Epidermal Changes following Application of 7,12-Dimethylbenz(a)anthracene and 12-O-Tetradecanoylphorbol-13-acetate to Human Skin Transplanted to Nude Mice Studied with Histological Species Markers

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ABSTRACT

Effects of the tumor initiator 7,12-dimethylbenz(a)anthracene (DMBA) and of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) on epidermis of human fetal and adult skin were studied in the nude mouse/human skin model. Human skin grafts on NC nude mice were exposed to two topical applications of 1 mg of DMBA in 50 μl of acetone with an interval of 3 days and/or to applications of 10 μg of TPA in 50 μl of acetone twice weekly. In some animals, it was attempted to augment the susceptibility of the grafts to the tumor-initiating effect of DMBA by pretreatment with TPA or ultraviolet light. The mice were sacrificed 8–32 wk after the initial treatment. Tumors did not appear in the central portions of any of the grafts, but epidermal tumors were seen at the graft border in 34.9% of the DMBA-treated animals. To identify human epidermis on the grafts and to determine the species origin of the induced tumors, two independently working histological marker methods were applied. (a) The first is detection of a human Blood Group B-like antigen present in mouse epidermis and in chemically induced murine epidermal tumors. This antigen cannot be demonstrated in human epidermis and in epidermal tumors of human patients. (b) The second is histological staining with the DNA-specific fluorochrome, bisbenzimide, displaying a characteristic pattern of 5–10 intranuclear fluorescent bodies in murine nonneoplastic epidermal cells and in murine epidermal tumor cells. Such a pattern is not seen in human epidermis and in epidermal tumors of human patients. The studies showed that TPA treatment resulted in epidermal hyperplasia in both the human epidermis and the adjacent mouse epidermis and that the induced tumors were derived from murine tissue. The mechanisms behind the DMBA action in the nude mouse/human skin model are discussed, and suggestions for future carcinogenesis studies on the model are given.

INTRODUCTION

In laboratory animals, administration of the polycyclic aromatic hydrocarbon DMBA2 to the skin is an extensively studied model of cutaneous tumorigenesis. DMBA is a potent initiator of this process, which may be facilitated by subsequent topical application of the diester of the diterpene alcohol phorbol, TPA (1–4). Such studies have given important and extensive insights into the mechanisms of carcinogenesis, but their significance for human skin cancer is unknown. When human skin is transplanted to nude mice, its main histological features are preserved (5), and the human epidermis can be identified until several months after the transplantation by means of cell markers (6–8). The purpose of the present investigation was to study the effects of DMBA and TPA on epidermis of human skin transplanted to nude mice. Fetal and adult human skin grafts were used, and in some cases it was attempted to modify a possible carcinogenesis response by exposing grafts to TPA or UV light before DMBA treatment. The model necessitated techniques to distinguish between murine and human epidermal tumors and between murine and human nonneoplastic epidermis. The quantity of tissue was rather small, and chromosomal species determinations were unfeasible, if the tissue additionally should be examined histologically. Therefore, two independent histological species marker methods were applied. One method is based upon a cell membrane marker, which is known to be present on murine epidermal basal cells (9) but is not demonstrable on human epidermal basal cells (10). This marker is a human Blood Group B-like antigen, which can be demonstrated by its ability to bind a human Blood Group B test serum or the lectin, GSA I (7–9). The other method, recently introduced for species identification in studies of developmental phenomena in chimeric organs (11), utilizes the different heterochromatin patterns in murine and human cells stained with the fluorochrome, bisbenzimide.

MATERIALS AND METHODS

Grants and Mice. Split skin measuring 0.5 mm in thickness was taken 6–12 h after death from 5 human corpses. From the corpses of 4 adults (3 men and 1 woman, 61–83 yr), skin was taken from the anterior aspects of the thighs, while skin from the thighs as well as the back was used from a male fetus (830 g, 27th gestational wk). The donors were Caucasians, and the skin was normal by microscopy. The skin was transplanted in 2- × 2-cm pieces to the midback region of 5– to 11-wk-old male nude mice, representing the first and the second cycle of a transfer of the nu gene to the NC mouse strain. The grafting technique and the housing of the animals in separate cages and in laminar flow racks have been described previously (5).

Chemicals and UV Light. DMBA and TPA were purchased from Sigma, St. Louis, MO; stored at −20°C; and dissolved in acetone immediately prior to use. Ultraviolet radiation was generated by a Hanovia Model 30620 lamp with a 616 A-13 100-W U-shaped quartz-mercury tube (Hanovia Lamp Division, Conrad Precision Industries, Inc., Newark, NJ). A beam-limiting cone with an opening diameter of 8 mm was mounted in front of the lamp. The intensity in front of the cone was 5.0 mJ/cm²/s in the UV-B spectrum. Acute effects of the irradiation generated by this lamp on human skin transplanted to nude mice have recently been described (12).

Experimental Design. The study comprised 10 groups of mice...
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(Groups 1–10) with nonulcerated human skin grafts. The different treatments listed in Table 1 were started 4–11 wk after the transplantation. All of the 5 donors were represented in the groups treated with DMBA and/or TPA. The mice of Groups 1 and 2 carried grafts from the fetus. Group 5 carried skin from a 61-yr-old woman, and the grafts of Groups 7 and 8 were from a 83-yr-old man. Each of the remaining groups includes grafts from all of the 4 adult donors.

DMBA was given in doses of 1 mg and TPA in doses of 10 μg. Each dose was contained in 50 μl of acetone. The chemicals were administered topically by slowly pipetting the acetone solutions on the grafts. The acetone dried a few seconds after the application. Care was taken to avoid application to the adjacent mouse skin. Transplants were irradiated with UV-B by gently pressing the grafts towards the lamp with the beam focused on the center of the grafts applicating a single dose of 0.1 J/cm².

Groups 1, 3, 4, 5, and 7 were treated with DMBA twice with an interval of 3 days. In Group 5, this was preceded by 2 weekly applications of TPA during 5 wk. In Group 7, UV-B irradiation was given 7 days before the first DMBA application. Two weekly applications of TPA were given to Groups 1, 4, 5, and 7 after the DMBA treatment. Group 8 was only treated twice a week with TPA, and Group 8 was only given a single dose of UV-B. Groups 2, 9, and 10 were controls.

A minimum 8-wk observation period was used.

 Autoradiography. From each of the 5 grafts of Group 8, a 5-mm-punch biopsy was taken from the central field 7 days after the UV-B irradiation. Similar control biopsies were taken from the grafts of 5 animals of the untreated Group 10. The grafts of these 5 controls originated from the same donor as the grafts of Group 8. One h prior to biopsy, these mice had been given an i.p. injection of [3H]thymidine (Amersham International, Amersham, United Kingdom) in 0.5 ml of sterile water (100 μCi; specific activity, 22 Ci/mm). The biopsies were prepared for histology and autoradiography, and the epidermal labeling index was determined as the number of labeled cells per mm of interfollicular epidermal surface. Details of the autoradiography procedure have been described in a previous paper.

 Detection of Human Blood Group B-Like Antigen. Expression of this antigen was demonstrated by staining with a HRP-conjugated lectin GSA I, which is a Blood Group B antigen reagent (13) and by double layer immunofluorescence.

 For lectin staining, histological sections were deparaffinized, hydrated, and washed 3 times for a total of 5 min. In PBS, whereupon they were incubated at 20°C for 20 min with HRP-conjugated GSA I (E-Y Laboratories, San Mateo, CA) at a concentration of 1 mg/ml. After rinsing 3 times in PBS for another 5 min, the sections were incubated with 3- amino-9-ethylcarbazol (0.4 mg/ml) and hydrogen peroxide (0.015%) at 20°C for 5 min. After a rinse in tap water for 10 min, a nuclear counterstain with hematoxylin was performed. The sections were mounted in Aquamount (BDH Chemicals, Ltd., Poole, England). Controls included: (a) lectin replaced with PBS; and (b) 0.15 m lactose or 0.15 m glucose added to the lectin solution 1 h prior to the tissue incubation to test sugar-specific inhibition.

In the HRP-GSA I-stained sections, the number of layers of nucleated epidermal cells was determined centrally in the grafts and in the adjacent epidermis 2 mm from the graft border.

In the immunofluorescence technique, the primary antibody was a human blood test serum against blood group antigen B provided by Statens Seruminstitut, Copenhagen, Denmark. The applied serum was chosen from a panel of test sera because of its ability to react strongly with Blood Group B substance in human and murine tissue in an immunofluorescence technique. Rehydrated paraffin sections were incubated with this serum diluted 1:5 in PBS at 4°C overnight, washed 3 times in PBS (pH 7.2), and incubated with FITC-conjugated rabbit antiserum to human IgG (DAKO; Glostrup, Denmark) diluted 1:20 in PBS at 20°C for 40 min. After being washed 3 times in PBS, the slides were mounted in PBS:glycerol (1:10; pH 8.0) to which p-phenylenediamine (10 mg/ml) had been added to reduce fading (14). For control, the primary antiserum was substituted by (a) normal serum, (b) anti-B serum absorbed with Blood Group B erythrocytes, or (c) PBS.

Bisbenzimide Stain. Rehydrated paraffin sections were stained for 1 min with bisbenzimide (Hoechst 33342 dye; Sigma) at 20°C, as described by others (11). The staining solution consisted of 4 μg of bisbenzimide per ml in Hanks’ balanced salt solution without phenol red. After rinsing for 1 min in running tap water, the slides were mounted with Mcllvaine’s buffer (pH 5.5). Like the immunofluorescence specimens, the bisbenzimide-stained slides were viewed and photographed on a microscope with epifluorescence and with filters appropriate for FITC.

Control Tissue. The species marker methods were applied to the following tissues of known species origin. (a) The first of these was mouse epidermal tumors. Among the above-mentioned 63 DMBA-treated mice, 14 mice developed 23 epidermal tumors outside the graft area (16 papillomas, 5 squamous cell carcinomas, and 2 keratoacanthomas). Other mouse skin tumors were obtained from 10 ungrafted NC nude mice. With an interval of 3 days, 2 doses of 1 mg of DMBA in 50 μl of acetone had been applied to the dorsal skin 8–12 wk prior to sacrifice. In 5 mice, the DMBA applications were followed by 2 weekly applications of 10 μg of TPA in 50 μl of acetone. All of these mice developed skin tumors (14 papillomas and 6 squamous cell carcinomas). After the animals had been sacrificed, the mouse tumors were removed and prepared for histology. (b) The second consisted of human epidermal tumors. Paraffin blocks of formalin-fixed human skin tumor samples were selected by means of hematoxylin: eosin-stained sections from the routine files of Pathological-Anatomical Institute, Kommunehospitalet, Copenhagen, Denmark. Each of the following 5 diagnostic categories was represented by 10 specimens: serborrhoic keratosis; verruca vulgaris; keratoacanthoma; squamous cell carcinoma; and basal cell carcinoma. (c) The third tissue group consisted of normal mouse skin, normal human skin, and normal human buccal mucosa of a secretor belonging to Blood Group B.

Statistical Method. Mann-Whitney’s rank sum test was used by the comparisons of the epidermal labeling indices and the numbers of nucleated epidermal cell layers.

RESULTS

Mice

The average observation length of the 103 grafted mice was 18.0 wk (range, 8–32 wk). There were no significant differences between the observation lengths in the 10 groups. By autopsy, it was found that 20.3% of the mice had normal internal organs, while the remaining had interstitial pneumonia (64.9%), hepatitis (24.3%), myocarditis (5.4%), and pyelonephritis (3.0%). The frequencies of these postmortem findings were not related to the specific treatments. Tumors in the internal organs, including the inguinal and axillary lymph nodes, were not observed. In 14 of the 63 mice treated with DMBA (22.2%), tumors were seen on the skin outside the graft area, e.g., head and legs. There were 16 papillomas, 5 squamous cell carcinomas, and 2 keratoacanthomas.
Macroscopic Examinations of the Grafts

Neither tumors nor ulcers were seen in the central zone of the grafts, but in 22 of the 63 mice treated with DMBA (34.9%), tumors arose at the well-defined graft margins (Fig. 1; Table 1). In 20 animals, the tumors emerged within 8 wk after the initial DMBA treatment. Nineteen animals had one tumor, while 2 tumors were seen in 3 animals. At the end of the experiments, the maximal size of the tumors was 5 mm. Untreated mice or mice treated with acetone, TPA, or UV-B alone did not develop tumors.

Microscopic Examinations of the Grafts

Nonneoplastic Epidermal Changes. The mouse skin muscle was lacking beneath the grafts, and the graft dermis was fibrotic and had few adnexal structures contrary to the adjacent mouse dermis. Two different nonneoplastic epithelia were seen at the margins. The cells of the (supposed human) epidermis covering the graft dermis expressed no Blood Group B-like antigen (Figs. 2 and 3), and their nuclei showed a uniform fluorescence in the bisbenzimide-stained sections (Fig. 4A). Contrary to this, the adjacent (supposed murine) epidermis expressed Blood Group B-like antigen at the plasma membranes of the cells of the basal and lower spinous layers (Figs. 2 and 3), and all of the nuclei of the cells of this epithelium contained 5–10 distinct brightly fluorescent bodies (chromocenters) (Fig. 4B). Premalignant lesions (dysplasia, carcinoma in situ) were not seen in the supposed human epidermis, but in some groups epidermal hyperplasia was found. The numbers of layers of nucleated epithelial cells in the 10 groups were determined in the HRP:GSA l-stained sections (Table 2). By comparing the groups transplanted with adult skin, it was found that the treatment with TPA significantly increased the number of layers in the supposed graft epidermis (P < 0.01, Group 6 versus Group 9). Previous exposure to DMBA or UV-B did not significantly change the result (P > 0.1, Groups 4 and 7 versus Group 6). DMBA or acetone given alone did not alter the number of cell layers in these experiments (P > 0.1, Group 3 and Group 9 versus Group 10). Also the fetal transplants showed epidermal hyperplasia after application of DMBA and TPA (P < 0.02, Group 1 versus Group 2). The grafts treated with TPA showed, in addition, pronounced hyperkeratinization compared to controls. This was not quantitated due to methodological difficulties. In all cases treated with TPA, the HRP: GSA l-stained epidermis was significantly thickened 2 mm from the unstained graft epidermis (P < 0.01, Group 1 versus Group 2 and Groups 4–7 versus Groups 3 and 9–10). At a 5-mm distance, the number of nucleated cell layers was equal to that of normal mouse epidermis.

Tumors. Microscopy of the 63 DMBA-treated grafts confirmed that tumors were present in 22 cases. Eighteen tumors were papillomas and 7 were squamous cell carcinomas (Table 1). Mesenchymal tumors were not observed. In 19 cases, only a single tumor was observed, but in 3 cases both a papilloma and a squamous cell carcinoma were seen. The tumors were situated at the graft margins, and the adjacent nonneoplastic epithelia showed different staining reactions as described above. Weak HRP: GSA l binding could be detected only in small areas of 13 of the 25 tumors, but Blood Group B-like antigen could be demonstrated in all of the tumors by the immunofluorescence technique. In the papillomas, the cells of the basal and spinous layers of the surface epithelium showed a bright fluorescence at the plasma membranes (Fig. 3). In the squamous cell carcinomas, unevenly distributed fluorescence was seen at the plasma membrane of more than 50% of the cells. The fluorescence was most pronounced in areas of poor cytological differentiation, and keratinized cells did not fluoresce. It was invariably found that the antiserum against B antigen, in addition, reacted with the cell membranes of the basal and lower spinous layers of the adjacent epidermis at one side of the tumors. At the other side of the tumors, the epidermis did not fluoresce.

In the bisbenzimide-stained sections, the tumor cells contained 5–10 intranuclear chromocenters (Fig. 5A).

Autoradiography. The average epidermal labeling index of the UV-B-irradiated grafts was 3 times higher than in the control group (5.2 versus 1.8 counts/mm). The difference was statistically significant (P < 0.01).

Control Reactions

Human Blood Group B-like Antigen. The controls verified the specificity of the primary antibody. GSA l binding was inhibited by lactose but not by glucose. In the immunofluorescence tech-

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**Table 1**

<table>
<thead>
<tr>
<th>Grafts</th>
<th>Group</th>
<th>Pretreatment</th>
<th>Initiation</th>
<th>Promotion</th>
<th>Total no. of mice</th>
<th>Papillomas</th>
<th>Squamous cell carcinomas</th>
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<tbody>
<tr>
<td>Fetal skin</td>
<td>1</td>
<td>DMBA, 2 treatments</td>
<td>TPA, twice weekly</td>
<td>10</td>
<td>5</td>
<td>1</td>
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<td>2*</td>
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<tr>
<td>Adult skin</td>
<td>3</td>
<td>DMBA, 2 treatments</td>
<td>TPA, twice weekly</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>DMBA, 2 treatments</td>
<td>TPA, twice weekly</td>
<td>18</td>
<td>7</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>TPA, twice weekly, for 5 wk</td>
<td>DMBA, 2 treatments</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td></td>
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<tr>
<td></td>
<td>6</td>
<td>UV-B (0.1 J/cm²) 1 wk before initiation</td>
<td>DMBA, 2 treatments</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>UV-B (0.1 J/cm²)</td>
<td>TPA, twice weekly</td>
<td>16</td>
<td>3</td>
<td>2</td>
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* Controls (acetone, twice weekly).

* Controls (no treatment).
Conventional histological staining did not allow species identification, and the marginal graft areas could result in such zonation. The absence of tumors from the central graft areas does not exclude human derivation, as possible different nutritive conditions. Absence of tumors from the central graft areas does not indicate the origin was identical with the expression in the tumors of the graft epidermis was unstained. In the human buccal mucosa from a normal human epidermis, from epidermis of grafts without demonstrable secretions, the tumors of the graft margins showed a nuclear staining pattern closely similar to the pattern in normal mouse epidermis and in tumors of known murine derivation. By presenting several intranuclear chromocenters, they differed clearly from normal epidermal tumors, the nuclei showed uniform or slightly irregular fluorescence of the basal and lower spinous layers. The bright fluorescence of the basal poorly differentiated cells was seen. In all cases, epidermis adjacent to the murine tumors showed fluorescence of the basal and lower spinous layers. Normal human epidermis was unstained. In the human buccal mucosa from a secretor belonging to Blood Group B, the cell membranes of the spinous layer of the epithelium were stained.

**Bisbenzimide Stain.** In bisbenzimide-stained sections of epidermal tumors and of normal epidermis of known murine derivation, the nuclei contained 5–10 chromocenters. In the epidermis of normal human skin, human buccal mucosa, and human epidermal tumors, the nuclei showed uniform or slightly irregular moderate fluorescence, but they were devoid of intensely fluorescent regions (Fig. 5A).

### DISCUSSION

The origin of the DMBA-induced tumors of the graft margins in the applied two-species model is the central issue of this study. Absence of tumors from the central graft areas does not exclude human derivation, as possible different nutritive conditions in the marginal graft areas could result in such zonation. Conventional histological staining did not allow species identification, but the results of the two-species marker examinations were clear, and for the following reasons we find it justified to regard the tumors at the graft margins as murine. (a) Like normal murine epidermis and like tumors of known murine origin, these tumors expressed Blood Group B-like antigen, which was neither detectable in normal human epidermis nor in epidermal tumors of known human derivation. (b) In the bisbenzimide-stained sections, the tumors of the graft margins showed a nuclear staining pattern closely similar to the pattern in normal mouse epidermis and in tumors of known murine derivation. By presenting several intranuclear chromocenters, they differed clearly from normal human epidermis, from epidermis of grafts without demonstrable Blood Group B-like antigen substance, and from tumors of known human origin. Analogously, the nonneoplastic epidermis covering the grafts must be regarded as human because of its lack of Blood Group B-like antigen and its lack of nuclear chromocenters.

Previous studies on non-trypsin-treated histological slides of formalin-fixed and paraffin-embedded material have shown that human Blood Group B-like antigen cannot be demonstrated in the epidermis of normal human skin, but it is expressed at the cell membrane in the basal and lower spinous epidermal layers of skin from mice of the NC and BALB/c strains (9, 10). These findings are confirmed by the present study, which additionally shows that chemically induced murine epidermal tumors express a B-like antigen at the plasma membrane of the cells constituting the presumed proliferative compartments. Thus, the cells of the basal spinous layers of papillomas and many carcinoma cells in areas of low differentiation showed membrane fluorescence. On the contrary, Blood Group B-like antigen was not detectable in epidermal tumors of human origin. This is in accordance with a previous study by us, showing that a Blood Group A and B antigen precursor (the H antigen), which is present in normal human epidermis, is lost in premalignant human epidermal lesions (15).

For future studies applying Blood Group B test sera for species identification purposes, it shall be stressed that careful selection of the sera is necessary, because some of these sera react only weakly in immunofluorescence staining methods (16). Furthermore, it is important in such studies to include positive control tissue from the involved species, as it cannot be excluded that the reactivity of such test sera does not parallel in tissues from different species.

In full accordance with previous examinations (7), the HRP:GSA I staining technique could be used for species identification of nonneoplastic epidermis. Because this technique is applied to histological slides prepared for conventional light microscopy, it is superior to fluorescence techniques for concomitant morphological studies, but in our hands, it is not sufficiently sensitive to allow species identification of tumors in the human skin/nude mouse model. Whether this is due to a modification of the binding sites of the lectin during the conjugation is unknown. In a previous histological study of formalin-fixed and paraffin-embedded normal human skin grafts on nude mice, we found that binding of another lectin, PNA, could be used for epidermal species identification, as PNA exclusively was bound to human epidermal cells (7). PNA staining was not used in the present study, because other studies have shown that PNA receptors are present in both human and murine epidermal tumors.

The experiments show that TPA induces hyperplasia in grafted adult human skin. This parallels the results of Yuspa (17) who found that TPA induces hyperplasia in neonatal foreskin transplanted to nude mice, and the results of Krueger and Shelly (18) who showed that human skin grafts on nude mice undergo a proliferative response after TPA application. Probably, TPA was also responsible for the hyperplasia in Group 1 of human fetal skin grafts treated with DMBA and TPA.

The formation of murine tumors at the margins of the DMBA-treated grafts may be due to three factors. (a) Mechanical...
transport of the carcinogen from grafts to mouse skin, e.g., by scratching is one of these. Presumably such transport was responsible for the tumors outside the graft area seen in 14 of the 63 DMBA-treated mice. (b) Others are indiscernible overflow of the graft margins of the carcinogen solution, which logically cannot be proved or disproved, and (c) lateral diffusion of the carcinogen through the grafts, which in this study only can be hypothesized.

Tumors did not appear in the grafted human skin within the 32-wk observation period. Fetal as well as adult skin was used as age-dependent different susceptibility to carcinogens might be expected (19–21). In some cases, DMBA treatment was preceded by exposure to TPA or UV-B. In previous animal studies, it has been shown that such treatments inducing cell proliferation may increase the susceptibility of tissue to an initiator (22–23). The resistance of the transplanted human skin to the carcinogenic effect of DMBA is hardly due to its state as a graft. Thus, in other experiments epidermal tumors arose in all of 10 skin grafts of BALB/c mice on NC nude mice 20 wk after application of 500 μg of DMBA. Absence of human tumors may be due to the relatively short observation period, but it cannot be excluded that the DMBA has not penetrated the human skin and reached the cells of the lower epidermis, from which epidermal tumors presumably are derived. It has recently been reported that the ability of chemicals to penetrate human skin and nude mouse skin may differ considerably (24).

Even though human tumors were not seen in the present study, the nude mouse/human skin model should not be given up for studies of human carcinogenesis. In future experiments, the grafts should be serially transplanted to allow longer observation periods, and methods for identification of carcinogens and carcinogen:DNA adducts should be applied. The model makes maintenance of relatively large quantities of skin possible, and this study has shown that simple histological techniques allow identification of the grafted epidermis and species determination of induced lesions.

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REFERENCES

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Fig. 1. NC nude mouse with human skin graft treated with DMBA and TPA. Ten wk after the initial DMBA application, two tumors have arisen at the graft border.

Fig. 2. Border of a graft of human adult skin treated with TPA twice a week for 14 wk. The graft epithelium (left) shows no affinity for GSA I. To the right, epidermis (murine) binding HRP-GSA I. The lectin stain allows identification of the two epithelia, which otherwise are much alike. Bar, 100 μm. HRP:GSA I and hematoxylin, × 160.
Fig. 3. DMBA-induced cutaneous papilloma at the margin of a human skin graft on a nude mouse. The papilloma (right) shows fluorescence in the basal and spinous layers. To the left, unstained graft epidermis. Bar, 100 μm. Stained with Blood Group B antigen test serum, × 160.

Fig. 4. A, epidermis of human skin on nude mouse. The nuclei show uniform fluorescence. B, adjacent mouse epidermis in which the nuclei contain 5–10 brightly fluorescent chromocenters. Bars, 50 μm. Bisbenzimide stain, × 250.

Fig. 5. A, cutaneous squamous cell carcinoma from a human patient. The nuclei show slightly irregular fluorescence. B, DMBA-induced cutaneous squamous cell carcinoma at the margin of a human skin graft on a nude mouse. The nuclei contain 5–10 chromocenters. Bars, 25 μm. Bisbenzimide stain, × 400.
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