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Original Article

Adult epidermal keratinocytes are endowed with pilosebaceous forming abilities

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ABSTRACT Pluristratified epithelia of adult vertebrate skin continuously regenerate from stem cells, and the question still arises as to whether those cells are committed to the production of only one cell lineage, or in contrast they conserve their embryonic pluripotentiality. In order to investigate the abilities of adult cultured as well as wound healing epidermis, heterospecific fibroblast-keratinocyte recombinations were performed, which allow unquestionable identification of the cells implicated in the structures that differentiate. Adult human cultured breast epidermal cells and full-thickness wound healing from human facial skin and foreskin were associated with either rabbit embryonic trichogenic dermis or cultured dermal papilla cells of adult rat, before grafting onto nude mice for two weeks to one month. *In situ* hybridization with a human specific sequence Alu probe labeled the human cells, whereas implanted rabbit or rat and host mouse cells were distinguished by the Hoechst staining of their nuclei. The results show that human adult cultured breast epidermal cells are able to form hair buds and to participate in hair follicle formation, while adult healing epidermis from a sparsely hairy skin as the human face or the dorsal skin of nude mouse, or even from a glabrous epidermis as the human foreskin, are able to differentiate pilosebaceous units. Although a follicular origin of the involved keratinocytes cannot be excluded in the three first cases, the formation of hair and sebaceous glands by foreskin keratinocytes of children 2 to 10 years-old establishes the cutaneous appendage ability of the interfollicular epidermal stem cells. The formation of interspecies mosaic follicles also highlights the fact that there must be a significant level of commonality in the interactive signaling molecules used by epithelial cells from different species.

KEY WORDS: *differentiation, cell interactions, keratinocyte, hair follicle, sebaceous gland*

Introduction

The epidermis differentiates during embryogenesis and postnatal life into cutaneous appendages such as glands and hair follicles. These keratinocyte populations continuously regenerate in the adult. The stem cells in these replicating tissues are the earliest progenitors that lead, through the joint processes of proliferation and differentiation, to transient cells and then to terminally differentiated cells (Potten and Morris, 1988). Stem cells are defined by a number of general features (for a review see Latjha, 1979 and Miller *et al.*, 1993). First they are ultrastructurally unspecialized and correspond to a small population of the tissue. Second, they are slow-cycling, but may be induced to proliferate more rapidly in response to wounding and to other growth stimuli. Lastly, they are usually found in well-protected areas. The heterogeneity in epidermal basal keratinocytes have been reported by several authors based on the expression of differentiation markers, on the capacity to retain a ^{3}H-TdR label and on proliferative abilities tested by clonal analysis (Lavker and Sun, 1982; Morris *et al.*, 1985; Regnier *et al.*, 1986; Barrandon and Green, 1987), as well as on their expression of high levels of functional ß1 integrin family receptors (Watt and Jones, 1993; Jones, 1996). Taken together, these studies suggest that epidermal stem cells represent between 1% to 10% of the basal layer and are preferentially distributed in the deep papilla ridges. With regard to hair stem cells in the mouse, a subpopulation of slow-cycling cells are located outside the hair matrix and located in the bulge, a portion of the upper follicle that is the follicular attachment site of the arrector pili muscle (Cotsarelis *et al.*, 1989, 1991; Yang *et al.*, 1993). In the case of humans, cells with a corresponding behavior have been located in a region of the outer root sheath below the midpoint of the hair follicle (Rochat *et al.*, 1994).

The question still arises as to whether the keratinocyte stem cell populations of the interfollicular skin or hair follicle are committed to the production of only one cell lineage, i.e. whether epidermal interfollicular stem cells give rise only to the epidermis and follicular

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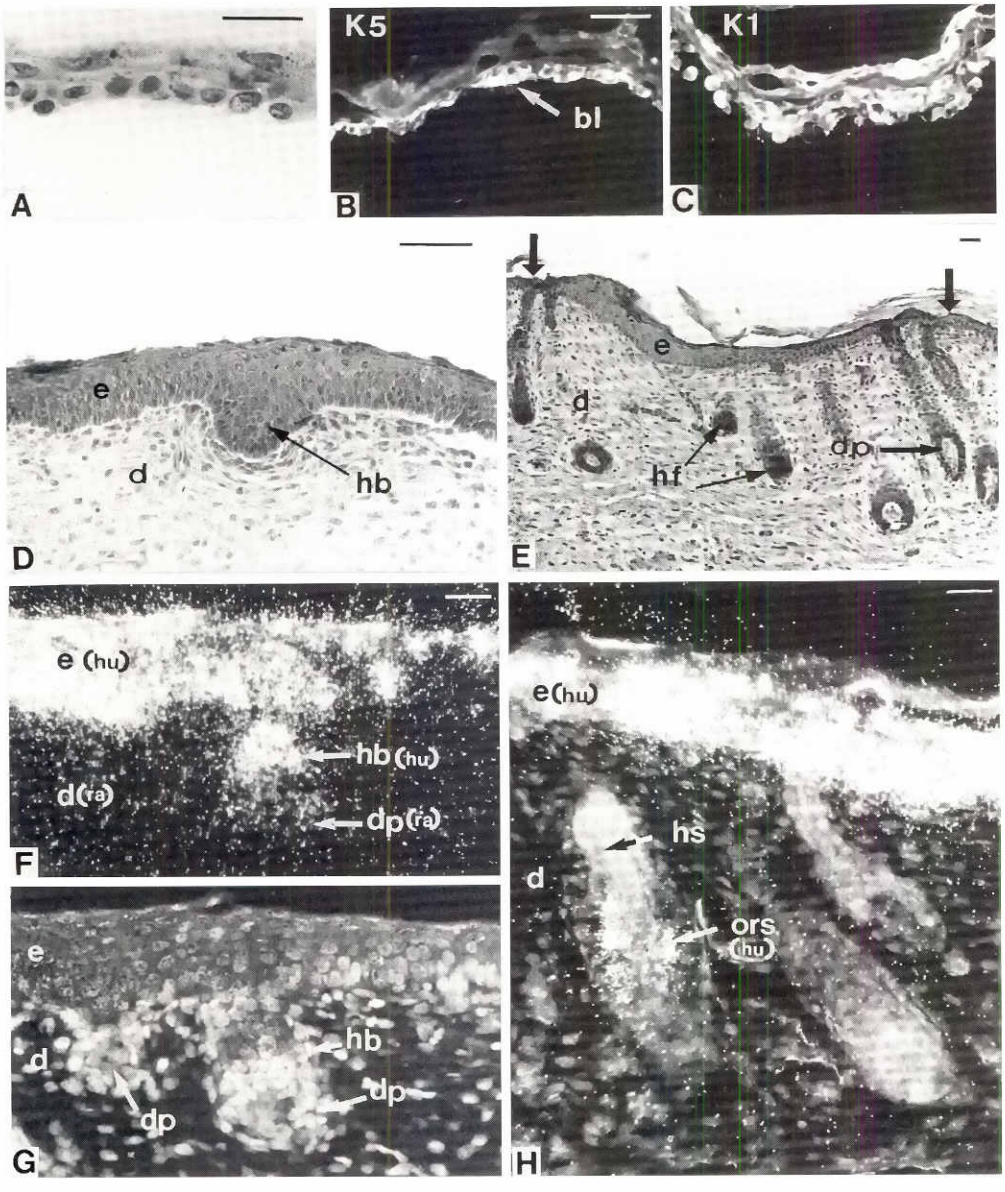

Fig. 1. Induction of hair follicles following the recombination of human *in vitro* cultured epidermis and dorsal rabbit embryo dermis, subsequently grafted onto the back of nude mice. **(A,B,C)** *Human cultured epidermis after 3 weeks of in vitro culture. Expression of the keratin K5 labeled with the AE14 monoclonal antibody in the basal cells* **(B)**, *and of the keratin K1 labeled with the AE19 monoclonal antibody in almost all the epidermal cells* **(C)**. **(D,E)** *Two weeks after recombination to the rabbit dermis (d) and grafting, the epidermis (e) formed stage 2 hair bud (hb)* **(D)** *or even stage 6 hair follicles (hf)* **(E)**. **(F,G,H)** *Same types of grafts than D and E, analyzed by in situ hybridization with the Alu probe. The epidermis (e), composed of human cells (hu) differentiated stage 2 to 4 hair buds (hb) associated to rabbit (ra) dermal papilla (dp)* **(F,G)**, *whereas stage 6 hair follicles* **(H)** *showed a mixed origin. The origin of the non-human keratinocytes implicated in such hair follicles has been elucidated by the use of Hoechst staining (compare with Fig. 3). hs, hair shaft; ors, outer root sheath. (A,D,E) Ehrlich hematoxylin/Biebrich scarlet staining. (B,C) Immunofluorescence with the AE14 and AE19 monoclonal antibodies. (F,H) Darkfield illumination on in situ hybridization using 35S-labeled Alu probe. (G) Fluorescent illumination on in situ hybridization, counterstained by propidium iodide. Bars: A, 25 μm; B-H, 50 μm.*

stem cells to hair follicle, or whether they are pluripotent and consequently have equivalent potentialities. In the second hypothesis, epithelial stem cells may still possess embryonic features and therefore their microenvironment, the fibroblasts to which they are associated, may play a crucial role in their differentiation. Indeed, during embryogenesis, the epidermis is dependent upon its associated dermis for its differentiation: heterotopic dermal-epidermal recombination experiments among mouse plantar, upper-lip and dorsal embryonic skin components (Kollar, 1970; Dhouailly, 1977a,b; Delorme and Dhouailly, 1989) have shown that the choice between a glabrous or a hairy skin, or between hair vibrissa and hair pelage-type follicles, is dictated by the origin of the dermis. In the adult, rat vibrissa follicle has long been employed as an experimental model, its dermal papilla appearing endowed with inductive properties, both within follicles and when associated with different rat epidermises, even from the plantar region (Reynolds and Jahoda, 1992; Jahoda *et al.*, 1993). On the other hand, outer root sheath cells of human hair follicle have been shown to be able to regenerate a fully differentiated epidermis *in vitro* (Limat and Noser, 1986; Lenoir *et al.*, 1988). Likewise, in case of skin injury follicular cells were shown to migrate upwards to cover the wound, suggesting that they can serve as a source for the epidermis (Lenoir *et al.*, 1988). From these results it can be assumed that adult epithelial cells can potentially be pluripotent. Here we investigated the capacity of the epithelial component of adult interfollicular skin to express an alternative differentiating pathway, i.e. to form hair follicles when confronted with trichogenic fibroblasts, either from an embryonic dermis or from cultured adult dermal papilla cells. The response of keratinocytes was studied in two different situations, such as in the recombination of an entire epithelial sheet after *in vitro* culture or in wound healing process. Cultured human epidermal cells originating from mammectomies were used in order to determine a possible effect of *in vitro* culture on the abilities of keratinocytes. For wound healing experiments, human face-lift

skin grafted onto nude mice was used in a first attempt, and then human foreskin was used, in order to eliminate a possible follicular origin of the wound healing keratinocytes.

Investigation in developmental biology in general (Le Douarin, 1973) and more recently of lens morphogenesis (Saha *et al.*, 1989) has underlined the necessity to identify the origin of the responding cells when exploring tissues interactions. Taking this into account, and in order to undoubtedly identify the origin of the differentiated structures, bispecific human/rabbit or human/rat epithelial-mesenchymal recombinants were performed and grafted onto a third species (nude mouse). Human cells were recognized from rat or rabbit cells by *in situ* hybridization with a human specific sequence Alu probe (Rubin *et al.*, 1980) and implanted rabbit cells from host mouse cells by the Hoechst staining of their nuclei (Cunha and Vanderslice, 1984; Ferraris *et al.*, 1994). This biological cell labeling technique is similar to that introduced by Le Douarin (1973) to distinguish quail and chick nuclei. We are thus able in the present experiments to distinguish between a differentiation obtained from the recombinant epidermis and an eventual regrowth from residual epithelial cells in the recombined dermis or even a host cell migration from the wound edges.

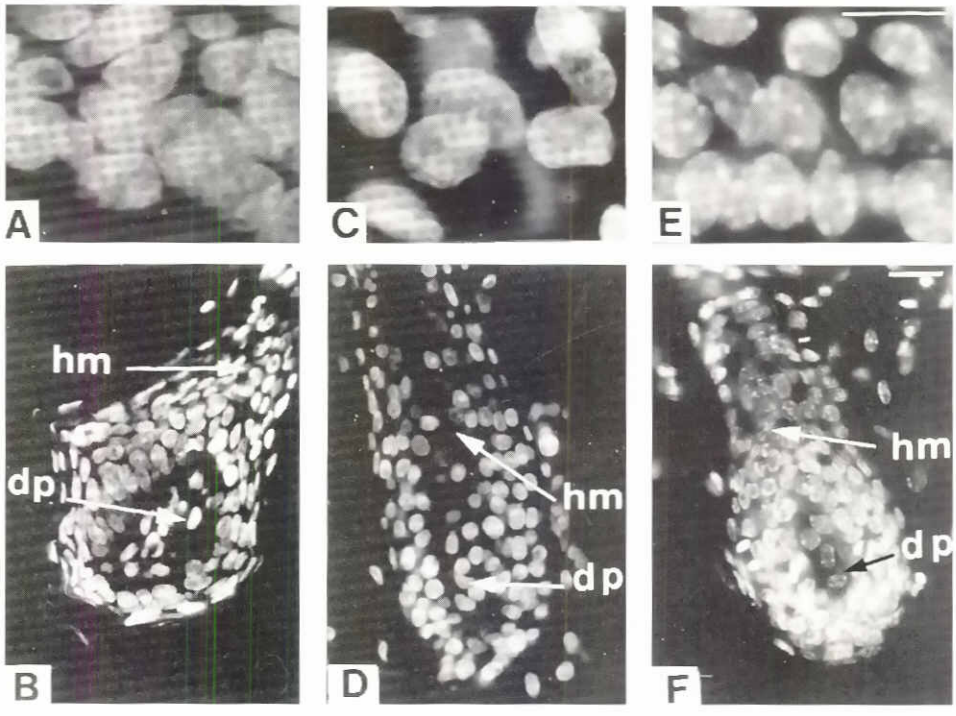

Fig. 2. Discrimination between rabbit (A,B), and human (C,D) or mouse (E,F) nuclei in skin, using Hoechst dye. *Mouse nuclei exhibit several fluorescent intranuclear bodies, as shown here in follicular dermal cells and keratinocytes, whereas rabbit and human nuclei are uniformly stained. dp, dermal papilla; hm, hair matrix. Bars: A,C,E, 10 μm; B,D,F, 50 μm.*

Results

Induction of hair follicles in human reconstructed in vitro epidermis and in wound healing host mouse epidermis by the rabbit embryonic dermis

When ready for recombination with dorsal dermis of a 19-day rabbit embryo, the human cultured epidermal sheets ranged in thickness from 2 to 4 living cell layers covered by flattened cells (Fig. 1A). The lowermost cells contain prominent large nuclei and minimal cytoplasm. These cells resemble basal cells of *in vivo* epidermis and may constitute the proliferative cell population. They were characterized, as the *in vivo* basal cells (Sun *et al.*, 1983), by the synthesis of keratin K5 (Fig. 1B). Nevertheless, most of them were already involved in the expression of keratin KI (Fig. 1C), a marker of epidermal-type differentiation, whose expression is known to start in transient cells (Sun *et al.*, 1983).

In a first series of experiments, the graft site was analyzed only by classical histology. After two weeks of grafting, among 10 obtained cases, one developed a few stage 2 hair buds (as defined by Hardy, 1968) (Fig. 1D), while three other cases showed several stage 6 or 7 hair follicles (Fig. 1E). In order to ascertain the species origin of the epithelial cells implicated in this morphogenesis, these experiments were repeated and analyzed both by *in situ* hybridization with the Alu probe and by Hoechst staining. Two weeks after grafting, in only 5 out of 16 cases, the human cultured epidermis was identified with the Alu probe. The epithelium thickened to 5 to 7 cell layers and developed both a granular and a horny layer. In one case the human cultured epidermis differentiated into stage 2 to 4 hair buds. These hair buds, which apparently were entirely constituted by human epidermal cells as specifically labeled with the Alu probe, were associated with a rabbit dermal papilla (Fig. 1F and G). In two more cases the human epithelial cells participated in the formation of stage 6 hair follicles of mixed origin including human cells (Fig. 1H). Furthermore, unlabeled hair follicles were found associated to the human epidermis. The species origin of the non-human cells which participated in the formation of the mixed or unlabeled hair follicles was identified on adjacent sections stained by the Hoechst dye. This staining allows an easy distinction (Fig. 2A to F) between mouse nuclei, characterized by multiple shining intranuclear bodies, and those of rabbit or human, which are less and almost uniformly stained. The non-human trichocytes of the mixed follicles or of the non-human follicles do not originate from residual rabbit epithelial cells with the implanted dermis, but were migrating from the mouse host epidermis (Fig. 3C). It should be noted that in those cases the basal epidermal layer was mostly constituted of host mouse cells, whereas only the epidermal upper strata were still formed by human cells. One month after grafting, numerous hair follicles with emerging hair shafts covered the graft site of all 15 cases (Fig. 3A). Some of the hair follicles were associated with sebaceous glands, and the epidermis involved a few cell strata (Fig. 3B). At this stage, the cultured human epidermis was totally replaced by a wound healing epidermis originating from the mouse host (Fig. 3D) in all the 27 cases analyzed. This mouse epidermis was thin (2 to 4 cell layers) and in all cases formed hair mouse follicles in contact with the implanted rabbit embryo dorsal dermis.

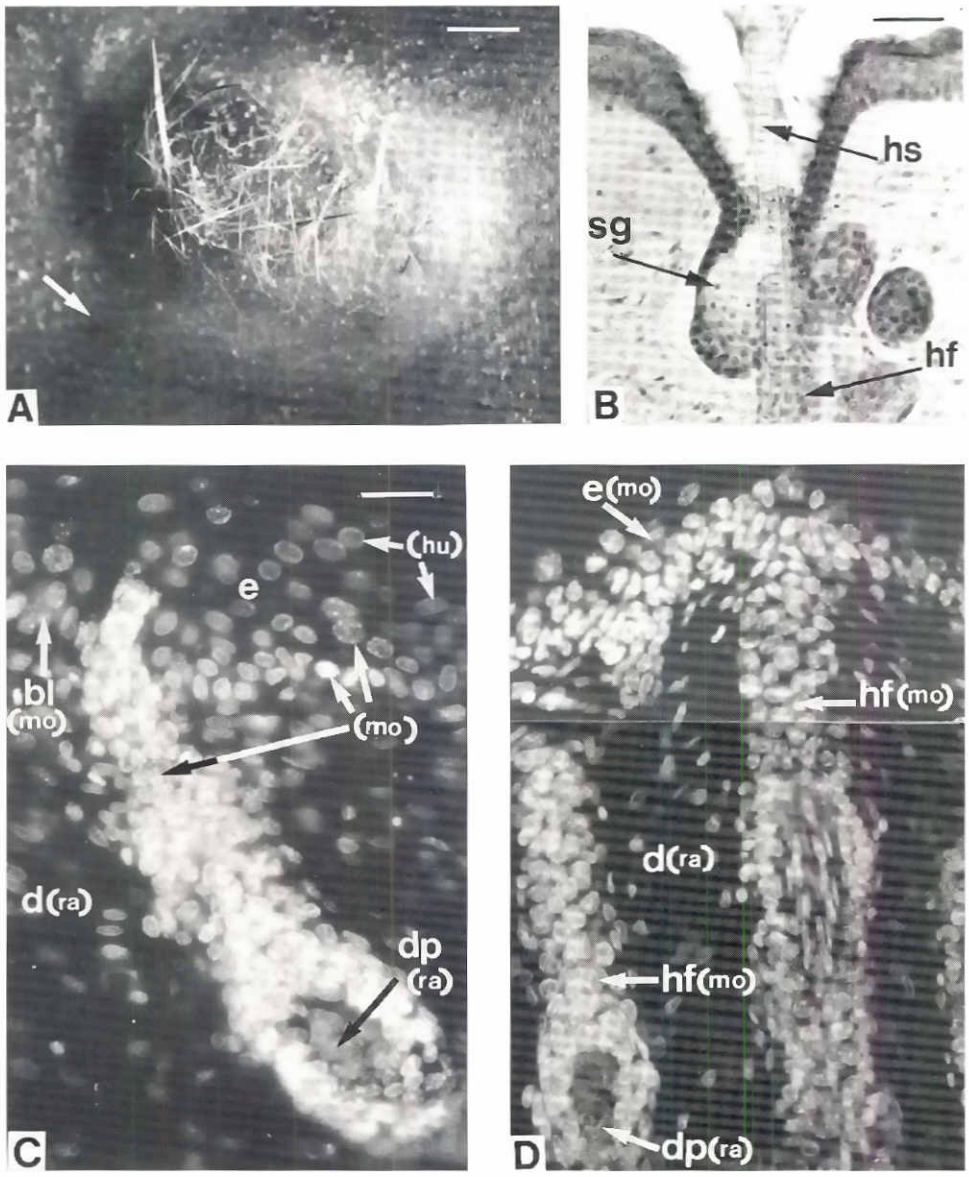

Fig. 3. Induction of hair follicles in wound healing host mouse epidermis by a dorsal rabbit embryo dermis. *After two weeks to one month of grafting, the analysis of the same grafts than those presented in Figure 1 showed that a migration of mouse keratinocytes from the edges of the graft bed lifted the cultured human epidermis and covered the implanted rabbit dermis.* **(A)** *After one month, numerous emerging hairs formed in the graft site, while the surrounding host skin (arrow) appears glabrous.* **(B)** *Section of the graft showed in* **(A)**. *Detail of the upper part of a hair follicle (hf) associated to a sebaceous gland (sg).* **(C)** *After two weeks, section of a graft similar to that shown in Figure 1H. This stage 6 hair follicle, induced by a rabbit (ra) dermal papilla (dp) is mostly composed of mouse (mo) keratinocytes, as shown by Hoechst staining. Note that the epidermis (e) appears composed of two strata of different origin. The basal cells display the intranuclear bodies which are characteristic of the mouse species, while the upper strata are composed of human (hu) cells whose nuclei are uniformly stained.* **(D)** *After one month of grafting, a mouse (mo) wound-healing epidermis totally replaced the human epidermis and developed hair follicles in contact with the rabbit (ra) embryonic dermis (d) and dermal papilla (dp). bl, basal layer; hf, hair follicle; hs, hair shaft; ors, outer root sheath; (B) Ehrlich hematoxylin/Biebrich scarlet staining; (C,D) Hoechst staining. Bars: A, 1 mm; B, 150 μm; C,D, 25 μm.*

Induction of hair follicles in human wound-healing face-lift skin and foreskin by cultured rat dermal papilla cells

Two months after grafting, control sections of graft sites of full-thickness human face-lift skin and foreskin were processed for histological studies. The grafted skin had maintained its morphology and was hyperpigmented. The human face-lift skin involved hair follicles with a large diameter characterized by a pluristratified outer-root sheath (Fig. 4A), associated with sebaceous glands. In contrast, the foreskin graft was constituted of a glabrous epidermis with deep rete ridges, which forms neither hair follicles nor sebaceous glands (Fig. 5A).

Six graft sites of split-thickness human face-lift skin implanted with cultured rat dermal papilla were analyzed after three months by both histology and *in situ* hybridization with the Alu probe. Human epidermis was usually thickened and often hyperkeratotic (6 to 7 cells layers), compared to native host murine epidermis (2 to 3 cells layers), which differentiated into sparse and thin hair follicles. The grafted human face-lift skin was entirely covered with large emerged hairs in 4 out of 6 cases. Those follicles were of two different origins. The pre-existing follicles (Fig. 4B) were identified by the fact that both the dermal papilla and the hair matrix were constituted by human cells as shown with the Alu probe, whereas additional induced hair follicles (Fig. 4C) were in contact with an unlabeled rat dermal papilla. It should be noted that nuclear density in the dermal papilla is similar to that of the hair bulb.

Seven graft sites involving foreskin were harvested three months after cultured dermal papilla implantation. Four grafts differentiated into stage 6 to 8 hair follicles (Fig. 5, B-F). The human origin of the differentiated hair follicles was attested by the *in situ* hybridization with the Alu probe (Fig. 5D and F). Longitudinal sections through the bulb (Fig. 5E and F) revealed the unlabeled dermal papilla of non-human (rat) origin. In most cases, sebaceous glands (Fig. 5B) were found associated to the hair follicles.

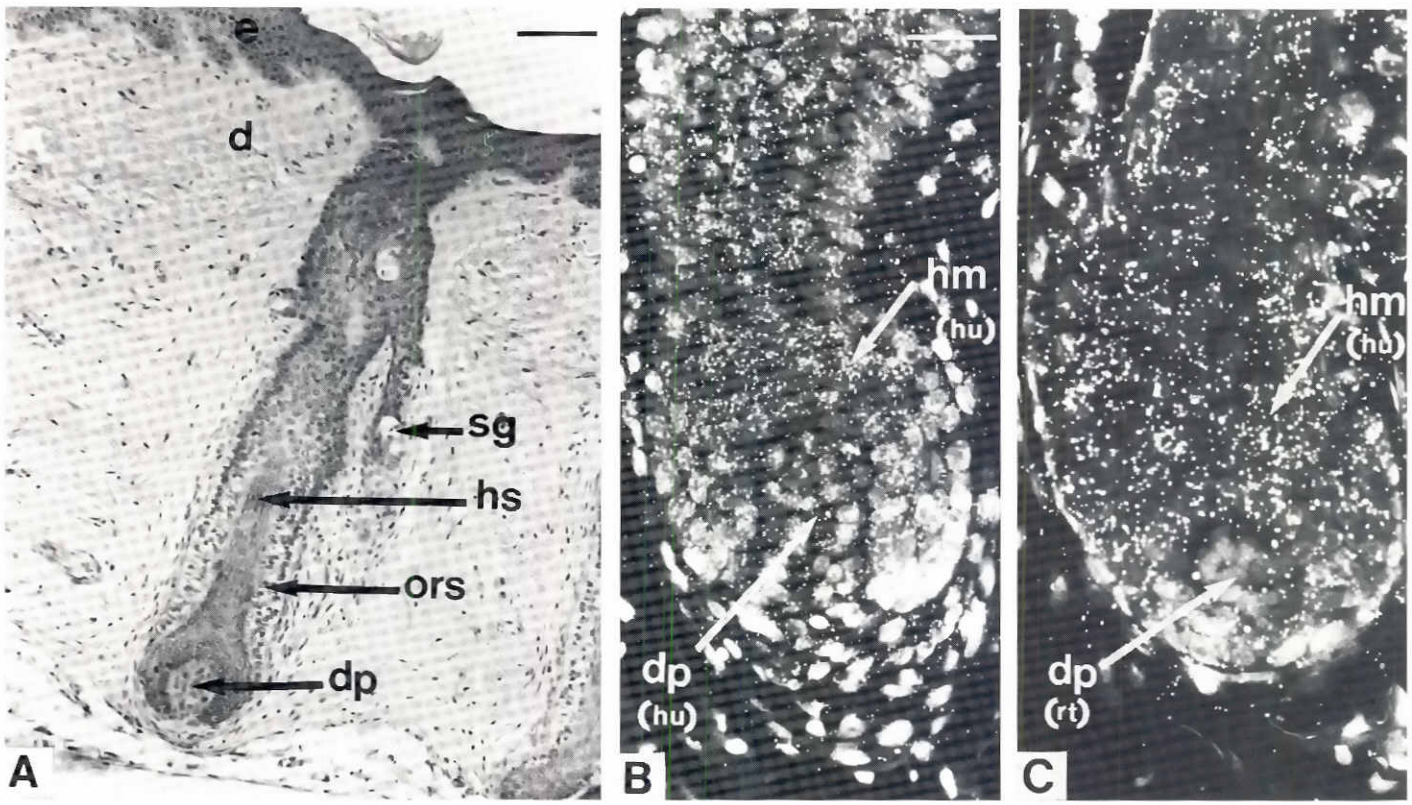

Fig. 4. Induction of hair follicles in human wound-healing face-lift skin after implantation of cultured dermal papilla cells of adult rat. (A) *Control two months after grafting of human face-lift skin onto nude mice, involves a few hair follicles associated to sebaceous glands (sg).* **(B,C)** *Three months after the implantation of cultured rat dermal papilla cells, the face-lift graft shows two kinds of hair follicles. Pre-existing follicles of human (hu) origin* **(B)**, *whose hair matrix (hm) and dermal papilla (dp) cells are labeled with the Alu probe, and newly induced follicles* **(C)** *constituted of human labeled keratinocytes associated with an unlabeled rat (rt) dermal papilla. d, dermis; e, epidermis; hs, hair shaft; ors, outer root sheath. (A) Ehrlich hematoxylin/ Biebrich scarlet; (B,C) both darkfield and fluorescent illumination on in situ hybridization using ^{35}S labeled Alu probe, counterstained by propidium iodide. Bars: A, 100 μm; B,C, 25 μm.*

Discussion

In the dermal-epidermal recombination experiments presented here, the nuclei of the two skin components, and those of the host which belong to three different species, are easily recognizable: human nuclei are identified by *in situ* hybridization with Alu probe, while Hoechst staining allows discrimination between mouse nuclei and rabbit or human nuclei. We clearly demonstrate that there was no contamination of the implanted inductive dermal cells by epidermal cells of the same origin, and that the mouse host keratinocytes can in some cases gradually replace the grafted epidermis. Indeed, in grafted recombinants involving a human cultured epidermis and a rabbit dermis, the implanted dermis remained in place, but the human cells were progressively replaced by a mouse wound healing epidermis, as shown by the Hoechst dye. The mouse cells progressed along the dermal-epidermal junction and lifted the human epidermal cells. In five cases, the human cultured epidermis was still in place after two weeks of grafting, but never after a longer grafting period. It may be assumed that *in situ* epidermal mouse cells have a higher proliferative potential than cultured human keratinocytes. This is reminiscent of the events which occur during the reepithelialization process in the case of allograft of human cultured epidermis to extensive burn injuries (Phillips *et al.*, 1990). One possibility is that the cultured keratinocytes used in our experiments are the so-called keratinocyte progenitor cells which are programmed to differentiate into mature keratinocytes and to express a progressively restricted proliferative potential (Asselineau *et al.*, 1986; Regnier *et al.*, 1986). This result raises the question of the required conditions for persistence of the stem cells after *in vitro* culture. Therefore, the environment of a stem cell (surrounding epithelial cells and underlying dermal cells or medium factors) may play a crucial role in preventing them from proliferating and/or differentiating and in maintaining their "stemness". This also suggests that in our experiments, despite the fact that stem cells may behave as clonogenic cells in culture (Barrandon and Green, 1985, 1987), they would probably rather act as transient amplifying cells than as truly stem cells. Indeed, in our experiments, only 3 cases differentiated into hair structures. In one case, the entire hair buds were apparently from human origin, whereas in the other cases, mosaic hair follicles formed, which were constituted with mouse and human epithelial cells. Incidentally, this last result clearly demonstrates that hair follicles have a polyclonal origin and that epithelial cells from two different species can homogeneously cooperate via their membrane receptors and form bispecific desmosomes.

This experimental series which have consisted in grafting human cultured epidermis/rabbit dorsal embryonic dermis recombinants in place of an excised piece of mouse skin have

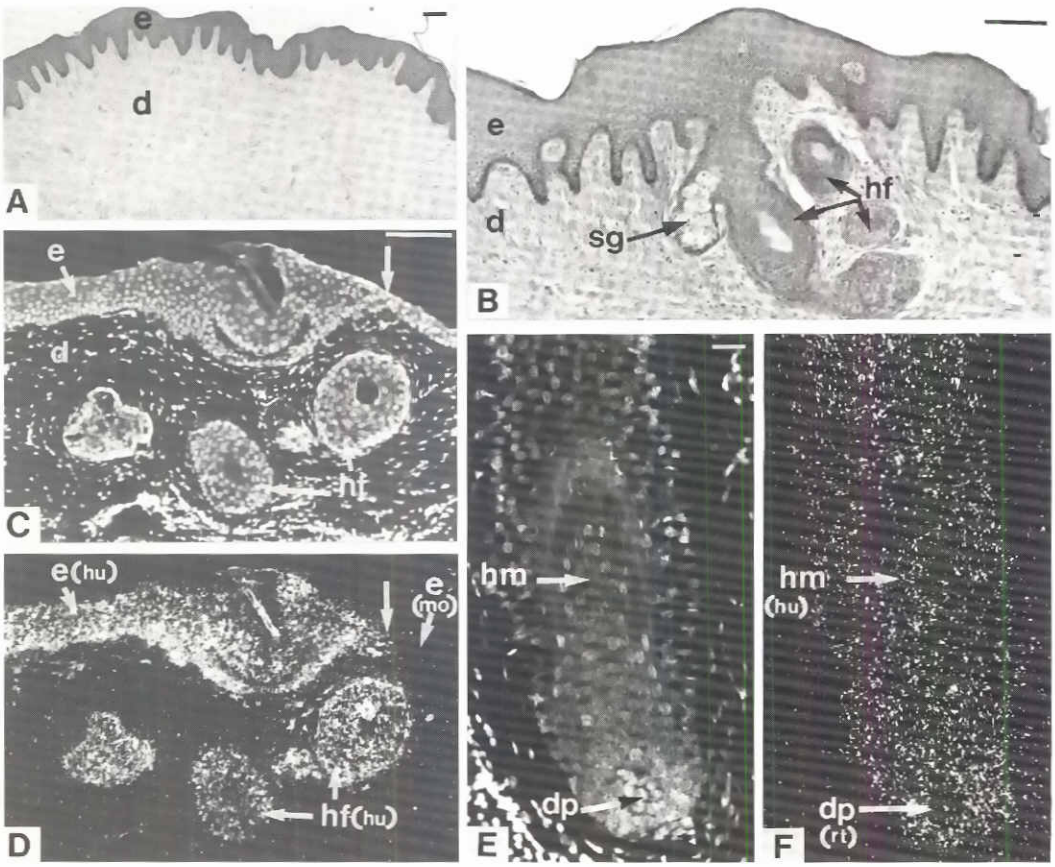

Fig. 5. Induction of hair follicles in human wound-healing foreskin after implantation of cultured dermal papilla cells of adult rat. (A) *A control two months after grafting of foreskin onto nude mice, is totally devoid of appendages.* **(B)** *Three months after implantation of rat papilla cells, induction of hair follicles (hf) and sebaceous glands (sg).* **(C)** *Transversal section of three hair follicles, which are labeled* **(D)** *with the Alu proble.* **(E,F)** *Longitudinal section showing the unlabeled implanted rat papilla cells (rt) and the hybridization of the Alu probe on hair follicles keratinocytes, which are of human (hu) origin. Note that the nuclei density is at least as intense in the dermal papilla (dp) as in the hair matrix (hm). d, dermis; e, epidermis; hf, hair follicle; hs, hair shaft. (A,B) Ehrlich hematoxylin/ Biebrich scarlet staining. (C,E) Propidium iodide staining. (D,F)Both darkfield illumination on in situ hybridization using ^{35}S-labeled Alu probe. Bars: A-D, 100 μm; E,F, 25 μm.*

moreover shown the hair-forming ability of the host wound healing epidermis. Indeed, this epidermis differentiated hair follicles in all cases under the influence of the implanted rabbit embryonic dermis. Thus, the embryonic dermis was able to successively induce at least two waves of hair follicle differentiation and to maintain its inductive capacities over a long period. However, the wound healing mouse epidermis may originate even in the nude breed either from follicular or interfollicular host cells. The same question arises in the case of hair follicles formed by wounded human face-lift grafted in contact with implanted rat dermal papilla cells. In order to definitively show that interfollicular postnatal cells are competent to form hair, foreskin of children aged 2 to 10 years was used in similar experiments. Hair follicles formed in 57% of the cases. Moreover, in both cases of dorsal mouse or human foreskin wound healing epidermis, entire pilosebaceous units were in fact induced, either by the embryonic dermis or the cultured dermal papilla.

In conclusion, adult epidermal keratinocytes, whatever the age from 2 years- to 69 years-old, or the regional or species origin, involve cells that are able to differentiate into pilosebaceous units. The ability to induce not only hair follicle, as already shown (Dhouailly, 1977b; Reynolds and Jahoda, 1992), but also sebaceous gland formation appears a property of both embryonic dermis and adult dermal papilla. Our data provide definitive arguments to support the previous work from Reynolds and Jahoda (1992), showing the induction of hair follicles in adult rat epidermis. Despite the conditions that may modulate their differentiation state *in vivo*, epidermal and hair keratinocytes have not diverged irreversibly during embryogenesis and share common potentialities.

Materials and Methods

The human skin originates from cosmetic surgery and the foreskin from circumcision, both practised in the Grenoble General Hospital. Mice, rats and rabbits were from "Iffa-Credo" and "Elevage Scientifique des Dombes". They were humanely euthanized. The nude mice, onto which the chimeric skin fragments were grafted, were prealably anesthetized with an intraperitoneal injection of 100 μl of a solution containing 50 mg of valium and 1 mg of ketamine hydrochloride (Imalgene, Rhône-Merieux). This was performed under French Animal Protection and Health Ministry authorization n° 04622 to Dr. Dhouailly.

Isolation of inductive dermal cells

Vibrissa dermal papillae were obtained from the upper-lip of adult Wistar rats. Follicles were isolated from the dermal tissue according to the procedure of Reynolds and Jahoda (1992). The lower portion of the bulb was cut open to release the dermal papilla, which at this stage is still attached to the dermal sheath. The isolated papillae were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with penicillin, streptomycin and 20% fetal calf serum. After reaching confluence in primary cultures within approximately 5 weeks, the cells were further subcultured once or twice before being used in association experiments. Embryonic trichogenic dermis was obtained from the dorsum of 19-day rabbit embryo. At this stage, the skin reaches stage 1 of hair formation (as defined by Hardy, 1968), which is characterized by a high hair inductive property of the dermis (Dhouailly, 1977a,b). The dermis was dissociated from its associated epidermis by enzymatic treatment (1.25% trypsin and 2% pancreatin) in Ca^{2+} and Mg^{2+} free Earle's solution.

Cultured epidermis/embryonic dermis recombinants

Human epidermal keratinocytes were isolated as previously described (Rheinwald and Green, 1975) from healthy female subjects aged 16 to 25 years who underwent mammary reduction. The keratinocyte suspension was seeded at a density of 1 to $2x10^5$ cells/cm^2 on a feeder layer of irradiated mouse 3T3 fibroblasts. Culture medium consisted of DMEM and Ham's F12 (3:1) media supplemented with 10% fetal calf serum, 0.4 mg/ml hydroxycortisone, 10 ng/ml EGF and 5 mg/ml insulin. After four weeks, epidermal sheets of stratified confluent cultures were harvested and associated with a dorsal dermis from 19-day rabbit embryo and then grafted for 2 to 4 weeks onto the anterolateral back of nude mice, in place of a piece of host skin, prealably excised.

Wound healing experiments

Full thickness foreskin of children aged 2 to 10 years and discarded face-lift skin obtained from a healthy 69 year-old woman were grafted onto nude mice. Rat cultured dermal papilla cells were implanted two months after skin transplantation. Confluent cultures of rat papilla dermal cells were collected into clumps with a rubber scraper and then transferred into an incision made in the middle of the graft through the entire thickness of the skin. After 3 months, the mice were humanely euthanized and the implantation sites were analyzed.

Immunological staining

Cryostat sections (7 μm) were labeled with the AE14 (anti keratin 5) and AE19 (antikeratin 1) monoclonal antibodies (a gift from Dr. T.T. Sun), themselves revealed by goat anti Ig (G+M) mouse conjugated with fluorescein isothiocyanate.

Identification of the species origin of the cells in the differentiated heterospecific recombinants

In order to distinguish the origin of the structures that we observed two different methods were used after standard fixation (formalin) and wax histology.

Fluorescent staining with Hoechst dye

The compound 33258 Hoechst is a fluorochrome that binds to the narrow groove of DNA. The staining procedure (modified from Cunha and Vanderslice, 1984) consisted of treating sections (7 μm) for 15 min with 4 mg/ml of Hoechst 33858 in phosphate buffered saline solution (PBS). Slides were mounted in 9:1 glycerol in PBS containing sodium azide and 1,4 diazabicyclo(2,2,2)octane (Dabco, Sigma) an antifading compound, and were observed under a 365 nm microscope.

Preparation of Alu probe and in situ hybridization

The specificity of the Alu probe that we used was first analyzed by Southern blotting (data not shown) and by *in situ* hybridization on control tissues from human, rabbit, mouse and rat. Sections (7 μm) were collected on gelatinized slides, treated with 2 mg ml^{-1} of proteinase K for 8 min at room temperature, immersed in 0.1 M triethanolamine pH 8.0/0.25% (v/v) acetic anhydride solution for 10 min, dehydrated and air dried. ^{35}S-labeled RNA probe (specific activity of $5x10^8$ pm min^{-1} mg^{-1}) was synthesized using a T3 polymerase *in vitro* transcription reaction, from pHum Alu clone which contains the fragment Bam/Bam (300pb) of the clone Blur 8 (Rubin *et al.*, 1980). The slides were heated at 73°C for 10 min in order to denature cellular DNA. After hybridization, sections were washed at 50°C in high-stringency conditions, with a final wash in 0.1xSCC at 37°C, and then dehydrated. The hybridized sections were coated with Kodak NTB-2 emulsion and exposed for one night. After hybridization, the slides were stained using a DNA fluorochrome (propidium iodide, 10 mg ml^{-1} in PBS).

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