Evidence for a Common Genetic Pathway Controlling Susceptibility to Mouse Skin Tumor Promotion by Diverse Classes of Promoting Agents


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INTRODUCTION

In mouse strains there are significant differences in susceptibility to two-stage skin carcinogenesis when phorbol esters, such as TPA, are used as promoters (1-3). For example, SENCAR, LACA, and CD-1 mice are reported to be relatively susceptible, whereas BALB/c mice are relatively resistant to TPA promotion (1-3). It has been suggested that a primary determinant in the observed strain differences toward two-stage skin carcinogenesis is related to the promotion stage (4-6). With Chr as the promoter, the order of sensitivity was SENCAR > SSIn > CD-1. Concurrent tumor promotion experiments examined the responsiveness of two common inbred mouse strains, DBA/2 and C57BL/6. The phorbol ester-responsive mouse strain, DBA/2, was more sensitive to skin tumor promotion by Chr than was C57BL/6 at all doses tested but was clearly less sensitive than both SENCAR and SSIn mice. Finally, DBA/2 and C57BL/6 mice were similar in their responsiveness to BzPo promotion, but again both of these inbred strains were significantly less sensitive than were SSIn and SENCAR mice to this organic peroxide type of skin tumor promoter. Histological evaluations comparing SENCAR and C57BL/6 mice revealed that a major difference between these strains in response to multiple Chr and BzPo treatments was in the inflammatory response (measured by edema formation). Unlike 12-O-tetradecanoylphorbol-13-acetate, Chr and BzPo did not induce dramatic differences in the epidermal hyperplasia (as measured by epidermal thickness) in these two mouse lines. The results presented in this paper suggest that there is a common pathway controlling susceptibility to skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate, BzPo, and chrysarobin. These results are discussed in terms of a possible genetic model(s) for skin tumor promotion in mice.

ABSTRACT

The present study has compared different mouse stocks and strains with known sensitivity to phorbol ester skin tumor promotion for their sensitivities to skin tumor promotion by a prototypic organic peroxide (benzoyl peroxide, BzPo) and anthrone (chrysarobin, Chr) tumor promoters. Following initiation with either 7,12-dimethylbenz(a)anthracene and/or N-methyl-N'-nitro-N-nitosoguanidine, groups of mice were promoted with several different doses of each promoting agent. Among mice selectively bred for sensitivity to phorbol ester promotion, the order of sensitivity to BzPo was inbred SENCAR (SSIn) > SENCAR > CD-1. With Chr as the promoter, the order of sensitivity was SENCAR > SSIn > CD-1. Concurrent tumor promotion experiments examined the responsiveness of two common inbred mouse strains, DBA/2 and C57BL/6. The phorbol ester-responsive mouse strain, DBA/2, was more sensitive to skin tumor promotion by Chr than was C57BL/6 at all doses tested but was clearly less sensitive than both SENCAR and SSIn mice. Finally, DBA/2 and C57BL/6 mice were similar in their responsiveness to BzPo promotion, but again both of these inbred strains were significantly less sensitive than were SSIn and SENCAR mice to this organic peroxide type of skin tumor promoter. Histological evaluations comparing SENCAR and C57BL/6 mice revealed that a major difference between these strains in response to multiple Chr and BzPo treatments was in the inflammatory response (measured by edema formation). Unlike 12-O-tetradecanoylphorbol-13-acetate, Chr and BzPo did not induce dramatic differences in the epidermal hyperplasia (as measured by epidermal thickness) in these two mouse lines. The results presented in this paper suggest that there is a common pathway controlling susceptibility to skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate, BzPo, and chrysarobin. These results are discussed in terms of a possible genetic model(s) for skin tumor promotion in mice.

INTRODUCTION

In mouse strains there are significant differences in susceptibility to two-stage skin carcinogenesis when phorbol esters, such as TPA,3 are used as promoters (1-3). For example, SENCAR, LACA, and CD-1 mice are reported to be relatively susceptible, whereas BALB/c mice are relatively resistant to TPA promotion (1-3). It has been suggested that a primary determinant in the observed strain differences toward two-stage skin carcinogenesis is related to the promotion stage (4-6). We reported previously that the inbred strain DBA/2 responded to a two-stage, initiation-promotion tumorigenesis protocol when high doses of DMBA or the alkylating carcinogen MNNG were used for initiation and subsequently followed by TPA for promotion (7). Conversely, C57BL/6 mice were relatively resistant to TPA promotion using standard promotion protocols regardless of the chemical class of initiator or the dose of initiator used (5-7). However, C57BL/6 mice were reported to be quite sensitive to complete skin carcinogenesis protocols using repetitive weekly treatments with DMBA or benz(a)pyrene and also sensitive to skin tumor promotion with both BzPo (5) and 1,8-dihydroxy-3-methyl-9-anthraquinone (Chr) (6) when very high initiating doses of DMBA were used. Thus, although strain differences appear to exist in sensitivity to phorbol ester tumor promoters, it is not clear to what extent such differences extend to other chemical classes of promoting agents.

Previous work from several laboratories, including our own, has demonstrated marked differences in the ability of TPA to induce inflammation and hyperplasia in different stocks and strains of mice (reviewed in Ref. 8). In general, there is a good correlation between the magnitude of these changes following repetitive treatments and the degree of susceptibility to phorbol ester-mediated skin tumor promotion. On the other hand, we reported very similar hyperplastic responses in DBA/2 and C57BL/6 mouse skin following repeated treatments with several non-phorbol ester tumor promoters including Chr and BzPo (9).

It should be emphasized that, although the current data discussed above suggest that C57BL/6 mice are partially responsive to certain non-phorbol ester tumor promoters, it is still not clear whether C57BL/6 mice are of generally lower sensitivity to organic peroxide and anthrone tumor promotion compared with SENCAR (or DBA/2) mice. This is admittedly due to the fact that, in the studies using BzPo (5) and Chr (6), different initiating doses of DMBA were used in SENCAR mice (10 nmol) compared with the DBA/2 and C57BL/6 mice (400 nmol). However, several investigators have provided evidence that certain mouse strains less sensitive to phorbol esters may also be less sensitive to other classes of promoting agents. For example, Bock and Burns (10) presented data suggesting that C57/st mice were less sensitive to both croton oil and anthralin (another anthrone derivative) than were Swiss mice initiated with the same dose of DMBA, although small numbers of animals were used in the experimental groups of this study. Ashman et al. (2) reported that LACA mice were more sensitive to anthralin than were BALB/c mice initiated with high doses of DMBA. Furthermore, CD-1 mice appeared to be less sensitive than were SENCAR mice to the promoting effects of 7-bromomethylbenz(a)anthracene (11) and UV light (12).

The present study has addressed several questions regarding cross-sensitivity and/or resistance among mouse stocks and strains to different classes of promoting agents. (a) We have examined in detail whether mice selectively bred for increased sensitivity to one chemical class of promoter (i.e., phorbol ester) are also more sensitive to other chemical classes of promoting agents (e.g., anthrones and organic peroxides). (b) We have compared the relative sensitivity of DBA/2 and C57BL/6 mice to skin tumor promotion by representatives from these two chemical classes, Chr and BzPo, respectively. The results dem-
onstrate that there may be a common genetic pathway controlling susceptibility to different chemical classes of promoters.

MATERIALS AND METHODS

Chemicals. TPA was purchased from LC Services (Woburn, MA). BzPo was obtained from the Aldrich Chemical Company, Inc. (Milwaukee, WI). Chr was purchased from ICN Pharmaceuticals, Inc., K and K Laboratories Division (Plainview, NY) and was purified using the procedure reported in a previous study (13). DMBA and MNNG were purchased from Aldrich Chemical Co. [3H]DMBA (specific activity, 20 to 50 Ci/nmol) was acquired from Amersham (Arlington Heights, IL). All other chemicals and reagents used were of the highest purity deemed necessary.

Animals. Female DBA/2N, C57BL/6N, CD-1, and SENCAR mice were purchased from the National Cancer Institute (Frederick, MD). Female SSIn mice were purchased from the University of Texas M. D. Anderson Cancer Center, Science Park-Veterinary Division (Bastrop, TX). At 7 wk of age, the backs of the mice were carefully shaved using surgical clippers. Mice were allowed to rest for 2 days, and only those mice in the resting phase of the hair growth cycle were utilized. All chemicals were applied to the shaved area in 0.2 ml of acetone. Mice received topical applications of various doses of promoters given once or twice weekly depending on the compound. Promotion was continued in each experimental group until a maximal response was achieved, and the incidence of skin papillomas was observed and recorded weekly. Control mice received only the vehicle, acetone (0.2 ml), at the time of initiation followed by applications of the appropriate promoter as indicated.

Tumor Induction Experiments. Groups of 30 mice each were initiated with either MNNG (2.5 μmol per mouse) or DMBA (25 nmol per mouse) unless otherwise specified. Two wk after initiation, mice received topical applications of various doses of promoters given once or twice weekly depending on the compound. Promotion was continued in each experimental group until a maximal response was achieved, and the incidence of skin papillomas was observed and recorded weekly. Control mice received only the vehicle, acetone (0.2 ml), at the time of initiation followed by applications of the appropriate promoter as indicated.

Histological Analyses. Groups of three to four female mice were treated with either single or multiple applications of acetone or acetone solutions of various tumor promoters, as indicated. At various times after the last treatment, mice were sacrificed. Skin from treated animals was excised, fixed in 10% formalin, and embedded in paraffin. Tissue sections were stained with hematoxylin and eosin. The measurements of the epidermal thickness (except horny layer) were performed as described before (9). The measurements of edema formation in skins from promoter-treated mice were performed also as previously described (14). Briefly, skins of uniform size (1 in²) were excised from promoter-treated mice at various times after the last promoter treatment. The skins were weighed and the weights were expressed as g/in² (mean ± SD). Statistical analyses of the differences between means were evaluated using Student’s t test.

Covalent Binding to Mouse Epidermal DNA in Vivo. Individual groups of five female mice were used for each experimental group. Mice received topical application of 4 to 25 nmol of [3H]DMBA (100 μCi per mouse). Twenty-four h following application of the hydrocarbon, mice were sacrificed. The isolation of DNA and quantitation of hydrocarbon binding followed procedures described previously (15).

RESULTS

Responsiveness of SSIn, SENCAR, CD-1, DBA/2, and C57BL/6 Mice to Skin Tumor Promotion by Chr. Table 1 summarizes dose-response studies on the sensitivity of female SENCAR and CD-1 mice to skin tumor promotion by TPA and Chr. For these experiments, MNNG (2.5 μmol per mouse) was used as the initiating agent. As shown, CD-1 mice were less sensitive to the skin tumor-promoting actions of both classes of promoting agents. It is of interest to note that CD-1 mice did not yield a significant papilloma response at doses of chrysarobin up to 440 nmol per mouse after 30 wk of promotion in this experiment. A dose of 220 nmol of Chr was a maximal promoting dose in SENCAR mice in the current experiments, a finding similar to our previous observations (16). Table 2 summarizes a separate dose-response experiment.

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Initiator (dose, nmol)</th>
<th>Papillomas/ mouse</th>
<th>% of mice with papillomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>SENCAR</td>
<td>DMBA</td>
<td>10</td>
<td>0.90 ± 0.57</td>
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<tr>
<td></td>
<td></td>
<td>25</td>
<td>5.20 ± 1.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>9.22 ± 0.95</td>
</tr>
<tr>
<td>MNNG</td>
<td>25</td>
<td>1.12 ± 0.89</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.54 ± 0.94</td>
<td>71</td>
</tr>
<tr>
<td>SSIn</td>
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<td>0.04 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.13 ± 0.10</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.83 ± 0.22</td>
<td>79</td>
</tr>
<tr>
<td>MNNG</td>
<td>25</td>
<td>0.08 ± 0.06</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.20 ± 0.06</td>
<td>21</td>
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<tr>
<td>DBA/2</td>
<td>DMBA</td>
<td>100</td>
<td>0.71 ± 0.29</td>
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<td></td>
<td></td>
<td>440</td>
<td>1.49 ± 0.63</td>
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<td>880</td>
<td>1.00 ± 0.32</td>
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<tr>
<td>MNNG</td>
<td>440</td>
<td>0.25 ± 0.03</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>880</td>
<td>0.32 ± 0.18</td>
<td>24</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>DMBA</td>
<td>100</td>
<td>0</td>
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<tr>
<td></td>
<td></td>
<td>440</td>
<td>0.50 ± 0.09</td>
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<td></td>
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<td>880</td>
<td>0.48 ± 0.12</td>
</tr>
<tr>
<td>MNNG</td>
<td>440</td>
<td>0.24 ± 0.14</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>880</td>
<td>0.25 ± 0.09</td>
<td>21</td>
</tr>
</tbody>
</table>

*a Mean ± SD.
*b Significantly greater (P < 0.05) than corresponding SSIn treatment groups.
Significantly greater (P < 0.05) than corresponding DBA/2 and C57BL/6 treatment groups.
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comparing the sensitivity of SSIn, SENCAR, DBA/2, and C57BL/6 mice to skin tumor promotion by Chr. In this experiment mice were initiated with either DMBA (25 nmol) or MNNG (2.5 \( \mu \)mol) and then promoted with the anthrone derivative. Surprisingly, SSIn mice were significantly less sensitive to skin tumor promotion by Chr regardless of the initiating agent used. In this regard, the average number of papillomas per mouse at all doses of Chr was significantly greater (\( P < 0.05 \)) in SENCAR than the corresponding groups of SSIn mice. This finding was surprising in light of previous studies demonstrating SSIn mice to be significantly more sensitive to skin tumor promotion by TPA (17). Therefore, a second experiment was performed simultaneously comparing the responsiveness of SSIn and SENCAR mice to both TPA and Chr. For this experiment, groups of 30 mice were initiated with 25 nmol of DMBA and promoted with 0.85 nmol of TPA or 100 nmol of Chr. After 15wk of promotion, TPA produced 13.32 (100%) and 3.52 (68%) papillomas per mouse, whereas Chr induced 0.24 (20%) and 2.32 (48%) papillomas per mouse in SSIn and SENCAR mice, respectively. The numbers in parentheses are the corresponding papilloma incidences at 15wk of promotion. These data confirm that SSIn mice are more sensitive than SENCAR mice to TPA promotion but less sensitive to Chr skin tumor promotion.

To further explore the strain distribution patterns for susceptibility to skin tumor promotion by different classes of promoters, we compared the sensitivity of DBA/2 and C57BL/6 mice to Chr. These results are also summarized in Table 2. Previous work from our laboratory demonstrated that DBA/2 mice were relatively sensitive to skin tumor promotion by TPA (6–8). Differences in TPA sensitivity of the DBA/2 and C57BL/6 mice used in the present study were initially reconfirmed. In groups of 30 mice following initiation with 25 nmol of DMBA and 27wk of promotion (twice weekly application) with 6.8 nmol of TPA, DBA/2 mice had 3.3 papillomas per mouse (90% incidence), whereas C57BL/6 mice had 0.10 papillomas per mouse (10% incidence). In groups of mice initiated with 25 nmol of DMBA, DBA/2 mice were more sensitive (\( P \leq 0.1 \)) than were C57BL/6 mice at all doses examined. The differences, however, were more evident at the lower doses of Chr used in the present experiments (i.e., 100 and 440 nmol per mouse). In mice initiated with MNNG (2.5 \( \mu \)mol) the tumor response was low in both strains and did not allow meaningful comparisons related to sensitivity. However, in two separate additional experiments DBA/2 mice initiated with 2.5 \( \mu \)mol of MNNG and promoted with either 100 or 220 nmol of Chr yielded significantly (\( P < 0.05 \)) higher tumor responses compared with similarly treated C57BL/6 mice (0.40 and 0.83 versus 0.05 and 0.17 papillomas per mouse, respectively). These values represent an average of both of the repeat experiments. A repeat experiment was also performed to confirm the data obtained with DMBA as the initiator and the 100- and 440-nmol doses of Chr giving essentially identical results (data not shown). It is interesting to note that both DBA/2 and C57BL/6 mice were considerably less sensitive than were SENCAR mice to skin tumor promotion by Chr (\( P < 0.05 \)). The SSIn mice, while less sensitive than were SENCAR mice to Chr promotion, were still more sensitive to this promoter than were DBA/2 mice (and C57BL/6) when comparing the groups with DMBA as the initiator. The data in this table are from the same overall experiment and therefore allow direct comparison. These data demonstrate that, although SSIn mice have lost their increased sensitivity relative to SENCAR mice to the anthrone promoter, they still retain a relatively high sensitivity in comparison with the other inbred strains of mice examined in our present study.

Responsiveness of SSIn, SENCAR, CD-1, DBA/2, and C57BL/6 mice to Skin Tumor Promotion by BzPo. The ability of BzPo to promote skin papillomas in SSIn, SENCAR, CD-1, DBA/2, and C57BL/6 mice is presented in Tables 3 and 4. Table 3 compares SENCAR and CD-1 strains at a 20-ng dose of BzPo and initiation with either DMBA (10 nmol) or MNNG (0.68 \( \mu \)mol); data generated are part of a previous study (18). SENCAR mice were more sensitive than were CD-1 mice to skin tumor promotion with BzPo, but this could only be assessed with the groups initiated with DMBA. The dose of MNNG (0.68 \( \mu \)mol) used in this particular experiment did not yield a significant papilloma response in SENCAR or CD-1 mice after 32wk of promotion with BzPo. In a separate experiment, SSIn mice were compared with SENCAR, DBA/2, and C57BL/6 mice using several different doses of BzPo in mice initiated with either DMBA (25 nmol) or MNNG (2.5 \( \mu \)mol) (Table 4). With this organic peroxide promoter, SSIn mice were more sensitive than were SENCAR mice, especially at the lower doses of BzPo used. Table 4 also summarizes the response to BzPo promotion in DBA/2 versus C57BL/6 mice initiated with DMBA (25 nmol). Little differ-

### Table 3 Response of SENCAR and CD-1 mice to benzoyl peroxide promotion following initiation with DMBA or MNNG

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Initiator</th>
<th>BzPo (dose, mg)</th>
<th>Papillomas/mouse</th>
<th>% of mice with papillomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>SENCAR</td>
<td>DMBA</td>
<td>20</td>
<td>1.38</td>
<td>45</td>
</tr>
<tr>
<td>CD-1</td>
<td>DMBA</td>
<td>20</td>
<td>0.07</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>MNNG</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Thirty mice were used for each experimental group. Mice were initiated with either 10 nmol of DMBA or 0.68 \( \mu \)mol of MNNG followed 1wk later by twice weekly applications of 20 mg of BzPo for 32wk. Survival was \( \geq 97% \) in all groups.

### Table 4 Dose response for BzPo promotion following DMBA or MNNG initiation in SENCAR, SSIn, DBA/2, and C57BL/6 mice

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Initiator</th>
<th>BzPo (dose, mg)</th>
<th>Papillomas/mouse</th>
<th>% of mice with papillomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>SENCAR</td>
<td>DMBA</td>
<td>5</td>
<td>4.40 ± 1.96( ^a )</td>
<td>60</td>
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<tr>
<td></td>
<td>MNNG</td>
<td>10</td>
<td>7.19 ± 1.06( ^b )</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>6.20 ± 2.35( ^c )</td>
<td>88</td>
</tr>
<tr>
<td>SSIn</td>
<td>DMBA</td>
<td>5</td>
<td>11.71 ± 0.11( ^d )</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>MNNG</td>
<td>10</td>
<td>14.25 ± 0.37( ^e )</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>13.13 ± 2.37( ^f )</td>
<td>100</td>
</tr>
<tr>
<td>DBA/2</td>
<td>DMBA</td>
<td>5</td>
<td>0.56 ± 0.31( ^g )</td>
<td>44( ^h )</td>
</tr>
<tr>
<td></td>
<td>MNNG</td>
<td>10</td>
<td>0.47 ± 0.20( ^i )</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>0.47 ± 0.11( ^j )</td>
<td>42</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>DMBA</td>
<td>5</td>
<td>0.36 ± 0.16( ^k )</td>
<td>18</td>
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<tr>
<td></td>
<td>MNNG</td>
<td>10</td>
<td>0.57 ± 0.33( ^l )</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>0.48 ± 0.22( ^m )</td>
<td>35</td>
</tr>
</tbody>
</table>

\( ^a \) Mean ± SD; 
\( ^b \) Significantly greater (\( P < 0.05 \)) than corresponding DBA/2 and C57BL/6 treatment groups; 
\( ^c \) Significantly greater (\( P < 0.05 \)) than corresponding SENCAR treatment group; 
\( ^d \) Significantly greater (\( P < 0.05 \), \( \chi^2 \) test) than corresponding C57BL/6 treatment group.
ences were noted in the promotion response to BzPo between these two inbred strains except at the lowest dose examined (i.e., 10 mg). At this dose, DBA/2 had approximately 1.6 times as many papillomas per mouse and approximately 2 times as many animals responding compared with the C57BL/6 mice. However, only the difference in the number of animals responding was statistically significant ($P < 0.05$, $\chi^2$ test). Although not as striking as with chrysarobin, these data suggest the possibility that DBA/2 mice may also be more sensitive to lower doses of BzPo. It is clear from the data presented in Table 4 that doses of BzPo above 5 mg were maximal promoting doses. Therefore, further work using lower doses of BzPo will be necessary to confirm any differences between DBA/2 and C57BL/6 strains in response to BzPo. Nevertheless, both DBA/2 and C57BL/6 mice were significantly less sensitive than were SENCAR and SSIn mice to skin tumor promotion by BzPo. Again, the data in these two tables are from the same overall experiment and can be directly compared.

Hyperplasia and Edema Formation. Fig. 1 shows the hyperplasia response in SENCAR and C57BL/6 mice after multiple treatments with both Chr (220 nmol) and BzPo (20 mg). The data presented are values obtained 48 h after the last four applications of each promoter. In this experiment, Chr was given once weekly, whereas BzPo was given twice weekly to exactly mimic the treatment protocols in the tumor experiments. These treatments are optimum for the induction of hyperplasia and promotion of skin tumors with both compounds (9). Also shown, for comparative purposes, is the hyperplasia response following multiple treatments with TPA (3.4 nmol given twice weekly for four applications). Unlike TPA, where there was a dramatic difference in epidermal thickness at 48 h after the last treatment (53.93 ± 2.60 versus 29.58 ± 1.63, respectively), much smaller differences were observed between SENCAR and C57BL/6 mice with Chr (33.4 ± 3.33 versus 26.06 ± 1.10, respectively). With BzPo, statistically significant differences in epidermal thickness values between SENCAR and C57BL/6 mice were not observed. Thus, there was not a strong correlation between magnitude of the sustained hyperplasia and promotion response to either Chr or BzPo between SENCAR and C57BL/6 mice. These data are similar to our previous studies comparing DBA/2 and C57BL/6 mice (9) but now extend to the more sensitive SENCAR mouse.

In contrast, when we examined edema in the skin of SENCAR and C57BL/6 mice, significant differences were observed with both Chr and BzPo. The data summarized in Figs. 2 and 3 demonstrate significantly greater edema formation in skins of SENCAR mice, especially 24 h after the last of four applications of either promoter. Interestingly, while Chr also produced significantly greater edema in SENCAR mice relative to C57BL/6 mice at 24 h following a single application, single treatments with BzPo produced only slight increases in edema formation in both strains. Thus, the differences in edema response observed between SENCAR and C57BL/6 mice with both classes of promoters were most apparent using the multiple treatment regimen.
Dose Response for DMBA:DNA Adduct Formation. A potential confounding variable in the interpretation of the tumor experiments in our present study involved possible genetic differences in the metabolic activation of DMBA. We previously suggested that genetic differences at the Ah locus could be easily overcome using relatively high initiating doses in mouse epidermis (7). Since the doses of DMBA used in the present study were intermediate initiating doses (i.e., 10 and 25 nmol), we examined the covalent binding of this hydrocarbon to epidermal DNA at several doses in DBA/2, C57BL/6, CD-1, and SENCAR mice. The results of these experiments are summarized in Table 5. As shown, DMBA:DNA adduct levels were essentially identical in all four stocks/strains of mice over the dose range examined.

DISCUSSION

The overall goal of the current investigation was to determine whether there was cross-sensitivity and resistance among inbred and outbred mouse lines to different classes of skin tumor promoters. The major finding from these studies is that mice less susceptible to phorbol ester promotion (e.g., DBA/2 and C57BL/6) are also generally less susceptible to at least two other classes of skin tumor promoters that appear not to work by directly activating PKC (19). These data suggest that there may be a common biochemical/molecular pathway mediating the promotion response to diverse promoting agents and that this pathway plays an important role in controlling promotion responsiveness among various stocks and strains of mice. Several genetic models could explain our current data, although it should be stressed that proof of any model will require additional genetic analyses. The first model is based on our recent studies examining F2 and backcross mice and BxD recombinant inbred strains derived from DBA/2 and C57BL/6 for responsiveness to TPA promotion (Ref. 20; Footnote 4). These studies determined that at least two genetic loci control responsiveness to TPA promotion in DBA/2 and C57BL/6 mice. Based on these earlier studies it is possible that one or more loci controlling responsiveness to TPA are also responsible for directly controlling responsiveness to other classes of skin tumor promoters such as BzPo and Chr. We have called this locus the Pms locus (for skin tumor promotion sensitivity). Alternatively, the current data could be interpreted as showing that different genes responsible for high sensitivity to promotion by diverse promoting agents act on a pathway(s) common to promotion in mouse epidermis. In this case, the Pms locus would represent a common biochemical/molecular pathway where different responses mediated by different types of promoters ultimately converge and lead to the process of tumor promotion in mouse skin. Directly testing either of the above models will require analyses of progeny from appropriate segregating crosses among the various stocks and strains used in the present study.

The latter model discussed above could help explain two additional observations in our present study. (a) SSIn mice, while still retaining a fairly high sensitivity to skin tumor promotion with Chr relative to other inbred strains (i.e., DBA/2, C57BL/6), have lost their increased sensitivity to this anthrone derivative relative to SENCAR mice. SSIn were developed through a process of inbreeding starting with the current outbred SENCAR and employing a selection scheme using DMBA initiation and TPA promotion similar to that originally devised by Boutwell (1, 17, and references therein). (b) C57BL/6 mice, although relatively resistant to all three types of promoting agents, are somewhat peculiar in their high resistance to standard promotion protocols using TPA (5-8). Indirectly, these observations suggest that different genes may regulate
some responses to particular types of promoting agents. A testable prediction from these observations is that it may be possible to selectively breed for mouse lines sensitive and/or resistant to specific classes of promoting agents. Such experiments are currently in progress.

Presently, little is known, at the biochemical or molecular level, about the genes controlling susceptibility to skin tumor promotion in mice. The majority of work to date has focused on potential differences related to phorbol ester actions (reviewed in Ref. 8). Potential mechanisms that have been explored include the following: (a) differences in phorbol ester metabolism (21); (b) differences in phorbol ester receptor number or affinity (22–24); (c) differences in epidermal PKC activity and major isozymes (24–26); (d) differences in arachidonic acid metabolism (27, 28); and (e) differences in epidermal oxidant response (29). Except for differences noted in the oxidant response induced by TPA in both epidermal cells (29) and peritoneal macrophages (27) from TPA-sensitive and -resistant mice, no other consistent differences have been noted. While differences in oxidant production correlated with susceptibility to TPA promotion among SSIn, SENCAR, and C57BL/6 mice, this correlation did not hold in F1 progeny between SSIn and C57BL/6 mice, raising questions about its relationship to genetic differences in response to TPA promotion (30). Furthermore, the generality of this response to other classes of tumor promoters that appear to work through PKC-independent pathways has not been demonstrated. Therefore, if the direct production of oxidants (presumably O2·−) by epidermal cells is important for genetically mediated differences in response to tumor promotion, this response may be specific for the phorbol ester class of promoters. This latter statement is further supported indirectly by two lines of evidence. (a) Lewis and Adams (27) showed that the differences in the release of hydrogen peroxide and metabolites of arachidonic acid from macrophages of SENCAR and C57BL/6 mice were observed primarily with phorbol esters and not with other stimuli. Our present data also demonstrate that C57BL/6 mice are less sensitive than are SENCAR mice to skin tumor promotion by two classes of tumor promoters that generate free radical intermediates directly (including reactive oxygen species). Therefore, C57BL/6 mice appear to also possess a more generalized variation in their response to tumor-promoting agents relative to SENCAR mice (see below).

At the tissue level, it has been known for some time that mouse stocks and strains vary widely in their inflammatory and hyperplasiogenic responses to phorbol ester treatment (reviewed in Ref. 8). In general, mouse stocks and strains that are fairly sensitive to TPA promotion (e.g., SENCAR, DBA/2, CD-1) respond with hyperplasia after a single application, but with a potentiation of this hyperplasia after multiple applications of TPA. In contrast, mice relatively resistant to TPA promotion respond with hyperplasia to a single application, but with multiple applications, either become refractory or fail to achieve a potentiated hyperplasia. Work in our laboratory extended these earlier observations by demonstrating, through dose-response experiments, that C57BL/6 × DBA/2 F1 (hereafter called B6D2F1) hybrid mice were less sensitive than were DBA/2 parental strains for induction of sustained epidermal hyperplasia by TPA (20). These data confirmed the results of tumor experiments showing that B6D2F1 mice were less sensitive to TPA promotion than were DBA/2 mice (20). A similar correlation was also observed for basal DC induction by TPA. Thus, the ability of TPA to induce these histological changes correlated closely with inherited susceptibility to promotion. In contrast to these studies with TPA, little differences were observed in the magnitude of the epidermal hyperplasia induced by Chr or BzPo in DBA/2 and C57BL/6 mice (9). In the present study, we further examined the production of epidermal hyperplasia in SENCAR and C57BL/6 mice by Chr or BzPo. The results, presented in Fig. 1, demonstrate that even in the SENCAR mouse, which is considerably more sensitive than is the DBA/2 mouse, these two promoters produced approximately equivalent hyperplastic responses. Thus, unlike the data obtained with TPA, the magnitude of the epidermal hyperplasia response did not correlate closely with responsiveness to promotion by Chr or BzPo.

Lewis and Adams (31) reported that TPA treatment of C57BL/6 mice induced the dermal infiltration of a very small number of PMNs compared with SENCAR mice. We also demonstrated a similar correlation between sensitivity to TPA promotion and infiltration of PMNs in the dermis of DBA/2, C57BL/6, and B6D2F1 mice, although this correlation was not as quantitative as with the hyperplasia response (including DC induction). The results presented in Figs. 2 and 3 of our current study demonstrate significant differences in the edema response to both Chr and BzPo between SENCAR and C57BL/6 mice. We previously noted a significant difference between DBA/2 and C57BL/6 for dermal infiltration of PMNs 48 h following the last of four treatments of Chr but not BzPo. However, we did not measure edema formation in this earlier study nor did we examine other time points. By examining this parameter and a more complete time course, we now report significant differences in a component of the inflammatory response (i.e., edema) between SENCAR and C57BL/6 mice that correlate with susceptibility to skin tumor promotion by both Chr and BzPo as well as TPA (31).

Until now, few studies have adequately compared different mouse stocks or strains for their susceptibility to different classes of skin tumor promoters. The majority of work reported to date has utilized the widely studied phorbol ester TPA (6). Reiners et al. (5) reported that C57BL/6 mice were quite responsive to skin tumor promotion by BzPo but relatively resistant to TPA promotion. The conclusion that C57BL/6 mice were quite responsive to BzPo promotion in their study is based on the observation that carcinomas apparently developed very rapidly in these mice in the absence of a significant papilloma response. For example, after 25 wk of promotion with 20 mg of BzPo, C57BL/6 mice initiated with either 400 or 800 nmol of DMBA had approximately one carcinoma per surviving mouse. In contrast, these same mice had ≤0.25 papillomas per mouse during the course of the same promotion period. This type of tumor response is very characteristic of a complete carcinogenesis regimen with either DMBA or B(a)P (4). The initiating doses of both DMBA and B(a)P used for the C57BL/6 mice in this study were very high; nevertheless, similar groups of mice promoted with TPA failed to yield this rapid production of carcinomas and, overall, appeared to be less sensitive to the phorbol ester. Our present data, although not totally different from these previous studies, do differ in several significant aspects. First, we used a much lower dose of DMBA (25 nmol) and also the direct acting initiator, MNNG. Under these conditions, SENCAR mice were clearly more sensitive to all tumor promoters examined, including BzPo, based on comparison of the papilloma response. Furthermore, after 30 wk of promotion with BzPo, only one carcinoma was present in SENCAR mice and no carcinomas were present in any of the C57BL/6 mice.
at this time point. These groups of C57BL/6 mice were further maintained on the BzPo protocol for an additional 8 wk. During this additional time period no unusual increase in the rate of carcinoma appearance was evident. Nevertheless, in our present study C57BL/6 mice were highly resistant to TPA promotion (using a twice weekly treatment regimen) as shown before (5, 7), and BzPo was capable of promoting a weak papilloma response in C57BL/6 mice. One possible mechanism for the differences between our current study and the study by Reiners et al. (5) could be that BzPo does possess very weak skin tumor-initiating activity. One report suggested that BzPo possessed very weak carcinogenicity (32), although this isolated observation has not been substantiated by other investigators (33, 34). A weak initiating activity coupled with the very high initiating doses of both B(a)P and DMBA used in the earlier study could conceivably have contributed to the significant carcinoma response observed. Alternatively, by virtue of its ability to produce certain types of DNA damage (35–37), BzPo may have exhibited a cocarcinogenic effect by modulating DNA replication and/or repair. In this latter case, one must assume that the time during which initiation took place was extended to the time at which BzPo treatment was begun (~1 wk). At present, these possibilities remain highly speculative and remain to be substantiated.

It should also be noted that Fischer et al. (38) recently reported that C57BL/6 mice responded to TPA promotion using relatively high initiating doses of DMBA (100 nmol) and more frequent applications of TPA (3 or 5 times/wk). However, it should be stressed that similar doses of DMBA and TPA given to SENCAR mice in a standard two-stage protocol would yield significantly more tumors than observed with C57BL/6 mice (39, 40). In addition, we have recently compared SENCAR and C57BL/6 mice initiated with 25 nmol of DMBA and treated with either 1.7 nmol or 6.8 nmol 3 times/wk, respectively.1 SENCAR mice had 13.0 papillomas per mouse (100% incidence), and C57BL/6 mice had 0.52 papillomas per mouse (36% incidence) after 25 wk of promotion. Thus, when compared under similar conditions, it is clear that C57BL/6 mice are still considerably less sensitive to TPA promotion than are SENCAR mice.

In conclusion, our current data support the hypothesis that there is a common genetic pathway(s) (Pms) controlling sensitivity of different mouse stocks and strains to a variety of skin tumor-promoting agents. The implication from these studies is that different classes of mouse skin tumor promoters may ultimately bring about some similar biochemical and molecular changes leading to the selective clonal expansion of initiated cells in mouse epidermis. The identity of this common biochemical pathway(s) remains to be determined.

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Evidence for a Common Genetic Pathway Controlling Susceptibility to Mouse Skin Tumor Promotion by Diverse Classes of Promoting Agents


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