Effect of Ultraviolet-B Radiation on the in Vivo Growth of Murine Melanoma Cells

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ABSTRACT

The role of UV radiation in the development of malignant melanoma has yet to be clearly defined. The purpose of these studies was to determine whether UV irradiation of mice produces local or systemic alterations that increase the in vivo growth of transplanted melanoma cells. K-1735 melanoma cells were injected into the external ears of syngeneic C3H mice. UV irradiation of the mice before or at the time of injection of the melanoma cells accelerated the appearance of the tumors. The effect was observed when melanoma cells were transplanted directly into the site of UV irradiation, but not when they were injected into an unirradiated site. The initial survival of radiolabeled melanoma cells at the site of inoculation was not altered by UV irradiation of the host, suggesting that the accelerated appearance of tumors was due to an increase in the clonogenic potential of cells injected into UV-irradiated skin. The effect of UV irradiation on the development of other syngeneic tumors was also investigated. The outgrowth of a second melanoma was also accelerated in UV-irradiated mice, whereas the growth of a UV-induced fibrosarcoma, a methylcholanthrene-induced fibrosarcoma, and a spontaneous hepatocarcinoma was not affected. These results suggest that, in addition to its carcinogenic activity, UV radiation may contribute to the incidence of cutaneous melanoma because of a local effect on the skin that stimulates melanoma development.

INTRODUCTION

Over the past several decades, the incidence of cutaneous melanoma has increased at an alarming rate both in the United States and in other countries (1–3). Exposure to sunlight, particularly the UV rays, has been implicated as a contributing factor in the etiology of this disease (4, 5). The exact role played by UV radiation in the induction of melanoma remains controversial, however, because unlike the common basal and squamous cell carcinomas of the skin, most cutaneous melanomas do not seem to be associated with a cumulative lifetime exposure to UV radiation (6, 7). The findings that UV irradiation has multiple effects on both systemic immunity (8, 9) and on local inflammatory processes in the skin (10), in addition to its carcinogenic activity, suggest that UV radiation might contribute to the pathogenesis of melanoma at stages other than the initial transformation event. The threat of increasing UV radiation in sunlight owing to depletion of stratospheric ozone (11) makes it important to determine whether UV radiation is involved in the pathogenesis of cutaneous melanoma. In these studies, we tested the hypothesis that exposure of the skin to UV radiation can affect the growth or the time of appearance of melanoma cells injected into the external ears of UV-irradiated mice.

The source of UV radiation used in these studies was the FS40 fluorescent sunlamp. This light source emits approximately 60% of its energy within the UV-B (280–320 nm) wavelength range (8). UV-B radiation was used because it is in precisely this region of the solar spectrum that the most significant increases in irradiance will occur should the ozone concentration decrease (11). Furthermore, UV-B radiation is biologically active, in that it induces sunburn and skin cancer and produces both local and systemic immunological changes in mice (8, 9, 12, 13).

MATERIALS AND METHODS

Animals. Specific pathogen-free female C3H/HeNcr (MTV−) mice were purchased from Charles River Breeding Laboratories (Wilmington, MA) or from the Animal Production Area of the Frederick Cancer Research Facility (Frederick, MD). Mice were 8–10 weeks old at the beginning of each experiment, and they were housed in a pathogen-free, barrier facility accredited by the American Association for Accreditation of Laboratory Animal Care, where ambient light was automatically controlled to produce 12-h light, 12-h dark cycles. Animal procedures were approved by the Institutional Animal Care and Use Committee.

UV Irradiation. The UV light source was a bank of six FS40 sunlamps (Westinghouse, Bloomfield, NJ) with a peak emission at 313 nm. The incident dose rate was approximately 4.7 J/m2/s over the wavelength range from 250 to 400 nm, and around 60% of the energy emitted was within the UV-B (280–320 nm) range (8). Unshaved mice were exposed twice weekly for 20 min to the sunlamp. In some experiments, the right ear was shielded from the UV radiation with opaque tape. The tape was removed after each UV treatment.

Tumor Cell Lines. The K-1735 melanoma arose in a C3H mouse that had been treated with UV-B radiation, followed by painting with the tumor promoter croton oil (14). Tumor CM3205 is a new C3H melanoma induced in our laboratory by 7,12-dimethylbenz(a)anthracene initiation and promotion with the tumor promoter 12-O-tetradecanoylphorbol-13-acetate, according to the method of Berkhammer et al. (15). The UV-2237 fibrosarcoma was induced in a C3H mouse by chronic UV irradiation and the MCA-3152 fibrosarcoma was induced in a C3H mouse by s.c. injection of 3-methylcholanthrene (16). Tumor HCA is a hepatocarcinoma that arose spontaneously in a C3H mouse; this tumor cell line was kindly provided by Dr. Luka Milas (University of Texas M. D. Anderson Cancer Center). Cell lines established from these tumors were used at passages 5 to 15. All cells were grown in EMEM, supplemented with 10% fetal bovine serum and L-glutamine (Gibco Laboratories, Grand Island, NY). Cells were routinely tested to demonstrate that they remained free of Mycoplasma. In addition, they were free of pathogenic murine viruses (Microbiological Associates, Rockville, MD).

For tumor cell injections, subconfluent monolayers were harvested after a 1-min treatment with 0.25% trypsin-0.02% versene (v/v). The trypsinized cells were washed and resuspended in HBSS (Gibco). Cell number was determined by using a hemocytometer, and viability was measured by the exclusion of trypan blue. The tumor cells were diluted to the desired concentration, and 100 µl of the cell suspension was injected s.c. into the pinna of the ear.

Survival of Radiotracely Labeled Tumor Cells. K1735 cells were seeded into T-75 tissue culture flasks at 1.5 x 10³ cells/flask in EMEM with 10% fetal bovine serum. Twenty-four h later, 0.3 µCi/ml of [3H]-5-iodo-2'-deoxyuridine was added (New England Nuclear, Boston, MA; 2000 mCi/µmol). After an additional 24 h, the cell monolayers were harvested.
rinsed twice with excess Ca²⁺- and Mg²⁺-free HBSS to remove non-bound label. Cell monolayers were then overlayed with a thin layer of 0.25% trypsin and 0.02% EDTA solution for 1 min. The cells were dislodged from the plastic and EMEM with 10% fetal bovine serum was immediately added. The cell suspension was washed and resuspended in Ca²⁺- and Mg²⁺-free HBSS at a final concentration of 1 × 10⁶ cells/0.05 ml for injection into the external ear. Groups of 10 C3H/HeN mice were killed at 2, 24, 48, and 72 h after injection. Ears were collected from each mouse and placed in test tubes containing 70% ethanol. The ethanol was replaced daily for 3 days to remove all soluble ¹³¹I label (17). The radioactivity in the samples was monitored in a Gamma Trac Model 1185 gamma counter (TM Analytic, Elk Grove Village, IL).

Tumor Measurements. Mice were individually numbered by toe clipping, and were examined weekly for tumor growth. Ear tumors were measured in two bisecting diameters by using vernier calipers. Tumor incidence was defined as the number of mice with palpable tumors greater than 2 mm in diameter, divided by the total number of mice in each group. Between 10 and 15 mice per group were used in each experiment.

Statistical Analyses. The significance of differences in tumor size between treatment groups was determined by using a two-tailed Student’s t test. Differences in tumor incidence were compared by using the Fisher exact test.

RESULTS

Effect of UV Irradiation on Development and Growth of Melanoma Cells. To determine if UV irradiation of mice could affect the latent period or growth rate of transplanted melanoma cells, C3H mice were exposed to UV radiation for 5, 10, or 20 min (282 J/m²/min), twice per week for 4 weeks. One day after the last UV treatment, groups of 10 mice each were given injections of 2 × 10⁵ or 1 × 10⁶ K-1735 melanoma cells into the pinna of the ear, which was the major exposed body site on the unshaved mice. Fig. 1, A and B, shows that the tumors appeared earlier and reached a higher incidence in mice treated with the highest dose of UV radiation. The lower doses of UV radiation had no detectable effect on tumor incidence. Measurements of tumor size (Fig. 1, C and D) failed to reveal an effect of UV irradiation on the higher inoculum of tumor cells (Fig. 1C). At the lower cell dose, however, tumor sizes were significantly greater in mice pretreated with 20-min UV exposures at weeks 3 and 5, compared to those in unirradiated mice. Calculation of the tumor growth rates in each group indicated that although the tumors appeared earlier in mice given 20-min exposures to UV radiation, once the tumors were established, their subsequent rate of growth was indistinguishable from that of tumors in the unirradiated group.

Having established the minimal dose of UV radiation that caused accelerated outgrowth of the melanoma cells, we next investigated the relationship between UV irradiation and the time of tumor cell inoculation. Groups of 15 mice each were exposed to UV radiation for 20 min twice per week for 4 weeks, beginning either 4 weeks before, at the time of, or 2 weeks after implantation of K-1735 melanoma cells. As illustrated in Fig. 2, the tumors appeared earlier and reached a higher final incidence in mice pretreated with UV or irradiated beginning at the time of tumor cell inoculation. UV irradiation beginning 2 weeks after injection of tumor cells only marginally affected the development of tumors. To determine whether the effect of UV irradiation persisted after cessation of the UV treatment, mice were pretreated with UV radiation for 4 weeks (20 min, twice per week) and challenged with K-1735 cells 1 day or 8 days later. As expected, the appearance of melanomas was accelerated in mice challenged 1 day after the course of UV irradiation, but tumor development in mice challenged 8 days later was indistinguishable from that in unirradiated mice (data not shown). This result implies that the effect of UV irradiation on tumor development is transient.

Growth of Melanoma Cells in Non-UV-irradiated Sites. The earlier appearance of the tumors in UV-irradiated mice was not due to a systemic effect of the UV irradiation but, rather, to a direct, local effect at the site of tumor implantation. This conclusion is based on an experiment in which 20 C3H mice were treated for 4 weeks with UV radiation (20 min, twice per week). The right ear of these mice was covered with opaque tape during irradiation. The right ear of 20 unirradiated control mice was taped at the same time to control for possible stress and epidermal damage. Half of the mice from each group were then given injections of K-1735 melanoma cells in the right (shielded) pinna and the remaining mice were given injections...
in the left (exposed) pinna. Accelerated development of the melanoma cells was observed only in the ears directly exposed to UV radiation (Table 1). In an additional experiment, mice were pretreated with UV radiation as described above, and melanoma cells were then injected s.c. on the flank, a site shielded by fur from UV radiation. The latent period of the tumors in these animals was not altered by UV irradiation (data not shown). Collectively, the data indicate that accelerated outgrowth of melanoma cells occurred only when tumor cells were implanted into skin directly exposed to UV radiation.

Effect of UV Irradiation on Initial Tumor Cell Survival. The earlier appearance of tumors in the ears of UV-irradiated mice could result from the survival of a greater number of tumor cells in the initial inoculum or to an increase in the clonogenic potential of the surviving cells, or both. To address this issue, K-1735 melanoma cells were labeled with $[^{125}]$-5-iodo-2'-deoxyuridine and injected into the ears of UV-irradiated (20 min/week for 4 weeks) and control mice. At various times thereafter, the mice were killed, and the amount of residual radioactivity associated with viable cells was determined. As shown in Fig. 3, the survival rate of the melanoma cells injected into UV-irradiated mice was indistinguishable from that of cells injected into unirradiated animals. This experiment was performed twice with nearly identical results. Therefore, the accelerated outgrowth of the melanoma cells in UV-irradiated mice cannot be attributed to an increase in the survival of the initial tumor cell inoculum.

Effect of UV Irradiation on Development of Other Tumors. To investigate whether UV irradiation affected the outgrowth of other tumors in addition to the K-1735 melanoma, mice were exposed to UV radiation for 20 min, twice per week for 4 weeks, and given injections of CM3205 melanoma cells, UV-2237 and MCA-3152 fibrosarcoma cells, or HCA hepatocarcinoma cells in the pinna. All tumors are syngeneic to C3H mice. The results of representative experiments are illustrated in Table 2, which shows that the CM3205 melanoma, like the K-1735 melanoma, exhibited accelerated development in the UV-irradiated mice. In contrast, the incidence of the other tumors was not significantly increased by prior UV irradiation of the host. There appeared to be an increase in the incidence of the UV-induced fibrosarcoma, but this was not statistically significant ($P = 0.06$).

DISCUSSION

These studies demonstrate that exposure of mice to UV radiation has a stimulatory effect on the outgrowth of melanoma cells. The effect was observed in animals pretreated with UV radiation, as well as in mice exposed to UV beginning at the time of tumor cell inoculation. Therefore, the effect is due primarily to the action of UV radiation on the host, rather than on the melanoma cells. The effect was observed only when melanoma cells were injected into the irradiated site, indicating that it was a local, not a systemic effect of UV radiation. The experiment in which tumor cells were injected into ears protected by opaque tape ruled out the possibility that the decreased tumor latent period in UV-irradiated mice was due to handling stress or heat encountered during the irradiation procedure. Although we have not yet defined the complete time course of the effect of UV irradiation, it appears to be transient because the outgrowth of tumor cells injected 1 week after cessation of UV treatment was no longer accelerated.

Exposure of the host to UV radiation affected the latent period of tumor development and the minimum tumorigenic dose of melanoma cells. The latter effect was most evident in the experiment with the CM3205 melanoma cell line, in which a dose of $3.5 \times 10^5$ tumor cells produced no tumors in normal mice but produced tumors in 60% of the UV-irradiated animals. These effects were most pronounced when small tumor cell inocula were used, and injection of larger numbers of cells tended to obscure the effect. Some variability was noted from experiment to experiment in the latent period and growth rates of the tumors. However, this is to be expected when using uncloned cell lines at early passages. The actual growth rate of the tumors did not appear to be accelerated by pretreatment of the host with UV irradiation. Once the tumors were apparent, they seemed to grow at the same rate, regardless of the pretreatment of the host. This phenomenon could result from either an increase in the clonogenic potential of the cells injected into UV-irradiated mice or to an enhanced survival or retention of cells at the site of inoculation. However, studies with radiolabeled cells indicated that there was no difference in the survival of the initial tumor cell inoculum in UV-irradiated and control mice. Therefore, we conclude that small numbers of melanoma cells have an increased clonogenic potential in UV-irradiated mice.

It is noteworthy that the local effect of UV irradiation on tumor development seemed to be limited to melanomas. Other tumors appeared to be unaffected, with the possible exception of UV-2237, a UV-induced skin cancer. This selectivity for some, but not all, tumors suggests that UV radiation might be inducing the production or release of tissue-specific growth factors. Such substances could act selectively on certain types of cells to increase their rate of division for a brief period of time. Because UV irradiation increases melanin production and
stimulates the division of melanocytes (18), it is not unreasonable to postulate that the release of melanocyte-stimulating growth factors is responsible for the local effect of UV irradiation on melanoma cells in vivo. An alternative hypothesis is that the effect of UV irradiation on melanoma development results from UV-induced damage to cutaneous immune cells. UV radiation damages epidermal Langerhans cells (12, 13) and causes activation of the suppressor cell pathway to antigens introduced via the skin (19). It is possible that exposure to UV radiation, followed by tumor implantation, may cause the induction of suppressor lymphocytes that prevent the development of immunity to antigenic tumors implanted into the skin. The outgrowth of nonimmunogenic tumors would thus not be affected by UV irradiation, and only immunogenic tumors would exhibit accelerated outgrowth. Experiments testing these hypotheses are currently in progress.

It is difficult to assess the relevance of the local effects of UV radiation on transplanted melanoma cells in mice to the possible effects of UV exposure on the growth and progression of primary melanomas in humans. Nonetheless, our results suggest that direct exposure of the skin to UV-B radiation may favor the outgrowth of small numbers of melanoma cells in vivo. The dose of UV radiation necessary to decrease the latent period for melanoma development in mice is quite low, corresponding to around 4 to 12 h of cumulative sunlight exposure at a latitude of 30 degrees north. The possibility that short-term exposure to sunlight may accelerate the outgrowth of melanoma cells in the skin may help to explain the curious observation that the majority of cutaneous melanomas in humans are diagnosed during the summer months (20–22). Moreover, the possibilities that factors released by UV-irradiated epidermal cells or damage to cutaneous immune cells might accelerate the outgrowth of melanoma cells suggest new avenues for the study of this complex disease.

ACKNOWLEDGMENTS

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REFERENCES


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**Table 2** Incidence* of tumors in ears of UV-irradiated* and control mice

<table>
<thead>
<tr>
<th>Tumor cell line (dose)</th>
<th>Host</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM3205 melanoma (3.5 × 10⁶)</td>
<td>UV-irradiated</td>
<td>0/10</td>
<td>0/10</td>
<td>3/10</td>
<td>3/10</td>
<td>5/10*</td>
<td>5/10*</td>
<td>6/10*</td>
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<tr>
<td></td>
<td>Control</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>UV-2237 fibrosarcoma (3.5 × 10⁶)</td>
<td>UV-irradiated</td>
<td>0/8</td>
<td>0/8</td>
<td>2/8</td>
<td>5/8</td>
<td>6/8</td>
<td>6/8</td>
<td>6/8</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0/10</td>
<td>0/10</td>
<td>3/10</td>
<td>3/10</td>
<td>3/10</td>
<td>3/10</td>
<td>3/10</td>
</tr>
<tr>
<td>MCA-3152 fibrosarcoma (5 × 10⁶)</td>
<td>UV-irradiated</td>
<td>0/10</td>
<td>0/10</td>
<td>2/10</td>
<td>2/10</td>
<td>2/10</td>
<td>2/10</td>
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<tr>
<td></td>
<td>Control</td>
<td>0/10</td>
<td>1/10</td>
<td>7/10</td>
<td>9/10</td>
<td>10/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCA hepatocarcinoma (7 × 10⁵)</td>
<td>UV-irradiated</td>
<td>0/10</td>
<td>1/10</td>
<td>7/10</td>
<td>9/10</td>
<td>10/10</td>
<td></td>
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<tr>
<td></td>
<td>Control</td>
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<td>7/10</td>
<td>9/10</td>
<td>10/10</td>
<td></td>
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</tr>
</tbody>
</table>

* Tumor incidence = number of mice with palpable tumor (>2 mm)/number challenged.

* UV-irradiated = 20 min twice per week for 4 weeks; last treatment received 24 h before tumor cell inoculation.

* P ≤ 0.01 versus control.
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