PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 219–222 (1975)

Inhibition by Levamisole of Metastases by Cells Transformed by Herpes Simplex Virus Type 1 (38776)

J. MICHAL SADOWSKI AND FRED RAPP

Department of Microbiology, College of Medicine, The Milton S. Hershey Medical Center of The Pennsylvania State University, Hershey, Pennsylvania 17033

Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) induce transformation of hamster cells (1–3) after exposure to ultraviolet (uv) irradiation or after photodynamic inactivation (4). Inoculation of the transformed cells into newborn or weanling hamsters results in tumor production accompanied by metastases (1, 3). In a previous report from this laboratory, immunologic manipulation changed the frequency of metastases due to herpesvirus-transformed cells (5); inhibition of metastases by previous immunization with SV40 virus and uv-irradiated tumor cells and enhancement after inoculation of herpesvirus were noted. Further investigation of the inhibition of metastases induced by HSV-1-transformed cells was conducted in this study using the drug Levamisole (6, 7), an immunopotentiator (8).

Materials and Methods. A total of 160 weanling Syrian hamsters (3 wk old) were divided into four groups, 40 animals per group. Hamsters in group A served as controls. Other groups consisted of eight animals injected subcutaneously with either 10^1, 10^2, 10^3, 10^4, or 10^5 HSV-1-transformed cells (line 14-012-8-1 T ※ 10). Each animal in group B was given an intraperitoneal injection of 2 mg of Levamisole (ORF-10321 from ORTHO Pharmaceutical Corporation) simultaneously with the transformed cells. Animals in group C were injected once with 2 mg of Levamisole 24 hr after inoculation of the transformed cells. The hamsters of group D were injected once a week with 2 mg of Levamisole as soon as a tumor was palpable at the site of inoculation. Experimental animals were checked for tumors twice a week to calculate the number required to produce tumors in 50% of the animals (TPD50). The TPD50 was calculated by the Reed-Muench method (9). All hamsters used in this study were sacrificed 13 wk after cell challenge. Each animal was checked for metastases at the time of postmortem examination. The χ^2 test with the Yates correction (10) was used in the statistical calculations.

TABLE I. INDUCTION OF TUMORS AT SITE OF INOCULATION BY HSV-1-TRANSFORMED CELLS IN UNTREATED AND LEVAMISOLE-TREATED HAMSTERS.

Transformed cells challenge	Number of challenged hamsters in each group	Group A[a] No. with tumors	Groyup B[b] No. with tumors	Group C[c] No. with tumors	Group D[d] No. with tumors
10^1	8	2	1	0	0
10^2	8	5	2	3	2
10^3	8	8	6	8	6
10^4	8	8	8	8	8
10^5	8	8	8	8	8

[a] Group A. Control without drug.
[b] Group B. Transformed cells and drug at the same time.
[c] Group C. Drug 24 hr after challenge with transformed cells.
[d] Group D. Drug once a week in hamsters with tumor.

Results and Discussion. The results presented in Fig. 1 show the TPD 50 values for each group of hamsters. Primary tumors developed in each group of hamsters at essentially the same rate. However, there are

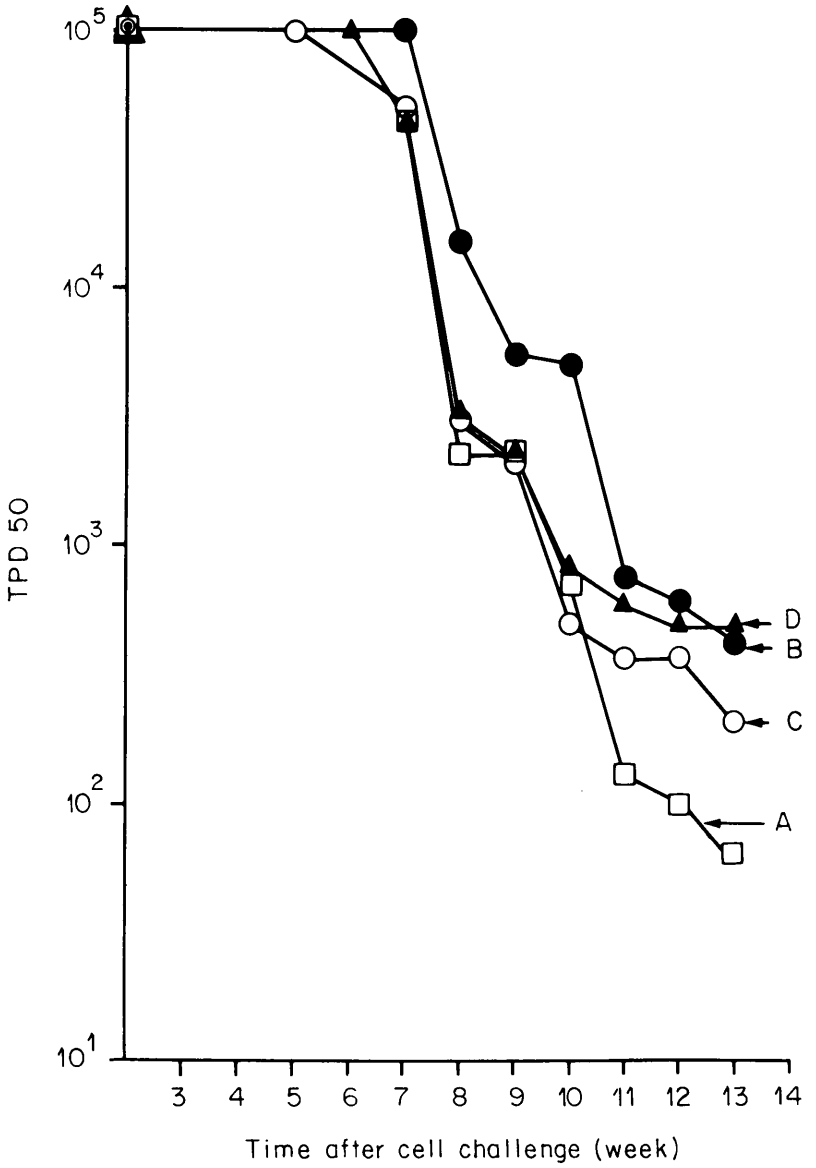

FIG. 1. TPD50 values in hamster inoculated with HSV-1-transformed cells and treated with Levamisole. Group A. Control without drug. Group B. Transformed cells and drug simultaneously. Group C. Drug treatment 24 hr after inoculation of transformed cells. Group D. Drug once a week after tumor development.

differences in the TPD50 values between control group A (without the drug) and groups, B, C, D treated with Levamisole. More than 200 cells were required per TPD50 in the experimental groups compared with less than 50 in the control group.

Results of the induction of tumors and metastases by the HSV-1-transformed cells in control and drug-treated hamsters are presented in Table I. There are differences in the frequency of tumor induction in the control group when compared to the drug-treated hamsters. However, these differences are not statistically significant.

The metastatic lesions observed were usually localized in the lungs (Fig. 2) and found rarely in the kidneys and liver. Table II contains the observations concerning

metastases in the hamsters. Significant differences were noted between the untreated and Levamisole-treated animals. Fewer metastases were noticed in drug-treated animals, especially in group D. Our initial assumption using the Null Hypothesis (N.H.) was that there would be no difference in tumor incidence between untested animals and groups treated with Levamisole. However, a N.H. level of 5% was calculated for the hamsters of group D which had been injected with 10^3, 10^4, or 10^5 HSV-1-transformed cells and treated once a week with the drug after tumor development. The χ^2 values in these cases are 6.66, 6.25, and 7.10, respectively.

Previous work in this laboratory had revealed that the hamster cells transformed by HSV-1 and used in these experiments carry a virus-specific glycoprotein at the cell surface (11). Transplant of these cells to hamsters elicits antibody capable of neutralizing the transforming virus (2) and results in appearance of lymphocytes specifically cytotoxic (12) for the homologous tumor cells. Despite this immune response, metastases develop and ultimately kill the host. It is possible that the immune response develops too late to prevent spread of the cells and is then ineffective in destroying the tumor mass that develops. The results reported here suggest that Levamisole can reduce the incidence

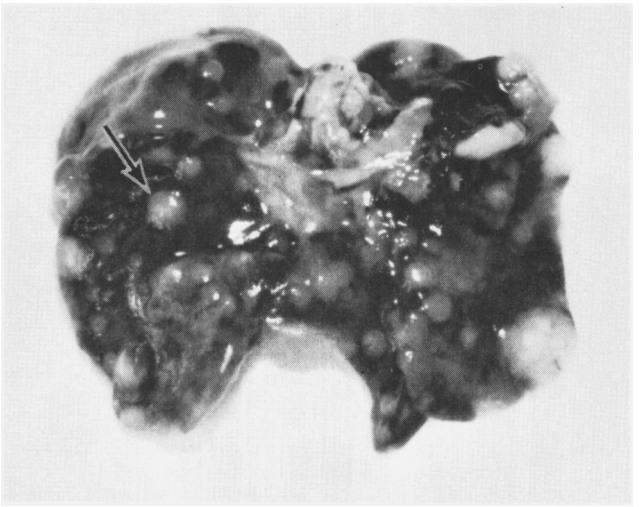

FIG. 2. Multiple metastases in lungs of hamsters inoculated subcutaneously with cells transformed by HSV-1. Arrow points to one of many metastases. Frequency of these metastases was reduced in animals injected with Levamisole.

TABLE II. OCCURRENCE OF METASTASES DUE TO HSV-1-TRANSFORMED CELLS IN UNTREATED AND LEVAMISOLE-TREATED HAMSTERS.

Challenge no. of transformed cells per hamster	Number of challenged hamsters in each group	Group A		Group B			Group C			Group D		
		No. without metastasis	No. with metastasis	No. without metastasis	No. with metastasis	$\chi^2[1]$	No. without metastasis	No. with metastasis	$\chi^2[1]$	No. without metastasis	No. with metastasis	$\chi^2[1]$
10^1	8	8	0	8	0	1	8	0	1	8	0	1
10^2	8	5	3	7	1	0.33	7	1	0.33	8	0	1.64
10^3	8	2	6	5	3	1.01	5	3	1.01	8	0	6.66*
10^4	8	1	7	2	6	0	4	4	0.5	7	1	6.25*
10^5	8	0	8	1	7	0	2	6	0.57	7	1	7.10*

* Statistically significant levels of the inhibition of metastases by the drug.

of tumor metastases in treated animals even after tumors are palpable. Presumably, the effect precedes spread of the cells from the initial site of inoculation. It should, therefore, be possible to determine when metastases occur by manipulating the drug regimen. Obviously, if drug treatment had been continued in the experimental groups given only one injection of Levamisole, metastases would probably also have been prevented in those animals. Tests to determine directly the effect of Levamisole on the specific immune response to virus and tumor antigens carried by the transformed cells should clarify the role of the drug in the altered pathogenesis of these tumors.

Summary. Levamisole was tested to determine whether the drug could reduce metastases by HSV-1-transformed cells in a model hamster system. The results presented reveal an inhibition of metastases to the lungs even when the drug is inoculated after development of subcutaneous tumors at the site of inoculation of the cells.

This study was conducted under Contract N01 CP 53516 within the Virus Cancer Program of the National Cancer Institute, National Institutes of Health, U.S. Public Health Service.

We are grateful to Myron Katz for his excellent technical assistance.

1. Duff, R., and Rapp, F., J. Virol. **8**, 469 (1971).
2. Rapp, F., and Duff, R., Cancer Res. **33**, 1527 (1973).
3. Duff, R., and Rapp, F., J. Virol. **12**, 209 (1973).
4. Rapp, F., Li, J-L. H., and Jerkofsky, M., Virology **55**, 339 (1973).
5. Duff, R., Doller, E., and Rapp, F., Science **180**, 79 (1973).
6. Renoux, G. and Renoux, M., Nature New Biol. **240**, 217 (1972).
7. Hoebeke, J., and Franchi, G., RES J. Reticulo Endothel. Soc. **14**, 317 (1973).
8. Chirigos, M. A., Pearson, J. W., and Pryor, J., Cancer Res. **33**, 2615 (1973).
9. Reed, L. J., and Muench, H., Amer. J. Hyg. **27**, 493 (1938).
10. Clarke, G. M., *in* "Statistics and Experimental Design", 57pp. American Elsevier, New York (1969).
11. Reed, C. L., Cohen, G. H., and Rapp, F., J. Virol. **15**, 558 (1975).
12. Lausch, R. N., Jones, C., Christie, D., Hay, K. A., and Rapp, F., J. Immunol. **114**, 459 (1975).

Received Nov. 11, 1974. P.S.E.B.M., 1975, Vol. 149