Cell Death by Apoptosis in Epidermal Biology

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Homeostasis in continually renewing tissues is maintained by a tightly regulated balance between cell proliferation, cell differentiation, and cell death. Until recently, proliferation was thought to be the primary point of control in the regulation of normal tissue kinetic homeostasis and as such has been the major focus of both understanding the etiology of disease and developing therapeutic strategies. Now, physiologic cell death, known as apoptosis (ă-pŏp-tō′sĭs, ă-pō-tō′sĭs [Thomas CL (ed.): Taber's Cyclopedic Medical Dictionary. F.A. Davis, Co., Philadelphia, 1989)] has gained scientific recognition as an active regulatory mechanism, complementary, but functionally opposite, to proliferation with important roles in shaping and maintaining tissue size and prevention of disease. In this review we will describe the concept of apoptosis and discuss possible molecular mechanisms of its regulation that may have implications for skin biology. *J Invest Dermatol 101:107–112, 1993*

HISTORICAL BACKGROUND AND DEFINITION OF APOPTOSIS

Two distinct forms of cell death, necrosis and apoptosis, are recognized (reviewed in [1]). Despite the early recognition of programmed cell death as a normal physiologic event in development, cell death historically has been considered to be primarily a pathologic process. Glücksmann [2] indicated the biologic significance of more than 50 individual instances of programmed cell death in embryogenesis but Kerr [3] was the first to emphasize that naturally occurring physiologic single-cell death exists also in adult tissues and to show in electron microscopy studies [4] that this form of cell death is clearly distinct from pathological cell death (necrosis). Although initially called "shrinkage necrosis," the term "apoptosis," from the Greek for "falling off" as in leaves or petals, was coined by Kerr *et al* [4] to indicate the orderly pattern of cytoplasmic and nuclear changes that leads to self-destruction of single cells and deletion from the tissues without injury to neighboring cells. Whether the term programmed cell death, applied in a developmental context, should be used synonymously with apoptosis is still under debate. They share many morphologic characteristics, and are more similar to each other than either is to necrosis, yet some differences between programmed cell death and apoptosis have been described. Because programmed cell death during development may take a slightly different form than apoptosis in adult tissues some consider it simply to be a special developmental form of apoptotic cell death (reviewed in [5]) that is initiated by as yet unknown spatially and temporally defined cues. However, both programmed cell death and apoptosis are clearly controlled cell death, in the sense that they each follow an intrinsic gene-directed pathway, initiated by physiologic or environmental stimuli, whereas there is no evidence that necrosis is programmed. Terminal differentiation is another example of cell death that may be considered a specialized form of the apoptotic program that has evolved to serve tissue-specific functions, perhaps best exemplified by the terminal differentiation of the erythrocyte and the epidermal keratinocyte.

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Abbreviations: BrdVrd, bromodeoxyuridine; NAD, nicotinamide adenine dinucleotide.

APOPTOSIS IN EPIDERMIS AND OTHER TISSUES

Apoptosis is a feature of many diverse biologic processes but in each case the common function of elimination of unnecessary, damaged, or harmful cells is served. In development programmed cell death removes cells during remodeling of a number of organs undergoing morphogenesis including the limb bud, nervous system, and the metanephros during mesenchymal-epithelial transition [1,2,6]. Tissue regression by apoptosis following hormone stimulation or deprivation occurs in a large number of hormone-sensitive tissues such as the prostate after androgen withdrawal [1,7] and in the atretic ovarian follicle [1]. Apoptosis contributes to the generation of diversity in the immune system by functioning in clonal selection of immature thymocytes, B-cells, and antigen-primed peripheral T cells and also protects us from autoimmunity by deletion of self-reactive cells. The mode of target cell killing by cytotoxic T lymphocytes in cell-mediated immune reactions is by apoptosis ([8] and references therein). In addition to the lymphatic tissues, other continually renewing tissues, especially epithelia such as those of the gastrointestinal tract, the liver, and the epidermis, have apoptotic cells within their normal cell populations. Apoptosis is thought to play a major role in regression of neoplasms, either spontaneous or induced by cytotoxic drugs or X-rays [1]. In the skin, cells dying by apoptosis have been found in Bowen's disease, squamous cell carcinoma, and malignant melanoma and cell death likely accounts for the slow growth rate of basal cell carcinoma, despite the high mitotic rate. Also in the skin an increase in apoptotic cells has been described in the epidermis in various lichenoid diseases such as lichen planus, in graft-versus-host disease, fixed drug eruptions, during regression of warts, in response to ultraviolet radiation (formation of "sunburn cells"), and in the hair follicle during catagen phase of the hair cycle (reviewed in [9]).

MORPHOLOGY OF APOPTOTIC CELLS

In a scientific discipline dominated by the techniques of molecular biology, it is telling that the original morphologic criteria are still heavily relied upon in the recognition and characterization of this newly appreciated process. Even though biochemical and molecular correlates, such as synthesis and activity of tissue transglutaminase [10] and a Ca^{++}/Mg^{++}-dependent endonuclease [11], are now becoming available, the investigator must look for progressive changes in cell structure (Fig 1) evident at the light- or electron-microscopic levels, to identify an apoptotic cell.

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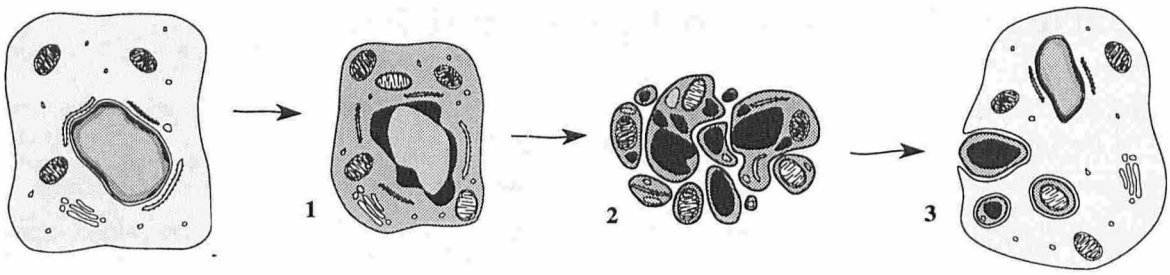

Figure 1. The first morphologic indications of apoptosis are cell shrinkage and loss of cell-cell contacts, accompanied by condensation of chromatin at the margin of the nuclear membrane 1. The cytoskeletal structure collapses and the cell often becomes rounded and detached from the pericellular matrix, although the plasma membrane remains intact and the apoptotic cell is impermeable to dyes such as trypan blue. In histologic sections, cells at this stage of apoptosis appear shrunken, with small amounts of eosinophilic cytoplasm and marginally condensed basophilic nuclei. Nuclear fragmentation (karyorrhexis) next occurs, followed by fragmentation of the entire cell without rupture of lysosomes or mitochondria 2. Cellular fragmentation results in the formation of apoptotic bodies, small membrane-bound vesicles containing nuclear fragments, and other cellular constituents, some of which are too small to be visualized at the light microscopic level. Apoptotic bodies are phagocytosed by neighboring cells or macrophages without evidence of inflammation 3. In contrast, necrosis is characterized by early cell swelling, loss of integrity of mitochondrial and plasma membranes and rupture of lysosomes leading to gross tissue inflammation [1]. The duration of apoptosis from initial cell shrinkage through removal of apoptotic bodies is short, requiring as little as 1–3 h in lymphocytes [11], but up to 48–72 h in epidermal keratinocytes [14]. Illustration and description adapted from [1].

In the skin, cells that conform to this pattern of morphologic changes and are now recognized as apoptotic have been independently described and named in the field of dermatopathology (reviewed in [9]). The term "Civatte bodies" was applied to the shrunken eosinophilic basal epidermal cells characteristic of lichen planus. Civatte bodies, as well as the abnormal cells of a number of other disorders, have also been referred to as "dyskeratotic" usually implying disordered or premature keratinization. The reader must be wary because "dyskeratotic cell" is a descriptive term and should not necessarily imply a specific cellular process. In fact, some, but not all, cells described in the literature as dyskeratotic are actually apoptotic [12]. Another descriptive term applied to keratinocytes undergoing apoptosis is "dark cell" because of the heavy staining properties of the condensed cytoplasm and nucleus ([13] and references therein). The "sunburn cell," used as an example of apoptosis by Kerr in his landmark paper [4], is a basal or suprabasal keratinocyte described as shrunken, with a densely stained glassy cytoplasm and hyperchromatic condensed pyknotic nucleus that appears following ultraviolet irradiation [14]. Apoptotic bodies have been identified in the epidermis and also within phagocytosing neighboring keratinocytes. Due to the abundance of tonofilaments in the keratinocyte, some apoptotic bodies are not easily phagocytosed and apparently become the basement membrane–surrounded "keratin bodies" or "colloid bodies" that have been described in the upper portion of the dermis [15].

CONTROL OF APOPTOSIS

Attention on the regulation of apoptosis has been increasing because of the important biologic implications of a gene-directed cell death process. Although most likely a general phenomenon, apoptosis has been studied predominantly in trophic-dependent tissues such as those of the hematopoietic system, the prostate and liver, and the developing nervous system, perhaps because in these tissues cell death occurs naturally at a high rate and because it is readily inducible in response to changes in trophic factors or other extracellular stimuli. Despite the rapidly increasing data in this field, the molecular mechanisms of apoptosis, the nature of the cell signaling, and the intrinsic factors that promote and regulate cell death, are still largely unknown. However, based on the constant morphologic changes (described above) and several common biochemical and molecular characteristics of dying cells, certain rules appear to govern the process of apoptosis.

Apoptosis Is an Active, Genetically Controlled Process Apoptosis is an energy-dependent process, requiring adenosine triphosphate synthesis from intact mitochondria [16]. Studies using inhibitors, actinomycin D, and cycloheximide, have demonstrated that in most cases, not always [17], apoptosis will not proceed in the absence of RNA and protein synthesis, supporting the argument that apoptosis is an active, genetically controlled process and suggesting that the cell carries an intrinsic capacity of self destruction.

Perhaps the best evidence that apoptosis is of genetic origin comes from the study of programmed cell death during development of the nematode *Caenorhabditis elegans* (reviewed in [18]), which also has provided further insight into the cellular, molecular, and genetic details of cell death, in general. The initiation, execution, and completion of the cell death program is regulated by a set of genes, some of which already have been identified by genetic analysis of *C. elegans* mutants. This simple organism is built of 959 somatic cells that remain to function in the adult whereas 131 of 1090 cells originally produced by the division of the fertilized egg are genetically programmed to die at specific times and locations in the developing worm. It appears that in the nematode the regulation of cell death is not simply a matter of turning on the death program. Rather, the major control is to prevent cell death. Cell death is suppressed in the presence of a functional ced-9 gene [19]. Loss of ced-9 function by mutation initiates the cell death program, the execution of which requires cell-type–specific activation of the wild-type ced-3 and ced-4 genes, whose gene products have not been fully characterized. The ced-3 and ced-4 mutants escape death and do not proliferate, but differentiate according to their normal lineage program indicating that they are beyond division and have entered the differentiation/cell death pathway. Programmed cell death in *C. elegans* culminates in the genetically controlled recognition and engulfment of the dying cell by its neighbor and digestion of its genome by the nuc-1–encoded DNA endonuclease.

The sequence of events and morphologic pattern of the dying *C. elegans* cell in many respects resembles apoptosis in mammals and suggests evolutionary conservation of the cell-death program. In fact, transfection [20–22] and transgenic mice studies [23] established that acquisition of constitutive expression of a protooncogene, bcl-2, that when altered correlates with human follicular B-cell lymphoma, promotes survival of affected cells. Significantly, expression of the human bcl-2 gene can inhibit programmed cell death in transfected *C. elegans* suggesting functional analogy between *C. elegans* ced-9 and human bcl-2 genes and implying that the molecular mechanism of cell death controlled by bcl-2 in humans and by ced-9 in nematodes might be the same [24].

The Intrinsic Program of Apoptosis Can Be Enhanced or Inhibited by Extrinsic Stimuli We now know many factors that can initiate or suppress the self-destruction program under experimental conditions (extensively reviewed in [25]). A large number of these are growth factors and hormones, which are also known

to affect cell proliferation. These factors can either stimulate apoptosis, as in the case of immature thymocytes treated with glucocorticoids [11], cytolytic cytokines such as tumor necrosis factor [26], and in primary cultures of uterine epithelial cells treated with transforming growth factor beta (TGFβ) [27] or they can inhibit apoptosis, as does nerve growth factor when added to growing neural cells [28] or granulocyte-macrophage colony-stimulating factor added to hematopoietic progenitor cells [29]. Another class of factors that affect apoptosis are agents that modulate intracellular Ca^{++} or cyclic adenosine monophosphate such as Ca^{++} ionophores that induce apoptosis in lymphoid cells [30] or 12-O-tetradecanoylphorbol-13-acetate that inhibits apoptosis probably through the protein kinase C pathway [31]. Pharmaceuticals used in cancer chemotherapy and physical agents such as ultraviolet light [32,33] or γ- and X- irradiation [34] used in radiation therapy arrest cell proliferation but also induce apoptosis possibly by interruption of chromatin structure, as inhibitors of topoisomerase II also can do [35], or by affecting the integrity of chromosomes and introduction of radiation-induced double-stranded DNA breaks [25,35]. Cell-surface proteins also appear to mediate the induction of apoptosis. This is exemplified by the APO-1/Fas antigen belonging to the tumor necrosis factor family of receptors. Apoptosis can be artificially triggered in T- and B-cell tumor lines by anti-APO-1/anti-Fas monoclonal antibody [36]. Finally, natural cell death during morphogenesis must be initiated by factor(s), in many instances unidentified, whose activation appears to be written into the developmental program. These factors are likely among morphogens and hormones already known to function in development. For example, Müllerian-inhibiting substance, a member of the TGFβ gene family, initiates regression of the Müllerian duct in males [37]. Similarly, retinoic acid likely plays an important role in programmed cell death through one of its specific receptors, RARβ. RARβ is expressed in tissues destined to die during mouse embryogenesis [38] and it has been suggested that the teratogenic effects of retinoids are due to expanded cell death in tissues that normally undergo limited cell death [39].

Components of the Apoptotic Pathway Function in Other Cellular Processes One can induce apoptosis and determine its outcome—the death of the cell—yet there is very little detailed understanding of the process. One common observation for most of the factors known to initiate apoptosis is that they alter the gene-expression pattern or activity of constitutively synthesized proteins in the cell promoted to die and these proteins may function in the apoptotic pathway as well as in other cellular processes. For example, UV radiation is known to introduce nicks in DNA that then activate a DNA repair enzyme, poly(ADP-ribose) polymerase. The process of extensive DNA repair by poly(ADP-ribose)polymerase, in turn, can deplete cellular nicotinamide adenine dinucleotide (NAD^+) and consequently adenosine triphosphate and finally the loss of energy may lead to cell death [25,35].

One of the strongest correlations of a specific biochemical activity with apoptosis is the activation or increased synthesis of a Ca^{++}/Mg^{++}-dependent endonuclease. Endonuclease activity has been shown to be associated with induction of apoptosis in lymphoid cells [11,40] and also in keratinocytes [41]. Activity of this enzyme results in DNA fragmentation into nucleosome-sized segments that can be visualized after electrophoresis as a ladder of fragments that differ in size by 180–200 basepairs. The DNA ladder is generally accepted to be a hallmark [1,40] as well as a cause of apoptosis in some cell types such as lymphocytes [17,40]. Endonuclease activity may function simply as a "cleaning-up" mechanism [18,42] because its loss by mutation does not prevent programmed cell death. In a number of other cases of programmed cell death during development internucleosomal DNA degradation does not occur [5] or it may be difficult to discern against the background of non-apoptotic cells [5,43]. Whether the endonuclease is a cause or consequence is still in question and complicated further by identification of two different types of endonuclease associated with apoptosis in mammalian cells: DNase I–like [42] and Ca^{++}–independent DNase II [30].

It appears that Ca^{++} is an important regulator of apoptosis. The activity and synthesis of another Ca^{++}-dependent enzyme, type II (tissue) transglutaminase, normally present in many cell types, is induced in apoptotic cells. Although high levels of type II transglutaminase do not necessarily lead to apoptosis, its cross-linking activity causes irreversible structural changes such as formation of the apoptotic envelope; a highly insoluble rigid structure that prevents leakage of cytoplasmic contents and development of an inflammatory response. Quantitation of these envelopes, the apoptotic index, is a measure of the rate of apoptosis. Transglutaminase may also be involved in membrane remodeling during apoptosis [10]. This and other cell-surface modifications such as expression of the sulfated glycoprotein encoded by the testosterone-repressed prostate message 2 gene and the cell-surface receptor for vitronectin appear to function in recognition of apoptotic cells during phagocytosis [37,43].

Perhaps most important is that the patterns of expression of the same protooncogenes/anti-oncogenes that control the cell cycle and proliferation are changed, suggesting that they play some regulatory role in the cascade of events during cell death. In regressing rat prostate glands, androgen withdrawal results in the sequential induction of the protooncogenes c-fos, c-myc, and the heat shock protein, hsp70, gene transcripts [44]. Depletion of growth factor induces c-fos and c-jun protooncogenes in dying lymphoid cell lines [45]. The intracellular level of p53, a tumor suppressor gene, increases dramatically in response to a variety of DNA damaging agents, including in human skin after exposure to ultraviolet radiation [46]. These correlations allow for one more general statement; the cell cycle and apoptosis share a common regulatory pathway that diverges at some point and the cell either divides or dies.

MODELS OF APOPTOSIS

Is the regulation of apoptosis in mammalian cells operating to prevent cell death and assure cell survival or to induce cell death and impose self destruction? In other words, from the practical point of view, to interfere with and study cell death experimentally, should we look for inhibitors of inducers or rather for activators of repressors of apoptosis? Arguments supporting both views can be found in the literature. For example, Raff in his review article [47] provides a variety of convincing evidence that the cell, once produced through cell division, would die in the absence of continuous suppression of the death program by survival signaling factors delivered by other cells. Dependence of hematopoietic cells on hormones and cytokines for survival and the developing nervous system on nerve growth factor are good examples of such a social control of cell survival. Again, genes such as bcl-2 in human cells or ced-9 in *C. elegans* appear to have an important role in prevention of apoptosis. In the presence of survival factors encoded by these genes a normal cell could proliferate and in their absence the cell would either differentiate or turn on the death pathway. In support of this model, bcl-2 expression has been localized to long lived cells, cells known to eventually undergo apoptosis, tissues with high cell turnover and in adult skin in the proliferating cell layer, suggesting suppression of the apoptotic program in these cells by bcl-2 [48]. The advantage of a mechanism that operates by suppression of a constitutively active cell death program by tissue-specific survival factors would be, according to Raff [47], to provide a simple way to eliminate overproduced, no longer needed, misplaced, or mutated cells (perhaps unable to recognize or respond to survival factors) and prevent metastasis. Conversely, the ability to synthesize cell-specific receptors would assure continued response to survival factor.

A different mechanism of "unbalanced signaling" is proposed by McConkey [49] to account for apoptosis in thymocytes induced by Ca^{++} ionophores, glucocorticoids, or tumor necrosis factor. In this model the extracellular inducers would activate the cell death program by a sustained increase of intracellular Ca^{++} and cyclic adenosine monophosphate, the second messengers of signal-transduction pathways, and subsequent activation of the factors, such as the Ca^{++}/Mg^{++}-dependent endonuclease, that execute cell death. Ele-

vated levels of Ca^{++} or cyclic adenosine monophosphate induce the endonuclease-dependent apoptosis pathway but they also are known inducers of cell proliferation in thymocytes. Whether the cell dies or proliferates would depend on the presence or absence of an appropriate second signal. For example, that could explain why a Ca^{++} signal present concomitantly with activation of protein kinase C inhibits DNA fragmentation. Thus, apoptosis either is triggered, as in the first model, by lack of survival factors, which can be the same growth factors and hormones that also regulate cell division, or by inducers that control proliferation or apoptosis, depending on the balance of other factors, as in the second model.

Importantly, the two models merge at the cell cycle. In fact studies with chemotherapeutic agents show that the cell must be in cycle to enter the apoptotic pathway [44]. This is supported by the finding that hormone-regulated apoptosis in prostate cells coincides with cell-cycle activities. Apoptosis induced by androgen depletion is preceded by expression of molecular markers of proliferation, including proliferating cell nuclear antigen expression and BrdUrd incorporation into DNA, suggesting that cells re-enter the cell cycle before progressing to DNA degradation. These dying cells also express the p53 tumor suppressor gene that inhibits S-phase progression [44].

How then can genes that control cell-cycle progression determine two opposing cellular activities, cell division and cell death? Evidence from prostate epithelia studies imply that apoptosis and mitosis occur through identical early molecular pathways but abnormal re-entry of the differentiated cell into S-phase and then inhibition by p53 results in activation of the apoptotic pathway because the differentiated cell has lost its capacity to divide. p53 appears to serve as a guard of the genome in response to stress [46]. If anything goes wrong in the cell cycle or DNA becomes intensively damaged beyond repair, p53 will abrogate the cell cycle and the abnormal cell will enter the death pathway. In the absence of p53 the abnormal cell will survive and eventually become tumorigenic. This model is supported by transfection studies with c-myc [50] and with p53 [51].

Upregulated c-myc expression in growth-arrested cells leads to cell death by apoptosis [50] perhaps because the block of the cell cycle together with lack of appropriate differentiation signals, suppression of differentiation by c-myc, or imbalance of regulatory proteins results in the activation of the program of apoptosis. Likewise, overexpression of wild type p53 in already-transformed cells results in induction of apoptosis [51]. A function of p53 in activation of apoptosis is consistent with induction of p53 in damaged cells and the fact that cancer cells often have mutated p53. In 58% of invasive SCC p53 was shown to be a target for ultraviolet-induced DNA damage [52] and p53 is a target for E6 protein in cells immortalized by human papillomavirus 16 [53].

Is there any particular point of the cell cycle at which entry into apoptosis is controlled? There is evidence that apoptosis can be promoted at any point of the cell cycle except perhaps mitosis but that the sensitivity of a given point of the cell cycle to induction of apoptosis is dependent on the nature of the inducing agent and the physiologic condition of the cell [25,50]. Ultraviolet induction of the sunburn cell in the epidermis is related to the cell cycle, occurring predominantly in S-phase cells [33]. Also overexpression of transfected c-myc will induce apoptosis in cells arrested in G_0 by serum deprivation, G_1 by interferon, late G_1 by isoleucine deprivation S-phase by an excess of thymidine, and S/G_2 by inhibition of topoisomerase II [47,50].

Apoptosis, then, could be pictured as abrogated cell proliferation and specific gene products associated with apoptosis, such as the endonuclease, transglutaminase, and vitronectin or modified glycoproteins on the cell surface [25,37] would be secondary effects resulting in physical disintegration of the abnormal cell, prevention of leakage of cell contents, recognition by phagocytosing cells, and deletion from the tissue. Thus specific "death genes" may be confined only to factors involved in the process of cell elimination rather than the genes that determine the onset of the apoptotic program. However, two mRNA species unique to dying thymocytes recently have been identified [54] but their roles in thymocyte apoptosis and their expression in other apoptotic cells have not yet been established to verify that they are "death genes."

To summarize, it appears that apoptosis is an intrinsically written program that shares a molecular pathway with the normal cell cycle and as such is regulated by the same molecular mechanisms that control cell growth and proliferation and is sensitive to the same environmental factors. Whether the cell divides or dies depends on the fine tuning of regulatory proteins and the health of the cell in general. Apoptosis is prevented by suppressors such as the bcl-2 gene product but cell damage or the onset of a developmental program can derepress the death program and the cell is destined to die. The inhibition of cell growth appears to be a prerequisite for apoptosis and perhaps under physiologic conditions is installed by either TGFβ or p53. The cell can either die rapidly by apoptosis or slowly by terminal differentiation, after serving tissue-specific function. In that sense terminal differentiation may be considered a specialized form of apoptosis. Tissue specificity and the existence of tissue specific genes may explain variations in the mode of apoptosis in different cell types.

IMPLICATIONS FOR SKIN BIOLOGY

For these studies to have bearing on skin biology it must be established that apoptosis is an important control point in normal epidermal homeostasis, not only an anomaly of certain skin diseases. In fact, Budtz [13] makes a strong case for such a role of apoptosis through a series of elegant studies of toad skin spanning more than a decade. Budtz convincingly demonstrated that the toad epidermis has an excessive mitotic rate, that cell deletion by apoptosis removes the excess cells, thus sculpturing the epidermal architecture, and that the rate of apoptosis is controlled by pituitary hormones. Pituitary ablation studies demonstrated that, like proliferation, there is a basal rate of apoptosis that is unaffected by hormonal control. Therefore, in amphibian skin apoptosis is a significant factor of the epidermal equation, balancing the difference between cell birth and cell loss through desquamation. These findings probably can be extended to mammalian skin as well because the apoptotic process described by Butdz was similar to that described for normal human epidermis [15] and recently cells that appear to be apoptotic have been localized in normal adult human epidermis [48] and developing human epidermis* by the terminal deoxynucleotidyl transferase–mediated dUTP-biotin nick end labeling [43] method.

Is there evidence that control of epidermal apoptosis conforms to the models of control proposed for other cell types? Gradient separation of mouse epidermal granular cells from basal cells allowed McCall and Cohen [41] to show that DNA fragmentation occurs specifically in the differentiating compartment by a Ca^{++}-activated endonuclease that is already present, but inactive, in the proliferating cell compartment. These authors proposed that in epidermis there are "death proteins" (such as the endonuclease) and inhibitors of these proteins and that the inhibitors have a shorter half-life so that when protein synthesis stops the death proteins can act [41].

What are these factors/inhibitors in epidermis? We propose that some of the same factors that control keratinocyte proliferation and differentiation, such as Ca^{++}, retinoic acid, TGFβ, and epidermal growth factor also may be involved in the initiation and progression of apoptosis in the epidermis. The pathway that a keratinocyte takes will depend, as in other tissues, on the balance of these factors perhaps according to the following model. As cells arise in the basal layer by cell division, they express Bcl-2 initially and are protected from death. Bcl-2 is expressed in the basal cell layer, but not suprabasal cells, of developing human fetal skin* and in the adult epidermis [48]. Our studies* showing differential intensities of staining in basal cells suggest that the level of Bcl-2 may decline in basal cells because of dilution through continued cell division or through degradation. Cells destined to die by apoptosis or undergo terminal differentiation move up to suprabasal layers (is loss of Bcl-2 involved in the signal to move up?). Suprabasal keratinocytes stop proliferating possibly in response to downregulation of c-myc or

* Polakowska RR, Piacentini M, Bartlett R, Goldsmith LA, and Haake AR (manuscript submitted).

synthesis of TGFβ [55,56]. TGFβ, synthesized in suprabasal cells, may be a prerequisite for the apoptotic pathway because it is expressed in cells just prior to the onset of apoptosis [37]. Following cell-cycle arrest most keratinocytes undergo terminal differentiation, express differentiation-specific genes, and eventually die, whereas some immediately die by apoptosis as a result of an intrinsic program, imbalance of signaling factors, or cell damage.

Is terminal differentiation a case of specialized apoptosis as suggested by some investigators [10,16] or are these two processes independent? In epidermis cellular and molecular events are shared by apoptosis and terminal differentiation. Our preliminary results† indicate that retinoic acid, 12-O-tetradecanoylphorbol-13-acetate, and TGFβ, in addition to affecting proliferation and differentiation of the epidermis, influence the number of apoptotic cells and their positions within the epidermis. Also, an increase in the Ca^{++} concentration induces apoptosis and induces keratinocyte terminal differentiation. Ca^{++}-activated endonuclease and transglutaminases may function both in keratinocyte apoptosis and terminal differentiation. In epidermis and differentiating cultured keratinocytes type I transglutaminase, rather than tissue type II, is the major form of the enzyme [57]. It is possible that type I functions in keratinocyte apoptosis as well as in terminal differentiation catalyzing the formation of the apoptotic envelope or analagous keratinocyte cell envelope. The nucleus of keratinocytes in the epidermal granular disintegrates, apparently by endonuclease digestion of chromatin into nucleosome size fragments [41], as in apoptosis. Specialization in terminally differentiating keratinocytes by expression of differentiation-specific genes, such as keratins, loricrin, and filaggrin, confers tissue-specific function. Thus, in agreement with Fesus' [58] model of molecular events in apoptotic cells of the immune system, we and others [10,16] suggest that terminal differentiation of cells in renewing tissues is a specialized form of apoptosis. Both share initial pathways that diverge to allow for functional specification of differentiated cells and again merge at the endpoint.

Finally, the cellular and molecular basis of some skin diseases can be viewed in a different context when apoptosis is emphasized. For example, apoptosis may play a role in psoriasis, traditionally approached as a hyperproliferative disorder [59]. The number of cells that undergo DNA synthesis in psoriasis exceeds the number that enter mitosis by four to five times, suggesting that there is also a high rate of cell death at the S or G2 phases of the cell cycle in psoriatic epidermis [13]. Targeting apoptosis for studies of alopecia, induced hormonally, by drugs or irradiation, may be productive because apoptosis occurs normally in the catagen phase of the hair cycle and apoptosis is modulated by these same factors. New avenues of research and treatment also are opened by the recognition that aberrations in the control of apoptosis may contribute to oncogenesis. For example, identification of mutations of p53 in skin tumors indicates possible unexplored regulatory pathways in skin carcinogenesis. A role of cell death in carcinogenesis also is proposed in the "two-signal" model [21,22], suggesting that c-Myc can provide the first signal leading either to apoptosis or proliferation. Acquisition of constitutive bcl-2 expression (second signal) promotes survival of affected cells and continued proliferation, increasing the chance for acquiring new mutations or fixation of an already mutated oncogene and finally resulting in carcinogenic evolution. Understanding the mechanisms that regulate apoptosis and identifying individual control points of the apoptotic pathway will undoubtedly have impact on future strategies for therapeutic intervention in treating skin disease.

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MEETING ANNOUNCEMENTS

The 8th Annual Dialogues in Cosmetic Dermatology will be held at the LeMeridien Resort San Diego, Coronado, California, Wednesday, October 27 to Sunday, October 31, 1993, sponsored by Northwestern University Medical School. Co-directors are J.B. Pinski, M.D., and Henry H. Roenigk Jr., M.D. Telephone, (312) 263-4625.

The Sixth International Psoriasis Symposium will be held at the Sheraton Chicago City Center Towers, Wednesday, July 20 to Sunday, July 24, 1994, sponsored by Northwestern University Medical School. Co-directors are Henry H. Roenigk Jr., M.D., and Howard Maibach, M.D.

The 18th Annual Hawaii Dermatology Pre-Seminar Business Meeting, Financial Planning, Coding, Medicare will be held February 17–19, 1994 at the Hyatt Wailea Resort, Maui, HI, sponsored by Northwestern University Medical School. Co-directors are Henry H. Roenigk Jr., M.D., and Howard Maibach, M.D. Telephone, (312) 908-8173; FAX, (312) 908-0664.

The 18th Annual Hawaii Dermatology Seminar will be held February 19–23, 1994 at the Hyatt Wailea Resort, Maui, HI, sponsored by Northwestern University Medical School. Co-directors are Henry H. Roenigk Jr., M.D., and Howard Maibach, M.D. Telephone, (312) 908-8173; FAX, (312) 908-0664.

The 18th Annual Hawaii Dermatology Post-Seminar will be held February 23–26, 1994 at the Ritz Carlton Mauna Lanai, Big Island, Hawaii, sponsored by Northwestern University Medical School. Co-directors are Henry H. Roenigk Jr., M.D., and Howard Maibach, M.D. Telephone, (312) 908-8173; FAX, (312) 908-0664.

The 2nd International Symposium on Cutaneous T-Cell Lymphoma will be held Wednesday, October 13 to Saturday, October 16, 1993 at the Northwestern University Medical School, Chicago, IL. Co-directors are Henry H. Roenigk Jr., M.D., Steven Rosen, M.D., and Timothy Kuzel, M.D.