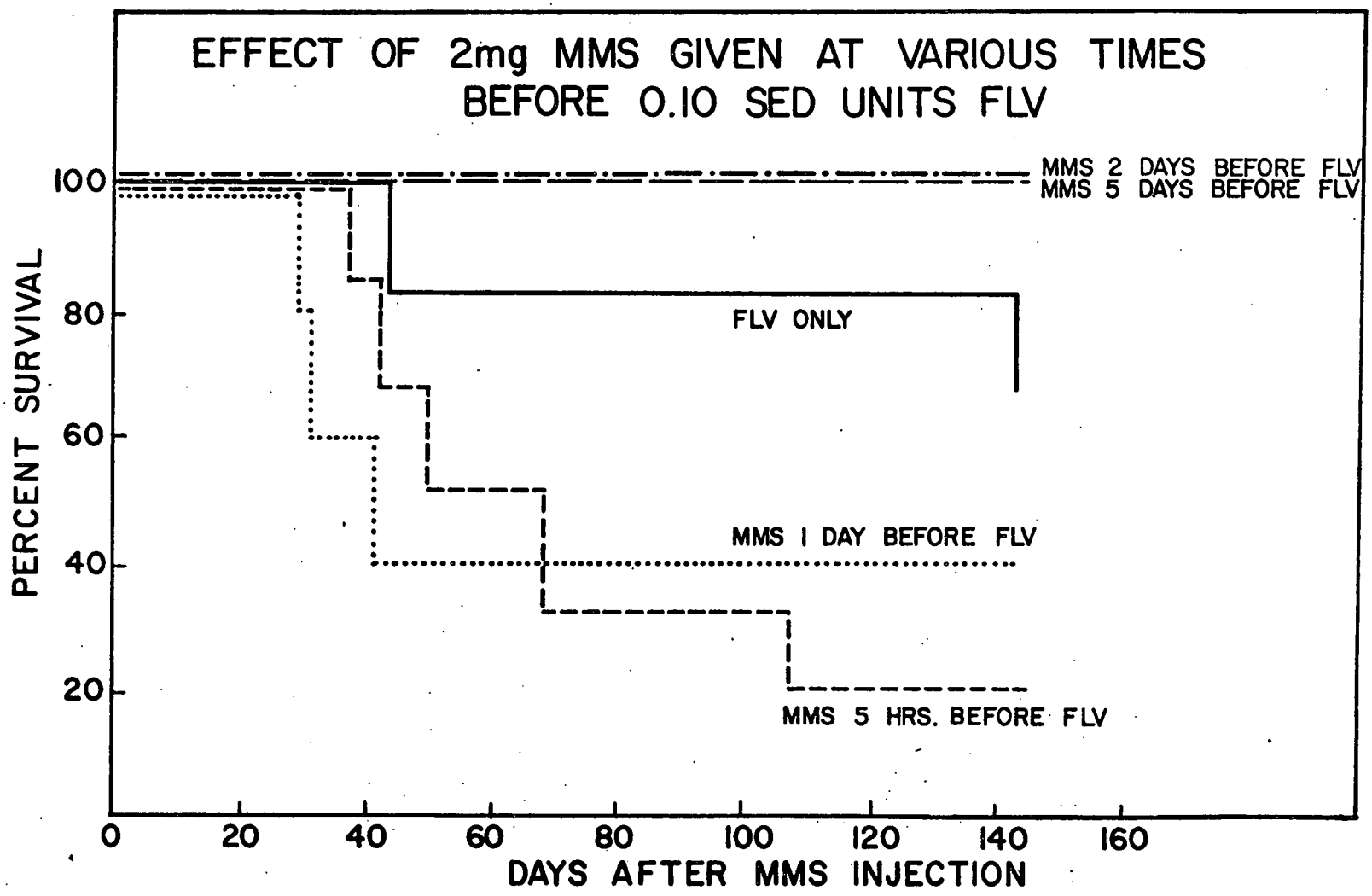

Fig. 3. SJL/J mice were injected IP with 2 mg MMS at 5 hrs, 1 day, 2 days or 5 days before 1.0 FLV. There were 6 mice per group.

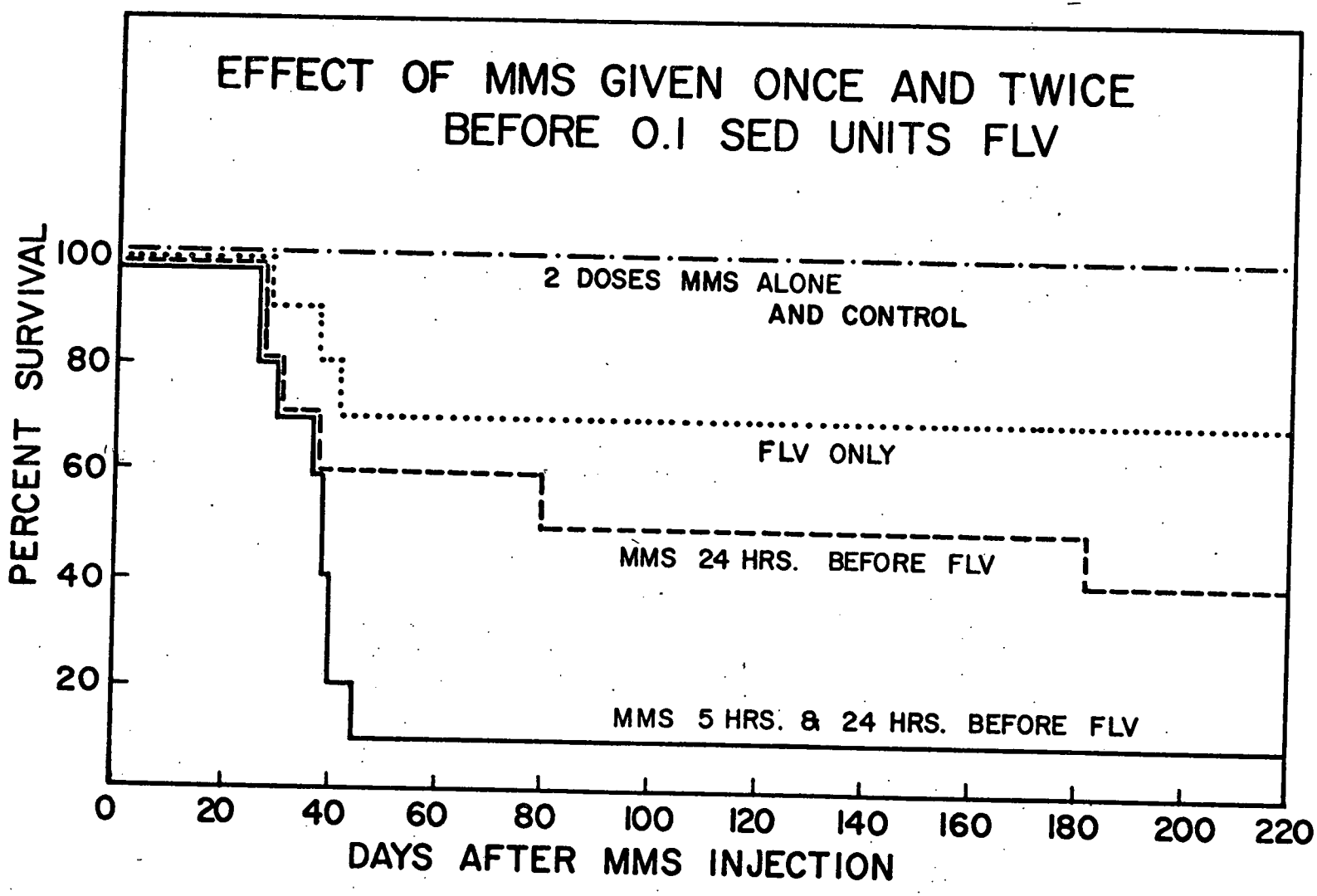

Fig. 4. 2 mg MMS was injected into SJL/J mice IP 24 hrs and/or 5 hrs before 0.1 SED FLV. There were 10 mice in each of the groups, except in the control group that received saline injection only which consisted of 5 mice.

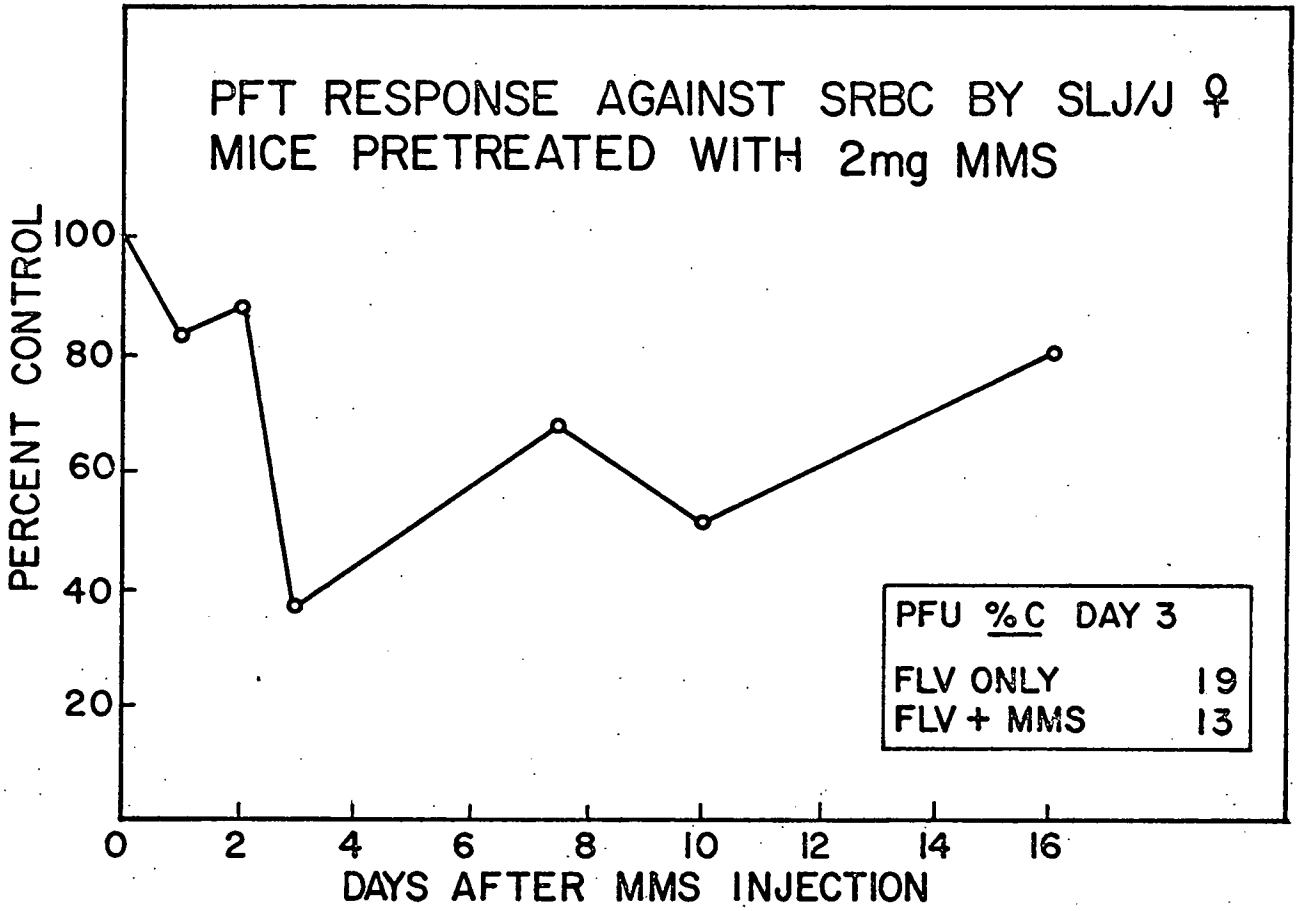

Fig. 5. SJL/J mice were injected with 2 mg MMS IP and then with SRBC on the designated days. Five days after SRBC injection the mice were sacrificed and their splenocytes were tested for ability to lyse SRBC in vitro. To obtain the data in the insert, SJL/J mice were injected with 2 mg MMS and then 5 hrs later with 50 SED FLV. Three days after the FLV injection these mice were injected with SRBC, and 5 days after SRBC they were sacrificed and their splenocytes isolated for in vitro assay.

day 300, with some leukemic death occurring as early as 40 days (figure 1). The lower dose of 0.015 SED used in the experiment of figure 2 appeared to be totally without effect when used alone until day 180. With either FLV dose the injection of MMS 5 hrs before the virus enhanced leukemogenesis, i.e. it decreased the number of long-term survivors. When 2 mg was injected 5 hrs after 0.1 SED of FLV, it appeared to have a slightly inhibitory effect on FLV leukemogenesis. However, when 3 mg MMS was given 5 hrs after 0.015 SED FLV, there was a small enhancement of viral action. It is unclear at present whether this discrepancy is due to the doses of virus or chemical carcinogen employed. However, it is clear that pretreatment with MMS is the most effective way of enhancing FLV leukemogenesis by this chemical.

MMS dose - As mentioned above, the 3 mg/mouse dose of MMS used in the experiments of figure 2 proved to be toxic. However, when a total of 4 mg was given in two doses separated by 24 hrs (figure 4), there were no early deaths, and such a double treatment with MMS was more effective than a single 2 mg dose. Thus the MMS effect does appear to be dose dependent.

Time of MMS pretreatment - Having established that pretreatment by MMS was effective in enhancing viral leukemogenesis, we varied the interval between the MMS pretreatment and the injection of virus in order to determine whether the MMS effect was reversible. As shown above, when given 5 hrs or 24 hrs before virus, MMS was approximately equally effective. However, MMS given 2 to 4 days before FLV did not enhance viral leukemogenesis at all. Thus it appears that the MMS caused lesion is repaired by 48 hrs.

Humoral immune function - The plaque forming assay used in the present study measures the ability of mice to produce lymphocytes that synthesize antibody against sheep red blood cell antigens. This function is apparently dependent not only on B cells but also requires T helper cells (9). It has been shown that FLV causes pronounced suppression of this function in virus susceptible mice (8), and, because the degree of this immunosuppression has been correlated with the degree of susceptibility of viral leukemia in various mouse strains, it was postulated that the virally induced immunosuppression may be an etiological factor in this disease. It was therefore of interest that MMS was also found to be an effective immunosuppressant by the plaque forming assay, and that when MMS was given in combination with FLV a lower level of immunosuppression was seen than when either agent was given alone. However, this effect does not appear to be the prime cause of the presently observed potentiation of viral leukemogenesis. We draw this conclusion from the following two points: 1) MMS is effective in enhancing leukemogenesis only if given within the 24 hrs preceding the virus, although the peak of immunosuppressive activity is not reached until 5 to 7 days after MMS; 2) Cytoxan, which is equally or more immunosuppressive when compared to MMS (10), has no effect on viral leukemogenesis (unpublished results). Moreover, the short-lived nature of the MMS effect suggests that the MMS induced damage is relatively quickly repaired. Thus it is reminiscent of DNA damage repair. There is evidence that MMS-augmented, adenovirus transformation of hampster cells is also dependent on short-lived DNA damage (6). Thus we suggest that such an MMS effect, as

previously demonstrated in vitro (3,6), is probably also the most important MMS action responsible for viral enhancement in vivo.

REFERENCES

1. Mishra, N.K., Pant, K.J., Thomas, F.O. and Price, P.J. (1976) Chemical-viral co-carcinogenesis: requirements for leukemia virus expression in accelerated transformation. Int. J. Cancer 18: 852-858.

2. Ide, T., Anzai, K. and Andoh, T. (1975) Enhancement of SV40 transformation by treatment of C3H2K cells with UV light and caffeine. I. Combined effect of UV light and caffeine. Virology 66: 568-578.

3. Casto, B. (1973) Enhancement of adenovirus transformation by treatment of hamster cells with ultraviolet irradiation, DNA base analogs, and dibez(a,h)anthracene. Cancer Res. 33: 402-407.

4. Cho, H.Y., Arnstein, P. and Rhim, J.S. (1978) Chemical transformation of human revertant cells induced by murine sarcoma virus. Int. J. Cancer 21: 22-26.

5. Salerno, R.A., Ramm, G.M. and Whitmire, C.E. (1973) Chemical induction of subcutaneous tumors in Balb/c and Swiss mice infected with wild type CRNA viruses derived from Balb/c tissues. Cancer Res. 33: 63-77.

6. Casto, B.C., Pieczynski, W.J., Janosko, N. and DiPaolo, J.A. (1976) Significance of treatment interval and DNA repair in the enhancement of viral transformation by chemical carcinogens and mutagens. Chem. Biol. Interact. 13: 105-125.

7. OKunewick, J.P., Meredith, R.F., Brozovich, B. and Weaver, E.V. (1978) Stimulation of immune response in hybrid mice following Rauscher virus infection. Proc. of the Soc. for Exp. Biol. and Med. 157: 448-451.

8. Ceglowski, W.S., Campbell, B.P. and Friedman, H. (1975) Immunosuppression by leukemia viruses. XI. Effect of Friend leukemia virus on humoral immune competence of leukemia-resistant C57B1/6 mice. J. Immunol. 114: 231-236.

9. Kennedy, J.C. and Axelrad, M.A. (1971) An improved assay for hemolytic plaque forming cells. Immunol. 20: 253-257.

10. Scott, J.R. (1977) The effect of immunosuppressive drugs and immunostimulative agents on reproductive performance. Transplant. Proc. 9: 1403-1407.