In this Issue

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Life and Death Signaling in Epidermis: Following a Planned Cell Death Pathway Involving a Trail That Does Not Lead to Skin Cancer

Creation of a cutaneous barrier requires production of the stratum corneum. Ironically, the vitality of skin depends on the properly regulated formation and function of dead keratinocytes (KC) in the exquisitely thin outermost layers of epidermis. The death of the corneocytes must be delayed until the KC undergo terminal differentiation. We recently coined the term ''planned cell death pathway'' to signify the temporal and spatially coordinated reactions that are operative in the epidermis giving rise to the orderly process of corneogenesis. As this concept implies, if there is premature keratinocyte cell death prior to terminal differentiation, this portends disastrous consequences for the epidermis; not only altering epidermopoiesis, but also the barrier function of skin. Such abnormalities render the patient highly susceptible to life-threatening infections. Whereas it is intuitively obvious that maintenance of a physiologic epidermal thickness requires a balance between cell proliferation and cell death, the precise biochemical mechanisms that govern this regulated balancing act are not known at this time. For example, we do not know if the death of KC under physiologic conditions is initiated by death ligands/receptors at the plasma membrane, or triggered by a primary event in the mitochondria or nucleus. Moreover, it is unknown if this specialized form of cell death requires specific caspases, or other effector enzymes. How can the entire collection of viable KC in the stratum granulosum within one cell layer have its organelles, not to mention its nuclear contents, including all of the DNA completely digested without provoking an inflammatory reaction, and creating such a highly organized structure of cross-linked lamellar bilayers on the outside despite the massive intracellular turmoil just a few microns away? These are just a few of the unanswered questions facing investigative skin biologists.

As a self-renewing tissue, the continual entry of transiently amplifying cells derived from stem cell precursors cannot be permitted to propagate unchecked. The initial cell cycle-mediated growth arrest, followed by early/late differentiation, with final induction of cell death and desquamation, represent the critical determinants that serve a subtractive function in normal epidermis to preserve homeostasis. On the one hand, imbuing KC with various cell survival genes provides a defense against premature apoptosis, or other forms of cell death, prior to terminal differentiation. Especially in skin that is subjected to a wide variety of potentially harmful or toxic stimuli (i.e., ultraviolet/ionizing radiation, chemical irritants, infectious organisms), KC have many molecular tools at their disposal to prevent or minimize cell death. On the other hand, if there are excessive pro-survival gene products operative in KC that can interfere with terminal differentiation and corneogenesis, there may also be pathologic consequences such as thickened skin with altered barrier properties (i.e., psoriatic plaques), or accumulation of KC with genetic abnormalities that result in skin cancer.

In this issue, Bachmann *et al* (p. 59) explore the role of several mediators of KC cell death with respect to the evolution of actinic keratoses (AK) and formation of squamous cell carcinoma (SCC). Specifically, they focus on the apoptotic response of KC involving sunlight, and two increasingly prominent members of the tumor necrosis superfamily CD95L and TRAIL. Both of these ligands can trigger cell death by engaging specific receptors on the surface of target cells. For CD95L, the ligand-receptor system is relatively straightforward; but the TRAIL pathway is more complex, as there are receptors that mediate cell death (i.e., TRAIL-R1 and -R2), but there are also so-called ''decoy'' receptors (i.e., TRAIL-R3 and -R4). These latter receptors can bind TRAIL, but rather than provoking cell death may either produce a null event, or signal a potentially important survival pathway involving NF-κB activation. There are several noteworthy observations contained within the Bachmann *et al* report. First, these investigators document the presence of CD95 and CD95L, as well as TRAIL and its receptors in skin. Whereas several previous groups have dissected out the epidermal location and change in expression for CD95/CD95L before and after UV-irradiation in mice and human subjects, this is one of the initial publications identifying the TRAIL family members in normal and diseased human skin. The nonrandom distribution patterns in normal skin for TRAIL and its death/decoy receptors is intriguing. Even though investigations into apoptotic processes are usually centered around various pathologic states (i.e., autoimmunity or malignancy), the presence of TRAIL and its receptors in normal skin appears to be strategically placed to also subserve a physiologic function, such as producing the stratum corneum to ensure the structural integrity of the epidermis. For example, the basal layers of epidermis that contain stem cells and transiently amplifying cells, contain high levels of the cell survival protein FLIP, and low or absent levels of the death receptors CD95 and TRAIL-R1 and -R2. Moreover, the decoy receptor TRAIL-R3 is strongly expressed in the basal layer. As one moves upward and outward in the epidermis, there are higher levels of the death receptors, TRAIL-R1 and -R2, accompanied by diminished to absent levels of the decoy receptors. Given the concomitant presence of both death ligands – CD95L and TRAIL – throughout the epidermis, and verification that they are expressed as functionally active at inducing apoptosis in various immunocytes *ex vivo*, it is tantalizing to speculate that these ligands also contribute to KC cell death leading to formation of stratum corneum. More definitive experiments are warranted to confirm this speculation, however, but it may be true that the constitutive expression and strategic location of the CD95L and TRAIL components are fundamentally important molecular determinants of the structural integrity of human epidermis. Secondly, response of KC within human skin to UV irradiation is examined with respect to these death ligands/receptors. UV light presents several challenges for the epidermis – it can induce DNA damage giving rise to premalignant and malignant cells with altered differentiation and growth characteristics. UV light can also directly induce premature apoptosis of KC. In both these scenarios barrier function is perturbed, and understanding the molecular mechanism underlying the UV light response of KC may shed new light on finding better

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methods to treat the many skin pathologies that arise following excessive solar exposure. As Bachmann *et al* discovered, UV light exposure directly influences KC expression of the aforementioned death ligands/receptors. Specifically they observed an initial increase in KC CD95L expression, followed by a decrease, accompanied by an increase in CD95 expression. Expression of TRAIL and both death/decoy receptors tended to diminish in acutely irradiated skin. Interestingly, chronically UV-exposed skin of two individuals with heavily sun-tanned areas revealed similar changes. Whereas it is difficult to draw definitive conclusions based on a single individual's acute response to UV light, or from two subjects with tanned skin, further studies are warranted to more precisely determine specific contributions of these TNF members' role in the apoptotic response of epidermal KC to UV light. It is likely that there will be cross-talk between p53, NF-κB, and the expression of various death ligands and death/decoy receptors that contribute to the life and death signaling responses in skin.

Thirdly, the expression of these death mediators is examined in 12 samples of AK and four cases of SCC. Whereas the precancerous AK failed to express CD95L, the invasive SCC strongly and diffusely were positive for CD95L, which was associated with an absence of CD95 by the tumor cells. The authors suggest that not only can the cancer cells avoid killing themselves, or their adjacent counterparts (they fail to express CD95), but the malignant cells could launch a pre-emptive strike by expressing CD95L against tumor infiltrating immunocytes that do express CD95. The malignant cells also expressed TRAIL and FLIP, which may operate in a similar fashion to preserve the viability of the tumor cell and providing it with a molecular sword to fend off tumor-seeking cytotoxic T cells. Once again, the transformed cells protect themselves, by down-modulating the TRAIL death receptors, just as they eliminated CD95 expression rendering them immune to killing via these important mediators of apoptosis in skin.

Taken together, these three observations add valuable data to the literature and advance our understanding of the role specific death ligands/receptors play in both normal and pathologic skin conditions. Following this line of inquiry may reveal new insights into immune privilege, autoimmunity, and carcinogenesis involving skin. Finding methods to selectively kill premalignant or transformed cells in the epidermis and/or dermis, but at the same time avoiding triggering premature apoptosis of normal cells, should be possible as we learn more about these mediators of life and death signaling in skin. Interestingly, in a completely different system involving retinoic acid-induced apoptosis of leukemia cells, Altucci *et al* (Nat Med 7:680–686, 2001) noted a key role for TRAIL in which an early and late differentiation program was followed by a post-maturation apoptotic event mediated by TRAIL. It will also be important to try and find methods to avoid premature apoptosis of normal KC without necessarily enhancing the survival of premalignant or transformed cells in the skin.

Sensing and Killing Bacteria by Skin: Innate Immune Defense System: Good and Bad News

The primary function of human skin is to produce a protective coat preventing excessive water loss (i.e., dehydration), and shielding against an infectious onslaught from a myriad of lethal bacteria, fungi, and viruses. This no easy task as the skin of terrestial inhabitants such as ourselves are constantly exposed to mechanical stress, thermal variations, changes in relative humidity, solar and ionizing radiation, as well as chemical irritants and toxins. The specialized barrier function of skin is accomplished by formation of the stratum corneum in the outermost layers of epidermis, when keratinocytes undergo terminal differentiation and cell death. Despite the deceptively simple light microscopic appearance and thinness of the stratum corneum, it harbors several elegant, complex, and dynamic processes designed to detect and then destroy potentially harmful bacteria. Not only does the lipid rich stratum corneum form a physical shield amongst the mummified and anucleated cells, but there is also an ''acid mantle'' produced by energy requiring mechanisms that can inhibit colonization by certain pathogenic bacteria such as *Staphylococcus aureus*. As reported by Fluhr *et al* (p. 44), generation of an acidic pH by free fatty acids regulates stratum corneum acidification and functional integrity. But there are other innate defense systems in the epidermis that contribute to this biologic ''Saran Wrap'' as demonstrated by Durschner *et al* (p. 91). Besides the physical barriers, including acidic pH, lipids, and various hydrolytic enzymes, epidermal keratinocytes also produce a wide variety of antibacterial peptides, including α-defensins, β-defensins, and cathelicidins. In general, this small cationic peptide-based defense system is better at destroying certain types of bacteria such as group A *Streptococcus*, but are relatively ineffective against *Staph. aureus*. Given the increasing incidence of antibiotic resistant strains of *Staph. aureus*, and the role *Staph. aureus* plays in several common and chronic skin diseases such as atopic dermatitis and psoriasis, further research to hunt for methods to bolster the innate immune response against *Staph aureus* are certainly warranted. We should not just think about finding our way to the local pharmacy for a new type of synthetic antibiotic, but scratching beneath the surface of our own skin may yields new insights from this evolutionarily ancient component of our innate defense system into treatment strategies to fight bacterial infections.

One way to search for new ways of treating cutaneous infections is to probe the molecular and cellular response a healthy individual mounts when the structural shield is compromised. Previous studies of murine and human subjects revealed that following perturbation of the barrier function, there is a rapid and prominent release of cytokines by epidermal keratinocytes, including TNF-α. This cytokine is of primary importance and can promote barrier repair. TNF-α initiates a cytokine cascade designed to attract and retain acute inflammatory cells such as neutrophils and monocytes, by releasing chemotactic polypeptides and expressing adhesion molecules. In addition, as Dorschner *et al* demonstrate, cutaneous injury also induces release of the cathelicidin antimicrobial peptides to bolster the initial keratinocyte-led battle against bacteria in the environment. Under most conditions, this rapid multimolecular response is highly effective at promoting wound repair and restoring cutaneous homeostasis. These and other studies have clearly established that we should not regard keratinocytes as only inert or passive contributors to cutaneous immunity, but as versatile active initiators and dynamic participants of the skin's immune system. Although this is the good news for the vast majority of healthy individuals, there is also some bad news that relates to the cutaneous innate immune response. The bad news is that some genetically based diseases are characterized by abnormal responses to skin infections. For example, patients with atopic dermatitis are frequently and heavily colonized by *Staph. aureus*. Not only is it possible that the inability of the host immune system to eradicate *Staph. aureus* is a critical contributor to the dermatologic problem, but the associated inflammatory reaction may actually drive or exacerbate the skin pathology by enhancing attachment of *Staph. aureus* to the keratinocytes (Cho *et al*, *J Invest Dermatol* 116:658–663, 2001), thus creating a vicious cycle. Another example of viewing the innate immune system as a foe rather than a friend is psoriasis. In this disease, there is actually an extremely low

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