Sensitivity to Two-Stage Carcinogenesis of SENCAR Mouse Skin Grafted to Nude Mice


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ABSTRACT

SENCAR mice are extremely susceptible to two-stage skin carcinogenesis, while BALB/c mice are relatively resistant. Skin grafts to BALB/c nude mice were performed with full-thickness skin from SENCAR and BALB/c donors, and tumor formation was monitored in grafted skin, surrounding host skin, and intact SENCAR, BALB/c and nude mice. Initiation was accomplished by exposure to 20 μg dimethylbenz(a)anthracene and promotion by repeated exposure to 12-O-tetradecanoylphorbol-13-acetate. SENCAR skin retained a high sensitivity to carcinogenesis when grafted to nude hosts, whereas BALB/c skin remained resistant. The donor type did not influence the tumor yield in surrounding nude host skin. The rate of tumor regression was not altered in SENCAR skin grafts on nude mice relative to intact SENCAR skin. These results indicate that the unusual sensitivity of SENCAR epidermis to chemical carcinogenesis is not due to altered systemic factors but is a property of the skin itself.

INTRODUCTION

Epidemiological and medical genetic data have indicated that major individual differences exist in cancer risk among humans. Increased risks in overall cancer susceptibility or in susceptibility at a particular target organ have been identified. In some cases, specific genetic alterations are linked to increased risk, but in many examples polygenic influences appear to be involved. To date, biochemical-epidemiological and laboratory studies on susceptibility factors have focused mainly on genetic differences in carcinogen metabolism (4). However, the complex multistage nature of carcinogenesis suggests that factors other than carcinogen metabolism play an important role in host susceptibility.

The development, through selective breeding, of animal strains with high susceptibility to carcinogenesis at a particular organ site provides an opportunity to study susceptibility determinants. SENCAR mice were developed for sensitivity to chemical carcinogenesis in the skin (3). SENCAR mice were derived by crossing STS males (1) with Charles River (Charles River Breeding Laboratories, North Wilmington, Mass.) CD-1 females and then breeding those mice which responded maximally to initiation by DMBA and promotion by TPA. Little is known of the cellular basis for susceptibility of SENCAR mice. This strain does not differ significantly from less susceptible strains in the metabolism of DMBA (3) and demonstrates enhanced sensitivity to initiation by benzo(α)pyrene and N-methyl-N'-nitro-N-nitrosoguanidine as well as DMBA (3, 5). It is not known for certain if the skin is a selective target site for carcinogenesis in SENCAR mice. In attempting to define the basis for susceptibility of SENCAR mice, it is important to distinguish between factors determined at the level of the host animal or at the target tissue. This study indicates that sensitivity to chemical carcinogenesis is a property of SENCAR skin.

MATERIALS AND METHODS

Animals. Male SENCAR mice were obtained from Dr. T. Slaga, Oak Ridge National Laboratories, Oak Ridge, Tenn. Male BALB/c mice were obtained from Charles River. Both strains were 8 to 12 weeks old at the start of the protocol. Female BALB/c nude (nu/nu) mice were obtained from the Animal Resources Program of the National Cancer Institute and maintained as described previously (11). Grafting. Donor mice were shaved, killed by cervical dislocation, and washed with Betadine and alcohol. Full-thickness dorsal skin was removed and cut into circles with a sterile cork borer. Graft beds were prepared on the right dorsolateral thorax of nude mice as described previously (10), and grafts were placed and wrapped with a bandage for 7 days. All grafts were performed over a 12-day period, and 4 weeks after the last animal was grafted the animals were randomly distributed into experimental groups.

Tumor Induction Experiments. DMBA (Eastman Chemical Co., Rochester, N. Y.) was dissolved in acetone, and 20 μg/0.1 ml was applied once to the graft site or an area of equal size on ungraded animals. One week after DMBA initiation, promotion with TPA was begun. TPA was obtained from Chemicals for Carcinogenesis Research (Eden Prairie, Minn.) and 2μg/0.1 ml acetone was applied weekly for 2 weeks, then twice weekly for 6 weeks, and then alternating once or twice weekly for 12 additional weeks. This protocol was used to lessen the skin damage which TPA produces on SENCAR mice (5). Papillomas were counted and recorded for each mouse at weekly intervals. After 20 weeks, all treatments were stopped, and the animals were observed for an additional 8 weeks prior to sacrifice. The treatment groups are shown in Table 1. Groups of intact (nongrafted) nude mice and SENCAR-autografted mice (Table 1 groups 14 to 17) were added at a later time and were treated by the same protocol.

Expression of Results. Only papillomas were considered in these experiments, although some carcinomas developed in the intact SENCAR group. Mean tumor incidence was compared for the same target site in grafted and nongrafted animals by measuring each graft or control-treated skin and calculating a tumor incidence per sq cm. Although localized application to the graft site or an equal size area in nongrafted animals was attempted, the use of acetone as a solvent resulted in diffusion beyond these limits. An estimate of the entire treatment area was made by applying 1% May-Grünwald stain in acetone (0.1 ml) onto 10 mice and excising the purple area. A filter paper copy of each skin was prepared and weighed, and an average weight was compared to the weight of standard 4-sq cm pieces of filter paper. The treatment area was determined to average 6.8 sq. cm. In
this way, the incidence of host tumors and graft tumors could be compared.

**RESULTS**

In these experiments, only mice receiving both initiator and promoter developed tumors. Chart 1 compares the tumor incidence of BALB/c and SENCAR mouse skin in situ and in grafts. As reported previously (5), SENCAR mice are highly susceptible to 2 stage skin carcinogenesis protocols, whereas BALB/c mice are relatively resistant. Chart 1 also shows that SENCAR skin retains its sensitivity when grafted to nude mice while BALB/c skin continues to be resistant. The decrease in tumor incidence between grafted and intact SENCAR skin and the longer latency period in grafted skin appears to be associated with the grafting itself, since SENCAR autografts (Table 1, Group 16) have a lower tumor incidence than do surrounding host skin (not shown). However, since Group 16 was a different

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of mice</th>
<th>Host mouse</th>
<th>Grafted skin</th>
<th>Initiator (Wk 0)</th>
<th>Promoter (Wk 1–20)</th>
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<tr>
<td>1</td>
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<td>Nude</td>
<td>SENCAR</td>
<td>DMBA</td>
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‡ Initiation and promotion were instituted at a different time for these groups although animal age was the same.

* Autografts.

Not grafted.

Chart 1. Papilloma incidence per sq cm in SENCAR and BALB/c donors and skin grafts. All mice were initiated with 20μg DMBA and promoted with TPA for 20 weeks, as described in "Materials and Methods" (arrow, end of promotion phase). Papillomas were counted on the grafted skin and on the surrounding host skin and calculated on the basis of the treatment area. O, intact SENCAR; A, SENCAR skin grafted to nude mice; Δ, intact BALB/c; △, BALB/c skin grafted to nude mice.

group of SENCAR mice, grafted at a different time, and not initiated and promoted simultaneously with the groups shown in Chart 1, the reduced tumor incidence cannot be compared directly with certainty. Also of interest in Chart 1 is the fact that tumor regression occurs equally in intact and grafted SENCAR skin. The regression of SENCAR tumors on nude hosts suggests that T-cell-mediated immune mechanisms are not a major determinant of papilloma regression. This is in agreement with the papilloma regression data reported by Burns et al. (2).

Chart 2 compares the tumor incidence of the graft to the surrounding treated host tissue. In Chart 2A, one can see that the SENCAR skin grafts are 6 times more susceptible to tumorigenesis than is the surrounding nude skin, while in Chart 2B the BALB/c graft and its nude (BALB/c derived) host have about the same tumor incidence, although the latency period in the grafted skin is significantly lengthened. Chart 2, A and B, also indicate that the host tumor incidence is not altered by the source of the graft. In Chart 2C, tumor incidences among intact nudes, nude grafts, and their nude hosts are compared and show little significant difference among the groups. The intact nude group in Chart 2C also was not a simultaneous control (Group 14) but has the same tumor incidence as all other nude groups.
DISCUSSION

These results indicate that major determinants for the sensitivity of SENCAR mouse skin to chemical carcinogenesis reside in the target tissue. Previous reports have indicated that DMBA metabolism does not differ in SENCAR and other strains and that SENCAR mice are sensitive to carcinogens not requiring metabolism. Thus, it can be concluded that other genetic factors regulate susceptibility in this strain. Since these factors are inherent to SENCAR skin, the use of cell culture to explore the basis for sensitivity in SENCAR epidermis is valid.

This laboratory has shown recently that carcinogens induce epidermal cells to resist a signal for terminal differentiation in vitro (6). We have also reported that resistance to terminal differentiation is characteristic of malignant epidermal cells (6) and cells cultured from skin initiated in vivo but not from control skin (9). These findings have led us to conclude that an alteration in response to differentiation signals is a fundamental change resulting from initiation. When untreated SENCAR skin in culture is tested for responsiveness to a differentiation signal, colonies resistant to terminal differentiation persist (9). In vivo, a relatively high tumor yield occurs in SENCAR mice treated with TPA only (without exogenous initiator) (5). Taken together, these results imply that SENCAR mouse skin contains constitutively initiated cells. This could result from a gene which directly determines initiation or which makes SENCAR cells more prone to undergo initiation spontaneously. Exposure of SENCAR mice to carcinogen markedly increases the number of initiated cells, as expressed in tumor experiments in vivo (5) or as altered differentiation in vitro (9). Thus, there may be several stages of initiation, with resistance to terminal differentiation a characteristic of an early stage and subsequent stages. In this case, initiation by a low dose of carcinogen would cause progression of constitutively initiated cells to a more advanced stage of initiation that still requires promotion for tumor formation. Alternatively, a small number of cells in SENCAR skin may be constitutively and completely initiated, but carcinogen exposure would initiate other particularly prone cells.

While constitutively initiated cells appear to exist in SENCAR skin, these mice also are more sensitive to tumor promoters. Thus, TPA administration must be kept at low levels to avoid severe cytotoxicity in SENCAR mice, whereas other mice tolerate higher exposures without overt cytotoxicity. It is conceivable that the same genetic factor(s) which control sensitivity to initiators also regulate sensitivity to tumor promoters (e.g., free radical generation). Recently, the level of phorbol ester receptors has been measured in SENCAR skin cells (7) and found to be in the range reported for other cells, suggesting that receptor level does not provide the basis for the sensitivity to TPA.

Our results indicate that a focus on the target tissue (skin) is likely to yield more information regarding determinants for susceptibility of SENCAR mice to 2-stage carcinogenesis. Since techniques for the culture of mouse epidermis are well established (8), future studies on the mechanism of sensitivity of SENCAR skin to multistage carcinogenesis can be conducted in vitro.

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REFERENCES

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