Regression of Induced Keratoacanthomas in Anagen (Hair Growth Phase) 
Skin Grafts in Mice

Cees G. Ramselaar,¹ E. Joost Ruitenberg, and Wim Kruizinga

ABSTRACT

Transplants of experimental keratoacanthomas induced in skin grafts which were in the growth phase of the hair follicle cycle (anagen phase) were carried out in immunocompetent and immunoinactive recipients (‘nude’ mouse, nu/nu). No differences in gross graft observations were noticed. More than 80% of all keratoacanthomas disappeared postgrafting. This percentage was the same for both groups of recipients. These data are in keeping with a nonimmunological regression of experimental keratoacanthomas. A possible correlation with the hair follicle cycle is suggested.

INTRODUCTION

The hair follicle cycle has an influence on skin grafts and skin cancerogenesis. Grafts of skin in the growth phase are more disturbed than are grafts carried out with skin in the resting phase of the hair follicle cycle. (6, 28).

It is well documented that carcinogens, like polycyclic hydrocarbons applied to a skin area in the resting phase (telogen phase), induce a significantly higher incidence of skin papillomas than when applied on skin in the growth phase (anagen phase) of the hair follicle cycle (2, 3, 6, 16, 28).

In mice, the appearance of hair follows characteristic patterns. Throughout life the skin is subject to changes, periods of growth of the hair follicle that alternate with periods of inactivity. This wave of growth moves down along the back and flanks (1, 5, 9, 15, 17, 24).

Irritation of telogen skin, like plucking or painting with a brush, results in the induction of a new hair follicle cycle; as a consequence, telogen skin turns into anagen skin (5).

Ghadially (13, 14) introduced the experimental keratoacanthoma, a benign tumor which originates from hair follicle epithelium induced by epicutaneous application of carcinogens. Several forms can be distinguished morphologically, depending on whether the hair follicle is in the anagen or telogen phase when proliferation starts. These tumors often regress spontaneously. Whether this regression is based on an immune response or has its origin in the hair follicle cycle is still a matter of discussion (3, 14, 19, 20, 25, 26).

In this study, the fate of this tumor was followed in grafts to determine whether their growth would be disturbed in anagen skin grafts or whether they are growing as a separate entity independently of the hair follicle cycle. Moreover, the influence of a possibly existing immune response was studied. For these purposes, skin grafts were carried out with keratoacanthomas induced in anagen skin and grafted either on recipients of the same inbred strain or on congenitally athymic (nude) mice (10), lacking functional T-cells.

MATERIALS AND METHODS

Donors

Sixty inbred male BALB/c mice were used. They were obtained from the Central Laboratory for the Breeding of Laboratory Animals, Zeist, The Netherlands. The animals were kept in 4 cages containing 15 mice each, fed with Muracon (Trout & Co., Putten, The Netherlands), and water ad libitum.

After the age of 11 weeks, both flanks of 45 mice were shaved weekly with electric clippers and painted with a 1% solution of 7,12-dimethylbenz(a)anthracene in lanolin with 3% soft paraffin. Fifteen mice were kept to serve as a control group. They were shaved and painted only with the non-carcinogen-containing vehicle. After about 3 weeks, the painted skin area was, macroscopically, in the anagen growth phase. Within 8 to 12 weeks, nearly all carcinogen-treated mice had tumors. Painting was discontinued when a mouse showed the first tumor. The tumors produced were papillomas, type I keratoacanthoma, type III keratoacanthoma, and possibly carcinomas, according to the classification of Ghadially (13). Keratoacanthomas for grafting were selected by macroscopic criteria.

Thirty-two type I keratoacanthomas and 8 type III keratoacanthomas were used for further graft procedures (Figs. 1 to 4). Most animals carried several tumors. From each animal, only one tumor was selected. The tumors were at least 3 weeks old and had a minimum diameter of 4 mm.

Recipients

First Graft. Twenty male BALB/c nu/nu mice, 6 weeks old, obtained from Dr. Friis, Gammel Bomholtgard Ltd., Aarhus, Denmark, and kept in laminar-flow cabinets, were used as recipients for grafting. Although these mice lack functional T-cells, they were treated s.c. at Days -3, 0 (day of grafts), 2, 4, and 7 with 0.5 ml anti-Ø serum each. The anti-Ø serum was prepared by Dr. J. G. Kreetenfberg, National Institute of Public Health, Bilthoven, The Netherlands, according to a published procedure (27). In addition, 20 male BALB/c mice, 6 weeks old, of the same inbred strain as the donor mice, served as recipients.

Second Graft. Tumors formed in the first graft were transplanted on new recipients. Male BALB/c nu/nu mice as well as immunocompetent male BALB/c mice were used. The same type of recipients served also for further transplants with tumors formed in the second graft.

Graft Procedures

The grafting procedures essentially followed the method described by Billingham et al. (3). Donor mice were killed

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Instantly by cervical dislocation. The tumor material was excised with surrounding skin, about 15 mm in diameter. The material was reversed and, as far as possible, all underlying fat, muscle, and fascia were scraped off with a scalpel. The prepared grafts were kept until use in Petri dishes on filter paper moistened with 0.9% NaCl solution.

The backs of recipient mice were shaved with electric clippers. Full-thickness graft beds, about 15 mm in diameter, were prepared under halothane anesthesia. The prepared grafts were carefully placed in the graft beds and fixed at the border with Ethicon Bucrylat (Ethicon GmbH, 2000 Norderstedt, Federal Republic of Germany). The grafts were covered with a square of Vaseline gauze, upon which fine tulle gauze was placed and secured with tape (Scotch 471 plastic film tape; 3M Company), placed as a circular bandage around back and abdomen. On Day 7 postgrafting, the tapes were removed. Grafts were observed every other day during the first week and twice a week thereafter.

**Histology**

Tumors from grafts and tissue specimens, including regional lymph nodes, lung, liver, and spleen, of autopsied animals were fixed in 10% buffered neutral formalin. Tissues were dehydrated and embedded in Paraplast according to standard procedures. Sections (4 µm thick) were stained with hematoxylin, eosin, and Weigert-van Gieson.

**RESULTS**

The gross graft observations were the same for both groups of recipients. On Day 10 postgrafting, a cast was formed. Sometimes the cast detached when the bandages were removed. The cast consisted of a thick layer of dead keratinized epithelial cells from tumor and surrounding skin. In general, the cast dropped off in the third week, exposing a skin surface which was firm and white. After 3 weeks postgrafting, new hairs reappeared in the graft. They were sparsely distributed over the graft surface in varying density. Tumor formation started in both groups at about 4 to 5 weeks after grafting. Table 1 gives the combined data for all tumors in both groups of recipients. Of a total of 40 keratoacanthomas, 32 tumors disappeared in the graft, while 8 tumors reappeared at about 4 to 5 weeks after grafting.

The grafts in the BALB/c recipients were followed for a period of 4 months. This was not possible for the nude recipients, since they died within 2 or 3 months after the graft procedures. The 4 keratoacanthomas formed in the grafts in the BALB/c recipients were removed with surrounding skin for a second transplant. In the second grafts, tumor growth was not observed in the 3 type I keratoacanthomas, while the type III keratoacanthomas resulted in tumor growth within 4 weeks. For further grafts, fragments of this tumor were transplanted in both BALB/c and nude BALB/c recipients. The tumor could be kept easily in serial transplants. The microscopic picture of this tumor (Fig. 5) revealed a well-circumscribed epithelial cell mass without signs of local invasion. At the periphery of the tumor, the epithelial cells were packed together, while more centrally the epithelial cells lay in smaller nests, forming anastomosing strands. Especially at the periphery of the tumor, many epithelial cells showed an atypical appearance with many mitoses and hyperchromatic nuclei (Fig. 6). Toward the center, there was some degree of maturation; and in the stroma between the epithelial cells, a rather dense infiltrate was seen, mainly composed of mononuclear cells. In 3 recipients, where the tumors reached a considerable size, nearly comprising the entire backs of the animals between hip and shoulder, no signs of metastasis could be found at autopsy in draining lymph nodes, lungs, liver, and spleen.

In the nude BALB/c recipients, tumor formation in a second transplant was seen only with one type III keratoacanthoma. This tumor could not be kept in serial transplants, because the recipient died and was too autolytic to use for further experiments.

**DISCUSSION**

When discussing the results described in this paper, it is important to pay attention to the behavior of the hair follicle in transplants. Previously, several authors reported an increasing disturbance of the graft and graft survival time in allografts with skin in a proceeding anagen phase of the hair follicle cycle (8, 28). In the anagen phase, a complete breakdown of follicle epithelium with related structures is seen. Reconstruction is completed by remnants of hair follicle cells which also reconstitute the original epidermis. This reconstitution takes place at the expense of the hair follicles themselves, seen morphologically as an irregular, mostly sparse, growth of new hair in the graft.

Experimentally induced keratoacanthomas originate from hair follicle epithelium. Their rapid onset and regression closely resemble the physiology of the hair follicle cycle, i.e., a rapid growth in the anagen phase and regression in the telogen phase of the hair follicle cycle (14). In grafts, the behavior of keratoacanthomas seems to have much in common with the hair follicle. The tumor is destroyed just like the hair follicle

## Table 1

<table>
<thead>
<tr>
<th>Recipients</th>
<th>No. of donor grafts from BALB/c</th>
<th>Keratoacanthomas in donor graft</th>
<th>Tumor formation in recipient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Type I</td>
<td>Type III</td>
</tr>
<tr>
<td>BALB/c</td>
<td>20</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>BALB/c nu/nu</td>
<td>20</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>32</td>
<td>8</td>
</tr>
</tbody>
</table>

- Tumors formed in first graft were transplanted on new recipients (in both immunocompetent and athymic recipients).
- Tumors formed in second graft were transplanted on new recipients.
- Number of tumors/total number of donor grafts.

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when skin is grafted in the anagen phase of the hair follicle cycle. What remains is grafts of transplantation is a graft with the same gross macroscopic appearance as in anagen skin grafts, without any sign of the original tumor. In our series, more than 80% of all grafted keratoacanthomas disappeared in the graft. The grafting procedure does not seem to be responsible for this phenomenon. All grafts were performed by the same person, following the same technique, for all tumors. Moreover, one tumor, grafted according to the same procedure, could be kept easily in serial transplants in immunocompetent and athymic BALB/c recipients. It might perhaps be concluded that this tumor was not a keratoacanthoma but a true carcinoma. The histological features support this view.

It is well known that the experimental type III keratoacanthomas and the skin carcinomas deriving from hair follicle epithelium are sometimes difficult to differentiate on macroscopic criteria (13). The same holds true for keratoacanthoma in man. Metastasis is a definite criterion for cancer. In our study, no metastases were found either in BALB/c or in nu/nu recipients. However, the fact that no metastases were observed does not mean that a tumor is nonmalignant, because it is known that metastases from mouse squamous cell carcinoma are relatively uncommon, even with expandingly growing tumors (13). Also, in nu/nu mice, implantation of malignant tumors or tumor cell suspensions of several kinds is seldom accompanied by metastases (16, 29, 31).

There was no difference in gross graft observations in both groups of recipients. The number of keratoacanthomas which disappeared in the graft was equal for both the immunocompetent recipients and for the recipients lacking functional T-cells. Furthermore, no time difference was observed in tumor formation. It was concluded that the behavior of the tumor in the grafts was possibly a thymus-independent phenomenon. This behavior resembles that of the hair follicle, since we observed previously no macroscopic and microscopic differences in normal skin grafts on both immunocompetent and athymic (nu/nu) mice (2).

Whether the regression of chemically induced keratoacanthomas is based on an immune response is still a matter of discussion. Andrews (3) reported an 80% regression of skin papillomas, derived from follicle epithelium, in immunosuppressed mice. For immunosuppression, recipients were thymectomized, irradiated, and treated with antilymphocytic serum. In rabbits given injections of autologous tumor extracts prepared from 7,12-dimethylbenz(a)anthracene-induced keratoacanthomas, we did not find evidence for a delayed hypersensitivity reaction or for a humoral antitumor response (26). In contrast to this, Lappe (19) reported a delayed regression in mice recipients which are partly immunologically suppressed. The experimentally chemically induced keratoacanthoma closely resembles keratoacanthoma in humans (14, 21). There is no agreement in the literature on whether the spontaneous regression of the human keratoacanthoma is based on an immune response or has its origin in the normal physiology of the hair follicle cycle (7, 22, 23, 25).

Flannery and Muller (11) presented data which failed to support the view that spontaneous regression of human keratoacanthoma is mediated by immunological mechanisms. This paper supports a non-T-cell-mediated immune regression, since the physiology of the behavior in grafts of both experimental keratoacanthoma and normal hair follicle is similar. The actual mechanism of the destruction of these tumors in grafts is not clear. Macrophages and/or natural killer cells may play a role. The regression is probably based on destruction of tumor follicle epithelium, just as an anagen hair follicle is destroyed when it changes from the anagen toward the telogen phase, whether or not induced by transplantation.

ACKNOWLEDGMENTS
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Fig. 1. Gross appearance of chemically induced keratoacanthoma in BALB/c mouse, graded as type I keratoacanthoma, bud shaped, according to the classification of Ghadially (14).

Fig. 2. Gross appearance of chemically induced keratoacanthoma in BALB/c mouse, graded as type III keratoacanthoma, berry or cyst-like shaped, according to the classification of Ghadially (14).

Fig. 3. Typical histological appearance of keratoacanthoma graded as type I. Tumor, BALB/c mouse, Paraplast section. H & E, × 32.

Fig. 4. Typical histological appearance of keratoacanthoma graded as type III. Tumor, BALB/c mouse, Paraplast section. H & E, × 32.
Fig. 5. Histological appearance of type III keratoacanthoma, which could be kept easily in serial transplants. Tumor, BALB/c mouse, Paraplast section. H & E, × 32.

Fig. 6. Periphery of tumor presented in Fig. 5, with histological signs of carcinoma. Tumor, BALB/c mouse, Paraplast section. H & E, × 320.
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