2 weeks, conditions that favor proliferation of keratinocytes but not of mesenchymal cells. Finally, combining LF24 cells with HF buds and FDCs yielded enlarged, densely haired grafts that were also wrinkled. The factors contributed by LF24 cells to achieve these effects are being investigated (Kartasova *et al*, this issue, p 21).

In addition, we have begun studies to provide insights into epidermal and dermal contributions to abnormalities in HF development and hair growth in mouse mutants by grafting HF buds with fresh dermal cell preparations from different mouse backgrounds. As anticipated, dermis-derived developing HF from transforming growth factor-alpha knockout mice, that were cultured as monolayer cells either overnight or for 1 week in low-calcium medium, when combined with FDCs from wild type mice in grafts yielded wavy hair characteristic of the donor mutant mouse. The reciprocal combination produced primarily straight hair.

The results presented here demonstrate the potential usefulness of a relatively simple grafting procedure in the elucidation of positive and negative influences on HF development and hair growth.

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Cultured Human Hair Follicles and Growth Factors

Michael P. Philpott, Debbie Sanders, and Terence Kealey

Department of Clinical Biochemistry, University of Cambridge, Addenbrookes Hospital, Cambridge, United Kingdom

The factors that regulate hair growth are still poorly understood and this has been due, in part, to the lack of good *in vitro* models. We have previously reported on the *in vitro* growth of isolated human hair follicles (see [1] for review) and shown that isolated human hair follicles can be maintained *in vitro* in a serum-free medium for at least 10 d during which time they continue to produce a keratinized hair fiber at *in vivo* rates.

Growth factors may play an important role in many aspects of hair-follicle biology both in normal and disease states. To explore this we have used our model to study the effects of exogenous growth factors on the *in vitro* growth of cultured human hair follicles. This paper is intended to review some of the key findings that have so far emerged from this study.

ISOLATION AND CULTURE OF HUMAN HAIR FOLLICLES IN A FULLY DEFINED SERUM-FREE MEDIUM

The methods developed in our laboratory for the isolation and culture of human hair follicles have been extensively reviewed elsewhere [1]. Briefly, human hair follicles are isolated from human facelift skin by microdisection. This is achieved by taking full-thickness skin and with a scalpel blade cutting through the skin at the dermo-subcutaneous skin interface. Then, under a stereo dissecting microscope, the intact hair-follicle bulb is removed from the fat by gently grasping the outer root sheath (ORS) of the hair follicle with watchmakers forceps and gently pulling the hair follicle from the fat. Isolated hair follicles are maintained in Williams E medium supplemented with 2 mM L-glutamine, 10 ng/ml hydrocortisone, 10 μg/ml insulin, 100 Units/ml penicillin, and 100 μg/ml streptomycin at 37°C in an atmosphere of 5% CO_2/95% air. Under these conditions hair follicles grow for at least 10 d during which time they continue to produce a keratinized hair fiber and maintain *in vivo* morphology and patterns of DNA synthesis. In our hands fetal bovine serum, HEPES, and low-calcium medium all inhibit hair-follicle growth.

Reprint requests to: Michael P. Philpott, Department of Clinical Biochemistry, University of Cambridge, Addenbrookes Hospital, Hills Road, Cambridge, CB2 2QR UK.

INSULIN-LIKE GROWTH FACTOR ONE (IGF-I) IS AN IMPORTANT REGULATOR OF HAIR-FOLLICLE GROWTH *IN VITRO*, A POSSIBLE MECHANISM FOR THE *IN VIVO* REGULATION OF THE HAIR-GROWTH CYCLE

We have shown [2] that insulin stimulates human hair follicle growth *in vitro* but only at supraphysiologic concentrations (10 μg/ml). In the absence of insulin, or at physiologic concentrations, there is a marked inhibition of hair-follicle growth; moreover, the hair follicles also show premature entry into a catagen-like state characterized by condensation of the dermal papilla, thickening of the glassy membrane, and the formation of a club hair-like structure. However, in the absence of insulin, both IGF-I and IGF-II stimulate hair-follicle growth in a dose-dependent manner. IGF-I is more potent than either insulin or IGF-II and stimulates maximum rates of hair-follicle growth at 10 ng/ml, whereas IGF-II gave maximum stimulation at 100 ng/ml. IGF-I was almost a thousand-fold more potent than insulin and tenfold more potent than IGF-II at stimulating hair-follicle growth. Both IGF-I and IGF-II were more potent than insulin at preventing hair follicles from entering catagen. This data suggests that IGF-I may be an important

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physiologic regulator of hair-follicle growth and the hair-growth cycle. This is supported by the recent observation of Granger *et al* (unpublished observation) that during the rat hair-growth cycle there is a marked decrease in expression of mRNA for the IGF-I receptor during late anagen early catagen. The source of IGF-I *in vivo* may either be from the circulation or from the dermal papilla; this remains to be elucidated.

EGF ACTIVATES A HIGHLY PROLIFERATIVE POPULATION OF CELLS IN THE ORS OF ISOLATED HUMAN HAIR FOLLICLES

When isolated human hair follicles are maintained *in vitro* in the presence of either 10 ng/ml epidermal growth factor or transforming growth factor-α there is a marked change in both the morphology and patterns of DNA synthesis [3]. These changes in morphology are characterized by a marked expansion of ORS-like cells in the lower hair-follicle bulb that appear to push the hair-follicle matrix cells upwards within the hair follicle away from the dermal papilla resulting in the formation of a club hair-like structure that remains connected to the dermal papilla via a strand of cells. Tritiated thymidine autoradiography showed that EGF inhibited thymidine uptake in the hair-follicle matrix but stimulated a marked increase in thymidine uptake into the upper ORS [3]. EGF would therefore appear to activate a highly proliferative population of cells in the ORS of human hair follicles. Moreover, we have also shown by immunohistochemistry that in the same region of the ORS in which we see increased thymidine uptake we also see increased expression of keratin 19. This is very interesting as keratin 19 has been suggested as a possible marker for stem cells in the ORS of hair follicles and it has been reported that a highly proliferative population of stem cells resides in the ORS of human hair follicles [4]. We suggest that it is these ORS stem cells that EGF activates *in vitro*. It appears, therefore, that EGF may play a physiologic role in the initiation of early anagen where an intense burst of ORS cell proliferation results in the formation of a new anagen hair follicle. Our data and that of the literature also suggests that EGF may play an important role in the transition from anagen to catagen.

IMMUNOMODULATORY CYTOKINES: INTERLEUKIN (IL)-1α AND TUMOR NECROSIS FACTOR (TNF)-α INDUCE AN ALOPECIA AREATA–LIKE MORPHOLOGY *IN VITRO*

There is evidence to suggest that the immune system is involved in the regulation of normal hair follicle growth as well as in the pathogenesis of some hair diseases. Immunomodulatory cytokines not only act as mediators of an immune response but also regulate some non-imune cell proliferation and differentiation and may, therefore, be important regulators of hair growth. We have investigated the effects of a number of interleukins (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-10), colony stimulating (G-CSF, GM-CSF) and tumor necrosis factors (TNF-α, TNF-β), and gamma interferon (γIFN) on hair follicle growth *in vitro*. Dose-response studies showed that IL-1α, IL-1β, and TNF-α were potent inhibitors of hair-follicle growth. Histology showed that inhibitory doses of all three cytokines had similar effects on hair-follicle morphology resulting in the formation of dystrophic anagen hair follicles characterized by the condensation of the dermal papilla and disruption and abnormal keratinization of the pre-cortical cells of the hair matrix. These changes in hair-follicle morphology are similar to those reported in alopecia areata [5] and suggest that IL-1α, IL-1β, and TNF-α may play an important role in the pathophysiology of inflammatory hair disease. This is supported by the recent observation that severity of alopecia areata is associated with a polymorphism in the IL-1 receptor antagonist gene [6].

In conclusion we suggest that the ability to culture isolated human hair follicles *in vitro* and the fact that they are so highly responsive to *in vitro* manipulation by growth factors represents an excellent model with which to study many aspects of hair follicle biology. Moreover, this model should also be ideal for studying the role of endogenous growth factors in regulating hair-follicle growth using neutralizing antibodies and antisense oligonucleotides.

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