Transplants from balding and hairy androgenetic alopecia scalp regrow hair comparably well on immunodeficient mice

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Human hair follicles were grafted onto 2 strains of immunodeficient mice to compare the regeneration potential of vellus (miniaturized, balding) and terminal (hairy, nonbalding) follicles from males and a female exhibiting pattern baldness. Each mouse had transplants of both types of follicles from a single donor for direct comparison. Grafted follicles from 2 male donors resulted in nonsignificant differences in mean length (52 mm vs 54 mm) and mean diameter (99 μm vs 93 μm) at 22 weeks for hairs originating from balding and hairy scalp, respectively, corresponding to 400% versus 62% of the mean pretransplantation diameters. Follicles from the female donor transplanted to several mice also resulted in nonsignificant differences in length (43 mm vs 37 mm) for hairs from balding and hairy scalp, respectively, during a period of 22 weeks. The mean diameter of the originally vellus hairs increased 3-fold, whereas the terminal hairs plateaued at approximately 50% of pretransplantation diameter, resulting in a final balding hair volume double that of the nonbalding hairs. This report shows that miniaturized hair follicles of pattern alopecia can quickly regenerate once removed from the human scalp and can grow as well as or better than terminal follicles from the same individual. (J Am Acad Dermatol 2003;48:752-9.)

There is a need for a reliable animal model to study the process of the common condition known as male pattern baldness, or androgenetic alopecia (AGA). In recent years, the athymic (nude) mouse1,2 and the severe combined immunodeficient (SCID)3,4 mouse have been used to study a variety of human dermatoses including AGA. Xenograft experiments to these animals allow the differentiation of extrinsic and/or systemic from intrinsic factors in the cause and pathology of these disorders.2

Van Neste5 reported on the use of the nude mouse model and its potential for screening hair-growth drugs. Grafting experiments onto female nude mice using balding scalp from human male and female donors showed that linear hair-growth rates of transplanted hair follicles, both terminal and vellus, were less than growth rates recorded in situ on the scalps of the patients with AGA but that the hair diameters in grafted specimens matched those observed in situ at 6 months posttransplantation. Thus, hair diameter and linear hair-growth rate can be regulated by independent mechanisms. It was noted, however, that some interaction is maintained because terminal follicles continued to synthesize thicker hairs more rapidly than did miniaturized anagen follicles.6 Testosterone (T)-conditioned female nude mice showed reductions in both linear growth rate and in the initiation of second hair-growth cycles.7 The inhibitory action of androgens in this model was blocked when the androgen receptor blocker RU58841 was administered concomitantly with T propionate; grafts treated in this manner showed increases in linear growth rate and in the number of second hair cycles but no effects on hair diameter.8

In a more recent and somewhat contradictory study, balding grafts from male donors were transplanted onto male SCID mice.9 Hair regrowth was compared, 68 days posttransplantation, between grafts on vehicle-treated controls and on mice treated topically with antiandrogenic formulations containing either finasteride, a 4-azasteroid inhibitor of 5α-reductase (5αR) type II, or flutamide, an androgen receptor blocker. Notably, the 2 treatments increased hair length and the number of hairs per graft, analogous to the results reported for T-
conditioned female mice treated with RU58841, but also significantly increased diameter measurements.9

We sought to optimize the procedure of grafting AGA hair follicles to immunodecient mice to have a highly reproducible system. To our surprise and contrary to the reports of Van Neste, et al.6 we found that miniaturized hair follicles of balding scalp, when removed from their natural human milieu, possess a remarkable ability to regenerate and produce terminal anagen hairs equal to or better than those produced by hair follicles from hairy scalp. This was demonstrated after comparing 2 different immunodecient mouse strains and controlling for extraneous variables by transplanting follicles from the balding and hairy scalp of the same donor to each mouse.

METHODS AND MATERIALS

Approval

All patients reviewed and signed an informed consent document before harvesting of tissue. All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee of the Orentreich Foundation for the Advancement of Science Inc.

Animals

Nude (Tac:N:NIH (S)-nu/nuDF) and SCID (Tac: Icr:Ha:(ICR)-scidDF) female mice were acquired as needed (Taconic Farms, Germantown, NY). The animals were 6 weeks of age at receipt and were allowed to acclimate to laboratory conditions for 2 weeks before the onset of studies. Study I consisted of 20 nude mice; study II used 7 SCID mice. All mice were singly housed in sterile polycarbonate microisolets and maintained on a photoperiod of 12 hours of light and 12 hours of dark. Food (Purina Autoclavable Chow 5010, Purina Lab Diet, St Louis, Mo) and sterile distilled water were provided ad libitum.

Preparation of hair grafts and transplantation onto nude or severe combined immunodecient mice

Tissue was removed by 2-mm, full-thickness, punch excisions from both balding and hairy areas of the scalp and stored in Hank’s balanced salt solution pH 7.4 (Gibco BRL, Rockville, Md) at 4°C no longer than 24 hours after harvesting. The tissue was trimmed and hair follicles dissected under a dissecting microscope as described by Gilhar and Krueger10 and Van Neste et al.6 Only gray and darkly pigmented hair follicles were collected for transplanting to distinguish human hairs from those of the SCID mouse.

All operations were performed under sterile conditions in a laminar flow unit. The mice were anesthetized (Metofane, Pitman Moore Inc, Mundelin, Ill) and each dorsum was cleansed with 10% povidone-iodine solution and 70% ethanol. After this, 4 to 5 small incisions (total: 8-10/animal) were made into the right and left dorsum. The trimmed grafts (containing no more than 2 follicles) were examined under magnification and then transplanted into the subcutaneous tissue. The right dorsum was transplanted with nonbalding anagen follicles whereas the left was transplanted with balding, primarily telogen, follicles. After the operation, 0.5% neomycin ointment was applied to the grafts and the animals were fitted with an Elizabethan collar to discourage biting at the sites. The animals were closely monitored throughout the postoperative period. The collar was removed 3 to 5 days after operation.

Both balding and hairy follicles transplanted onto an individual mouse were obtained from the same person with patterned baldness. Hair follicles from 2 male patients were used for study I. The males were 37 and 54 years of age with frontal balding (3+ on a scale of 0-4) and had been bald in excess of 12 years. One female, aged 38, with frontal balding (3+ on a scale of 0-4) served as the donor source for hair follicles in study II. AGA was confirmed in this donor by histologic examination of hematoxylin-eosin-stained, paraffin-embedded biopsy sections.

Evaluation and measurement

In both studies, we observed that all grafts underwent a postoperative effluvium and initial regrowth within the first 7 weeks after transplantation. In study I, the hairs were allowed to grow undisturbed for the 22-week experiment period. The length of each hair type was measured periodically throughout the study. At the end of the study, the animals were killed, and the diameter and length of the hair was measured under original magnification X5.

In study II, the diameter and length of periodically cut hairs were used as parameters of hair growth. The growing hairs were cut at the level of the epidermis at 6.5, 9, 13.5, 18, and 22 weeks posttransplantation. Hair diameters (from balding and nonbalding areas) had been measured before transplantation. In both studies hair volume was determined using the formula (πd/4) × l, where d is equal to hair diameter and l is equal to hair length.

Statistical analysis

All data are expressed as mean ± SEM or mean and range of values. Student t test was used to determine the significance of the data. Critical levels less than .05 were considered significant.
RESULTS

Fig 1 shows a representative nude mouse with terminal hairs growing from both balding (arrowbeads) and hairy (arrows) grafts from scalp of male donor 5 months posttransplantation.

Fig 2. Photomicrograph of human hair regeneration 6 months posttransplantation in skin of nude mouse. There are 2 fully developed anagen follicles, 1 longitudinal (arrowbead), 1 cross-sectional (arrow). (Hematoxylin-eosin stain; 5-μm paraffin section at original magnification ×65.)

The results of study I are presented in Fig 3. Nearly 50% of the grafts gave rise to viable follicles after the postoperative effluvium period, which lasted about 7 weeks. A total of 36 hairs from balding scalp and 45 hairs from nonbalding scalp were measurable at the conclusion of the experiment (15/35 balding and 16/35 nonbalding for male No. 1; and 21/60 balding and 29/60 nonbalding for male No. 2.) Before transplantation, the combined mean diameter of the hairs from the balding scalp was $24 \pm 2.3 \, \mu m$ (range: 10-50 μm). At 22 weeks posttransplantation, the combined mean diameter of these hairs had increased to $99 \pm 2.2 \, \mu m$ (range: 71-147 μm, $P < .01$). In the case of the terminal hairs, the mean diameter at baseline was $151 \pm 11.6 \, \mu m$ (all hairs $>100 \, \mu m$). The final diameter of these hairs averaged $93 \pm 1.7 \, \mu m$ (range: 71-115 μm), a 38% reduction from the baseline value ($P < .05$). Although the final mean diameter of the balding hair follicles was slightly greater (6.0%) than the nonbalding, the difference was not significant. The hair length of the balding follicles increased on average by 2.26 mm/wk, whereas the terminal follicles grew at a rate of 2.44 mm/wk. This is comparable with the
rate of human hair growth, which averages 2.5 mm/wk (.35-.38 ± .03 mm/d). Greater variability was observed in the hair-length measurements (coefficient of variation ~60%) compared with diameter measurements (coefficient of variation ~11%-13.5%). There were no statistically significant differences in final hair length or diameter measurements between male No. 1 and male No. 2, nor between balding and terminal hairs at the conclusion of the experiment ($P > .24$ for all comparisons). The calculated mean volume growth rates per week were essentially equal (.0085 vs .0080 mm$^3$) for balding and terminal hairs, respectively.

In study II, balding and hairy-area hair follicles were harvested from 1 female human patient and were transplanted onto female SCID mice. Of 31 initially transplanted hairs from balding scalp, 17 were growing and measurable at 22 weeks, as were 14 of 22 hairs from nonbalding scalp. Before grafting (Fig 4), the mean diameter of the balding hairs was $26 ± 1.7 \mu m$ (range: 11-38 \mu m), whereas terminal hairs averaged $108 ± 3.5 \mu m$ (range: 75-165 \mu m). The balding area hairs reached a maximum diameter of 83 \mu m at 22 weeks posttransplantation. This corresponds to an increase of more than 300% from the time of transplantation. In contrast, terminal follicles transplanted onto SCID mice gave rise to hairs having smaller diameters (55 \mu m) at 22 weeks when compared with pretransplant values (108 \mu m), a reduction of 50%. Interestingly, during the first 13.5 weeks, the diameters of the hairs from balding and hairy areas were similar. The balding-area hairs, however, had a final diameter greater than the hairs from the nonbalding scalp ($P < .01$) because the balding-area hairs continued to increase in diameter at a reduced rate during the last 5 weeks, whereas the hairs from nonbalding scalp stopped increasing in diameter by week 18.

The rate of hair growth for balding and hairy grafts for study II was determined from the mean cumulative hair length of the 5 time intervals (Table I). The cumulative hair length for each type of graft was calculated by adding the average growth for the time interval to the sum of the growth for the previous intervals. At 6.5 weeks posttransplantation, the average hair length of the balding grafts was 1.5 mm compared with 1.2 mm for the hairy grafts, but the number of hairs present, irrespective of origin,

![Fig 4. Shown, for study II, are comparison of diameter changes with time for balding and terminal hairs for grafts from female where time 0 corresponds to diameters at transplantation. Diameters no longer increase after week 17 and are significantly different from at transplant: thicker for balding hairs (*$P < .01$) and thinner for terminal hairs (**$P < .05$).](image)

<p>| Table I. Mean cumulative hair length (millimeters) for study II |
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<th>Weeks</th>
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was insufficient for analysis at this early time point. The small number of measurable hairs corresponds to the postoperative effluvium period and subsequent lag phase in hair growth that have been reported for this animal model.4,6 In general, the hairs originating from nonbalding scalp grew more slowly than hairs from balding scalp. At 9 weeks, the difference in cumulative hair length between the 2 types was more than 30%. However, by the final 8.5 weeks of study (13.5-22 weeks posttransplantation), the difference between the groups had diminished and ranged from 10% to 14%, a nonstatistically significant difference.

The comparison of mean changes in hair length and volume per week during these intervals for study II are shown in Fig 5. The initially higher growth rate per week of the balding-area hairs from weeks 6.5 to 9 accounts for the balding-area hairs being longer until week 18. The reduced growth rate for terminal hairs after week 18 minimizes the possibility of the terminal hairs catching up. The early plateau for diameter increase for terminal hairs (Fig 4) resulted in significant differences in volume of hair produced per week after week 13 (Fig 5). As a result, the calculated mean volume growth per week of balding-area hairs (0.0084 mm³) was double that of hairs from nonbalding scalp (0.0041 mm³).

**DISCUSSION**

The original intent of this study was to optimize the procedure of grafting human hair follicles onto immunodeficient mice to have a highly reproducible model system for evaluating treatments for AGA. To that end, various methods of transplantation were compared (data not shown), as were several strains of mice (National Institutes of Health Swiss nude and SCID) both male and female, and scalp skin from balding and hairy regions of numerous AGA male and female donors (28 males; 11 females). What became apparent, contrary to the results reported by Van Neste et al,6 was that regardless of the method used and sex or strain of mice, grafts from balding scalp produced pigmented terminal hairs equal in length and diameter to those produced by transplanted hair follicles from hairy, nonbalding scalp. We report here the results from 2 representative male donors in an experiment using female nude mice (Fig 3).

To minimize extraneous variables and to further verify these results, we then transplanted follicles from both the balding and hairy scalp areas from the same individual, a female with clinically prominent AGA, to each of 7 female SCID mice. The results show that the regrowth of hair, irrespective of follicle origin, is similar after transplantation onto immu-
nodeficient mice (Fig 4). To our knowledge, this is the first study that compares the hair growth of balding and terminal scalp follicles from the same human being transplanted onto the same mouse and for both sexes with AGA.

A key difference between this study and those previously reported appears to be the characteristics of the pretransplantation hairs present on the selected scalp tissues. Van Neste, De Brouwer, and Dumortier specified the use of balding human scalp tissue only; the study then compared the growth characteristics of harvested hairs, postransplantation, onto female nude mice, with the hairs growing in situ on the scalps of the male and female AGA donors. Information about the grade and duration of AGA in these donors was not provided. From the reported wide distribution of diameters of the hairs harvested from these balding sites, however, it appears that transitional scalp areas (ie, in the early process of balding yet still possessing a considerable number of terminal hairs) were the preferred sampling sites. On the basis of our observation that postransplantation vellus hairs increase in diameter and terminal hairs decrease in diameter, a starting population of mixed hairs (vellus, terminal, thick terminal) as described would likely yield a final mean diameter close to the pretransplantation mean diameter for the population, leading to the conclusion that pretransplantation diameters are maintained. In our study, balding scalp tissue had only vellus hairs and hairy tissue had only thick, terminal hairs allowing a direct comparison of these hair types, pretransplantation and postransplantation.

Sintov et al also specified balding grafts but did not report the mean diameter of the pretransplantation hairs. The pregrafted hairs probably constituted a more homogeneous population of fine, vellus hairs than those of Van Neste et al because at 68 days postransplantation the grafted hairs on untreated male SCID mice had diameters from 10 to 20 μm versus 30 to 40 μm on antiandrogen-treated mice. Although this study is difficult to evaluate relative to our data because of its shorter duration and the lack of information regarding pretransplantation diameters, it suggests that the males of some strains of mice have systemic androgen levels high enough and/or the grafts retain sufficient 5α-R activity to impede the growth of androgen-sensitive hair follicles. In our experience, vellus hairs regrew as well on male as on female nude mice when evaluated at 6 months postransplantation (data not shown). Female mice were preferred because previous studies to which we were comparing, specified females.

Human hair growth is subject to both inhibitor and promoter factors, but each follicle appears to be genetically programmed to be more sensitive to one or the other of these factors and can be transplanted without losing this donor-dominant genetic characteristic. The hormonal environment of childhood allows all scalp hairs to grow to normal anagen size. At sexual maturity, the hormonal environment changes, and AGA commences in the genetically predisposed. AGA is not the result of the loss of hair follicles, but rather a systematic involution of the follicle, which results in the transformation of terminal follicles to vellus follicles with no appreciable reduction in density. The volume of the dermal papilla, which depends largely on its cell number and the amount of extracellular matrix, correlates with the size of the hair fiber produced. Several reports suggest that androgen-mediated alterations in hair size occur through interaction with the dermal papilla.

In human males, dihydrotestosterone (DHT) is required for AGA to occur in the genetically predisposed. Adults with pseudohermaphroditism type 2 have normal levels of T but significantly lower levels of DHT and do not show balding as do their healthy siblings. Systemic treatment to inhibit the metabolism of T to DHT in combination with local therapy to reduce the conversion by 5α-R of T to DHT has produced hair regrowth in bald human scalp both in length and diameter. The process, however, is slow and not necessarily effective in all individuals.

The phenomenon occurring in the xenograft experiments reported here is quite different and dramatic: hypotrophic anagen and telogen hairs from balding scalp exhibiting only vellus hairs in situ regenerate very quickly. By 6 months, the ratio of the diameters of grafted to pretransplant vellus hairs exceeds 3:1 (Fig 3). Histologic examination of postransplantation follicles from balding scalp also shows fully developed anagen follicles at 6 months (Fig 2). The regeneration of vellus follicles occurs just as quickly on male as on female mice (data not shown); this suggests that a factor or factors other than androgen withdrawal may be involved but does not necessarily rule out that differences in androgen levels, availability, or both between human beings and mice account in part or entirely for the rapid vellus-to-terminal transformation of balding follicles. For instance, the activity of the 5α-R enzyme(s) may be greatly reduced or absent in the transplanted follicles, thereby, limiting exposure of the follicles to DHT. The accelerated transformation of vellus follicles on immunodeficient mice might then correspond to responses seen in balding men treated with oral finasteride who are exceptionally good responders. However, in our clinical experi-
ence, females with AGA, including the female in study II, frequently have normal androgen and androgen-binding globulin levels for their age and sex. It is difficult to argue that lower systemic androgen levels in the female mouse environment (or higher in the case of the male mice) causes the rapid regeneration of vellus hair follicles from the human female. Therefore, the existence of an inhibitor factor other than androgens, particularly in women showing diffuse/pattern alopecia, is that lacking in the nude mouse seems plausible. This could be some other steroid, hormone, cytokine, neuropeptide, or an immunologically related factor.

The one-hair-cycle reversal of the miniaturized hair follicles of AGA, as demonstrated in this study, supports the hypothesis that miniaturization does not occur gradually over many hair cycles, as conventionally thought, but may, in fact, be an abrupt, large-step process that can potentially reverse just as quickly. The prompted new anagen as a result of postoperative effluvium that the transplanted follicles undergo in this experimental system may be an added advantage, accelerating or even initiating the regeneration of vellus follicles.

The data also indicate that the mouse environment is less than optimal for the growth of follicles originating from hairy scalp. The rate of increase in length of these hairs appears to be close to normal or only slightly diminished, but the mean diameter is markedly reduced. Currently, there is no explanation for the diameters of the hairs from the balding area continuing to increase, whereas those from the nonbalding area plateau after 17 weeks. It is important to note that the slope of mean diameter growth in the balding hairs diminishes at the same time that the nonbalding hair mean diameter stops increasing (Fig 5). Nonspecific factors such as differences in temperature, protein availability, or both between human beings and mice would be expected to affect balding and nonbalding grafts equally and may account for the fact that neither type of graft produces hairs equal to that found growing in situ in optimal regions (occipital) of the scalp. However, it is also possible that a growth factor or growth promoter originally available to the nonbalding hair follicles in situ may be lacking or reduced in these mice. The same factor that inhibits AGA follicles may act as a trophic factor for non-AGA follicles.

In summary, this report demonstrates that balding, miniaturized follicles possess the potential to quickly regenerate once removed from the human scalp and that the phenomenon applies equally to follicles from both men and women with AGA. These findings expand the use of this model beyond a tool for screening potential therapies to one that may yield insight into the mechanisms involved in AGA induction in both men and women.

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