6. B.St.L.977; WGA-HRP (0.2 μl) was injected four times in the posterior lobe.
7. J. D. Coulter, M. C. Sullivan, M. A. Ruda, *Soc. Neurosci. Abstr.* **6**, 339 (1980); S. K. Itaya and G. W. van Hoesen, *Brain Res.* **236**, 199 (1982).
8. G. C. Bangma and H. J. ten Donkelaar, *J. Comp. Neurol.* **207**, 255 (1982).
9. H. Künzle, *Exp. Brain Res.* **49**, 1 (1983).
10. A potential reciprocal circuit between the ventral cortex and hypothalamus may be completed by cerebellar corticonuclear fibers to the fastigial nucleus.
11. I am indebted to Drs. Alf Brodal, Per Brodal, Eric Rinvik, and Fred Walberg for their valuable criticism during the preparation of this report.

25 October 1982; revised 11 January 1983

Antigenic Similarity Between Cells Transformed by Ultraviolet Radiation in vitro and in vivo

Abstract. *Cells of the 10T 1/2 mouse fibroblast line transformed in vitro by ultraviolet radiation are antigenically similar to those from skin cancers produced in mice by repeated exposure to ultraviolet radiation. Both types of tumor cells grew preferentially in ultraviolet-irradiated syngeneic mice relative to untreated animals, and both were recognized by ultraviolet radiation–induced tumor-specific suppressor lymphocytes. These properties were not shared by 10T 1/2 cells transformed in vitro by x-rays or 3-methylcholanthrene.*

Skin cancers induced in mice by repeated exposure to ultraviolet (UV) radiation have two unusual immunological properties. First, these tumors are highly antigenic, in that most are rejected immunologically when transplanted into normal syngeneic mice. Second, they grow progressively in syngeneic mice that have been given a small number of exposures to UV radiation (*1*). The susceptibility of the UV-irradiated mice to transplanted tumors is caused, at least in part, by the suppressor T lymphocytes in their lymphoid organs, which prevent immunological rejection of the tumors (*2*). These lymphocytes are specific for UV radiation–induced tumors as a group, as evidenced by the finding that they do not decrease the resistance of the host to transplanted syngeneic tumors induced by other carcinogens (*3*). We investigated whether the antigenic determinant on UV radiation–induced tumor cells that is recognized by the UV radiation–induced suppressor lymphocytes arises as a consequence of exposing the cells to UV radiation or whether this determinant is present normally on cells of a particular lineage in the skin that are highly susceptible to transformation by UV radiation but less susceptible to transformation by other carcinogens.

We used tumor cell lines produced by in vitro transformation of cloned mouse fibroblasts by UV radiation, 3-methylcholanthrene (MCA), or x-rays. The development of these cell lines was compared in normal, UV-irradiated, and immunosuppressed mice. Only the tumor lines produced by transformation with UV radiation grew preferentially in UV-irradiated mice relative to untreated animals, and this preferential growth was attributable to the activity of the UV radiation–induced suppressor T lymphocytes. The results suggest that exposure of cells to UV radiation induces a carcinogen-associated determinant (or set of determinants) against which the UV radiation–induced suppressor T lymphocytes react, and that there is a similarity between UV radiation–induced transformation in vitro and UV carcinogenesis in vivo.

The mouse fibroblast cell line used was C3H 10T 1/2 clone 8 (*4*). Transformants were generated independently by a single exposure to x-rays, MCA, or 254-nm UV radiation (20 or 30 J/m^2 from a germicidal lamp) (*5*). Clones TU2 and TU3 were derived from type III foci, which are usually highly tumorigenic; clone TU4 was derived from a type II focus. Type II foci generally are composed of cells that are less tumorigenic than those in type III foci (*4*). Both of the cell lines obtained from x-ray–transformed clones were derived from type III foci generated by 600 R of x-radiation. The four MCA-transformed cell lines also originated from type III foci. Transformants, which grew as dense foci on top of a contact-inhibited monolayer of normal cells, were isolated with stainless steel cloning cylinders. These cells were propagated in culture to provide sufficient quantities for injection into mice. The minimum tumorigenic dose of

Table 1. Growth in syngeneic mice of C3H 10T 1/2 cells transformed in vitro by MCA, x-rays, or UV radiation.

Carcinogen	Cell line	Number of cells injected	Tumor incidence in syngeneic mice		
			Untreated	UV-irradiated*	Thymectomized and x-irradiated (450 R)
None	10T 1/2, clone 8	1×10^8	0/10	0/10	0/10
MCA	PT	1×10^3	3/10	3/10	9/10
	16	1×10^3	2/10	3/10	10/10
	609C-2	1×10^5	1/10	0/10	6/10
	5	1×10^4	4/10	3/10	10/10
Total (%)			10/40 (25)	9/40 (23)	35/40 (88)
X-radiation	F11	5×10^3	5/10	5/10	10/10
	F17	1×10^5	0/10	0/10	4/10
Total (%)			5/20 (25)	5/20 (25)	14/20 (70)
UV radiation	TU2	1×10^6	0/10	9/10	8/10
	TU3	1×10^4	1/10	6/10	10/10
	TU4	5×10^6	0/10	2/10	2/10
Total (%)			1/30 (3)	17/30 (57)	20/30 (67)

*Exposed to sunlamp radiation three times per week for at least 12 weeks.

Table 2. Effect of UV radiation–induced suppressor lymphocytes on the growth of C3H 10T 1/2 fibroblasts transformed in vitro.

Cells used to reconstitute lethally x-irradiated mice	Tumor incidence in mice challenged with		
	UV radiation–induced sarcoma cells (1×10^6)*	UV radiation–transformed 10T 1/2 cells (1×10^6)†	MCA-transformed 10T 1/2 cells (1×10^4)
Normal lymphoid cells (5×10^7)	0/10	0/10	4/10
UV-irradiated lymphoid cells (5×10^7)‡	10/10	8/10	4/10
Normal cells (5×10^7) plus UV-irradiated lymphoid cells (5×10^7)‡	5/10	5/10	5/10

*Derived from a tumor induced by repeated irradiation of a C3H mouse with FS40 sunlamps. †10T 1/2 cells transformed in vitro with 254-nm UV radiation. ‡Spleen and lymph node cells from mice exposed to sunlamp radiation four times per week for at least 12 weeks.

cells from each transformed line was estimated from titration experiments in which graded doses of each cell line were tested for growth in normal mice. The appropriate dose of cells was suspended in 0.1 ml of Hanks balanced salt solution and injected subcutaneously.

Recipient animals were specific pathogen–free female mice of the inbred strain C3H/HeN(MTV$^-$), obtained from the Frederick Cancer Research Facility. Immunosuppression of one group of animals was achieved by thymectomy at 4 weeks of age, followed by the administration of 450 R of whole-body x-radiation 24 hours before the inoculation of transformed cells. Mice in a second group were shaved weekly and exposed to UV radiation from Westinghouse FS40 sunlamps at an average cosine-corrected incident dose rate of 4 J/m^2-sec for 1 hour three times per week, beginning at 8 weeks of age. Approximately 80 percent of the radiation emitted by these lamps is composed of wavelengths between 280 and 340 nm. The mice were irradiated for a minimum of 12 weeks before being used in the experiments. A third (control) group of mice, from the same age-matched pool of mice as the UV-irradiated animals, was not treated. After being injected with the transformed cells, the animals were examined weekly for tumor formation at the injection site for 6 to 9 months or until they died.

Injection of 1×10^8 cells of the parent 10T 1/2 line did not produce tumors in any of the recipient mice, whereas all the lines transformed in vitro produced tumors (Table 1). At a minimum tumorigenic dose, all these cell lines grew in a larger proportion of immunosuppressed than untreated mice, implying that all the lines exhibited some degree of antigenicity. However, only the cell lines produced by transformation in vitro by UV radiation also grew more efficiently in the UV-irradiated mice.

To ascertain whether the preferential development in UV-irradiated mice of cells transformed in vitro by UV radiation is attributable to suppressor lymphocytes, a cell transfer study was performed. C3H mice that had been lethally x-irradiated (850 R) were reconstituted 24 hours later with 5×10^7 spleen and lymph node cells from normal or UV-irradiated donors, or with a mixture of these populations. These animals were challenged subcutaneously 24 hours later with cells from a fibrosarcoma induced in a C3H mouse by repeated sunlamp irradiation (UV-2240), with 254-nm UV radiation–transformed 10T 1/2 cells (TU2), or with MCA-transformed 10T 1/2 cells (MCA5). Lymphoid cells from the UV-irradiated donors could not mediate the rejection of either UV radiation–induced tumor, and they reduced the ability of normal lymphoid cells to effect rejection (Table 2). In contrast, the MCA-transformed 10T 1/2 cell line grew similarly in the three groups of mice, indicating that its growth was unaffected by the presence of the UV radiation–induced suppressor lymphocytes. The experiment was repeated by using a different 10T 1/2 cell line transformed in vitro with UV radiation (TU3), with the same results.

These findings indicate that cells transformed in vitro by UV radiation have an antigenic determinant that is the same as, or similar to, one that is otherwise found only in skin cancers induced in vivo by UV radiation and that is recognized by the antigen-specific, UV radiation–induced suppressor lymphocytes. Thus, this antigenic determinant is related in some way to the action of the carcinogen and is not a unique property of the particular cells transformed in vivo by UV radiation. It is not known whether this determinant is associated with the transformation event itself or whether it results from some heritable effect of UV radiation on the target cells. Nonetheless, it is interesting that this same change is produced in cells after exposure to either the shorter UV wavelength (254 nm) or to wavelengths in the mid-UV range (280 to 320 nm). One question that has arisen concerning in vitro transformation by 254-nm radiation is its relevance to the induction of skin cancers in mice by repeated exposure to mid-UV radiation. Our finding that both a single exposure of fibroblasts to 254-nm radiation in vitro and repeated irradiation of mouse skin with mid-UV wavelengths induce the same antigenic determinant supports the validity of the in vitro transformation system as a model for UV radiation carcinogenesis.

MICHAEL S. FISHER
MARGARET L. KRIPKE*
Basic Research Program–LBI, Frederick Cancer Research Facility, Frederick, Maryland 21701
GERALD L. CHAN
Laboratory of Radiobiology, Harvard School of Public Health, Boston, Massachusetts 02115

References and Notes

1. M. L. Kripke, *J. Natl. Cancer Inst.* **53**, 1333 (1974); *Cancer Res.* **37**, (1977); _____ and M. S. Fisher, *J. Natl. Cancer Inst.* **57**, 211 (1976).
2. M. S. Fisher and M. L. Kripke, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1688 (1977); C. W. Spellman and R. A. Daynes, *Transplantation* **24**, 120 (1977); M. S. Fisher and M. L. Kripke, *Science* **216**, 1133 (1982).
3. M. S. Fisher and M. L. Kripke, *J. Immunol.* **121**, 1139 (1978); M. L. Kripke *et al.*, *Transplantation* **28**, 212 (1979); M. L. Kripke *et al.*, *J. Natl. Cancer Inst.* **68**, 685 (1982).
4. C. A. Reznikoff, J. S. Bertram, D. W. Brankow, C. Heidelberger, *Cancer Res.* **33**, 3239 (1973).
5. G. L. Chan and J. B. Little, *Nature (London)* **264**, 442 (1976).
6. Research sponsored by the National Cancer Institute under contract N01-CO-23909 with Litton Bionetics, Inc., and by NIH grants CA-09078 and CA-11751.
* Present address: Department of Immunology, Box HMB 178, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

28 October 1983; accepted 21 December 1983

Adaptive Response of Human Lymphocytes to Low Concentrations of Radioactive Thymidine

Abstract. *When human lymphocytes were cultured with [^{3}H]thymidine, which acts as a source of low-level chronic radiation, and then exposed to 150 rad of x-rays at 5, 7, 9, or 11 hours before fixation, the yield of chromatid aberrations was less than the sum of the yields of aberrations induced by [^{3}H]thymidine and x-rays separately. Often fewer aberrations were found after exposure to radiation from both sources than were found after exposure to x-rays alone. At the same fixation times, nonradioactive thymidine did not affect the yield of x-ray-induced aberrations. The same phenomenon occurred at earlier fixation times, after exposure to 30 or 40 rad of x-rays and [^{3}H]thymidine. This response is analogous to the adaptive response to alkylating agents whereby prior treatment with small doses for a long period reduces the damage occurring from large doses of similar agents given for a short time.*

Low doses of radiation have frequently been reported to affect the response of cells to subsequent doses (*1*), but this phenomenon is still a matter of controversy [for example, see (*2*)]. Incorporation of [^{3}H]thymidine ([^{3}H]dThd) into chromosomes causes various kinds of genetic damage, including chromatid aberrations (*3*), which also can be induced by x-rays. To study the interaction of low chronic doses of radiation with subsequent higher acute doses in the production of chromatid aberrations, we carried out experiments in which human peripheral lymphocytes in culture were first labeled with [^{3}H]dThd and then exposed to x-rays 3 to 11 hours before fixation. Radiation from incorporated