Differential Response of Skin Epithelium to Growth-promoting Effects of Subcutaneously Transplanted Tumor*

THOMAS S. ARGYRIS AND BERTIE F. ARGYRIS

(Department of Zoology, Syracuse University, Syracuse, New York)

SUMMARY

Subcutaneously transplanted Ehrlich ascites tumor invaded the subcutis of the overlying skin and resulted in epidermal enlargement, consisting of cell hyperplasia and hypertrophy. The resting hair follicles, on the other hand, were completely unaffected by the invading tumor. The growing tumor eventually invaded the dermis, resulting in a further increase in the thickness of the epidermis. However, the hair follicles, even though completely surrounded by the invading tumor, remained unaffected.

The cells of the resting hair follicles and those of the basal layer of the epidermis had the same developmental potentiality and were interchangeable. Therefore, the results indicate that the physiological condition of a tissue, or its competence, is important in determining a positive outcome in a growth-promoting interaction.

We report the results of an investigation which demonstrates that subcutaneously transplanted Ehrlich ascites tumor stimulates the epidermis of the skin but does not affect the resting hair follicles. These results are important, because they demonstrate that two tissues—namely, the epidermis and resting hair follicles—which are developmentally equivalent and are interchangeable (12, 14), respond differently, presumably to the same growth-promoting influences.

MATERIALS AND METHODS

In total, 189 young adult C57BL mice of both sexes were used in this investigation. All mice came from our own inbred colony or from the Jackson Memorial Laboratory, Bar Harbor, Maine. The mice were kept in an air-conditioned animal room, fed Purina Laboratory Chow and water ad libitum, and lettuce and cod liver oil-soaked pellets, weekly.

The hair follicles of mice undergo cycles of growth and rest (12, 14). To be certain that the hair follicles would be in the resting phase throughout the course of investigation, all mice were plucked 21 days previous to inoculation of the tumor. Plucking initiated hair growth in the plucked area only (13). Upon completion of the growth phase of the hair growth cycle the hair follicles again entered the resting phase and remained there throughout the course of the experimental period; if not, the animals were discarded.

To study the effects of the Ehrlich ascites tumor on the skin, 69 mice were given inoculations subcutaneously of 0.1 ml. of Ehrlich ascites tumor. For details of the technic, precautions taken to maintain sterility, and source of tumor, see Argyris and Argyris (2, 3). Biopsy specimens of the tumor and overlying skin were taken at 2, 4, 7, 10, 14, 21, and 28 days. Six hours prior to removal of the tumor and the overlying skin, the mice were given injections Subcutaneously of 0.1 mg. colchicine in 0.25 ml. of saline. As controls, six mice whose skin was in the resting phase were given injections of colchicine, as described above, and biopsy specimens of the skin were taken 6 hours later.

To determine the effect of mild epidermal damage, twenty mice with resting hair follicles were shaved. Biopsy specimens were taken at 2, 3, 4, 7, and 9 days. These mice were pretreated with colchicine prior to removal of the biopsy specimens as described above.

All biopsy specimens were fixed in 10 per cent formol-calcium, dehydrated and cleared in dioxan,

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embedded in paraffin, and semi-serially sectioned at 6 μ. Sections were stained with toluidine blue, pH 3.5.

To relate the histological changes with tumor growth, about 60 mice were given inoculations of 0.1 ml of Ehrlich ascites tumor as described above. Tumors were dissected free and their wet and dry weights determined at 2, 4, 7, 10, 14, 21, and 28 days. Similarly, 40 mice were given subcutaneous inoculations of 0.05 ml of tumor, and the tumors were dissected out and their wet and dry weights determined at 2, 7, 10, and 14 days.

RESULTS

Histology of mouse skin.—A bare outline of the histological structure and mitotic pattern of mouse skin with resting hair follicles, pretreated with colchicine, is presented for purposes of orientation. Details may be found in Chase (12) and Montagna (14).

The epidermis is one or two cell layers in thickness. The basal layer shows occasional mitotic figures. The resting hair follicles were made up of a single epithelial sheath, the external root sheath, a sebaceous gland, and a dermal papilla. The resting hair follicle sheath below the level of the outlet of the sebaceous gland rarely shows mitotic activity, but the upper part of the hair follicle, from the level of the sebaceous gland to the epidermal surface, has mitotic activity similar to the basal layer of the epidermis. It also has the same diurnal mitotic rhythm as the epidermis (9-11), and it responds similarly to the epidermis to a variety of damaging agents. 1 Indeed, some suggest that it is reasonable to consider the upper sheath of the resting follicle functionally as a continuation of the basal layer of the epidermis (9), a convention which we shall arbitrarily use.

There is considerable mitotic activity in the sebaceous glands but none in the dermis. Mitotic activity is also absent in the panniculus carnosus, the muscle layer of the skin. Beneath the panniculus carnosus is a thin layer of loose areolar connective tissue, in which mitoses also are rare.

Histological changes in Ehrlich ascites tumor and mouse skin following tumor inoculation.—Within 2 days after tumor inoculation the tumor showed considerable growth (Table 1). It was composed of two areas: the outer area was deeply basophilic and had a number of layers of cells in which mitoses were frequent; the central area was undergoing necrosis, but mitoses might also be seen.

The skin was not invaded and was normal, but the subpapillary connective tissue was stimulated, showing a considerable amount of mitosis and metachromasia.

Four to 6 days after tumor inoculation, the tumor did not show any significant increase in mass (Table 1); however, both outer and central areas of the tumor appeared thicker. In ten cases the skin was normal except for a mild cellular infiltration in the subcutis. However, in three biopsy specimens the tumor had invaded the skin as far as the dermis. In these cases there was a definite enlargement of the epidermis, owing to an increase in cell number and size; but the resting hair follicles were unaffected (Fig. 2).

Tumor mass was increased by 10 days (Table 1). Correspondingly, both the outer area and the central necrotic area were bigger. Most of the sections showed normal skin, with the exception of a definite cellular infiltration in the panniculus carnosus and subcutis. The underlying subpapillary connective tissue continued to be enormously thickened, had many mitotic figures, and was metachromatic.

In a few cases, the subcutis of the skin was invaded by tumor cells. As before, the epidermis, but not the hair follicles, appeared to be stimulated. However, adjacent skin areas, where tumor invasion had not occurred, did not show epidermal stimulation, even though the tumor beneath the skin had reached a considerable size.

The situation at 14 and 21 days was essentially the same, except that the tumor was much larger (Table 1), and the number of biopsy specimens showing tumor invasion and epidermal stimulation had increased. In almost all cases tumor invasion

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Notes:
1 T. S. Argyris, unpublished observations.
2 H. B. Chase, personal communication.
continued to be limited to the subcutis, although in some cases the tumor had begun to invade the dermis (Fig. 3). By 28 days the tumor had further increased in size (Table 1) and in some cases had resulted in skin ulceration. Marked tumor invasion of the skin was prominent. In many cases the tumor had completely invaded the subcutis and dermis and might reach the epidermal-dermal junction (Fig. 4). In such cases the epidermal stimulation was usually maximal. The epidermis might contain five to eight cell layers of enlarged basophilic cells. The hair follicles, however, remained completely devoid of mitotic activity even though they might be not only completely surrounded by the tumor but actually in the process of degeneration. In the cases where the tumor had resulted in ulceration, microscopic examination showed the epidermis gone and only remnants of the hair follicles left. The epidermis immediately next to the ulcer was not stimulated but actually showed atrophic degeneration, a result which was opposite to that seen in epidermis adjacent to all other kinds of wounds studied (5, 6, 8). Progressing further away from the ulcer, but still in the area of tumor invasion, the epidermis showed marked stimulation. As before, in areas where tumor invasion had not occurred, the epidermis was not stimulated (Fig. 1).

We point out in passing that the sebaceous glands did not show obvious qualitative changes, even in areas of complete dermal invasion (Fig. 4). Only in areas of ulceration did they show degenerative changes.

Histological changes in skin damaged at the time of tumor inoculation.—The above results show a strong correlation between skin invasion and epidermal stimulation. If stimulation of the epidermis is dependent on tumor invasion, then we should be able to artificially speed up stimulation by enhancing invasion. This has been done in nine mice by damaging the panniculus carnosus with the needle of the syringe just prior to tumor inoculation. It is well known that damage can enhance tumor invasion (17). In all cases tumor invasion occurred within 4 days, and concomitantly the epidermis showed definite enlargement. By 7 days the tumor had invaded the dermis up to the level of the epidermis, a result usually not observed in undamaged skin of mice until at least 21 days after tumor inoculation.

Effects of damage on mouse skin.—Since it is well known that handling or mild surface damage of mouse skin stimulates the epidermis (10, 12), the question arises whether the epidermal enlargement we observed was due to nonspecific surface damage. To test this possibility the effects of shaving have been studied.

Shaving produced epidermal enlargement; but, in addition, hair follicle stimulation also occurred even though histological examination showed that shaving had not resulted in any open wounds or damage to the hair follicles. Moreover, the resting hair follicles had their club hairs intact as late as 9 days after shaving. This indicates that dislodgment of club hairs, which can be sufficient stimulus for hair growth, had probably not occurred, since club hairs which have been dislodged usually fall out within 5–6 days. Finally, epidermal enlargement was transient. It began at about 2–3 days and was gone by 9 days.

DISCUSSION

We may conclude that the Ehrlich ascites tumor, subcutaneously inoculated, can result in the enlargement of the epidermis of the overlying skin. This epidermal enlargement is a function of both cell hypertrophy and hyperplasia. Apparently epidermal stimulation occurs over considerable distances, since the epidermis is enlarged when tumor invasion is limited to the subcutis, which is at least 100–200 μ from the epidermal-dermal junction. On the other hand, the resting hair follicles are completely unaffected, as far as any increases in cell size or number are concerned. This refractoriness is most obvious when the tumor has invaded the dermis and completely surrounds the hair follicles.

It may be asked whether the epidermal stimulation is due to nonspecific damage, since it is well known that many forms of mild damage can stimulate skin epithelium (7, 10, 12). This seems unlikely, because in many cases only a small part of the skin shows epidermal stimulation, and we would have to hypothesize that wherever we have epidermal stimulation, by coincidence that point on the skin has been damaged. We feel this improbable, especially since a close inspection was always made of the overlying skin. Our results also show that mild damage due to shaving results in epidermal stimulation and, in addition, growth of the resting hair follicles. Similarly, small bites due to fighting or mites, small enough to be invisible to the unaided eye, also result in hair growth stimulation (18). Finally in skin wounds, epidermal stimulation, irrespective of wound size or depth, is never more than 1 mm. (10). Tumor stimulation may extend, however, as much as 8 mm. over the surface of the skin. Thus, we feel that even mild damage produces different and additional effects from those of the tumor, thereby

* T. S. Argyris, unpublished results.
enabling us to rule out surface damage as the cause of epidermal stimulation.

Neither the size of the inoculum nor large amounts of tumor growth are important in determining epidermal stimulation. Inoculation of one-half the amount of Ehrlich ascites tumor results in sufficient tumor growth (Table 1). Tumor growth per se beyond a certain minimal amount is not critical, because epidermal stimulation can be observed as early as 3–4 days when little tumor growth has occurred. On the other hand, the tumor may grow for as long as 28 days and achieve a large size without any epidermal stimulation.

Similarly, increases in blood supply, connective tissue proliferation, inflammation, or massive tumor necrosis are not directly responsible for epidermal stimulation. All these factors are present within a few days after tumor inoculation, and they increase in magnitude with increased tumor growth. Yet, just like tumor growth, there appears to be no direct correlation between them and epidermal stimulation. A lack of correlation between these variables and growth promotion is seen also in the stimulation of the mammary gland duct epithelium by the Ehrlich ascites tumor in ovariectomized, normal, pregnant, and lactating C37 mice (3, 15), and in the stimulation of hair growth in wound-healing (7).

Pressure which has also been associated with growth promotion (1, 16) cannot be responsible for epidermal stimulation, even though there is considerable distention of the skin as the tumor grows. We have had cases in which the tumor has grown for 30 days, without skin invasion, resulting in a considerable pressure on the skin, yet epidermal stimulation has not been seen. Conversely, we have obtained perfectly good stimulation within 4 days after tumor inoculation.

It appears to us that the most critical factor determining epidermal stimulation is invasion of the skin by the tumor cells. Indeed, very little invasion is necessary for epidermal stimulation to occur. We speculate that tumor invasion of the skin is necessary because the panniculus carnosus and/or the hyperplastic sub-pannicular connective tissue acts as a barrier to diffusible growth-promoting substances released by the tumor. We have previously suggested that muscle or connective tissue may act as a barrier to the stimulation of the mammary gland ducts by transplanted Ehrlich ascites tumor (4). The possibility that tumor invasion is critical, because it simply damages the connective tissue of the skin, which in turn releases growth-promoting substances which stimulate the epidermis, we believe is mitigated by the fact that Bullough and Laurence (10, 11) have shown that damage to the connective tissue of the skin does not result in epidermal stimulation. It is possible, however, that the damage to connective tissue produced by the invading tumor releases different substances than those released by mechanical damage.

The resting hair follicles and the basal layer of the epidermis have been shown to be experimentally interchangeable—i.e., the cells of the hair follicles can be used to make epidermis, and vice versa (12, 14). In other words, these tissues are developmentally equivalent. Therefore, a profound difference in the physiological condition must exist between the epidermis and resting hair follicles, since only the epidermis responds to the growth-promoting effects of the Ehrlich ascites tumor. This focuses our attention on the fact that the role of the receiver, or its competence, is important for a positive outcome in any growth-promoting interaction. The question arises why the epidermis is competent to respond and the hair follicles not. We have no answers to this question at the moment, but we offer the following speculations.

One obvious difference between the epidermis and the resting hair follicles is the fact that the epidermis is an actively dividing tissue, whereas the resting hair follicles are not. Perhaps the reason that the epidermis can respond is because some of its cells are already dividing, whereas the resting hair follicles rarely, if ever, contain mitoses (12). If this is the case, then the growth-promoting message(s), presumably liberated by the tumor, is sufficient only for stimulating tissues in which

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Fig. 1.—Section of skin 21 days after the subcutaneous inoculation of Ehrlich ascites tumor. The skin is normal. Arrow points to some of the growing tumor cells that are just beginning to invade, but have not entered in sufficient numbers to cause stimulation of the overlying epidermis. X188.

Fig. 2.—Section of skin 7 days after tumor inoculation. Note the large number of mitotic figures in the surface epidermis and in the part of the epidermis which dips toward the sebaceous gland. Note also the absence of mitoses in the hair follicles. The tumor has invaded the subcutis of the skin but not the dermis. X188.

Fig. 3.—Section of skin 21 days after tumor inoculation. X230.

Fig. 4.—Section of skin 28 days after tumor inoculation. Tumor has completely invaded the dermis. Note the marked epidermal enlargement and intact sebaceous glands. X188.
some cells already are dividing. This speculation is supported by the fact that the Ehrlich ascites tumor implanted in the mouse kidney does not stimulate adjacent kidney cells to divide.4 Mouse kidney cells are also essentially “differentiated” cells which normally show little mitotic activity (16). However, it is probably more than the mere absence of mitosis in a tissue which places it in a class unable to respond to the tumor, since mammary gland ducts of ovariectomized mice are also essentially devoid of mitosis, but they are stimulated by the growing Ehrlich ascites tumor (2). In this case it may be argued that, although the mammary gland ducts in ovariectomized mice are devoid of mitosis, the machinery for cell division has been previously synthesized. On the other hand, the fact that a tissue shows mitosis does not guarantee it will respond to the tumor-growth-promoting influences. Recent work of Simmons (15) has suggested that, in mice, ducts of lactating mammary glands exhibit mitoses, but they are not stimulated by transplanted Ehrlich ascites tumor. If these speculations are correct, then the growth-promoting influences released by the Ehrlich ascites tumor must be different from those observed after damage, since wound healing stimulates not only the epidermis but also growth of resting hair follicles (7), and damage to the kidney results in an intense mitotic proliferation around the damaged area.4

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REFERENCES


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Thomas S. Argyris and Bertie F. Argyris


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