Recovery of Transformed Nodule and Ductal Mammary Cells from Carcinogen-treated C57BL Mice

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ABSTRACT

Transformed nodule and ductal mammary cells were recovered by cell dissociation and transplantation of mammary cells from C57BL/Crgl mice treated with 7,12-dimethylbenz(a)anthracene. Four-week-old mice were divided into the following groups: Group A, not treated; Group B, 2 pituitary isografts under the kidney capsule; Group C, 1.0 mg 7,12-dimethylbenz(a)anthracene intra-gastrically at 5 and 6 weeks of age; and Group D, 2 pituitary isografts and 1.0 mg 7,12-dimethylbenz(a)anthracene intra-gastrically at 5 and 6 weeks of age. At 10, 14, 18, and 22 weeks of age, the mammary glands were enzymatically dissociated, and 10⁵ cells were injected into the gland-free mammary fat pads of 3-week-old syngeneic mice. After 10 weeks, the outgrowths were examined and classified as ductal, ductal dysplasia, or hyperplastic alveolar nodule. Ductal dysplasias and hyperplastic alveolar nodule outgrowths were recovered from carcinogen-treated mice. Pituitary isografting enhanced the recovery of ductal dysplasia. Five serially transplanted dysplastic outgrowth lines were established and are in their fifth and sixth transplant generations. The data demonstrate that transformed mammary gland cells can be recovered from carcinogen-treated mice by means of cell dissociation and transplantation.

INTRODUCTION

Recently, we developed a method for recovering transformed mammary epithelial cells from mouse mammary glands having no morphologically apparent dysplasias. DeOme et al. (2) have enzymatically dissociated the mammary glands of BALB/c fC3H (MTV + ) virgin mice at an age when no HAN or mammary tumors were apparent. When these dissociated cells were injected into the gland-free fat pads of syngeneic hosts, CD-derived outgrowths which contained HAN and tumors developed. In addition, when DeOme et al. (3) studied BALB/cfC3H (MTV + ) parous mice, the recovery of outgrowths containing transformed populations was enhanced. These CD-derived transformed outgrowths have been characterized as lobuloalveolar (nodule-transformed) and tumor (tumor-transformed) or mixtures of these outgrowth types. The outgrowths have been shown to have the morphological and biological properties of HAN and mammary adenocarcinomas. Medina et al. (6) have also demonstrated that, if hyperplastic nodule outgrowths were dissociated and injected in a similar manner, the tumorigenic potentials were enhanced over that produced when pieces of the same tissues were transplanted.

The primary aim of this study was to determine whether mammary dysplasias could be recovered by cell dissociation and injection of mammary cells from C57BL (MTV − ) mice treated with chemical carcinogens prior to development of tumors.

MATERIALS AND METHODS

Animals. The C57BL/Crgl mice were obtained from the inbred mouse colony maintained at the Cancer Research Laboratory, University of California, Berkeley, California. They were housed in plastic cages on wood shavings in temperature-controlled rooms with a fixed light cycle. Food pellets (Wayne Lab-Blox F-G., Allied Mills, Inc., Chicago, Ill.) and water were available ad libitum.

Carcinogen. The treated mice were fed DMBA i.g. once a week for 2 consecutive weeks. The carcinogen was dissolved in sesame seed oil, and 0.1 ml was fed in 1.0-mg doses.

Experimental Protocol. Four-week-old mice were treated in the following manner: Group A, untreated controls; Group B, 2 pituitary isografts under the kidney capsules of each mouse at 4 weeks of age; Group C, DMBA given i.g. at 5 and 6 weeks of age; Group D, 2 pituitary isografts under the kidney capsule at 4 weeks of age and DMBA i.g. given at 5 and 6 weeks of age (Table 1). At 10, 14, 18, and 22 weeks of age, 3 mice from each group were sacrificed, and all their mammary glands were used as donors. After 10 to 12 weeks, the host mice were anesthetized with Nembutal, the inguinal and thoracic mammary glands were exposed by means of midventral incisions, and the mammary gland outgrowths in the inguinal fat pads were identified and tentatively classified by examination at x 6.3 with a dissecting microscope. Pieces of outgrowth tissues were removed and transplanted into the gland-free fat pads of syngeneic 3-week-old female hosts. Donor mice having grossly visible mammary tumors were not used as donors. After 10 to 12 weeks, the host mice were given i.p. injections of 0.75 ml of 0.5% trypan blue in 0.85% NaCl solution. Twenty-four hr later, the host mice were anesthetized with Nembutal, the inguinal and thoracic mammary glands were exposed by means of midventral incisions, and the mammary gland outgrowths in the inguinal fat pads were identified and tentatively classified by examination at x 6.3 with a dissecting microscope. Pieces of outgrowth tissues were removed and transplanted into the gland-free fat pads of syngeneic 3-week-old female hosts when unusual dysplasias were encountered. The remaining inguinal fat pads and pieces of the host thoracic mammary glands were removed, spread in Tissue-Tek capsules, fixed in Tellyesniczky's fixative, defatted in acetone, stained in iron hematoxylin, and examined in methyl salicylate by means of a dissecting microscope. Samples of selected outgrowths from the fixed and stained whole mounts were cut out, sectioned at 7 μm, and stained with hematoxylin and eosin for histological examination.

1 This investigation was supported by Grants CAO5388 and CAO9041 awarded by the National Cancer Institute, Department of Health, Education and Welfare.

2 To whom requests for reprints should be addressed.

3 The abbreviations used are: MTV + , mammary tumor virus expressed; HAN, hyperplastic alveolar nodules; CD, cell dissociation; MTV − , mammary tumor virus unexpressed; DMBA, 7,12-dimethylbenz(a)anthracene; i.g., intra-gastrically.

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ductal outgrowths that were morphologically distinct from the normal ductal pattern of the host's own glands were classified to the scheme used by DeOme et al. (2) with the addition that the injection of dissociated cells were classified according to the host mice, pieces of outgrowth tissues were retransplanted into the gland-free fat pads of syngeneic female hosts. Numerious samples of the 4 types of outgrowths found were transplanted: normal ducts, ductal dysplasias, lobuloalveolar structures, and tumors. These transplantation studies were done to verify classification of unusual outgrowths, to determine neoplastic potential of the outgrowths, and to establish serial transplant lines of the outgrowths containing both ductal and lobuloalveolar structures.

Classification of Outgrowths. The outgrowths found following the injection of dissociated cells were classified according to the scheme used by DeOme et al. (2) with the addition that ductal outgrowths that were morphologically distinct from the normal ductal pattern of the host's own glands were classified as ductal dysplasias. These dysplasias were observed as differences in branching patterns, distances between branches, cysts, or increases in apparent cellularity.

Transplantation of Outgrowths. At the time of sacrifice of the host mice, pieces of outgrowth tissues were retransplanted into the gland-free fat pads of syngeneic female hosts. Numerous samples of the 4 types of outgrowths found were transplanted: normal ducts, ductal dysplasias, lobuloalveolar structures, and tumors. These transplantation studies were done to verify classification of unusual outgrowths, to determine neoplastic potential of the outgrowths, and to establish serial transplant lines of carcinoma-induced abnormal outgrowths.

RESULTS

Incidence of CD-derived Lobuloalveolar (HAN) and Ductal Dysplastic Outgrowths in Untreated Mice and Untreated Mice Bearing Pituitary Isografts. At all 4 time points examined, the mammary glands of untreated virgins (Group A) and untreated virgins bearing pituitary isografts (Group B) produced only ductal outgrowths following dissociation and injection (Table 1). These CD-derived outgrowths were similar in morphology to the virgin hosts' own mammary glands (Fig. 1).

Incidence of CD-derived Lobuloalveolar (HAN) and Ductal Dysplastic Outgrowths in DMBA-treated mice. The data in Table 1 demonstrate that nodular and/or ductal dysplastic outgrowths were recovered from DMBA-treated mice with or without pituitary isografts (Figs. 2 and 3). The incidence of recovered nodule outgrowths was variable for the time points assayed with no apparent relationship to donor age after treatment. However, both the number of donor groups yielding nodule outgrowths and the incidence of these outgrowths were greater in mice bearing pituitary isografts than in mice without pituitary isografts. Outgrowths with ductal dysplasias were recovered from all treated donor groups. The incidence of outgrowths with ductal dysplasias was greater when treated donors had pituitary isografts, and the incidence increased with donor age.

Table 2 presents the types of outgrowths recovered when pieces of 4 types of CD-derived outgrowths were transplanted into gland-free fat pads. In general, the transplantation of CD-derived outgrowths indicated that the abnormal morphological types were maintained. Selected outgrowths were serially transplanted, resulting in outgrowth lines. Five such lines were established. Three lines were established from mice treated with 1.0 mg DMBA. One line is lobuloalveolar, has a high tumor potential (50% at 15 weeks), and is in the fifth transplant generation. The other 2 lines are ductal dysplastic lines and are in the fourth and fifth transplant generation, respectively. Two other ductal dysplastic lines were derived from a similar experiment in which the mice were treated with 10 mg DMBA, twice at 1-week intervals. These 2 lines have produced tumors within a period of 8 to 11 months.

DISCUSSION

The data reported herein demonstrate that transformed mammary gland cells capable of producing alveolar and ductal dysplasias can be recovered from DMBA-treated C57BL (MVT−) mice by means of the CD assay. These data also indicate that the procedure of CD did not induce nodule-transformed or ductal dysplastic-transformed cells since donors that were not treated with DMBA did not produce either lobuloalveolar or ductal dysplasias. Pituitary isografting also had no effect on the occurrence of CD-derived dysplastic outgrowths from mice not treated with DMBA since the outgrowths from mice with or without pituitary isografts had only ductal morphologies resembling those of the virgin hosts. In this study, as in many other reported studies (11), pituitary isografts have been shown to be an effective means of enhancing the mammary transformation process. We do not yet know whether the high levels of prolactin provided by pituitary isografts are important in the initiation of dysplastic or tumor-transformed cells, in the emergence of overt lesions, or on both of these events.

The mammary glands of BALB/cfC3H (MVT+) mice contain nodule-transformed cells which produce lobuloalveolar out-

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Table 1

<table>
<thead>
<tr>
<th>Donors</th>
<th>Outgrowths</th>
<th>No. of transplant/</th>
<th>DuD (%)</th>
<th>HAN (%)</th>
<th>DuT (%)</th>
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<td>10</td>
<td>44/43</td>
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<tr>
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<td>14</td>
<td>48/45</td>
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<tr>
<td>A None</td>
<td>22</td>
<td>42/43</td>
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<td></td>
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<tr>
<td>B Pit. + DMBA</td>
<td>10</td>
<td>46/45</td>
<td>100</td>
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<tr>
<td>B Pit.</td>
<td>14</td>
<td>48/47</td>
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</tr>
<tr>
<td>B Pit.</td>
<td>18</td>
<td>44/42</td>
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<tr>
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<td>22</td>
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<tr>
<td>C DMBA</td>
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<td>46/46</td>
<td>96</td>
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<tr>
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<td>38/33</td>
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<td>42/41</td>
<td>48</td>
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<td>D Pit. + DMBA</td>
<td>22</td>
<td>44/33</td>
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Table 2

<table>
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<tr>
<th>Donors</th>
<th>No. of transplant</th>
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<td>Du</td>
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<tr>
<td>Du*</td>
<td>5</td>
<td>34</td>
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<td>LA</td>
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<tr>
<td>LATu</td>
<td>1</td>
<td>8</td>
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See "Materials and Methods."
growths when assayed by the CD method (2, 3). Evidence that
dysplastic lesions induced by DMBA in C57BL mice contain
transformed cells was provided by the behavior of the 4 serially
transplanted outgrowth lines. Each line maintained its particular
type of morphology during 5 to 6 transplant generations. In
addition, each line appears to have an infinite life span. Trans-
formed mammary gland cells possess the ability to grow indef-
inently as expressed by their ability to fill the host fat pads in
each transplant generation. In contrast, normal cells have a
finite life span and fill smaller percentages of the host fat pads
in each transplant generation until growth ceases at the fifth to
seventh generation (2, 3).

Histopathological studies of human breast samples by sev-
eral investigators (10) have suggested that the site of origin
of human mammary carcinoma may be in lobules and terminal
ductules. It has been reported that, in rats treated with chemical
carcinogens, mammary carcinomas may arise in terminal mam-
mary ducts (4, 8). In GR (MTV +) mice, ductal dysplasias have
been implicated as a site of the development of both preg-
nancy-dependent (plaques) and pregnancy-independent mam-
mary tumors (1). BALB/c (MTV −) mice treated neonatally with
estrogens exhibit an increase in type and incidence of ductal
dysplasias (9). It has also been demonstrated that DMBA-
treated mice develop ductal hyperplasias and that these hy-
perplasias give rise to mammary carcinomas in situ or when
transplanted to the gland-free mammary fat pads of syngeneic
mice (5, 7).

Both the lobuloalveolar outgrowth line and 2 ductal dysplasia
lines have been demonstrated to be tumorigenic. The ability to
readily recover these different types of lesions by the use of
the CD technique provides a useful method for studying the
incidence, morphology, growth behavior, and response to
agents which promote or induce mammary tumorigenesis, such
as hormones, chemical carcinogens, or viruses.

ACKNOWLEDGMENTS

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pathology of the human breast with special reference to possible precanc-
Fig. 1. Whole-mount preparation of a CD-derived outgrowth from a mouse bearing pituitary isografts (Group B). This outgrowth was classified as normal ductal and resembles the untreated glands of the host. × 7.

Fig. 2. A CD-derived outgrowth from a mouse bearing pituitary isografts and treated with DMBA. The outgrowth has areas of both HAN outgrowth (arrow) and ductal dysplasia with many lateral buds. × 7.

Fig. 3. A CD-derived outgrowth from a mouse bearing pituitary isografts and treated with DMBA. There are numerous end bud-like structures (arrow) in an outgrowth which has already filled the host fat pad. The end bud-like structures often contain localized epithelial hyperplasias. Cysts are often present at the terminal ends of ducts. × 16.
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