In This Issue . . .

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Hair Dermal Papilla Cells Produce Vascular Endothelial Growth Factor

In this issue, Lachgar *et al.* (p. 17) report that hair follicle dermal papilla cells (DPCs) produce an autocrine growth factor that is indistinguishable from vascular endothelial growth factor (VEGF). Normal hair follicles progress through three basic stages of cyclical growth: anagen, an active growth stage, in which the cells are actively dividing and synthesizing; catagen, in which growth stops and the follicle shortens to one fourth of its previous length; and telogen, a quiescent. DPCs, the dermal component of the hair follicle that may program hair type, growth, and cycling, may regulate these stages by releasing diffusible factors such growth factors. In the course of searching for growth factors in conditioned medium from cultured DPCs, the authors purified an activity and found that it was indistinguishable from VEGF. Supporting a role for VEGF, DPCs from anagen hair follicles bound antibody to VEGF. In contrast, DPCs from catagen and telogen follicles did not. VEGF, a potent angiogenic factor, has been considered mitogenic only for vascular endothelial cells and some lymphocytes. With this report, Lachgar and colleagues show that DPCs in culture actively synthesize and release VEGF into the medium and that VEGF stimulates DPC division and migration. Anti-VEGF abolished this stimulation. Because the onset of anagen is accompanied by an increase in the number of blood vessels in the perifollicular dermis, VEGF released by the DPCs may be an initiator of hair follicle growth. Conversely, a decrease in VEGF may lead to catagen. In support of this theory, VEGF immunoreactivity is decreased in hair follicles from patients with alopecia. An increase in this growth factor might stimulate follicles to enter anagen by acting directly on the DPCs or by stimulating local vascularization.

Melanocytes in the Outer Root Sheath of Hair Follicles Differ From Other Melanocytes in Human Skin

Horikawa and co-workers (p. 28) find that the melanocytes of the outer root sheath (ORS) of hair follicles in human skin contain premelanosomes or premelanosome-related products, but not the melanosome-related proteins found in other skin melanocytes. ORS melanocytes are believed to be the major reservoir from which melanocytes are recruited to repopulate the epidermis. This occurs, for example, during re-epithelialization after loss of the epidermis and during repigmentation of the skin when vitiligo is treated. Repigmentation requires recruitment of melanocytes and usually occurs first around the hair follicles, suggesting that inactive ORS melanocytes are activated and move into the surrounding epidermis. ORS melanocytes can be identified by staining with toluidine blue but not by DOPA staining; they become DOPA positive only after some kind of stimulation, such as after dermabrasion or ultraviolet irradiation. In hunting for possible structural and biochemical differences between these two types of melanocytes, the authors found that the majority of ORS melanocytes are located in the mid to upper region of the follicle and, although dendritic and nonpigmented, can be distinguished from DR+ Langerhans cells. ORS melanocytes had a distinctive pattern of expression of melanocyte-related proteins. They stained for premelanosome-related proteins but failed to react with antibodies to a cytoplasmic antigen associated with melanosomes (the pigment organelles), with antibodies to tyrosinase (the rate-limiting enzyme in melanin synthesis), or with antibodies to "tyrosinase-related proteins", TRP-1 and TRP-2. ORS melanocytes thus appear to contain the "early", or possibly more basic, structural proteins, but not the "later" proteins farther along the pigment pathway. The transition from ORS melanocytes to epidermal melanocytes may involve synthesis of structural proteins and enzymes in the ORS melanocytes.

Transforming Growth Factor-β Modulates Endothelial Cell Interactions With Dermal Matrix

Frank *et al.* (p. 36) report that transforming growth factor-β (TGF-β) reduces expression of integrins on human dermal microvascular endothelial cells, thereby affecting the ability of the cells to adhere to extracellular matrix proteins. Integrins are cell surface glycoproteins made up of two different subunits, α and β, and are therefore referred to as heterodimers. Type 1 integrins share a common $\beta 1$ subunit. Which heterodimers are expressed on the dermal cell's surface affects the cell's affinity for the surrounding matrix proteins, such as fibronectin, laminin, and different types of collagen. Because microvascular endothelial cells are exposed to several matrix proteins, changes in cell surface integrin expression during development or repair could provide a way to modulate migration or other functions. Both fibronectin and TGF-β have been detected near newly forming vessels after wounding, and it has been suggested that TGF-β induces angiogenesis. This concept was not easily reconciled with the fact that TGF-β inhibits endothelial cell proliferation and migration in culture. In order to clarify the role of TGF-β, Frank and colleagues investigated its effect on endothelial cell functions. They found that TGF-β reduced the expression of type1 integrins on the endothelial cell surface and also decreased integrin mRNA, resulting in reduced endothelial cell attachment to matrix proteins, especially fibronectin. This change in integrins affected another function as well; TGF-β decreased the chemotaxic effect of fibronectin on endothelial cells. These effects of TGF-β may modulate blood vessel growth by decreasing both cell attachment to matrix and cell migration at the site of newly forming vessels.

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Nitric Oxide Mediates Vasodilation in Human Skin

Goldsmith and co-workers (p. 113) demonstrate that nitric oxide mediates vasodilation in erythema induced by ultraviolet B radiation and also in response to the neuropeptide, calcitonin gene related peptide (CGRP). Nitric oxide is a strong dilator of blood vessels and is synthesized by a family of enzymes, nitric oxide synthases, one of which is found in the endothelium. Inhibition of these enzymes affects blood flow in both animal and human tissues. Since inhibitors of nitric oxide synthase reduce edema caused by histamine injection as well as in skin exposed to ultraviolet radiation, the authors thought that nitric oxide might mediate vasodilation in inflammation. Also implicated in the control of cutaneous blood flow are neuropetides, which are involved in axon reflex vasodilation. One potent neuropeptide, calcitonin gene related peptide (CGRP), is present in blood vessels in human skin. The authors examined the effect of nitric oxide synthase inhibitors on resting cutaneous blood flow, after local warming or ultraviolet B (UVB) irradiation, and after stimulation of blood flow by CGRP and found that intradermal injection of a specific inhibitor produced visible pallor and reduced resting blood flow. After warming or UV-B irradiation of the skin, inhibitors increased visible pallor and decreased measured blood flow, and both inhibitors reversed the increase in blood flow caused by CGRP. These data implicate nitric oxide in maintaining resting blood flow in human skin as well as in mediating the vasodilator responses to local warming, UVB irradiation, and CGRP. Drugs affecting the nitric oxide pathway may be useful in the treatment of Raynaud's phenomenon, which is associated with a deficiency of CGRP.

Autoantibodies to BP180 in Three Disorders

Balding *et al.* (p. 141) report that like bullous pemphigoid (BP) and herpes gestationis (HG), cicatricial pemphigoid (CP) is an autoimmune disease in which the major target for the IgG autoantibodies is the extracellular domain of the epidermal antigen BP180. BP and HG are associated with autoantibodies directed against two hemidesmosomal polypeptides, BP180 and BP230, found in the basement membrane zone. BP230 is intracellular and is part of the hemidesmosomal plaque. BP180 is a transmembrane protein, the extracellular domain of which is recognized by BP and HG autoantibodies. Binding of these antibodies is thought to lead to a reaction that results in separation of the epidermis and dermis at the basal lamina. In CP, blisters are usually observed in mucosal tissues and often heal with scarring. IgG, can be demonstrated at the epithelial-stromal junction in perilesional tissue of CP patients. In recent studies, the sera from a subset of CP patients were shown to contain autoantibodies to laminin-5; but most CP sera reacted with a 180 kD epidermal antigen, possibly BP180. To identify the antigens recognized by autoantibodies in CP sera, Balding and colleagues tested the sera for reactivity with a panel of bacterial fusion proteins containing segments of human BP180. Seventy percent of CP patient sera reacted with one or two different sites on the extracellular domain of BP180, one located in the same noncollagenous domainthat was previously shown to be reactive with BP and HG autoantibodies, and the other site located at the carboxy-terminal end of the protein. The authors suggest that BP180 may also harbor additional CP-reactive sites. Based on these new findings, there are now three bullous diseases associated with an IgGautoimmune response directed against the extracellular domain of BP180. Future studies will address the pathogenic relevance of specific autoantibody populations.

New Control Points for Formation of Cornified Envelopes by Transglutaminase

The cornified envelope of keratinocytes is produced by cross-linking of proteins such as involucrin by the enzyme, transglutaminase, and the major signal that stimulates the formation of the keratinocyte envelope in skin is thought to be calcium ion. Gibson *et al.* (p. 154) studying keratinocytes in vitro, report that in the case of transglutaminase, there is an unexpected dissociation between transcription of the mRNA and production of protein on the one hand, and activation of the protein on the other.

It has been shown that calcium induces the transcription of both involucrin and transglutaminase mRNA, and it was assumed that the mRNA led to an active product. In the studies of Gibson *et al*, however, even though relatively low concentrations of calcium ion stimulated production of transglutaminase and involucrin protein, the transglutaminase was not active, so that it did not cross-link proteins to make envelopes. Activation is associated with a shift from the cytosol to the membrane, where the protein apparently becomes activated, and requires concentrations of calcium ion higher than those needed at the earlier steps. In cultured squamous cell carcinoma cells, neither involucrin nor transglutaminase mRNA was regulated by calcium, suggesting that failure to form protein in the tumor cells is a fundamental flaw in the RNA transcriptional machinery. These studies indicate that calcium concentrations have differential effects on protein pathways involved in terminal differentiation, allowing for control of differentiation at many points.