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Effect of Polycations on Sensitivity of BALB/3T3 Cells to Murine Leukemia and Sarcoma Virus Infectivity

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Polybrene or diethylaminoethyl dextran, when added with murine leukemia or sarcoma virus inocula, significantly increased virus infectivity of BALB/3T3 cells. Residual polycation concentrations were nontoxic.

Pretreatment of cells in tissue culture with the polycation diethylaminoethyl dextran (DEAE-D) increases the sensitivity to infection by several ribonucleic acid (RNA) tumor viruses (2, 4, 6-8). However, concentrations of DEAE-D required for maximum enhancement are toxic to cells upon prolonged exposure (1, 2, 4, 7). Cell toxicity is routinely avoided by extensively washing the cells before virus inoculation. In contrast, the polycation hexadimethrine (polybrene) which substantially enhances Rous sarcoma virus (RSV) infection is nontoxic to the cellular substrate (7). This paper describes the increased sensitivity of BALB/3T3 cells to murine leukemia virus (MuLV) and murine sarcoma virus (MSV) infection obtained when polybrene is added to cultures at the time of virus inoculation. We also compare polybrene enhancement of MSV focus formation with that of DEAE-D and propose a simple procedure to avoid the toxic effects of DEAE-D.

Stocks of MuLV and MSV (Moloney strain) were obtained from the National Cancer Institute and were propagated in a high-passage mouse whole embryo cell line (HPME) developed in this laboratory and derived from random bred Swiss mice. BALB/3T3 cells (kindly provided by R. V. Gilden, Flow Laboratories, Rockville, Md.) were used for both MSV and MuLV infectivity assays. BALB/3T3 cells were seeded at a density of 10^6 cells in 250-ml plastic flasks and grown in antibiotic-free growth medium (GM) consisting of Eagle's minimum essential medium supplemented with 10% fetal calf serum. The next day monolayers were inoculated with 0.5 ml of a virus-polycation admixture. Virus was mixed with polybrene (Abbott Laboratories, North Chicago, Ill.) or DEAE-D (Pharmacia Fine Chemicals, Piscataway, N.J.), which had been dissolved in GM. Virus was adsorbed to cells at 37 C for 90 min, and then 24.5 ml of GM was added without removing residual polycation or unadsorbed virus.

MuLV infectivity was assayed with the XC cell plaque technique described by Rowe et al. (5). MSV infectivity was assayed by the focus-forming method of Hartley and Rowe (3).

The sensitivity of BALB/3T3 cells to infection with MuLV or MSV was markedly increased by the addition of polybrene to the virus inoculum. Maximum enhancement for both viruses corresponded to the lowest concentration tested, 2 μg/ml. As illustrated in Fig. 1, concentrations of polybrene greater than 2 μg/ml also substantially increased the sensitivity of cells to virus infection. The size of plaques or foci was not altered by the different polybrene concentrations tested. In addition, toxic effects were not apparent.

The inclusion of DEAE-D in the virus inoculum significantly enhanced MSV focus formation. However, as seen in Fig. 2, the concentration of DEAE-D required to obtain the maximum effect, 20 μg/ml, was 10-fold higher than that for polybrene. The infectivity-DEAE-D concentration profile which we report for the Moloney strain of MSV is similar to that reported elsewhere for Kirsten and Harvey strains (2, 6). As with the polybrene study, no evidence of cellular toxicity or change in focus size was observed for any of the DEAE-D concentrations tested.

These results demonstrate that the sensitivity of BALB/3T3 cells to infection by MuLV and MSV is markedly increased by the inclusion of

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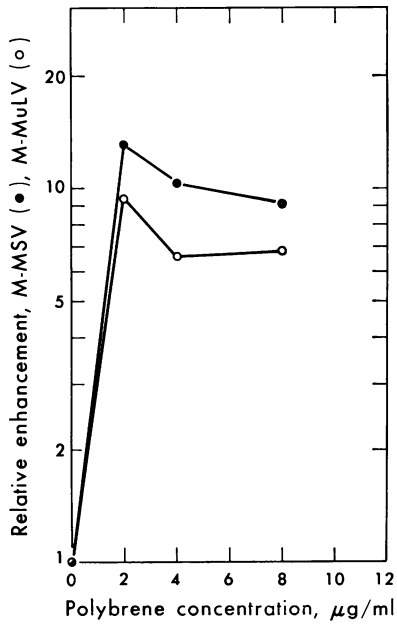

FIG. 1. *Influence of polybrene on MuLV plaque formation (○) and MSV focus formation (●) in BALB/3T3 cells. MuLV-infected cells were overlaid with 10^6 XC cells 3 days postinfection. Seven days postinfection, cells were fixed with methanol, stained with Giemsa, and scored for plaques without optical magnification. MSV infectivity was assayed by scoring foci of transformed cells without optical magnification 5 days postinfection. Relative enhancement represents the estimated plaque- or focus-forming unit (PFU, FFU) titer in polycation-treated cultures divided by that in untreated cultures. Projected MuLV and MSV titers of untreated cultures were 1.7 × 10^6 PFU/ml and 9.2 × 10^5 FFU/ml, respectively.*

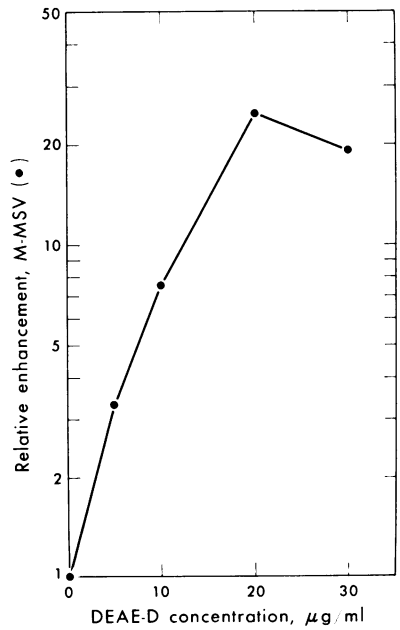

FIG. 2. *Relative enhancement of DEAE-D on MSV focus formation in BALB/3T3 cells. Assay conditions are as described in Fig. 1.*

polybrene or DEAE-D in the virus inoculum. The finding that MSV enhancement by polybrene requires significantly lower concentrations than DEAE-D is similar to that previously reported elsewhere for RSV (7). The induction of cellular toxic effects by DEAE-D was effectively eliminated by diluting the reagent-virus inoculum 50-fold immediately after the adsorption period. This procedure which eliminates the necessity of polycation pretreatment and subsequent extensive washing may be applicable to in vitro viral assay systems other than MuLV and MSV. The use of either polybrene or DEAE-D as described here may be of particular importance in enhancing detection of low concentrations of virus.

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