Abnormal Wound Healing in UV-Irradiated Skin of Sencar Mice

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Sencar mice demonstrate an unusual sensitivity to epidermal carcinogenesis by initiation-promotion or single high-dose exposure to ultraviolet radiation (UVR). These mice exhibited an exaggerated and persistent epidermal hyperplasia in response to tissue damage caused by UVR. The persistent hyperplasia was not present in similarly treated BALB/c mice, a strain that is relatively resistant to skin carcinogenesis by initiation-promotion or single-exposure UVR. Epithelial cell proliferation and migration were examined by autoradiography to determine the cellular basis for the persistence of hyperplasia in Sencar mouse skin. Twelve weeks after irradiation, the rate of epidermal basal cell proliferation was approximately 4 times greater in Sencar mice than in BALB/c mice, whereas epidermal cell transit times were similar in the two strains. This result indicated that persistent hyperplasia was due to sustained epithelial cell division rather than delayed cell maturation. Surgical incision of Sencar skin did not cause abnormal hyperplasia, nor did this procedure enhance the induction of tumors by UVR. These findings suggest that Sencar mice may possess a heritable defect that mediates both tissue regeneration and tumorigenesis in UV-irradiated skin.


 MATERIALS AND METHODS

Animals Female Cr:ORL Sencar and BALB/cAnNCr mice were obtained from the National Cancer Institute-Frederick Cancer Research Facility Animal Resources Program and maintained as described previously [4]. BALB/c mice were used as the resistant albino strain in the present study, rather than CD-1 mice as in previous studies, because of the considerable amount of information available on skin carcinogenesis by UVR in this strain [10,11]. Animals were used for experiments at 8–10 weeks of age, and dorsal hair was removed with electric clippers prior to irradiation.

UV Radiation UV-radiation lamps and monitoring equipment were as described previously [4]. Mice were exposed to a bank of 6 FS40 sunlamps (8.0 J/m²/s) (Westinghouse, Bloomfield, New Jersey) situated 20 cm above the animals.

Epidermal Wound Healing Groups of 5 Sencar or BALB/c mice were sham-irradiated or UV-irradiated (8.64 × 10⁴ J/m²). Dorsal skin biopsies were taken 3, 8, and 12 weeks after irradiation, fixed in Bouin’s solution, sectioned (4 μm), and stained with hematoxylin and eosin for histologic examination. Additional groups of animals were UV-irradiated as above and injected with [3H]thymidine (New England Nuclear, Boston, Massachusetts) 12 weeks after irradiation. Tissues were processed as described in the legend of Table I.

Tumor Induction Five groups of 20 Sencar mice (8 weeks old) were treated with either a single UVR exposure (8.64 × 10⁴ J/m²) or 6 UVR exposures (1.44 × 10⁴ J/m² each) on alternate days, or they were sham-irradiated. The single UVR exposure causes ulceration of the dorsal skin, whereas the 6 UVR exposures do not. Four weeks later the animals in 2 of these groups received 6 scalp incisions (1 cm in length; full skin thickness; longitudinal) on the dorsum while under anesthesia. Thus, treatment groups were as follows: (a) sham; (b) sham plus incision; (c) 6 UVR exposures; (d) 6 UVR exposures plus incision; and (e) single UVR exposure. All animals were inspected weekly for dorsal skin tu-
mors. A life table analysis was used to describe tumor development as the probability that an animal will develop a tumor as a function of time after irradiation [12]. Differences in tumor incidence between groups were tested for statistical significance by the \( \chi^2 \) test [13]. At the conclusion of the experiment, tumors were excised and processed for histologic examination as described above.

RESULTS

In normal mouse skin, the tissue response to ulceration caused by UVR is similar to the response to ulceration caused by other means and includes increased proliferation of epithelial cells, epidermal hyperplasia, and thickening of the dermis accompanied by an infiltrate of inflammatory cells [14]. Wound healing is usually complete 4–6 weeks after irradiation, and by 10–12 weeks, epithelial cell proliferation returns to normal and hyperplasia decreases. In the present study, the epithelial changes caused in mouse skin by a single exposure to UVR (8.64 × 10\(^4\) J/m\(^2\)) from a sunlamp were examined in Sencar and BALB/c mice, 8–10 weeks old. This dose of UVR is sufficient to induce papillomas and squamous cell carcinomas in Sencar mice but is not tumorigenic in normal albino (e.g., BALB/c or CD-1) mice [4; unpublished data].

Initial gross skin reactions in Sencar and BALB/c mice exposed to UVR were comparable. Ulceration of exposed dorsal skin occurred by 7 days in both strains, and wound healing by inward migration of epithelial margins was complete by 5–6 weeks. However, the margins of the healing wound in Sencar skin appeared coarse and raised, in contrast to those of the BALB/c skin wound, which were smooth and not raised. Light microscopic examination of tissue samples taken from the borders of the healing wounds revealed an unusually marked hyperplasia in Sencar skin (Fig 1a) compared to BALB/c skin (Fig 1b). In addition, hyperkeratosis with follicular occlusion and keratin cysts were common in exposed Sencar skin but not in exposed BALB/c skin. The difference in degree of hyperplasia between BALB/c and Sencar epidermis was best demonstrated 8 weeks after irradiation (Fig 1c, d). At this time the wounds had healed in both strains and hyperplasia had greatly decreased in BALB/c mice (epidermal thickness 3–5 cell layers) but not in Sencar mice (epidermal thickness 5–20 cell layers). In Sencar mice, cells resembling basal cells formed several layers in the hyperplastic epidermis in addition to the layer adjacent to the basement membrane. The exaggerated hyperplasia was present 3 weeks after irradiation and in some animals (~50%) persisted until 12 weeks after irradiation (Table I).

The hyperplasia was accompanied by enhanced proliferation of basal cells, which also persisted for 12 weeks. The percentage of basal cells synthesizing DNA in the exposed skin of Sencar mice was approximately 4 times that observed in exposed BALB/c skin as determined by autoradiography in samples taken on the

Figure 1. Epithelial changes in Sencar and BALB/c mouse skin following UVR exposure. Groups of 5 Cr:ORL Sencar or BALB/cAnNCr mice (8–10 weeks old) were sham-irradiated or UV-irradiated (8.64 × 10\(^4\) J/m\(^2\)) with a bank of 6 FS40 sunlamps. This treatment is tumorigenic in Sencar but not in BALB/c mice. Dorsal skin biopsies were taken 3 and 8 weeks after irradiation and processed as described in Materials and Methods. a, Sencar skin 3 weeks after irradiation, \( \times 30 \). b, BALB/c skin 3 weeks after irradiation, \( \times 30 \). c, Sencar skin 8 weeks after irradiation, \( \times 125 \). d, BALB/c skin 8 weeks after irradiation, \( \times 125 \). Note marked hyperplasia, hyperkeratosis with follicular occlusion, and keratin cysts in Sencar skin.
day of radiolabeling (Table I). Also, an increase in the size of the proliferative cell compartment in hyperplastic Sencar skin was suggested by the presence of DNA synthesis in suprabasal cells (not shown). Alternatively, labeled basal cells may have moved off the basal layer during the 4-h [3H]thymidine pulse. Further evidence for enhanced basal cell proliferation in Sencar skin is the rapid movement of labeled cells off the basement membrane in UV-irradiated Sencar skin than in similarly treated BALB/c skin (Table I). Epidermal cell transit times were estimated from movement of labeled cells through the epidermis in tissue samples taken at various times after radiolabeling. Transit times in the exposed skin of Sencar and BALB/c mice were similar (Table I), indicating that the abnormal hyperplasia in Sencar mouse skin was due to increased basal cell proliferation rather than inhibition of cell maturation.

In an effort to clarify the role of tissue damage and wound healing in UVR carcinogenesis in Sencar mice, an experiment was performed in which surgical incision was used to determine the effect of non-UVR-induced tissue damage on skin carcinogenesis. Sencar mice were treated with either a single exposure to UVR (8.64 × 10^4 J/m^2) or multiple exposures (6 times 1.44 × 10^4 J/m^2) given on alternate days, or they were sham-irradiated. The ulceration caused by the single exposure to UVR does not occur with the multiple-exposure regimen, although the total UVR doses are the same. Four weeks later, half of the animals in the sham-irradiated and the multiple-exposure groups received 5 scalp incisions on the dorsum while under anesthesia. The wounds caused by the incision were not sutured closed and thus were allowed to heal in a manner resembling that of UVR-induced ulcerations. This protocol was used rather than long-term wounding or mechanical abrasion in order to simulate as closely as possible the ulceration caused by single UVR exposure. All animals were inspected weekly for skin tumors. The multiple-UVR-exposure regimen was not tumorigenic at 30 weeks, in contrast (p < 0.01) to the single-exposure regimen which caused a 40% probability of tumor development by 30 weeks (Fig 2). Thus, a single large exposure to UVR was both carcinogenic and ulcerogenic in Sencar mice. The same dose of UVR when divided into 6 equal exposures administered on alternate days was found to be noncarcinogenic and nonulcerogenic. In addition, the multiple-exposure regimen followed by incision was not tumorigenic. Thus, under these experimental conditions, incision was clearly ineffective in enhancing the tumorigenic potential of UVR exposure. This indicates that multiple-exposure plus incision is not equivalent to the single-exposure regimen with regard to tumorigenesis. Histologic examination of the epithelial response of Sencar skin to surgical incision showed that persistent hyperplasia was not induced by this procedure. The results of these experiments are summarized in Table II. An additional group (n = 20) of Sencar mice was administered a thermal treatment which has been shown [15] to mimic UVR-induced ulceration. These mice also did not exhibit persistent hyperplasia or develop tumors. As in previous carcinogenesis experiments in Sencar mice, all of the tumors induced by a single UVR exposure were papillomas [4] or squamous cell carcinomas.

**DISCUSSION**

These results demonstrate that Sencar mouse skin responds differently to UVR-induced ulceration than does BALB/c mouse skin. The epithelial hyperplasia normally associated with wound healing is unusually severe and persistent in Sencar mice compared to BALB/c mice. The persistent hyperplasia is due to sustained basal cell proliferation, which is not present in BALB/c mice. Thus the basal cells in UV-irradiated Sencar skin appear unable to return to a normal rate of proliferation after wound healing is complete. It is not clear whether this deficiency is related to the observed hypersusceptibility of Sencar mice to carcinogenesis. Sustained basal cell proliferation could predispose Sencar mice to tumor susceptibility by UVR by causing continual division of tumors.

### Table I. Cell Kinetics in Sencar and BALB/c Epidermis 12 Weeks After Exposure to UVR

<table>
<thead>
<tr>
<th>Exposure to UVR</th>
<th>Strain</th>
<th>Epidermal Thickness</th>
<th>Percent Basal Cells Labeled</th>
<th>Transit Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12 Weeks Postirradiation</td>
<td>Days</td>
<td>0</td>
</tr>
<tr>
<td>−</td>
<td>BALB/c</td>
<td>1–3 cells</td>
<td>2.9 ± 0.24</td>
<td>1.8</td>
</tr>
<tr>
<td>+</td>
<td>BALB/c</td>
<td>2–5 cells</td>
<td>4.6 ± 0.6</td>
<td>3.3</td>
</tr>
<tr>
<td>−</td>
<td>Sencar</td>
<td>1–3 cells</td>
<td>2.6 ± 0.3</td>
<td>3.3</td>
</tr>
<tr>
<td>+</td>
<td>Sencar</td>
<td>5–20 cells</td>
<td>17.5 ± 7.9</td>
<td>9.5</td>
</tr>
</tbody>
</table>

Sencar or BALB/c mice were UV-irradiated as described in Fig 1 and injected with [3H]thymidine (50 μCi/mouse, i.p.) 12 weeks after irradiation. Dorsal skin biopsies were taken 4 and 2, 4, 6, and 8 days later, fixed in Bouin's solution, sectioned, deparaffinized, and exposed to NTB-2 emulsion for 3 weeks at 4°C. Samples were developed in D-19, fixed in Cresor, and stained with eosin. Percentages of heavily labeled (>20 grains per nucleus) cells were determined by counting 500–1000 interfollicular cells adjacent to the basement membrane in tissue samples from each of 3 animals. Cell transit times were estimated from the position of heavily labeled cells in the epidermis at various times after injection of [3H]thymidine. The approximate time required for labeled cells to traverse the epidermis and reach the keratin layer is listed in the last column.

*Days after injection of [3H]thymidine when tissue biopsy taken.

*Mean ± SD (n = 3); difference between strains not significant.

*Mean ± SD (n = 3); difference between strains significant (p < 0.025).
basal cells. Indeed, it has been postulated [9,16] that untreated Sencar mouse skin contains a population of cells constitutively initiated for tumorigenesis which are resistant to terminal differentiation. This hypothesis is based on 2 lines of evidence. First, Sencar mice often develop papillomas with repeated treatment with promoter alone [3], whereas BALB/c mice rarely develop tumors in the absence of exogenous initiation. Second, resistance to forced terminal differentiation in vitro by modulation of Ca"+ concentration in the media is a characteristic of chemically initiated basal cells from normal mice and untreated basal cells from Sencar mice [16]. Although no evidence of altered terminal differentiation was found in the present study in vivo, the sustained proliferation of a high-risk population of cells could conceivably lead to tumorigenesis.

On the other hand, sustained basal cell proliferation could be an independent manifestation of a genetic defect in Sencar mice which mediates susceptibility to skin carcinogenesis. Several recent studies have shown important links between tissue regeneration and cellular transformation. First, polypeptide transforming growth factors, which are capable of transforming the growth characteristics of nonneoplastic cells in soft agar, have been shown to accelerate wound healing in rat skin [17]. Second, platelet-derived growth factor, which is involved in tissue repair and wound healing, has been shown to possess extensive amino acid sequence homology with the transforming protein derived from the simian sarcoma virus oncogene, v-sis [18,19]. If Sencar mice do indeed possess a heritable defect that mediates both tissue regeneration and tumorigenesis in UV-exposed skin, then characterization of this defect may help to illuminate the relationship between tissue regeneration and cellular transformation.

In subsequent experiments, the role of tissue damage and wound healing in UVR carcinogenesis in Sencar mice was investigated. Tissue damage caused by surgical incision after multiple UVR exposures did not affect tumor incidence (Fig 2), as might be expected if abnormal responses to tissue damage were related to the hypersusceptibility of Sencar mice to skin carcinogenesis. There are several possible explanations for the inability of incision to enhance the tumorigenic potential of UVR in these experiments: (a) tissue damage and regeneration may not influence susceptibility to UVR tumorigenesis in Sencar mice; (b) the tissue damage caused by incision in this experiment may not be comparable to the UVR-induced tissue damage with regard to tumorigenesis; or (c) the single UVR exposure may be more effective than the multiple exposures in transforming epithelial cells. The epithelial response of Sencar skin to surgical incision did not include persistent hyperplasia (Table II), a finding that supports the second hypothesis. In addition, an ulcerogeneric thermal treatment also did not produce persistent hyperplasia (Table II). Thus, the abnormal response of Sencar skin to UVR-induced tissue damage is not a general response to all forms of tissue damage, but perhaps may be limited to tissue damage caused by certain carcinogens.

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