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Photocarcinogenesis and Persistent Hyperplasia in UV-Irradiated SENCAR Mouse Skin

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Susceptibility to photocarcinogenesis has been examined in several mouse strains and stocks including SENCAR, CD-1, BALB/c, C3H, C57Bl, and NZB. SENCAR mice are hypersusceptible to tumorigenesis caused by single high dose exposures to ultraviolet (UV) radiation but not by chronic low-dose exposures. SENCAR mice also exhibit an exaggerated and persistent epidermal hyperplasia in response to UV-induced tissue damage. The persistent hyperplasia is apparently due to a sustained proliferation of the epithelial basal cells, rather than to delayed cell differentiation. SENCAR mice did not exhibit persistent hyperplasia following other forms of tissue damage (surgical or thermal). In related studies, the levels of thymine dimers induced in SENCAR epidermis by UV radiation were comparable to those observed in BALB/c epidermis. In addition, no differences were found in the tissue distribution or persistence of thymine dimers in SENCAR and BALB/c skin.

The development of SENCAR mice, which are highly susceptible to skin carcinogenesis, has provided a useful model for investigating factors that modulate hypersusceptibility as an approach to the study of mechanisms of skin neoplasia (*1–3*). SENCAR mice are selectively bred to be hypersusceptible to chemically induced two-stage skin carcinogenesis, and this unusual sensitivity has been shown to be an inherent characteristic of the skin of these animals rather than an altered systemic response of the host (*4*).

We have been investigating certain aspects of photocarcinogenesis regarding susceptibility to tumor induction in mouse skin. A number of experiments have compared strain susceptibility to photocarcinogenesis using single and chronic exposure protocols, and these are summarized in Table 1. These experiments have shown that a single treatment of ultraviolet (UV) radiation ($5.8–11.5 \times 10^4$ J/m^2) from FS 40 sunlamps, without subsequent chemical or physical promotion, results in the dose-dependent induction of epithelial tumors (papillomas and squamous cell carcinomas) in SENCAR mice (*5*). No tumors of any kind are produced in comparably treated CD-1 or BALB/c mice. This finding demonstrates that the susceptibility determinants selected in SENCAR mice are not specific for chemical carcinogenesis, but can also potentiate photocarcinogenesis. In contrast, chronic exposure to UV radiation ($1.5–2.9 \times 10^4$ J/m^2 three times per week) is not unusually carcinogenic in SENCAR mice compared to BALB/c mice (Table 1). Thus the exhibition of hypersusceptibility depends on how the UV radiation is administered, suggesting that other factors (e.g., tissue damage) may be involved. Three pigmented mouse strains (C3H, C57Bl/6, and NZB/N) are somewhat more resistant than the two albino strains to the carcinogenic effects of chronic UV radiation exposure, based on latency period for tumor appearance. It is noteworthy that the tumors induced in SENCAR mice by either exposure protocol (single or chronic) are primarily of epidermal origin and, in this respect, are similar to most human skin tumors.

Much of our effort has focused on investigating the biological basis for the hypersusceptibility of SENCAR mice to single treatment photocarcinogenesis. One approach we have used to address this question is to examine UV-induced DNA damage in mouse skin to determine whether damage and repair varies among mouse strains. Since several lines of evidence suggest that cyclobutane–pyrimidine dimers produced in DNA by UV radiation may be the initial lesion involved in photomutagenesis and photocarcinogenesis, we have produced monoclonal antibodies specific for thymine dimers for use in immunological assays (*8,9*). Using these assays, we examined the distribution and repair of thymine dimers in the skin of three different strains of mice (SENCAR, BALB/c, and C3H) exposed to UV radiation. In preliminary studies, an indirect immunofluorescent assay (*10*) was used to examine the cellular and tissue distributions of thymine dimers in frozen skin

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Table 1. Variations in strain susceptibility to photocarcinogenesis.

	Latency period, wk	Anatomical site	Histological tissue	Reference
Single UVR[a]				
SENCAR	6–12	Dorsum (92%)[c]	Epidermis (88%)[d]	(5)
BALB/c	> 50	—	—	Unpublished
CD-1	> 50	—	—	(5)
Chronic UVR[b]				
SENCAR	18–20	Ear (81%)	Epidermis (80%)	Unpublished
BALB/c	18–20	Ear (85%)	Dermis (71%)	(6)
C3H	26–30	Dorsum (68%)	Dermis (77%)	(6)
C57BL/6	26–30	Ear (58%)	Dermis (88%)	(6,7)
NZB	26–30	Ear/dorsum (50%)	Dermis (64%)	(7)

[a] Single UV radiation = 8.64×10^4 J/m^2 single exposure.
[b] Chronic UV radiation = lifetime exposure (2.88×10^4 J/m^2 each) three times per week.
[c] Percent of tumors at this site.
[d] Percent of tumors in this tissue.

Table 2. Thymine dimer levels in UV-irradiated mouse skin.

	(T[ ]T/T) × 1000[a]	
Tissue	$t < 1$ hr[b]	$t = 24$ hr[b]
Dorsal epidermis[d]		
BALB/c	0.49 ± 0.05[c]	0.40 ± 0.21
SENCAR	0.52 ± 0.07	0.54 ± 0.05
Dorsal dermis[e]		
BALB/c	0.25 ± 0.09	0.23 ± 0.02
SENCAR	0.44 ± 0.12	0.38 ± 0.01
Ear (epidermis + dermis)[d]		
BALB/c	0.31 ± 0.22	0.35 ± 0.20
SENCAR	0.35 ± 0.13	0.34 ± 0.02

[a] Proportion of thymidines (T) that have formed dimers (T[ ]T).
[b] t = time after irradiation (1.4×10^3 J/m^2) at which tissue samples were taken.
[c] Mean of two samples ± range.
[d] No significant difference between strains by t test.
[e] Difference between strains is significant at $p = 0.10$ ($t < 1$ hr) and $p < 0.01$ ($t = 24$ hr) by t-test.

Table 3. Cell proliferation in SENCAR and BALB/c epidermis 12 weeks after exposure to UV radiation.

UV[a]	Strain	Epidermal thickness 12 weeks after irradiation (range)	% basal cells labeled[b]	Transit time, (days)
−	BALB/c	1– 3 cells	2.9 ± 0.2[c]	8
+	BALB/c	2– 5	4.6 ± 0.6[d]	6–8
−	SENCAR	1– 3	2.6 ± 0.5[c]	8
+	SENCAR	5–20	17.5 ± 7.9[d]	6–8

[a] SENCAR or BALB/c mice were UV-irradiated (8.5×10^4 J/cm^2) and injected with [^{3}H]thymidine (50 microcuries [μCi]/mouse, intraperitoneally) 12 weeks after irradiation. Dorsal skin biopsies were taken 4 hr and 2, 4, 6, and 8 days later, and processed for autoradiography. Percentages of heavily labeled (> 20 grains per nucleus) cells were determined by counting interfollicular cells adjacent to the basement membrane. The approximate time required for labeled cells to traverse the epidermis and reach the keratin layer (cell transit time) was estimated from the position of heavily labeled cells in the epidermis at 2-day intervals after injection of [^{3}H]thymidine.
[b] At 4 hr after injection of [^{3}H]thymidine.
[c] Mean ± SD ($n = 3$); difference between strains not significant by t-test.
[d] Mean ± SD ($n = 3$); difference between strains significant ($p < 0.025$) by t-test.

Table 4. Summary of SENCAR skin responses to nonchemical procedures.

	Ulceration	Persistent hyperplasia	Latency period for tumor appearance, wk
UV (single exposure)[a]	+	Severe	6–12[e]
UV (multiple exposure)[b]	−	—	> 50
UV (chronic exposure)[c]	−	Mild	18–20[f]
Surgical incision[d]	+	—	> 50
Thermal treatment	+	—	> 50
UV (multiple) + incision	+	—	> 50

[a] One exposure (8.64×10^4 J/m^2).
[b] Six exposures (1.44×10^4 J/m^2 each) on alternate days.
[c] Lifetime exposures (2.88×10^4 J/m^2 each) on alternate days.
[d] Unsutured.
[e] Compared to > 50 wk for BALB/c or CD-1.
[f] Compared to 18–20 wk for BALB/c.

sections; no major differences were observed among the three mouse strains (unpublished observation). This finding suggests that the optical properties of the skin that govern penetration and absorption of UV radiation are similar in these mouse strains and probably do not contribute to variations in susceptibility to photocarcinogenesis among the strains.

A radioimmunoassay was used to quantitate thymine dimer induction and persistence in DNA extracted from mouse skin at various times after UV irradiation (*11*). Initial yields of thymine dimers in DNA extracted from ear and dorsal epithelium were similar in the two mouse strains tested, SENCAR and BALB/c (Table 2). In addition, thymine dimer yields measured 24 hours after UV irradiaiton were similar to initial yields in the epidermis of the two strains. This persistence of thymine dimers in mouse skin is consistent with other studies, indicating that murine skin cells are deficient in excision repair of these photoproducts (*12,13*). A lower initial yield of dimers was detected in BALB/c dermis than in SENCAR dermis. However, this finding did not correlate with the preponderance of dermal tumors (fibrosarcomas) arising in BALB/c mice compared to SEN-

CAR mice, in which the tumors are mainly of epithelial origin. Thus no clear correlation can be drawn between the distribution, induction, or persistence of thymine dimers in the UV-exposed tissues of these mouse strains and their varying susceptibilities to photocarcinogenesis. These data suggest that SENCAR mice do not suffer from abnormalities that alter either the levels of DNA photoproducts induced in their epidermis or the rate of removal of thymine dimers. Consistent with this conclusion is the recent report (*14*) that host cell reactivation of UV-irradiated herpes simplex virus by primary epidermal cells from SENCAR, BALB/c, and CD-1 mice is similar. These data argue against a defective DNA repair mechanism for the observed hypersusceptibility of SENCAR mice to photocarcinogenesis.

It has been postulated (*4,15*) that untreated SENCAR mouse skin contains a population of cells, constitutively initiated for tumorigenesis, that are resistant to terminal differentiation *in vitro*. In our studies, SENCAR mice exhibited an unusual wound healing pattern following single exposure to UV radiation (*5,16*). These treatments are ulcerogenic, and the gross appearance of the healing wound was unusual in that it was exophytic and very coarse–somewhat reminisicent of hypertrophic scarring in humans. When we examined this abnormality in detail, an exaggerated and persistent epithelial hyperplasia was evident in the exposed skin of SENCAR mice but not BALB/c mice. Epithelial cell proliferation and migration were measured *in vivo* by autoradiography to determine the cellular basis for the persistence of hyperplasia. Twelve weeks after irradiation (when tissue regeneration is normally completed), the rate of epidermal basal cell proliferation in SENCAR mouse skin was approximately four times the rate in BALB/c mouse skin that had been identically treated (Table 3). On the other hand, epidermal cell transit times (time for labeled cells to traverse the epidermis) were similar in the two strains of mice. These results suggest that the abnormal hyperplasia in SENCAR mouse skin is due to a sustained increase in basal cell proliferation rather than to inhibition of cell differentiation.

To determine whether the abnormal hyperplastic response of SENCAR skin is specific for UV-induced tissue damage, two other types of tissue damage, surgical and thermal, were examined. Neither full thickness scalpel incision, nor a thermal treatment, which has been shown (*17*) to mimic UV-induced ulceration, were effective in producing persistent hyperplasia in SENCAR epidermis. Thus persistent hyperplasia is not a general response of SENCAR skin to all forms of tissue damage. In addition, tissue damage caused by surgical incision administered after subcarcinogenic exposures to UV radiation did not enhance the tumorigenic potential of the UV radiation. These results are summarized in Table 4.

The precise relationship between sustained basal cell proliferation and hypersusceptibility to photocarcinogenesis remains to be determined. However, three hypotheses are indicated: (1) that sustained basal cell proliferation predisposes SENCAR mice to tumor susceptibility by causing repeated replication of basal cell DNA; (2) that sustained proliferation is an independent manifestation of a genetic defect in SENCAR mice that also mediates susceptibility to skin carcinogenesis; or (3) that sustained proliferation is a premalignant state in which the cells are partially transformed.

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