Inheritance of Susceptibility to Phototumorigenesis and Persistent Hyperplasia in F1 Hybrids between SENCAR Mice and BALB/c or C57BL/6 Mice

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ABSTRACT

SENCAR mice are selectively bred for hypersusceptibility to two-stage chemical skin carcinogenesis. They are also hypersusceptible to UV radiation tumorigenesis with single high-dose, but not chronic low-dose, exposures. In addition, SENCAR mice exhibit an exaggerated and persistent epidermal hyperplasia (due to sustained proliferation of the basal cells) in response to UV-induced tissue damage. In the present study, we have examined the inheritance of susceptibility to both phototumorigenesis and persistent hyperplasia in the F1 offspring of SENCAR mice crossed with either of two inbred strains (BALB/c or C57BL/6) which are relatively resistant to phototumorigenesis. A total of 428 mice from the parental strains and reciprocal F1 crosses were given a single high dose (8.64 × 10^4 J/m^2) of UV radiation (FS40 sunlamps) which causes persistent hyperplasia and tumorigenesis in many SENCAR, but no BALB/c or C57BL/6, mice. F1 hybrids between SENCAR and C57BL/6 mice did not develop persistent hyperplasia or skin tumors, although at a much lower incidence than the SENCAR mice, indicating that susceptibility to both traits is only partially (incompletely) recessive to the BALB/c genotype. Thus, in either F1 cross, susceptibility to phototumorigenesis decreased in parallel with persistent hyperplasia. These results are consistent with the hypothesis that the two characteristics are mechanistically related.

INTRODUCTION

Exposure to exogenous carcinogens is believed to cause the majority of human cancers. However, the considerable variation within the human population for susceptibility to genotoxic agents and carcinogenesis suggests that certain host factors mediate this response (1, 2). Abnormalities in DNA repair or oxidative metabolism have been associated with increased susceptibility to carcinogenesis in some cases (3–5). One approach directed toward understanding the mechanisms responsible for susceptibility has been the study of animal strains unusually susceptible to carcinogenesis. SENCAR mice are selectively bred for hypersusceptibility to two-stage chemical skin carcinogenesis (6–8) and are also hypersusceptible to UV radiation carcinogenesis (9) with certain exposure protocols. While the biological basis for this sensitivity is unclear, it probably does not involve abnormalities in carcinogen metabolism (7, 8), DNA repair (10, 11), epidermal growth factor receptors (12), phorbol ester receptors (13), or systemic immunological factors (14).

On the other hand, two anomalous properties of SENCAR skin have been reported. (a) Cultured keratinocytes from SENCAR skin are resistant to forced terminal differentiation by modulation of Ca^2+ concentration in the medium (15), whereas keratinocytes from other (resistant) mouse strains are susceptible to forced differentiation. (b) SENCAR mice exhibit an exaggerated and persistent epidermal hyperplasia (which is due to sustained proliferation of epithelial basal cells) in response to tissue damage caused by UV radiation (16). It is not known whether these two properties are causally related to each other, or to the hypersusceptibility of SENCAR mice to skin carcinogenesis.

In the present study, the inheritance of susceptibility to phototumorigenesis and persistent hyperplasia has been examined in the F1 offspring of SENCAR mice crossed with either of two inbred strains (BALB/c or C57BL/6) which are relatively resistant to phototumorigenesis. In addition, the distribution of skin tumors and persistent hyperplasia among individual SENCAR mice exposed to UV radiation was analyzed.

MATERIALS AND METHODS

Animals. Parental strains of mice [C57BL/6NCr (C57BL/6), SENCAR AnNCr (SENCAR), and BALB/cAnNCr, and C57BL/6NCr] were obtained from the National Cancer Institute-Frederick Cancer Research Facility Animal Resources Program and maintained as previously described (9). Five breeding pairs of each of the following crosses were initiated: BALB/c x SENCAR F1, (hereafter called CSF1); SENCAR x BALB/c F1, (hereafter called SCF1); C57BL/6 x SENCAR F1, (hereafter called B6SF1); and SENCAR x C57BL/6 F1, (hereafter called SB6F1). All breeding pairs produced offspring with the exception of 3 of the 5 pairs in the C57BL/6 x SENCAR cross. The mean size of 36 litters produced was 10.1 ± 3.9 with a range of 3 to 19 offspring. Coat colors of the offspring were white for CSF1 and SCF1; mice and black or gray for B6SF1 and SB6F1 mice. F1 offspring were used for experiments at 8 to 10 wk of age, and dorsal hair was removed with electric clippers prior to irradiation. The appearance of the skin of the mice with pigmented hair was similar to that of the albino mice. The small amount of residual hair left after clipping was more visible in the pigmented mice. The hair growth cycle was in the resting phase at treatment (8 to 10 wk), and the growing phase began 1 to 2 wk later. Animals were routinely monitored and found to be free of murine viruses, parasites, Mycoplasma, and pathogenic bacteria.

UV Radiation. Mice were exposed to a bank of six FS40 sunlamps (Westinghouse, Bloomfield, NJ) situated 20 cm above the animals. During irradiation the animals were housed in wire-topped cages partitioned into compartments for individual animals. The average fluence rate at the position of the animals was 8.0 J/m^2/s as measured by an IL700 photometer with PT171C detector.

Persistent Hyperplasia and Tumor Induction. A total of 428 mice from the parental strains and F1 crosses (Table 1) were given a single high dose (8.64 × 10^4 J/m^2) of UV radiation which is known to cause ulceration, persistent hyperplasia, and tumorigenesis in SENCAR mice (16). All animals were inspected weekly, and gross skin reactions and tumors were recorded. The incidence of persistent hyperplasia was determined from the weekly observations at 4, 5, and 6 wk after treatment, since the response was most prominent during this period. Twenty-eight wk after irradiation the animals were sacrificed, and samples of all skin tumors were taken for histopathological examination. The probability of tumor development was determined by a life-table analysis (17), and differences in tumor development between...
percentage observed for SENCAR mice. The F₁ hybrids between which caused ulceration of the dorsal skin in the majority of were tested by standard x² analysis. The dependence of tumor appearance (SB6F₁/gray). These values do not differ significantly from that 
mals responding: 0% (B6SF₁); 7.7% (SB6F₁/black); and 6.7% (SB6F₁). In all cases, the probabilities were significantly reduced from that observed in SENCAR mice, but not significantly different from the appropriate resistant parental strain, BALB/c or C57BL/6. However, when the tumor development data from reciprocal crosses were combined, the (CS plus SC) F₁ hybrids were more susceptible (P < 0.05) to tumor development than the (B6S plus SB6) F₁ hybrids. Thus, the genotype controlling hypersusceptibility to phototumorigenesis in SENCAR mice is completely recessive to the C57BL/6 genotype and partially recessive to the BALB/c genotype, behaving in much the same way as the persistent hyperplasia trait (Table 2). This finding suggests that the two traits are mechanistically related, either directly or indirectly.

As in previous experiments using a single UV radiation exposure, most of the skin tumors induced were benign epidermal papillomas. Squamous cell carcinomas developed only in the SENCAR mice (4 carcinomas of a total of 22 tumors). The

**RESULTS**

Mice were exposed to a single high dose of UV radiation which caused ulceration of the dorsal skin in the majority of mice. The percentages of mice with ulceration were similar (range, 56 to 81%) in the different parental strains and F₁ crosses. In two of the F₁ crosses, CSF₁ and SB6F₁/black, male mice were more susceptible to UV-induced skin ulceration than female mice of the same cross (data not shown).

As previously reported (16), many (47%) of the SENCAR mice develop a persistent hyperplasia of the dorsal epidermis 4 to 6 wk after irradiation (Fig. 1), whereas BALB/c and C57BL/6 mice similarly treated do not develop persistent hyperplasia (Ref. 16, Fig. 1). When F₁ hybrids between SENCAR and BALB/c mice were exposed to the same dose of UV radiation, the percentage of mice developing persistent hyperplasia was 22% (CSF₁) and 24% (SCF₁), about half the percentage observed for SENCAR mice. The F₁ hybrids between SENCAR and C57BL/6 mice were even less susceptible to UV-induced persistent hyperplasia based on the percentage of animals responding: 0% (B6SF₁); 7.7% (SB6F₁/black); and 6.7% (SB6F₁/gray). These values do not differ significantly from that observed for the parental C57BL/6 strain (0%). These results indicate that the genotype controlling susceptibility to persistent hyperplasia in SENCAR mice is recessive to the C57BL/6 genotype, but only partially recessive to the BALB/c genotype.

Dorsal skin tumors began to develop in SENCAR mice within 6 wk of irradiation as expected, and the probability incidence reached 29% by 28 wk after irradiation (Fig. 2). In contrast, neither BALB/c nor C57BL/6 mice developed any skin tumors in the same period. The probability of tumor development in the F₁ hybrid mice was 6% (CSF₁), 6.3% (SCF₁), 0% (B6SF₁), and 0.6% (SB6F₁). In all cases, the probabilities were significantly reduced from that observed in SENCAR mice, but not significantly different from the appropriate resistant parental strain, BALB/c or C57BL/6. However, when the tumor development data from reciprocal crosses were combined, the (CS plus SC) F₁ hybrids were more susceptible (P < 0.05) to tumor development than the (B6S plus SB6) F₁ hybrids. Thus, the genotype controlling hypersusceptibility to phototumorigenesis in SENCAR mice is completely recessive to the C57BL/6 genotype and partially recessive to the BALB/c genotype, behaving in much the same way as the persistent hyperplasia trait (Table 2). This finding suggests that the two traits are mechanistically related, either directly or indirectly.

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**INHERITANCE OF SUSCEPTIBILITY**

**Table 1** Numbers of mice tested for susceptibility to UV radiation effects

<table>
<thead>
<tr>
<th>Strain/hybrid</th>
<th>Coat color</th>
<th>Female</th>
<th>Male</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SENCAR</td>
<td>White</td>
<td>25</td>
<td>20</td>
<td>45</td>
</tr>
<tr>
<td>BALB/c</td>
<td>White</td>
<td>24</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>Black</td>
<td>0</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>CSF₁</td>
<td>White</td>
<td>31</td>
<td>36</td>
<td>67</td>
</tr>
<tr>
<td>SCF₁</td>
<td>White</td>
<td>59</td>
<td>36</td>
<td>95</td>
</tr>
<tr>
<td>B6SF₁</td>
<td>Black</td>
<td>14</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>SB6F₁</td>
<td>Black</td>
<td>30</td>
<td>46</td>
<td>76</td>
</tr>
<tr>
<td>SB6F₁</td>
<td>Gray</td>
<td>29</td>
<td>48</td>
<td>77</td>
</tr>
</tbody>
</table>

| Total         |           | 212    | 216  | 428   |

![Graph](image)

Fig. 1. Percentage mice exhibiting persistent hyperplasia. Mice were irradiated by a single exposure to FS40 sunlamps as described in text and scored for persistent hyperplasia 4 to 6 wk after irradiation. Mouse strain and hybrid designations are defined in text. F(PH), female mice exhibiting persistent hyperplasia; M(PH), male mice; F+M(PH), all mice.

**Table 2** Summary of inheritance of susceptibility to phototumorigenesis and persistent hyperplasia in F₁ hybrids

<table>
<thead>
<tr>
<th>Strain/stock</th>
<th>% of mice with persistent hyperplasia (4-6 wk)</th>
<th>% of probability of tumor development (28 wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SENCAR</td>
<td>46.5</td>
<td>28.6</td>
</tr>
<tr>
<td>CSF₁</td>
<td>21.7</td>
<td>6.0</td>
</tr>
<tr>
<td>SCF₁</td>
<td>23.9</td>
<td>6.3</td>
</tr>
<tr>
<td>B6SF₁</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SB6F₁</td>
<td>7.2</td>
<td>0.6</td>
</tr>
<tr>
<td>BALB/c</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

![Graph](image)

Fig. 2. Percentage of probability of tumor development with time after irradiation. Mice were irradiated by a single exposure to FS40 sunlamps as described in text and scored weekly for tumor appearance on the dorsal skin. Mouse strain and hybrid designations are defined in text.

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distribution of skin tumors and persistent hyperplasia among individual SENCAR mice was examined to determine if tumorigenesis was dependent on persistent hyperplasia. A dependence of the two responses would be expected if they were directly (causally) related, e.g., if persistent hyperplasia was predisposing skin to tumorigenesis by means of continuous cell division. Alternatively, the two responses would be independently distributed if they were indirectly related, e.g., if they were independent mitogenic responses associated with a particular altered phenotype. As shown in Table 3, tumorigenesis was not directly associated with persistent hyperplasia in individual SENCAR mice ($\chi^2 = 0.065$), suggesting that these traits are related by an indirect mechanism.

**DISCUSSION**

SENCAR mice exhibit two unusual responses to a single high dose of UV radiation that are not exhibited by other strains or stocks of haired mice. (a) They develop an exaggerated and persistent epidermal hyperplasia, which is due to sustained proliferation of the epidermal basal cells (16); and (b) they develop skin papillomas and carcinomas (9). We suspect that these two characteristics may be mechanistically related, perhaps through an aberrant mitogenic response.

In the present study we have examined the inheritance of susceptibility to both of these traits, phototumorigenesis and persistent hyperplasia, in F1 hybrids between SENCAR mice and either of two inbred strains of mice (BALB/c or C57BL/6) which are resistant to both traits. Reciprocal crosses were used in the off chance that the traits under study were sex linked. F1 hybrids between SENCAR and C57BL/6 mice (both reciprocal crosses) were as resistant as C57BL/6 mice to both phototumorigenesis and persistent hyperplasia following UV radiation exposure. These results indicate that susceptibility to either trait is (completely) recessive to the C57BL/6 genotype.

On the other hand, F1 hybrids between SENCAR and BALB/c mice exhibited intermediate tumorigenic and hyperplastic responses to UV radiation. While tumorigenesis and persistent hyperplasia were less common in these hybrids than in SENCAR mice, the two traits were not completely eliminated as in the hybrids between SENCAR and C57BL/6. These results indicate that susceptibility to phototumorigenesis and persistent hyperplasia is only partially (incompletely) recessive to the BALB/c genotype.

The differing ability of the BALB/c and C57BL/6 genotypes to suppress the SENCAR hypersusceptibility phenotype in F1 crosses is interesting. The pigmentation contributed to F1 hybrids by C57BL/6 mice may afford some protection from UV radiation; however, this point was not investigated in detail. It is clear that pigmentation is not an absolute requirement for suppression of hypersusceptibility, since partial suppression occurs in the absence of pigmentation (in the F1 hybrids with BALB/c). The fact that both traits, susceptibility to phototumorigenesis and persistent hyperplasia, appear to be linked in four different F1 crosses suggests that they share a common mechanism.

Our previous studies (16) have shown that the UV-induced persistent hyperplasia exhibited by SENCAR mice is due to the inability of epithelial basal cells to cease proliferating following wound healing. This indicates that the biochemical processes that regulate cell proliferation during tissue regeneration are not operating properly. Deregulation of basal cell proliferation could directly predispose SENCAR mice to tumor susceptibility by causing continued proliferation of carcinogen-damaged cells. However, we show (Table 3) that persistent hyperplasia and tumorigenesis are independently distributed among individual SENCAR mice, suggesting an indirect mechanism.

One possible indirect mechanism is that sustained basal cell proliferation during wound healing and abnormal cell growth during skin tumorigenesis are independent mitogenic responses associated with the same altered genotype. Further studies are in progress to test this hypothesis.

**ACKNOWLEDGMENTS**

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**REFERENCES**


Table 3 Tumor formation and persistent hyperplasia in SENCAR mice

<table>
<thead>
<tr>
<th>No. of mice</th>
<th>+PH</th>
<th>–PH</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>With tumor</td>
<td>6</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Without tumor</td>
<td>15</td>
<td>19</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>24</td>
<td>45*</td>
</tr>
</tbody>
</table>

* +PH, number of mice with persistent hyperplasia; –PH, number of mice without persistent hyperplasia.

\chi^2 = 0.065 (not significant).
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