Histologic and Cell Kinetic Studies of Hair Loss and Subsequent Recovery Process of Human Scalp Hair Follicles Grafted onto Severe Combined Immunodeficient Mice

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To establish a model for studying human scalp hair, individually isolated hair follicles were grafted onto back skin of severe combined immunodeficient mice. Histologic changes and cell kinetics in the hair loss and subsequent recovery process were investigated. In the dystrophic stage (from day 7 to 30), all the hair shafts became dystrophic and were shed. Thickening and corrugation of vitreous membrane, apoptosis, and regression of the lower part were observed in the grafted hair follicles. 5-bromo-2′-deoxy-uridine-labeled cells were not detected in the lower end of the follicles, and keratin 19-positive cells appeared there. At the end of this stage their lower part was maximally retracted, secondary germ remained beneath the bulge, and the vitreous membrane disappeared. In the regeneration stage (from day 30 to 50), the same histologic findings as those at the end of the dystrophic stage were observed. The keratin 19-positive cells in the secondary germ, however, were replaced with keratin 19-negative and 5-bromo-2′-deoxy-uridine-labeled cells. Then, differentiation into an inner root sheath and a hair shaft began, and apoptosis was terminated. In the stable growth stage (from day 40 to at least 150), the grafted follicles were immunohistochemically and light microscopically identical with the normal anagen hair follicles except for the presence of melanin incontinence. These findings suggest that the grafted hair follicles entered into dystrophic catagen, subsequently dystrophic telogen, then returned to normal anagen follicles, and that stem cells or their close progeny in the secondary germ play an important part in the recovery process. ***Key words: apoptosis/keratin 19/secondary germ/stem cells. J Invest Dermatol 115:200–206, 2000***

In hair biology research, cell and organ culture systems have most often been used (Kobayashi *et al*, 1993; Westgate *et al*, 1993; Philpott and Kealey, 1994; Rochat *et al*, 1994; Williams and Stenn, 1994; Moll, 1995; Williams *et al*, 1996). These simple systems do not seem to be sufficiently helpful for investigating the complexities of the hair cycle. Mouse pelage has been used for the investigation of not only hair biology but also hair diseases (Cattaneo *et al*, 1960; Paus *et al*, 1994). Studies of this kind have provided much information, although this research may have limited value for understanding human hair biology and diseases, as there are some important differences in the hair cycle between human scalp hair and mouse pelage (Abell, 1994; Cotsarelis, 1997; Lavker *et al*, 1999). The hair cycle of adult human scalp hair is asynchronous, whereas that of mouse pelage is synchronous especially in the first two cycles. In addition, anagen is much longer in the former (several years or more) than in the latter (less than a month). In these respects human scalp skin transplanted to immunodeficient animals seems to be an excellent model for investigating human hair follicles. In this model the transplanted hairs are shed within a month (Gilhar *et al*, 1988; Lyle *et al*, 1998), and its regrowth begins within a few months of transplantation (Van Neste *et al*, 1991). As the hair loss and subsequent recovery process has not been histologically examined, the pathophysiology of the process remains unknown. To utilize this model effectively, information about histology and cell kinetics of the entire course of the process is necessary.

The main purpose of this study was to establish a useful experimental model of human scalp hair follicle by modifying the previously reported model. We isolated hair follicles from human scalp and grafted them on to the back skin of severe combined immunodeficient (SCID) mice. As longitudinal tissue sections of the grafted follicles are easily obtained in this model, detailed histology of the follicles were analyzed. The hair shafts became dystrophic, are mostly shed within 20 d and regrow within 70 d after transplantation. The second purpose was to study histologic changes and cell kinetics of the hair loss and subsequent recovery process. Here, we report light and electron microscopic, and immunohistochemical findings from the whole process. The histologic study showed that the grafted hair follicles enter into dystrophic catagen, subsequently dystrophic telogen, then return to normal anagen follicles. The immunohistologic study suggests that

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Abbreviation: SCID, severe combined immunodeficient.

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stem cells or their close progeny in secondary germ of dystrophic telogen follicles play important roles in the regeneration process.

MATERIALS AND METHODS

Isolation of hair follicles Human terminal hair follicles were isolated from surgically obtained scalp specimens under a WILD M32 dissecting microscope (Leitz, Welzlar, Germany) within 24 h after surgery. The specimens were stored at 4°C in DMEM (Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum, 2.5 μg amphotericin B per ml, 100 U penicillin per ml and 100 μg streptomycin per ml (GIBCO BRL, Grand Island, NY) until the isolation was performed in the culture medium at room temperature.

The scalp samples were cut into small pieces containing a bundle of hair follicles with scissors under the dissecting microscopy. Most of the dermal and subcutaneous tissue was abraded with scalpels and forceps. To preserve the integrity of the connective tissue sheath, the dermal tissue closely attached to the hair follicles was not removed, which should avoid injury to the follicular epithelium and the dermal papilla. The arrector pili muscle was difficult to identify among the dermal tissue and therefore could not be left uninjured. Most of the sebaceous glands, the interfollicular epidermis and the upper part of the infundibular epithelium were removed. The bundled hair follicles were divided into single ones with a scalpel. The isolated single hair follicles were stored in the culture medium at 37°C in 95% air/5% CO_2 until they were grafted.

Transplantation Only anagen hair follicles were selected under the dissecting microscope, and transplanted within 96 h after the isolation. The follicles that contained well-pigmented hair matrix enveloping the upper part of the dermal papilla were identified as anagen follicles (**Fig 1**). C.B17-SCID mice aged 4–8 wk were used as recipients. Under general anesthesia with sodium pentobarbital, the back skin of each mouse was shaved with a razor and bored with a no. 18 injection needle. Gripping the hair shaft with a forceps, an isolated hair follicle was inserted through the pore into the subcutaneous space, and its upper end of the follicular epithelium was adjusted to the level of the mouse epidermis. The hair shaft was left on the skin surface and fixed with a surgical adhesive (Vetbond, 3M Animal Care Products, St. Paul, MN) and a film (Tegaderm, 3M Canada Inc., Ontario, Canada). Two to eight follicles were grafted on each mouse. One hundred and fifty-six follicles were grafted on to 34 recipient mice.

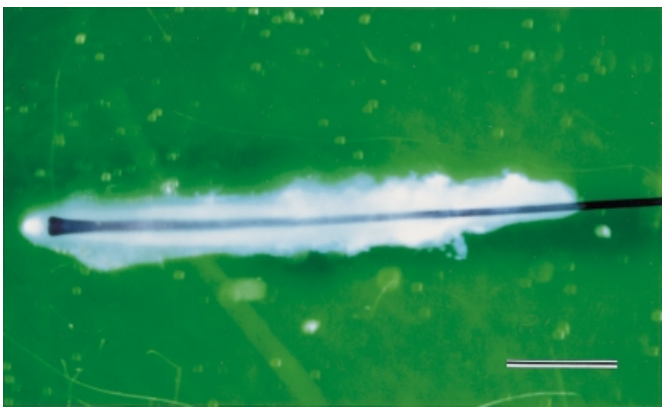


Figure 1. A macrograph of an isolated human scalp hair follicle for transplantation. A small amount of the dermal connective tissue remains around the anagen hair follicle. Therefore, the connective tissue sheath is preserved. *Scale bar.* 1 mm.

Histology and immunohistochemistry Biopsies of the grafted follicles were taken on day 7 (n = 8 follicles), 20 (n = 6), 25 (n = 3), 30 (n = 13), 35 (n = 2), 40 (n = 17), 45 (n = 5), 50 (n = 9), 55 (n = 4), 60 (n = 7), 70 (n = 4), 100 (n = 2), and 150 (n = 7) after transplantation. The specimens were fixed in 10% neutralized buffered formalin or 70% ethanol and embedded in paraffin. Deparaffinized sections were examined histologically with hematoxylin–eosin staining. Some sections obtained from each grafted follicle were incubated with monoclonal antibody against keratin 19 (K19) (clone Ks19.1, Progen Biotecnik GMBH, Heidelberg, Germany) or involucrin (Biomedical Technologies Inc., Stoughton, MA), and stained with a streptavidin–biotin peroxidase system (Histofine, Nichirei, Tokyo, Japan).

Some mice were intraperitoneally administered 400 μg 5-bromo-2′-deoxy-uridine (BrdU) (Sigma, St Louis, MO) in 0.5 ml normal saline 90 min before biopsies on days 7 (n = 5 follicles), 20 (n = 2), 30 (n = 13), 40 (n = 6), and 50 (n = 4). These specimens were fixed in 10% neutralized buffered formalin and embedded in paraffin. Deparaffinized sections were treated with 0.1% trypsin at 37°C for 5–10 min. Some sections of each follicle were immersed in 2 M HCl for 90 min to denature DNA, followed by incubation with monoclonal antibody against BrdU (Becton Dickinson, San Jose, CA). The other sections were incubated with monoclonal antibody against K19. All these sections were stained with the streptavidin–biotin peroxidase system.

Scanning and transmission electron microscopy Two specimens obtained on day 40 were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 and postfixed in 1% osmium tetroxide in the same buffer, dehydrated in graded alcohol and propylene oxide, and embedded in Epon 812. Thick sections were cut in a MT 5000 ultramicrotome (Du Pont Instruments, Newtown, CT), stained with Toluidine Blue solution, and observed under a light microscope to select sections suitable for electron-microscopic examination. The selected sections were embedded in Epon 812 again. Ultrathin sections were cut, double-stained with 4% uranyl acetate and lead citrate solutions. They were observed in a JEM 1010 electron microscope (JEOL DATUM, Tokyo, Japan). Some of the hair shafts that had been shed before day 20 were coated with a layer of gold on a JFC-1100 ion sputter (JEOL DATUM) at 1200 V and 5 mA for 12 min, and observed in a JSM 840 scanning electron microscope (JEOL DATUM).

RESULTS

Rate of successful engraftment Eighty-seven of 156 transplanted follicles were successfully engrafted (51%). Most of

Table I. Summary of histologic and immunohistochemical findings of grafted hair follicles at each stage[a]

	Degeneration stage (Days 7–30)	Regeneration stage (Days 30–50)		Stable growth stage (Days 40–150)
		Early	Late	
Bulb-like structure[b]	+	+	+	+
Melanin incontinence	+	+	+	+
Vitreous membrane	+ or –	–	–	–
Epithelial protuberance	+ or –	+	+	+
IRS/hair shaft	–	–	–	+
Apoptotic cells[c]	+	+	+	–
K19-positive cells[b]	+	decreased[d]	decreased[d]	–
BrdU-labeled cells[b]	–	–	+	+

[a]+, presence or positive; –, absence or negative.
[b]Findings in the follicular bottom.
[c]Findings in the lower hair follicle.
[d]The number and the staining intensity of the positive cells.

the unsuccessfully transplanted hair follicles were identified by the disappearance of their hair shafts on day 7, when all the adhesive films were scheduled to peel off. As the hair shafts disappeared with the cover films, the grafted hair follicles were probably pulled out by mice before completion of their attachment. It was confirmed by histologic examination that the hair follicles whose hair shafts had remained on day 7 retained their viability by the time of biopsy.

Histology of the hair loss and subsequent recovery The histologic and immunohistochemical findings in the hair loss and subsequent recovery process are summarized in **Table I**. In most of the successfully grafted follicles, the infundibular epithelium usually had a smooth connection to the mouse epidermis (**Fig 2*a***). The other follicles were entirely engulfed in the subcutaneous space and had no connection with the mouse epidermis. There were no differences in histologic and immunohistochemical findings in the entire experimental period between these two groups. The experimental period could be divided into four stages: the dystrophic stage, the early and the late regeneration stages, and the stable growth stage. The grafted hair follicles entered into a dystrophic form of catagen and subsequently that of telogen; their bulb lost proliferation activity and regressed, and the hair shafts became dystrophic soon after transplantation and were shed (the dystrophic stage: days 7–30). The grafted hair follicles recovered proliferation activity in their lower end (the regeneration stage: days 30–50) and reconstructed normal anagen follicles and hair shafts, which continued to grow until day 150 after grafting (the stable growth stage: days 40–150). In the dystrophic stage matrix cells and melanocytes in the bulb of the grafted follicles were decreased in number, which caused atrophy of the bulb (**Fig 2*b***). Eosinophilic shrunk keratinocytes with picnotic or fragmented nuclei, which correspond to apoptotic cells, were scattered in the lower part of the follicles (**Fig 2*b***). The regression of the lower part of the follicles progressed during this stage. There was a reduction of melanin content in the follicular bottom, melanin incontinence in the dermal papilla, and melanin clusters in the lower part of the follicle. Differentiation into a hair cortex and an inner root sheath (IRS) was interrupted, and the hair shafts became dystrophic in all the grafted follicles examined on days 7 and 20. The contour of the hair shafts was rough, and their end was tapered (**Fig 2*c***, ***d***). Then, most of the hair shafts were lost before day 20. The contour of the lower part of the hair follicle was smooth on day 7 (**Fig 2*a***) and undulated on day 20 (**Fig 2*c***), and several epithelial protuberances were formed there on day 30 (**Fig 2*e***). The vitreous membrane in the lower part of the follicles were thickened and corrugated on day 7 (**Fig 2*b***). On day 20 this change was advanced, and the membrane was left at the area where the lower part of the follicles had disappeared by its shrinkage (**Fig 2*c***). The vitreous membrane became inconspicuous or disappeared on day 30 (**Fig 2*e***, ***f***). At the end of this stage a cup-shaped bud of follicular epithelium and the underlying dermal papilla remained beneath the epithelial protuberances (**Fig 2*e***, ***f***). Many dilated capillary lumina were newly formed in the connective tissue enveloping the lower part of the follicle on day 20, and then decreased in number on day 30 (data not shown).

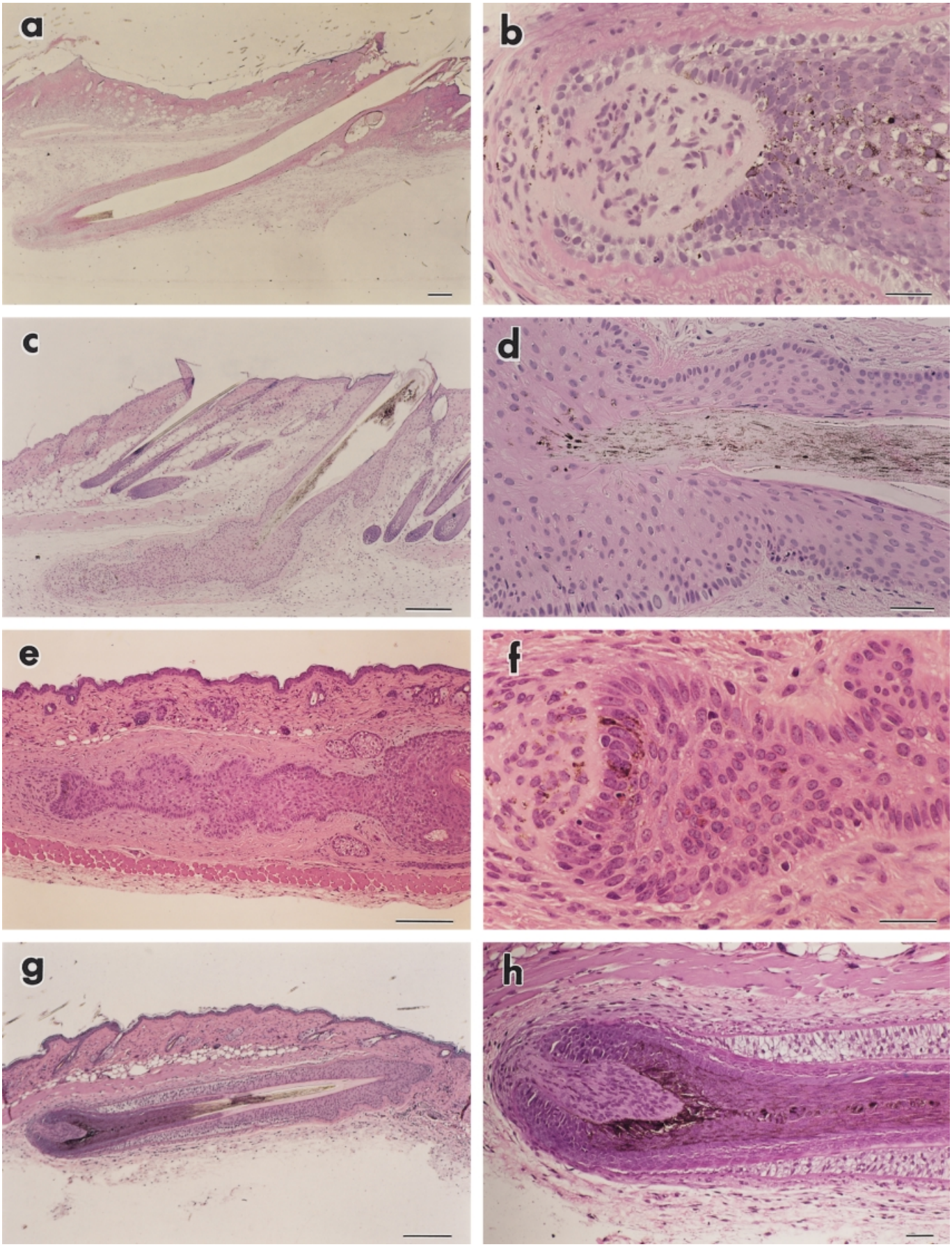
As shown later the immunohistochemical findings suggest that regeneration of the hair follicles started from day 30 to 50; however, histologic findings of the grafted follicles during this period were the same as those at the end of the dystrophic stage. In most of the follicles that lacked an IRS and a hair shaft, apoptosis was still found in their middle and lower parts. In contrast, in all grafted follicles that showed differentiation into both an IRS and a hair shaft, apoptosis was not detected. The latter follicles were classified as those in the stable growth stage.

On day 40 the grafted hair follicles in the stable growth stage appeared, although the number was small. From day 70 onward all the grafted follicles were histologically those of anagen VI (**Fig 2*g***, ***h***), and their hair shafts grew out from skin surface of mice. All of the hair shafts were well pigmented. No apoptotic cells were found in the hair follicles. The melanin incontinence in the dermal papilla and some protuberances on the outer root sheath (ORS) were, however, still preserved. Even at this stage the sebaceous glands were small or absent, and the arrector pili muscle was not found.

Immunohistochemistry of K19 and BrdU-labeled cells in the hair loss and subsequent recovery At the dystrophic stage BrdU staining showed that several positive cells were found in the basal and the first suprabasal cell layers of the upper part of the grafted follicles, and the peripheral cell layer of the sebaceous glands. The positive cells were not detected in the follicular bottom on days 7 and 20 (data not shown). K19 staining showed that it was expressed on the basal cell layer of the lower part of the follicles, including their lower end (**Fig 3*a***, ***b***), and the undulated portions on day 20 or protuberances on day 30 (data not shown). Although histologic findings of the grafted follicles were the same as those at the end of the dystrophic stage, immunohistochemical examination showed that dynamic changes in cell kinetics started in the regeneration stage. K19 expression was decreased in the cup-shaped epithelial bud at the follicular bottom: the number of K19 expressing cells was decreased, and the intensity of its positive cells became weak in this area (**Fig 3*c***, ***e***). The hair follicles that showed the decreased K19 expression were classified as those in the regeneration stage. In some follicles examined with BrdU-labeling, although a few labeled cells were occasionally observed in the lateral side of the follicular bottom, they were not detected in the basal layer adjacent to the dermal papilla (**Fig 3*d***). In the other follicles, the labeled cells appeared not only in the former site but also in the latter site (**Fig 3*f***). Development of an IRS and a hair shaft was observed only in the follicles that showed both the decreased K19 expression and the appearance of BrdU-labeled cells in the latter site. These findings indicate that the reduction of K19 expression in the cup-shaped epithelial bud begins at first (the early regeneration stage), then the number of BrdU-labeled cells in this portion is increased (the late regeneration stage), thereafter an IRS and a hair shaft reappeared, and apoptosis ceased (the stable growth stage). Either the decreased K19 expression or increased BrdU labeling was not observed in the protuberances above the epithelial bud throughout this stage. Both hair follicles in the dystrophic stage and those in the early regeneration stage were observed on day 30. Similarly, hair follicles in the early and the late regeneration stages, and those in the stable growth stage were observed on day 40. Therefore, the recovery process of each hair follicle did not progress in the same phase.

In the stable growth stage K19-positive cells in the hair bulb disappeared completely from all the grafted follicles, and most of

Figure 2. Histologic findings of grafted hair follicles. Hematoxylin and eosin stain. (*a*, *b*) Early degeneration stage (day 7). The upper part of the hair shaft has been artificially lost during sectioning. The outline of the hair follicle remains to be smooth. The hair bulb shows atrophy, and differentiation into either a hair shaft or an IRS is not observed. Not only reduction of melanin content but also melanin incontinence and clustering are observed at the lower end. (*c*, *d*) Early degeneration stage (day 20). A thick and corrugated vitreous membrane is shown around the lower part of the follicle. The lower portion of the ORS shows an irregular outline. The bottom end of the hair shaft is tapered in shape. (*e*, *f*) Late degeneration stage (day 30). The dermal papilla exists in contact with the follicular bottom, which keeps a bulb-like shape. In the mid part of the follicle many epithelial protuberances are observed. Vitreous membrane around the follicular bottom has disappeared. Melanin incontinence and apoptotic cells are still observed. (*g*, *h*) Stable growth stage (day 70). The regenerated hair follicle is histologically the same as a normal anagen hair follicle except for several protuberances in the upper portion. *Scale bars*: (*a*, *c*, *g*) 250 μm; (*b*, *d*, *e*, *h*) 50 μm; (*f*) 10 μm.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the positive cells were localized on the protuberances (**Fig 3*g*, *h***). Many BrdU-labeled cells were observed in the bulb (data not shown). Human involucrin was detected in the suprabasal layer of the ORS and an IRS (data not shown), indicating the reconstructed follicle was not composed of mouse keratinocyte but human keratinocytes derived from the grafted follicle.

Scanning and transmission electron microscopy Some hair shafts that had been shed before day 20 were observed with scanning electron microscopy. They did not form a club-shaped end, but were constricted in the proximal portion and broken at the end (**Fig 4**). Two grafted hair follicles obtained on day 40 were examined with transmission electron microscopy. In these samples condensed cellular fragments with a picnotic nucleus were observed to be ingested by keratinocytes (**Fig 5**). This finding confirms that apoptosis continued from the dystrophic stage through the regeneration stage.

DISCUSSION

Histologic information about the whole structure of hair follicles is required for their precise evaluation. It can be obtained by observation of their longitudinal sections. From our experience, it is difficult to obtain longitudinal sections of hair follicles from full-thickness scalp skin grafted on to SCID mice, as the hair follicles are bent in random directions probably due to shrinkage of the dermal connective tissue. In the present model single hair follicles with a minimal amount of dermal connective tissue were grafted. They were not bent, and their longitudinal sections were easily obtained. The second advantage of the present method is that only anagen hair follicles can be selected for grafts. Catagen and/or telogen hair follicles seem to be often bundled with anagen hair follicles in full-thickness skin grafts. The third advantage is that a sufficient number of grafts can be obtained from a small skin specimen. At least 30 terminal anagen hair follicles are gained from a 1 cm^2 scalp skin. In these respects, the present model is considered useful for studying human hair follicles, especially for morphologic evaluation.

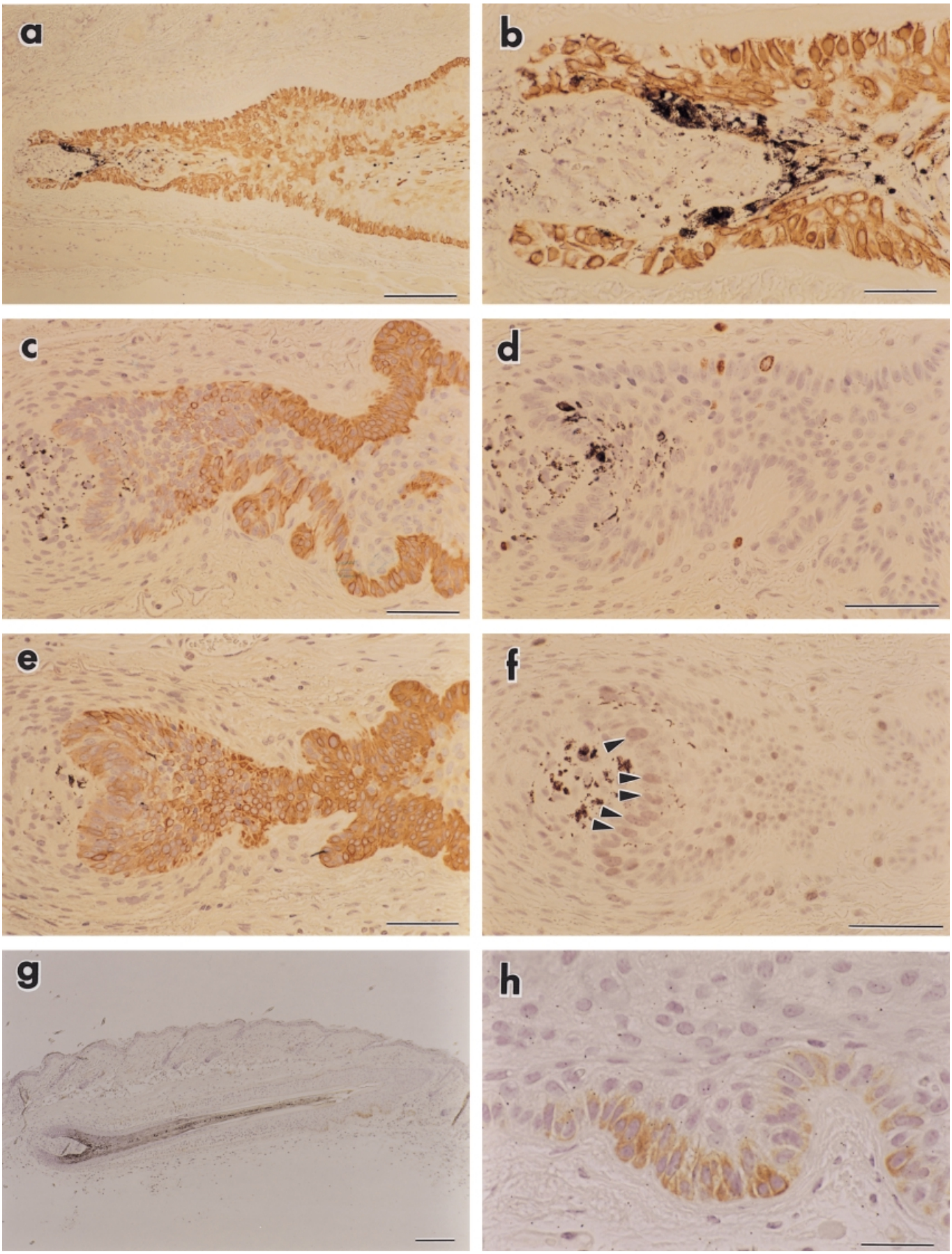
In the present model sebaceous glands and arrector pili muscles were mostly removed. Organ culture studies suggest that sebaceous glands play important parts in hair growth (Williams and Stenn, 1994; Williams *et al*, 1996). The fate of the grafted follicles, however, was essentially in accord with full-thickness scalp grafted on mice (Gilhar *et al*, 1988; van Neste *et al*, 1991), and their structure in the stable growth stage was almost normal except for the presence of melanin incontinence and the protuberances on the ORS. This finding indicates that the present isolation procedure does not affect the process of hair loss and subsequent recovery, and the morphology of the grafted hair follicles. The essential part of the sebaceous glands for hair growth seems to have survived the isolation process of the follicles. As the bulge of hair follicles is often inconspicuous in the adult human scalp (Abell, 1994;), arrector pili muscle has been used for a morphologic marker to identify the bulge (Sperling, 1991). In the present model the grafted hair follicle had several protuberances on the upper ORS below the sebaceous glands. In the stable growth stage, these protuberances were the main portions immunohistochemically positive for K19, which is a marker for the bulge in the normal hair follicle (Lane *et al*, 1991; Narisawa *et al*, 1994; Moll, 1995; Michel *et al*, 1996). Therefore, the protuberances seem to correspond to the bulge, although they were rather conspicuous compared with normal hair follicles in adult human scalp. The similar structures of the ORS are observed in regenerating follicles in full-thickness human scalp grafted on the SCID mice skin (Lyle *et al*, 1998), in injured skin by abrasion (Eisen *et al*, 1955) or suction blister (Lane *et al*, 1991), and in the lesional skin of alopecia areata (Gollnick and Orfanos, 1990) or psoriasis vulgaris (Wilson *et al*, 1994). These findings indicate that the formation of the protuberances in the present model may be an ordinary response of hair follicles to some kinds of stimuli, and not a specific feature induced by the present transplantation.

In the dystrophic stage of the present model all the hair shafts became dystrophic and were shed, and the follicles lost proliferation activity in their lower end. Early in this stage the lower part of the follicles regressed, and vitreous membrane was thickened and corrugated. At the end of this stage the follicles maximally retracted, and vitreous membrane disappeared. In these follicles a cup-shaped epithelial bud that corresponds to secondary germ (Kligman, 1959; Sperling, 1991; Cotsarelis, 1997) and the underlying dermal papilla remained beneath the bulge. In the regeneration stage the follicles recovered proliferation activity, and in the stable growth stage they returned to normal hair follicles and hair regrew. The histologic findings indicate that the grafted hair follicles entered into dystrophic catagen, and subsequently dystrophic telogen, then returned to normal anagen follicles, and suggest that human hair follicles have the ability to repair severe damage completely and rapidly through the same recovery process as ''secondary recovery'' in chemotherapy-induced alopecia in mouse pelage (Paus *et al*, 1994). Two possible factors that induce the dystrophic changes in the grafted follicles are speculated. First, the blood supply for grafted hair follicles had been stopped until capillary vessels were reconstructed around them, which meant metabolic damage to the matrix cells. Alternatively, some factors in fetal bovine serum supplemented to the culture medium (Westgate *et al*, 1993), or the medium itself, in which the scalp samples were immersed before transplantation, might affect the proliferation activity.

We analyzed the cell kinetics in the recovery process in the present model. At the end of the dystrophic stage K19-positive cells were localized in the secondary germ and the bulge. K19-positive cells in the hair follicles have been thought to be follicular stem cells or their close progeny (Lane *et al*, 1991; Narisawa *et al*, 1994; Michel *et al*, 1996; Lyle *et al*, 1998). The present finding suggests that these cells reside not only in the bulge but also in the secondary germ. In the regeneration stage the reduction of K19 expression in the secondary germ occurred at first. Next, the number of BrdU-labeled cells was increased there. Then, an IRS and a hair shaft reappeared. This finding suggests that stem cells or their close progeny in the secondary germ play an important part in the development of the new anagen follicle, and seems to be compatible with a central dogma that stem cells (K19-positive cells) are converted to transient amplifying cells (K19-negative and BrdU-labeled cells), which supply postmitotic cells that differentiate into an IRS and a hair shaft (Cotsarelis *et al*, 1990). Either the reduction of the K19 expression or the increased BrdU-labeling were not observed in the bulge. The dermal papilla may induce the conversion of the adjacent K19-positive cells in the secondary germ.

In this model apoptosis was observed not only in the dystrophic stage but also in the regeneration stage. In the latter stage apoptotic

Figure 3. Immunohistologic findings of grafted hair follicles. (*a–c*, *e*, *g*, *h*) K19 staining. (*d*, *f*) BrdU staining. Detection of antibody reaction was accomplished by streptavidin–biotin-peroxidase complex technique using diaminobenzidine (brown: *a–e*, *g*, *h*) or diaminobenzidine with nickel and cobalt (brownish black: *f*) as a substrate. (*a*, *b*) Early degeneration stage (day 7). In the lower portion of the follicle the basal and the suprabasal layers express K19, whereas in the mid portion only the outermost cell layer expresses the keratin. (*c*, *d*) Early regeneration stage (day 40). K19 expression is decreased at the lower end (*c*). In the same hair follicle BrdU-labeled cells are not detected in the epithelial cells adjacent to the dermal papilla (*d*). (*e*, *f*) Late regeneration stage (day 40). K19 expression is decreased at the lower end (*e*). In this hair follicle several BrdU-labeled cells are detected in the epithelial cells adjacent to the dermal papilla (*arrowheads*) (*f*). (*g*, *h*) stable growth stage (day 70). K19 expression is restricted in the basal layer of the epithelial protuberances in the upper part of the follicle. *Scale bars*: (*a*, *g*) 200 μm; (*b*, *h*) 50 μm; (*c–f*) 20 μm.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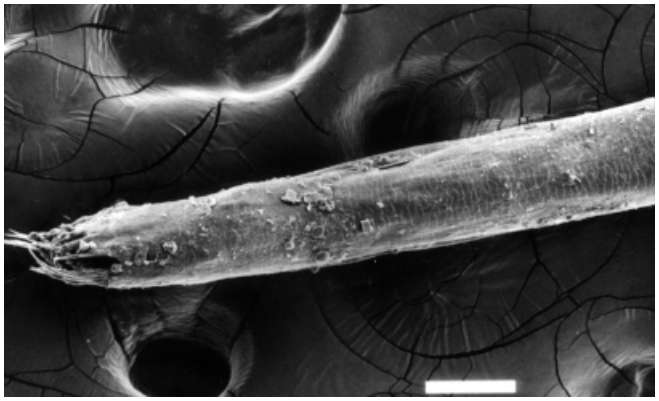

Figure 4. Scanning electron micrograph of a shed hair shaft. The proximal end of the hair shaft is constricted and broken. *Scale bar*: 100 μm.

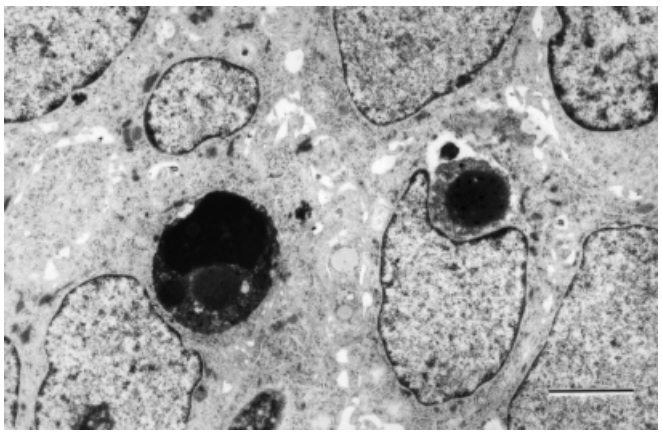

Figure 5. Transmission electron micrograph of the lower part of a grafted hair follicle (day 40). Phagocytosed apoptotic bodies are seen. *Scale bar*: 2 μm.

cells were detected simultaneously with actively proliferating cells. As apoptosis has not been detected in the hair bulb in either normal human telogen or anagen (Tamada *et al*, 1994; Kishimoto *et al*, 1999), the present finding may be an additional distinctive feature of the present recovery process. Apoptotic cells were not detected after differentiation into an IRS and a hair shaft begins, suggesting that they may be derived from matrix cells that fail to differentiate into an IRS or a hair shaft.

In the stable growth stage the grafted hair follicles histologically corresponded well to the normal anagen hair follicles except melanin incontinence and prominent bulge formation (epithelial protuberances). In addition, the immunohistologic findings in this stage coincided with those in hair follicles of the normal human scalp: most of the K19-positive cells were localized in the bulge, and many BrdU-labeled cells existed in the hair bulb. These results indicate that the grafted follicles return to normal anagen in this stage in terms of their morphology and cell kinetics. Therefore, for investigation of normal anagen scalp hair follicle, experiments should be designed to use the grafted hair follicles in this stage.

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