In Vivo Enhancement of a Murine Sarcoma Virus by Diethylaminoethyl-Dextran (35567)

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The Moloney strain of murine sarcoma virus (M-MSV) rapidly induces tumors in mice of all ages (1, 2). In newborn mice, tumor growth is usually progressive and results in death, but in the majority of immunologically competent mice tumor regression occurs spontaneously (2, 3). M-MSV readily propagates in murine tissue culture cells; and, at appropriate dilutions, it produces discrete focal lesions (4). When mouse cells are infected with serial dilutions of certain stocks of M-MSV which contain limiting amounts of murine leukemia virus (MuLV), the number of foci decreases as the square of the virus dilution (4). Addition of optimal amounts of MuLV (helper virus) to the assay plates results in an increase in focus formation in such a manner that the number of foci are proportional to the virus dilution, indicating that dual infection of mouse cells with both M-MSV and MuLV is necessary for focus formation under the usual assay conditions (4).

The uptake of proteins by tissue culture cells is enhanced by polycations (5). Polycations also enhance the sensitivity of tissue culture assays for infectious viral nucleic acid, as well as certain intact viruses (6–9). Viral enhancement is often due to the neutralization of polyanionic inhibitors, but also occurs in their absence (10). The polycation diethylaminoethyl-dextran (DEAE-D) increases cell sensitivity to certain RNA leukemia and sarcoma viruses of avian, murine, and feline origin, including M-MSV (10-14). Enhancement is maximal if the cells are pretreated 30–90 min before MSV infection. The concentration of DEAE-D used is critical, as high concentrations are toxic to cell cultures. The enhancement of MSV focus formation in mouse cells by DEAE-D is approximately 10- to 30-fold (13, 14). Although enhancement of virus activity in cell culture systems by DEAE-D is well documented, comparatively little is known about the *in vivo* effects. This report describes the effects of DEAE-D on M-MSV tumor induction in mice.

Methods and Results. Virus preparations. A single stock of M-MSV was used for both *in vivo* and *in vitro* experiments. This preparation was obtained from M-MSV infected monolayer cultures of 3T3FL cells 96 hr after inoculation with M-MSV derived from mouse tumor material. When titered in 3T3FL cells as previously described (15), this virus gave a "two-hit" titration pattern indicating that it contained defective M-MSV together with endogenous MuLV, but that the latter was not present in sufficient concentration to infect all the cells in the assay plate (4) (Fig. 1). Addition of optimal amounts of MuLV to this M-MSV stock resulted in an enhancement of focus formation in such a way that the titration pattern became "one-hit," *i.e.*, the number of foci was proportional to the virus dilution. The virus pool contained 5.3×10^5 focus-forming units (FFU)/ml when assayed in the presence of an optimal concentration of helper virus.

The helper virus used was the IC isolate of MuLV (16), and was kindly provided by Dr. Peter Fischinger.

Focus assay. Focus formation by M-MSV in 3T3FL cells, a subline of the original 3T3 NIH Swiss mouse cell line (17), was determined as described previously (15). DEAE-D was added to assay dishes at a concentration of 30 μg/ml, optimal for this cell line. Immediately prior to infection of cells, 2 ml

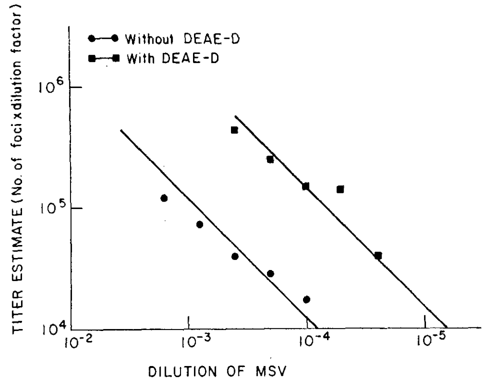

Fig. 1. Enhancement of M-MSV focus formation in 3T3 FL cells by DEAE-D.

of DEAE-D diluted in complete tissue culture medium were added to each dish, and the plates were incubated at room temperature for 20 min. The cells were rinsed once and infected with virus.

Mouse inoculations. NIH Swiss mice were supplied by the Animal Production Unit, National Institutes of Health, Bethesda, Maryland. DEAE-D was obtained from Pharmacia, Uppsala, Sweden, and had a molecular weight of approximately 2 × 10^6. McCoy's modified 5A medium (Grand Island Biological Company, Grand Island, New York) was used for preparing virus and DEAE-D dilutions, and for inoculation of control mice. Virus was inoculated intramuscularly (im) into the left thigh. Mice were examined daily, and tumor size was estimated according to the criteria of Blumenschein and Moloney (18).

Effect of DEAE-D on focus formation. Pretreatment of 3T3FL cells with 30 μg of DEAE-D enhanced focus formation by M-MSV approximately 10-fold (Fig. 1), an enhancement comparable to that previously reported (13, 14). Helper assays in 3T3FL cells using IC virus were performed as described previously (15). No enhancement of helper activity in cells pretreated with 30 μg/ml of DEAE-D was noted.

Toxicity of DEAE-D in vivo. The doses of DEAE-D used for the *in vivo* studies (50 and 200 μg) caused no mortality when inoculated intraperitoneally (ip) into newborn and 4-week-old mice, and had no effect on weight gain. Doses greater than 300 μg caused runting in newborn mice.

In vivo *titration.* Figure 2 demonstrates the effect of DEAE-D on the latent time to tumor induction by serial dilutions of M-MSV in 4 week old mice. Two hundred μg of DEAE-D were inoculated with the virus. At all the virus dilutions, DEAE-D significantly shortened the latent time to tumor appearance ($p < .01$). Moreover, a 10-fold enhancement was noted in the minimal tumor inducing dose, as calculated by the Reed–Muench formula (19). Data on tumor regression are presented in Table I. All tumors in the control group regressed, but in the lower dilutions of the DEAE-D treated group some mice died from progressive tumor growth. The tumor regression times of the DEAE-D treated and control groups were not significantly different.

Effect of pretreatment. The effect of pretreatment with DEAE-D on M-MSV tumor induction in 4-week-old mice is presented in Table II. The pretreated mice were inoculated with 200 μg of DEAE-D into the left thigh 1 hr prior to inoculation of 2.5 × 10^2 FFU of M-MSV at the same site. Controls received pretreatment with an equal volume (0.2 ml) of diluent. Other groups received M-MSV alone or M-MSV plus DEAE-D inoculated together. Tumors appeared in both of the DEAE-D-treated groups at a significant-

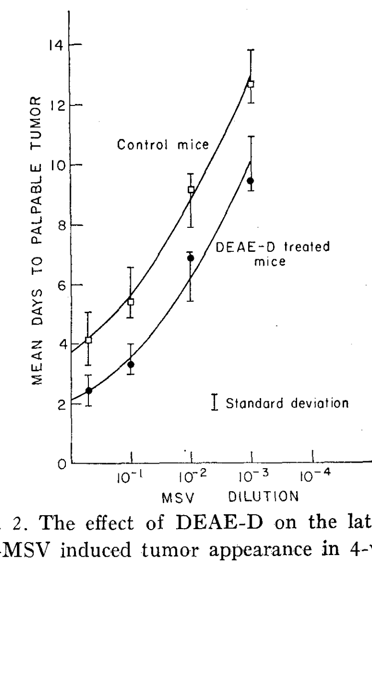

Fig. 2. The effect of DEAE-D on the latent time to M-MSV induced tumor appearance in 4-week-old mice.

TABLE I. Effect of DEAE-D on Regression of M-MSV-Induced Tumors in 4-Week-Old Mice.[a]

Group	Virus dilution	No. of regressions/ no. of tumors	Mean time in regression (days)
Control	$10^{-0.3}$	9/9	27.2
	10^{-1}	8/8	31.0
	10^{-2}	9/9	18.9
	10^{-3}	3/3	24.3
	10^{-4}	0/0	—
DEAE-D[b] treated	$10^{-0.3}$	4/9	33.8
	10^{-1}	0/9	33.5
	10^{-2}	8/8	22.9
	10^{-3}	9/9	11.0[c]
	10^{-4}	3/3	8.1[c]
	10^{-5}	0/0	—

[a] Eight or 9 mice inoculated in each group.

[b] 200 μg of DEAE-D inoculated simultaneously with M-MSV.

[c] Several tumors were of a relatively small size.

ly earlier time ($p<.001$) than in their respective control groups. The 2 control groups did not differ significantly ($p>.1$) in latent period to tumor appearance while the two DEAE-D groups differed significantly from one another ($p<.001$). Table II also presents data on tumor weight. Mice were sacrificed 5–7 days after apparent tumor growth had ceased, prior to the commencement of regression. Tumor weights in the two DEAE-D-treated groups were not significantly different from their respective control groups.

Effects of additional helper virus. The effects of added MuLV helper virus (10^6 helper units) on DEAE-D-mediated enhancement of M-MSV in 4-week-old mice are shown in Table III. All groups were inocu-

TABLE III. Effect of Additional Helper Virus on DEAE-D Enhancement of M-MSV Tumor Induction in 4-Week-Old Mice.[a]

Group	No. of tumors/no. inoculated	Mean time to palpable tumor (days) (coefficient of variability)
M-MSV	9/9	7.7 (18.2%)
M-MSV + helper[b]	9/9	8.2 (14.6%)
M-MSV + DEAE-D[c]	9/9	5.1 (17.6%)
M-MSV + helper[b] + DEAE-D[c]	9/9	5.4 (9.3%)

[a] All inoculations consisted of 8×10^2 FFU of M-MSV inoculated im, in a volume of 0.2 ml.

[b] 10^6 helper units of IC virus inoculated with M-MSV.

[c] 200 μg of DEAE-D inoculated with M-MSV.

lated im with 8×10^2 FFU of M-MSV. Both groups inoculated with DEAE-D developed tumors at a significantly earlier time ($p<.001$) than the control group. Addition of helper virus had no significant effect ($p>.1$) on the latent period whether M-MSV was injected with or without DEAE-D.

Route of inoculation. The effects of different routes of inoculation and doses of DEAE-D on M-MSV tumor induction in newborn mice are presented in Table IV. DEAE-D was inoculated ip 1 hr prior to virus inocula-

TABLE II. Effect of DEAE-D Pretreatment on M-MSV Tumor Induction in 4-Week-Old Mice.[a]

Group	No. of tumors/ no. inoculated	Mean time to palpable tumor (days) (coefficient of variability)	Mean tumor wt (g)
DEAE-D pretreatment[b]	11/11	6.4 (21.2%)	2.44
Pretreatment control[c]	8/11	10.3 (32.0%)	2.91
DEAE-D + M-MSV inoculated simultaneously	22/22	8.1 (12.2%)	1.15
Control for simultaneous inoculation	15/22	11.2 (14.4%)	1.32

[a] All virus inoculations consisted of 2.5×10^2 FFU of M-MSV inoculated im in a volume of 0.2 ml.

[b] 200 μg of DEAE-D in a volume of 0.2 ml inoculated im 1 hr prior to virus inoculation at the same site.

[c] 0.2 ml of diluent inoculated im 1 hr prior to virus inoculation at the same site.

TABLE IV. Effects of Inoculation of DEAE-D by Different Routes and Different Doses on M-MSV Tumor Induction in Newborn Mice.

Dose of DEAE-D (μg)	Inoculation route	Inoculum vol[a] (ml)	No. of tumors/no. inoculated	Mean time to palpable tumor (days) (coefficient of variability)	Mean time to death (days) (coefficient of variability)
50	ip	0.1	12/12	10.1 (8.9%)	17.7 (21.5%)
200	ip	0.1	12/12	8.2 (18.3%)	15.3 (17.7%)
Control	ip	0.1	14/14	15.0 (41.3%)	28.1 (39.5%)
50	im	0.2	17/17	10.4 (14.4%)	17.4 (21.2%)
200	im	0.2	18/18	10.1 (6.9%)	18.9 (11.6%)
Control	im	0.2	17/17	16.3 (39.9%)	31.4 (45.5%)

[a] All mice received 2×10^2 FFU of M-MSV, inoculated im.

tion, or im together with M-MSV (2×10^2 FFU). Two doses of DEAE-D, 50 and 200 μg, were used. All the mice devloped progressively growing lethal tumors. The four DEAE-D-treated groups of mice developed tumors after a significantly shorter latent period ($p<.01$) than their respective control groups and these treated mice died at a significantly earlier time. The coefficients of variability for both latent period and time to death was considerably smaller in the DEAE-D-treated groups than in the control groups, indicating a more uniform response. There was no significant difference in enhancing ability between 50 and 200 μg of DEAE-D when DEAE-D was inoculated im, but when inoculated ip, the 200-μg group developed tumors significantly earlier than the 50-μg group.

Discussion. Much data exist concerning the *in vitro* enhancement of viruses by DEAE-D, but the *in vivo* effects have seldom been investigated. Craighead (20) reported that DEAE-D increased the pathogenicity and dissemination of the r^+ variant of encephalomyocarditis virus. The results presented in this report indicate that DEAE-D enhances the pathogenicity of M-MSV as measured by several parameters. Significant enhancement occurred regardless of virus dilution and followed either systemic or local administration of DEAE-D. As with *in vitro* enhancement, pretreatment of mice with DEAE-D prior to virus inoculation had a greater enhancing effect than simultaneous administration with virus. While the two systems are not strictly comparable, it is of interest to note that the simultaneous administration of DEAE-D with virus in mice resulted in a 10-fold increase in virus potency, an enhancement comparable to that obtained in tissue culture using pretreatment. DEAE-D did not increase the helper effect of added MuLV on M-MSV either *in vivo* or *in vitro*. These results indicate that the DEAE-D mediated enhancement of focus formation by defective stocks of M-MSV may be due to a potentiation of the M-MSV component alone. It cannot be excluded, however, that potentiation of different preparations of MuLV by DEAE-D may vary. It should be noted that conclusive evidence of an *in vivo* helper effect of MuLV on tumor formation by M-MSV has yet to be demonstrated.

It appears that enhancement by DEAE-D of virus infectivity in cell culture systems is at least in part due to an increased adsorption rate of virus to cell (10, 12). However, the possibility that the enhancement of DEAE-D of M-MSV tumor induction described in this report could be due to other actions of DEAE-D in the intact animal cannot be excluded. These possible actions include effects on the immune and interferon-inducing systems and tumor cell metabolism. With regard to the latter possibility, it is of interest to note that DEAE-D has been reported to have an inhibitory effect on tumor growth (21).

These studies show that DEAE-D may be of use in the detection of activity in virus preparations having a relatively low oncogenic potential in the *in vivo* system under investigation.

Summary. The polycation diethylaminoethyl-dextran (DEAE-D) enhanced the oncogenicity of Moloney sarcoma virus (M-MSV) in mice as measured by several parameters including shortening of the latent time to tumor appearance, reduction of the minimal tumor inducing dose, decreased survival time, and decreased incidence of spontaneous regressions. Enhancement occurred following both local and systemic administration of DEAE-D, and was greater when DEAE-D was administered prior to virus inoculation. The degree of *in vivo* enhancement of M-MSV by DEAE-D was similar to the enhancement of focus formation in tissue culture.

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