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Light and Death: Photons and Apoptosis

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Phototherapies like photodynamic therapy (PDT), UVA1, UVB, and PUVA treat skin diseases. These phototherapies work because they alter cytokine profiles, change immune cytotoxicity in the skin, and directly kill diseased cells by apoptosis. Apoptosis is a term that only describes the morphologic changes a cell undergoes during this mode of cell death. The terms "immediate", "intermediate", and "delayed" apoptosis segregate the different apoptotic mechanisms into three kinetic categories, whereas the terms preprogrammed cell death (pre-PCD) and programmed cell death (PCD) describe the underlying mechanisms. Immediate apoptosis (T ≤ 0.5 h post-exposure) is triggered by singlet-oxygen damage that opens the mitochondrial megachannel, which can be mediated by PDT or UVA1 radiation. It is a pre-PCD mechanism of apoptosis, i.e., protein synthesis is not required post-insult, because all the necessary components are constitutively synthesized and only need to be activated. Intermediate apoptosis (T ≤ 4 h > 0.5 h) is initiated by receptor cross-linking on the plasma membrane, which can be achieved using high doses of UVB or UVC radiation. It is also a pre-PCD mechanism. Delayed apoptosis (T > 4 h) is induced by DNA damage that can be caused by X-rays, PUVA, UVC, UVB, UVA, and PDT. It is a PCD mechanism of apoptosis, i.e., protein synthesis is required post-insult. These three apoptotic mechanisms each access one of two "points-of-no-return" located on the mitochondrial membrane, which activate different, but not mutually exclusive, final pathways of apoptosis. This review discusses the latest findings on these apoptotic mechanisms and their implications in phototherapies. *Key words: phototherapy/radiation/reactive oxygen species/ultraviolet. Journal of Investigative Dermatology Symposium Proceedings 4:17–23, 1999*

Photons across the electromagnetic spectrum are used to treat various diseases. The longer wavelength photons are used to treat skin diseases because they penetrate to the dermal layer more efficiently than the shorter wavelengths (Bruls *et al*, 1984). Visible light wavelengths (400–700 nm) with one of several photosensitizers, i.e., photodynamic therapy (PDT), are used to treat cancers (Schuitmaker *et al*, 1996; Kato, 1998) and various skin diseases (Fritsch *et al*, 1998). The longest ultraviolet (UV) wavelengths (UVA; 320–400 nm) with a photosensitizer, such as 8-methoxypsoralen (PUVA), i.e., photochemotherapy, are used to treat psoriasis (Lauharanta, 1997; Gasparro *et al*, 1998) and cutaneous T cell lymphomas (Nestle *et al*, 1997). UVA1 wavelengths (340–400 nm) without any photosensitizer, i.e., UVA1 phototherapy, are used to treat various inflammatory skin diseases, such as atopic dermatitis (Krutmann, 1996, 1997). UVA1 phototherapy is also being investigated for the treatment of systemic lupus erythematosus (McGrath, 1997) and cutaneous T cell lymphomas (Krutmann, personal communication). The UVB wavelengths (290–320 nm), i.e., narrowband UVB (311 nm) phototherapy, are the shortest wavelengths used to treat a variety of skin diseases (El-Ghorr and Norval, 1997). All these phototherapies work because they alter cytokine profiles, modulate immunologic cytotoxicity in the skin (Norris *et al*, 1997), and directly kill diseased cells into apoptosis: the focus of this review.

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Abbreviations: AIF, apoptosis-inducing factor; Apaf-1, apoptosis protease-activating factor 1; cyto c, cytochrome c; PCD, programmed cell death; PDT, photodynamic therapy; pre-PCD, preprogrammed cell death.

WHAT IS APOPTOSIS?

Apoptosis is the "black hole" of cell death; it draws everything inward and nothing escapes its' biochemical event horizon.

It is one of two modes of cell death, necrosis is the other. The apoptotic cells in UV-irradiated skin are called "sunburn cells" (Daniels *et al*, 1961; F. Daniels Jr., see acknowledgment; Young, 1987). The term "apoptosis" only refers to the morphologic changes (Kerr *et al*, 1972) that can be seen using light, fluorescent, or electron microscopy (**Fig 1**). During apoptosis, the cell draws inward shrinking dramatically, the plasma membrane "blebs", the endoplasmic reticulum forms vacuoles, and the chromatin is digested and condenses along the nuclear membrane, often forming spheres and/or crescent shapes. In the final stages, the nucleus and the cell fragment form smaller compact units called apoptotic bodies. These apoptotic cells and bodies are primarily removed by nonprofessional phagocytes, e.g., keratinocytes in the skin; however, if they are not readily removed by phagocytosis, they undergo "secondary necrosis". During secondary necrosis, the lysosomes rupture and release hydrolytic enzymes into the cytoplasm causing further destruction of internal components including the plasma membrane. Once the plasma membrane loses integrity, the cell lyzes, releasing hydrolytic enzymes into the extracellular space guaranteeing an inflammatory response with possible tissue damage. The virtue of apoptotic cell death is that it usually does not result in an inflammatory response or tissue damage.

The term apoptosis was originally coined to describe only the morphologic and ultrastructual changes that occurred during this mode of cell death. However, this term did not include any biochemical events or mechanistic differences, because at that time they were not

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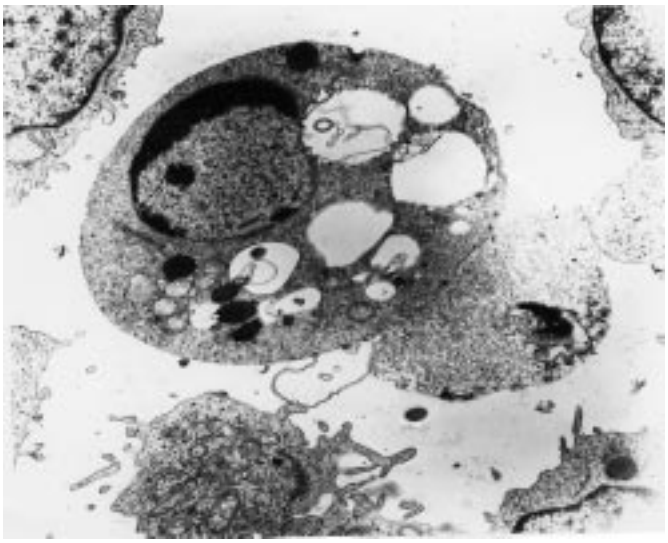

Figure 1. An example of an apoptotic, or sunburn, cell showing some characteristic morphologic changes associated with this mode of cell death. Note the condensed chromatin along the nuclear membrane forming sphere and crescent shapes and the dilated endoplasmic reticulum forming vacuoles. The most prevalent characteristic of apoptosis is cell shrinkage. This UVA1-irradiated Daudi B cell has about half the volume of its' nonapoptotic counterpart. Micrograph by D.P.Thomas.

known (Kerr *et al*, 1972). The biochemical events are now used to characterize and quantitate apoptotic cells, and to classify the different mechanisms. When cells undergo apoptosis, they dramatically shrink in size, one of the many characteristics that can be used to quantitate the percentages of these cells. This can be accomplished by counting the cells using either various microscopic methods or flow cytometry based on differences in their forward (size) *versus* side (granularity) light-scattering profiles (Dive *et al*, 1992). Apoptotic cells also express phosphatidylserine residues on the outer leaflet of their plasma membranes, which can be tagged with labeled Annexin V. When combined with dye exclusion (propidium iodide), the apoptotic cells can be quantitated using either fluorescent microscopy or flow cytometry, for unlike necrotic cells, they exclude vital dyes until their plasma membranes lose integrity. During apoptosis, the mitochondrial transmembrane potential drops, either immediately (Kroemer *et al*, 1997; Godar, 1999a) or sometime after 2 h (Bossy-Wetzel *et al*, 1998; Godar, 1999a), and can be measured using specific fluorescent dyes, such as JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide), along with flow cytometry (Salvioli *et al*, 1997) or confocal microscopy. Activation of "initiator" caspases, like caspase 8, can be monitored during some mechanisms of apoptosis, whereas activation of some of the "executioner" caspases, like caspase 3, can usually be detected (Thornberry and Lazebnik, 1998). Most of these cysteine proteases can be specifically inhibited and/or assayed using specific colorimetric- or fluorogenic-peptide substrates that contain aspartate residues. The executioner caspases take charge of the destructive phase, or final frontier of apoptosis. Like a preplanned military operation they strategically cut, modify, and destroy vital cellular components, such as Bcl-2, retinoblastoma-associated protein (Rb), nuclear lamina, U1 70-kDa small nuclear ribonuclear protein (involved in RNA splicing), focal adhesion kinase, p21-activated kinase 2, protein kinase C-γ, protein phosphatase 2A, poly(ADP-ribose) polymerase (PARP), fodrin, gelsolin, and many others. Their activation and/or destructive force can be documented with western blots. One of their manoeuvres usually involves cleaving inhibitors of and/or possibly directly activating both endogenous endonucleases that digest the genomic DNA, a relatively late biochemical event. One endogenous endonuclease only forms high molecular weight DNA fragments (50–300 kilobase pairs) that can be resolved using pulsed-field gel electrophoresis. The other endonuclease cleaves between nucleosomes forming multiples of 200 bp units that can be observed as a characteristic DNA "ladder" after agarose gel electrophoresis. Alternatively, the DNA strand breaks can be tagged using terminal deoxynucleotidyl transferase-mediated UTP nick end labeling (TUNEL), which is a good approach for identifying the apoptotic cells in tissue sections. Recently, antibodies to ε(γ–glutamyl)lysine, a proteolytic resistant product of transglutaminase, can allow assessment of various therapies by monitoring the amount of ε(γ– glutamyl)lysine present in patients' blood, urine, or tissue-sectioned biopsies. Caution is advised, however, because it is not yet known if this product is formed during all apoptotic mechanisms. These different approaches represent only a few ways to quantitate apoptotic cells: the rest are beyond the scope of this review (see Cotter and Martin, 1996).

WHY IS APOPTOSIS MEDICALLY IMPORTANT?

Apoptosis is receiving a lot of attention in the medical community because it is involved in development, differentiation, maintenance of homeostasis of the tissues and the immune system, and every altered cellular state, or disease. An example of one important disease is cancer, where cells continually divide and also ignore certain apoptotic death signals and/or strategically evade immune surveillance. They can ignore various death signals because specific genes required for the different apoptotic mechanisms are mutated, such as p53 (Fisher, 1994), or upregulated, such as Bcl-2 (Adams and Cory, 1998). Alternatively, they can evade immune surveillance by expressing high levels of the Fas ligand (FasL) on their membranes, thereby killing their approaching immunologic opponents by engaging their Fas receptor (FasR) first (Green and Ware, 1997). On the other hand, autoimmune diseases can occur when either functional mutations in FasR/FasL or increased levels of soluble FasL allow thymocytes to escape negative selection (Nagata, 1998; Suzuki *et al*, 1998). To kill these cells requires a therapeutic approach that will trigger an apoptotic mechanism that is downstream of the FasR/FasL pathway. Thus, in order to assure a favorable outcome when treating various diseases, a complete understanding of the different apoptotic mechanisms is essential for choosing the best therapeutic approach.

THE THREE KINETIC MECHANISMS OF APOPTOSIS: IMMEDIATE, INTERMEDIATE, AND DELAYED. PREPROGRAMMED AND PROGRAMMED CELL DEATHS

The terms "immediate", "intermediate", and "delayed" apoptosis assemble the different pre-PCD and PCD mechanisms into three kinetic categories (**Fig 2**). Immediate apoptosis is triggered in less than half an hour and is a pre-PCD, or constitutive, mechanism, i.e., protein synthesis is not required post-insult (Godar, 1996). Examples of some insulting agents that trigger immediate pre-PCD apoptosis include PDT, UVA1, and evidently any system that generates singlet-oxygen damage to mitochondrial membranes (Ball *et al*, 1998; Kessel and Luo, 1998; Godar, 1999a). Intermediate apoptosis occurs to a significant extent within 4 h (Wang *et al*, 1997), but requires more than half an hour, and is a pre-PCD mechanism as well. Examples of some insulting agents that initiate intermediate pre-PCD apoptosis include high doses of either UVB (Martin *et al*, 1995; Aragane *et al*, 1998; Bossy-Wetzel *et al*, 1998; Godar, 1999a, b) or UVC (200–290 nm; Rehemtulla *et al*, 1997) radiation, and any agent that activates a membrane receptor containing a death domain, such as Fas/CD95/APO-1 (Ashkenazi and Dixit, 1998; Godar, 1999b). Once cross-linked, these receptors usually transduce a signal to the mitochondria through the proteolytic action of caspases. Delayed apoptosis occurs well after 4 h (can take days) and is a PCD, or inducible, mechanism, i.e., protein synthesis is required post-insult (Godar, 1996). Examples of agents that induce primarily delayed PCD apoptosis are UVB, UVC (Godar, 1996; Godar *et al.*, 1994), PUVA (Marks and Fox, 1991; Vowels *et al*, 1996; Yoo *et al*, 1996), X-rays (Olive and Durand, 1997; Godar, 1999a), and any agent that causes significant DNA damage (Godar and Lucas, 1995; Kasibhatla *et al*, 1998). DNA damage leads to the upregulation of certain gene products, some of which are known to participate in apoptotic cell death mechanisms; the death receptor ligand, FasRL, and the mitochondrial pore-forming protein, Bax, and close relatives (e.g., Bid).

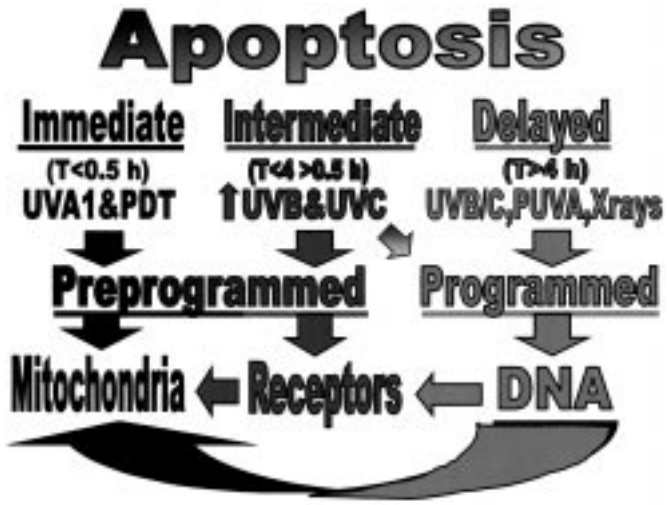

Figure 2. The three kinetic categories of apoptosis and underlying biochemical mechanisms: pre-PCD and PCD. Immediate apoptosis is triggered within half an hour by UVA1, PDT, or any singlet oxygen generating system. It is a pre-PCD mechanism of apoptosis, i.e., it does not require protein synthesis post-insult. Immediate apoptosis is triggered by immediate ($T < 0.5$ h) depolarization of the mitochondrial transmembrane potential. Intermediate apoptosis occurs rapidly ($T \leq 4$ h but > 0.5 h) and is initiated by high doses of UVB or UVC radiation, cross-linking of certain death receptors, such as FasR, and any superoxide anion generating system. It is also a pre-PCD mechanism of apoptosis. The mitochondria are depolarized 2–4 h after exposure by the action of caspases through receptor signaling. Moreover, high doses of UVB and UVC also cause DNA damage and delayed apoptosis. Delayed apoptosis is induced after 4 h by DNA damage that can be caused by PDT, UVA, UVB, UVC, PUVA, or X-rays or any ROS-generating system. Delayed apoptosis is a PCD mechanism, i.e., protein synthesis is required post-insult. DNA damage either activates the transcription factor, AP-1, increasing expression of the receptor Fas, or activates the transcription factor, p53, increasing expression of the mitochondrial pore-forming protein Bax. In addition, though it is not shown in the figure, UVA1 radiation gives a mixed apoptotic mechanism that not only triggers immediate pre-PCD apoptosis, but also induces delayed PCD apoptosis to some extent from DNA damage.

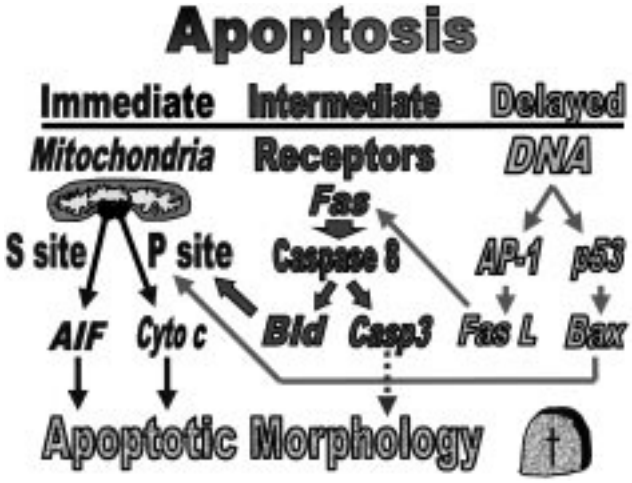

Figure 3. Model for all three kinetic mechanisms of apoptosis. The left side of the figure shows two pathways for immediate pre-PCD apoptosis. The far left-hand side of the figure shows what happens if singlet oxygen damage causes the megapore to open at the "S" site, releasing AIF. Evidently another immediate mechanism can be triggered when overwhelming amounts of superoxide anions or other agents cause Bax/Bid to rapidly form a channel at the "P" site. In this pathway, cyto c is released. The middle of the figure shows two pathways for intermediate pre-PCD apoptosis initiated by high doses of UVB or UVC radiation and any agent that causes cross-linking of a receptor with a death domain, e.g., the Fas receptor (CD95/APO-1). It is known that high doses of UVB or UVC radiation cause cross-linking of the Fas receptor and recruitment of FADD, which in turn clusters procaspase 8 that becomes an active protease. What is not known is whether or not Bax (or Bid), cyto c, or postmitochondrial caspases, other than caspase 3, are involved in photon-initiated intermediate apoptosis. This portion of the figure is somewhat speculative but is based on knowledge from other systems. The other receptor pathway involves caspase 8 directly cleaving procaspase 3 yielding active capsase 3 (Casp3) and apoptotic morphology. The far right side of the figure shows two pathways for delayed PCD apoptosis induced by any agent that causes DNA damage, such as PDT, UVA, UVB, UVC, PUVA, and X-rays. DNA damage can activate the transcription factor, AP-1, increasing FasL expression that engages FasR on the plasma membrane activating an intermediate pathway. DNA damage can also activate the transcription factor, p53, increasing Bax expression that directly activates an immediate pathway on the mitochondrial membrane, bypassing the intermediate mechanisms altogether. Other pathways may also be activated and many other gene products are known to be upregulated; however, which genes belong to what delayed PCD apoptotic mechanism and which genes belong to what survival mechanism remains to be determined. Because transcription and translation are required for apoptosis to proceed, these PCD mechanisms require some time to produce the products that will either trigger (Bax) or initiate (Fas) the apoptotic morphology. In these delayed apoptotic mechanisms, the mitochondrial transmembrane potential does not depolarize for many hours (>4 h) and they cannot be inhibited with CsA. These findings suggest that the "P" site of the megapore is ultimately involved in DNA damage-induced PCD mechanisms and that the final pathway may involve the pore-forming ability of Bax, or close relatives (e.g., Bid), and subsequent release of cyto c.

PHOTON-MEDIATED IMMEDIATE, INTERMEDIATE, AND DELAYED APOPTOSIS: MITOCHONDRIA, RECEPTORS, AND DNA

Immediate pre-PCD apoptosis can be triggered either by singlet-oxygen or superoxide-anion damage to mitochondrial membranes (**Fig 3**, left-hand side). Both UVA1 radiation and PDT primarily mediate singlet-oxygen production triggering immediate pre-PCD apoptosis by immediately depolarizing the mitochondrial transmembrane potential. Evidently, the megapore opens at the "S" site (sulfhydryl sensitive) releasing apoptosis-inducing factor (AIF) unless cyclosporine A (CsA) is present (Godar, 1999a). Immediate apoptosis can also be triggered at the "P" site (pyrimidine nucleotide sensitive) by high levels of superoxide anions (e.g. 1 mM vitamin K_3); however, this mechanism evidently involves release of cytochrome c (cyto c) because it is not inhibited by CsA (Godar, unpublished observations). UVA1- (Pourzand *et al*, 1997) and PDT- (He *et al*, 1996) triggered immediate pre-PCD apoptosis can be "inhibited", i.e., stopped or slowed down, by overexpression of Bcl-2, an antioxidant protein. Some of these Bcl-2 overexpressing cells survive and reproduce, as measured by clonogenic platting; however, most of these cells still died a clonogenic death, indicating that other cell death mechanisms are also activated, such as intermediate and/or delayed apoptosis, and possibly necrosis as well. Antioxidants, like glutathione (Godar, 1999a) and vitamin E (Godar and Lucas, 1995), can inhibit UVA1-triggered immediate pre-PCD apoptosis by scavenging most of the ROS before critical damage occurs to the mitochondrial megapore. Antioxidants, however, only slow the apoptotic process down because very few of the cells actually survived. Thus, clonogenic survival data are important for determining if an agent really stops apoptosis or simply delays the process. Regardless of the immediate mechanism that is triggered, i.e., "S" or "P" site, the apoptotic morphology only begins to occur after either AIF or cyto c is released from the mitochondria.

Intermediate pre-PCD apoptosis is usually initiated by receptor cross-linking that can be achieved using high doses (>99.9% clonogenic death) of UV radiation (**Fig 3**, middle to left). High-dose UVB- or UVC-initiated intermediate apoptosis has a kinetic profile that matches anti-Fas-initiated apoptosis (Martin *et al*, 1995; Rehemtulla *et al*, 1997; Aragane *et al*, 1998; Bossy-Wetzel *et al*, 1998; Godar, 1999a). The kinetics match because both mechanisms involve cross-linking of the FasR (Rehemtulla *et al*, 1997; Aragane *et al*, 1998). Exactly how high doses of UV cross-link the FasR is not yet known, but this process may involve superoxide anions and/or hydroxyl radicals (Gorman *et al*, 1997). It is known, however, that FasL does not engage FasR because apoptosis is not inhibited by a blocking antibody, such as ZB4. Moreover, confocal microscopy, limiting immunoprecipitation, and coimmunoprecipitation with FADD (Fas-associated death domain)

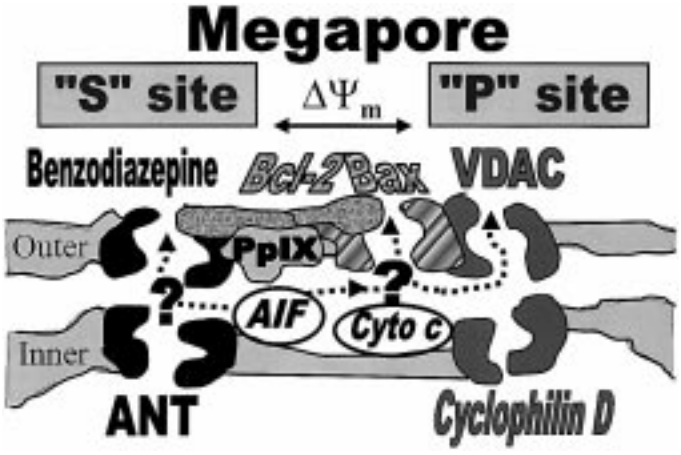

Figure 4. Hypothetical model of the two "points-of-no-return" located on the mitochondrial membrane. The figure shows the components of the mitochondrial megachannel or megapore. Located on the outer mitochondrial membrane of the megapore is the benzodiazepine receptor, protoporphyrin IX (PpIX) is a ligand, and voltage dependent anion channel (VDAC) or porin, along with Bcl-2 and/or Bcl-xL (antioxidant and inhibitory proteins), and Bax and/or Bid (pore-forming proteins). Located on the inner mitochondrial membrane is the adenine nucleotide transporter (ANT) and cyclophilin D, which can bind cyclosporin A. In functioning cells, cytochrome c (cyto c) is not released into the cytosol and apoptosis-inducing factor (AIF) is also maintained inside the mitochondria. The "S" site evidently contains the benzodiazepine receptor and ANT, while the "P" site contains VDAC and cyclophilin D along with the translocated pore-forming protein, Bax, and its inhibitory partner, Bcl-2. Although it is not currently known how Bcl-2 inhibits both the "S" and "P" site mechanisms, it may do so through close proximity to the benzodiazepine receptor where it could offer protection from ROS damage while it could also inhibit pore formation by Bax. How cyto c is released from the "P" site is not known (? in the figure), but it might exit through a very large pore created by Bax and/or VDAC. Likewise, how AIF is released from the "S" site is not known (? in the figure), but it might exit through the benzodiazepine receptor and/or Bax. In addition, the exact arrangement of the megapore components in three dimensions is not known and other known components have been omitted from the figure. Thus, the benzodiazepine receptor and VDAC may actually be next to each other with Bcl-2 and Bax surrounding the exterior (i.e., fold the figure in half and look from above). Furthermore, the arrangement of components is probably dynamic rather than static, for under certain conditions Bax can translocate from the outer to the inner member and cyclophilin D can translocate from the matrix to the inner membrane at different sites.

confirmed that FasR was directly cross-linked by UV radiation. After the FasR is cross-linked, FADD recruits procaspase 8 that becomes active caspase 8, a premitochondrial initiator caspase. Caspase 8 can either cleave Bid, which then forms a pore in the mitochondrial membrane (Li *et al*, 1998), or it can by-pass the mitochondria by directly cleaving procaspase 3 (Stennicke *et al*, 1998). Other receptors with a death domain, like TNF, can also initiate intermediate apoptosis (Ashkenazi and Dixit, 1998), and apparently play a role in some photon-initiated apoptotic mechanisms (Schwarz *et al*, 1995; Sheikh *et al*, 1998). In addition, another intermediate mechanism evidently involves p53, which somehow causes translocation of preformed Fas to the plasma membrane (Bennett *et al*, 1998). Although this mechanism is not currently understood or reported for photon-mediated apoptosis, it may be involved in the intermediate expression of surface FasL after UVA1 irradiation of T cells (Morita *et al*, 1997). When these intermediate mechanisms access a mitochondrial pathway, they only do so at the "P" site because they cannot be inhibited by CsA.

Delayed PCD apoptosis is induced by DNA damage that can be produced using visible light, UVA, UVB, UVC, or X-rays (Ley and Applegate, 1985; Jones *et al*, 1987; Peak and Peak, 1991; Godar and Lucas, 1995; Godar, 1999a). Besides mitochondrial membrane damage, UVA radiation also causes DNA damage and some delayed apoptosis (Godar and Lucas, 1995). Moreover, high doses of UV radiation not only affect cellular targets on the plasma membrane and in the cytoplasm (Schwarz, 1998), resulting in intermediate apoptosis, but also cause DNA damage resulting in delayed apoptosis (Godar, 1999a). These other affects confuse the interpretation of results and provide challenging complications when designing experiments to understand the mechanisms of DNA-damage induced delayed apoptosis; however, in spite of these challenging complications, two delayed PCD apoptotic mechanisms induced by DNA damage have emerged so far (**Fig 3**, right to left). One DNA-damage-induced delayed apoptotic mechanism involves the transcription factor AP-1, whereas the other involves the transcription factor p53. In the first case, DNA damage leads to activation of the transcription factor, AP-1. AP-1 binds to the FasL promoter increasing FasL mRNA and subsequently FasL protein expression, which is translocated to the plasma membrane (Kasibhatla *et al*, 1998). Note that the opposite can occur in FasL-expressing cells (Giordano *et al*, 1997). This DNA damage-induced apoptotic mechanism is partly understood for UVB radiation and some other DNA damaging agents, such as etoposide and teniposide, which damage the DNA by inhibiting topoisomerase II (Kasibhatla *et al*, 1998). A similar delayed apoptotic mechanism may also occur with therapeutically relevant doses of UVA1 radiation both *in vitro* and *in vivo*, because delayed upregulation of FasL expression and apoptosis have also been noted (Morita *et al*, 1997). Moreover, DNA damage from X-rays has recently been reported to result in delayed upregulation of FasL and subsequent apoptosis (Belka *et al*, 1998). Upregulating Fas expression allows this delayed apoptotic mechanism to initiate apoptosis by accessing an intermediate pre-PCD pathway. The other DNA damage-induced apoptotic mechanism involves the transcription factor, p53. Unlike UVA1 radiation, which predominately induces a p53-independent mechanism, both UVB (Wang *et al*, 1998) and UVC (Kimura *et al*, 1998) radiation primarily induce a p53-dependent apoptotic mechanism (Godar, unpublished results). Accumulation of p53 is linked to the delayed formation of UVB-induced sunburn cell formation in skin, and mutations in p53 to the development of skin cancer (Zeigler *et al*, 1994; Brash *et al*, 1996). Moderate to high doses (<99.9% clonogenic death) of UVB and UVC radiation cause accumulation of p53 and its alternatively spliced form, p53as, which results in cell cycle arrest in G1 and G2, respectively (Godar, unpublished results). Along with multitudes of other gene products, UVB-induced p53 accumulation leads to increased expression of Bax both *in vitro* and *in vivo* (Selvakumaran *et al*, 1994; Reinke and Lozano, 1997; O'Grady *et al*, 1998). In addition, UVC radiation also leads to increased expression of Bax (Kimura *et al*, 1998). Bax can form a pore in the mitochondrial membrane that somehow leads to the release of cyto c and subsequent apoptotic morphology. PUVA also causes DNA damage (through psoralen cross-linking and adduct formation), accumulation of p53, cell cycle arrest, and is a delayed PCD mechanism of apoptosis (Marks and Fox, 1991; Johnson *et al*, 1996; Vowels *et al*, 1996; Yoo *et al*, 1996; Godar, 1999a). Ionizing radiation-induced DNA damage also causes accumulation of p53, delayed PCD apoptosis (Clarke *et al*, 1993; Lowe *et al*, 1993), and induction of Bax (Zhan *et al*, 1994). The p53-dependent delayed PCD mechanism of apoptosis, unlike the AP-1-dependent mechanism, bypasses the intermediate pathways altogether by directly accessing the immediate pathway at the "P" site. Moreover, there appear to be other delayed apoptotic mechanisms induced by DNA damage that do not involve either p53 or AP-1; however, these other DNA damage-induced delayed PCD apoptotic mechanisms are not very well understood (Evan and Littlewood, 1998) and require future studies that are carefully planned and executed to unravel these different pathways.

Each of the three apoptotic mechanisms have at least two alternate pathways. These multiple, or "fail-safe", mechanisms and pathways probably coexist to assure that the damaged cell does not survive to reproduce genetic errors. All but one of these apoptotic pathways either originate or culminate at one of two "points-of-no-return" located on the mitochondrial membrane.

THE TWO "POINTS-OF-NO-RETURN"

Photon-mediated apoptotic mechanisms all access one of two "points-of-no-return", beyond which the cell cannot be rescued from death. One point-of-no-return, or commitment site, involves the opening of the mitochondrial megapore (Kroemer *et al*, 1997) at the "S" site,

whereas the other involves pore formation by either Bax (Jurgensmeier *et al*, 1998) or Bid (Li *et al*, 1998) at the "P" site. The opening of the megapore at either the "S" or the "P" commitment sites is controlled by dithiol oxidation and pyrimidine nucleotide status, respectively (Costantini *et al*, 1996). The sulfhydryl-sensitive "S" site is inhibited by CsA or increased levels of reduced thiols, like glutathione (Marchetti *et al*., 1997), whereas the pyrimidine nucleotide-sensitive "P" site is not inhibited by CsA but is inhibited by increased levels of reduced pyrimidine nucleotides, like NADH.

The mitochondrial megapore is comprised of a large complex of proteins that spans the outer and inner membranes (**Fig 4**). The proteins located on the outer membrane of the megapore include the benzodiazepine receptor (protoporphyrin IX, PpIX, is a ligand) and the voltage-dependent anion channel (VDAC). The proteins located on the inner membrane of the megachannel include the adenine nucleotide transporter and cyclophilin D. In addition, other proteins are associated with the cytoplasmic face of the megapore, like hexokinase (not shown in the figure), or purify along with the megapore, such as Bcl-2, Bax, Bag-1, and F_1-ATPase (Marzo *et al*, 1998). When the large megapore opens at the "S" site, the mitochondrial transmembrane potential immediately drops and apoptosis-inducing factor (AIF), is released unless CsA or bongkrekic acid are present (Kroemer *et al*, 1997; Susin *et al*., 1999). Singlet oxygen-generating systems, such as UVA1 or PDT, can immediately depolarize the mitochondrial transmembrane potential ($\Delta\Psi_m$) and evidently open the megapore at the "S" site, because these systems can be inhibited by CsA or reduced glutathione (Godar, 1999a). The other point-of-no-return involves a pore created by either Bax or Bid at the "P" site, which somehow leads to translocation of cyto c from the mitochondria to the cytosol (Jurgensmeier *et al*, 1998; Li *et al*, 1998). All but one signaling pathway initiates the release of cyto c (Kluck *et al*, 1997; Green and Reed, 1998), and evidently so can superoxide-anion generating systems, like menadione (vitamin K_3; Godar, 1999a). High levels of superoxide anions produced from vitamin K_3 (e.g., 1 mM) can also trigger immediate apoptosis (<15 min), but this immediate mechanism occurs at the "P" site as well, because it cannot be inhibited by CsA (Godar, unpublished results). Excessive production of superoxide anions by pyruvate with UVA1 radiation evidently also affects the "P" site, because CsA has no affect on that immediate apoptotic mechanism. Ethanol, like reduced glutathione, reduces this impressive effect (Godar, 1999a), because the activity of alcohol dehydrogenase increases the reduced pyrimidine nucleotide pool, which regulates the "P" site. The mitochondrial megapore "S" and "P" sites can both be inhibited by Bcl-2 or Bcl-X_L (Susin *et al*, 1996; Kluck *et al*, 1997; Marzo *et al*, 1998); however, once either AIF is released from the "S" site, or cyto c is released from the "P" site, one or the other final pathway of apoptosis begins, and the cells fate is sealed.

THE TWO FINAL APOPTOTIC PATHWAYS

Although not much is known about the final pathways of photon-induced apoptosis, other systems give clues as to what these mechanisms may involve. The two points-of-no-return and corresponding final pathways of apoptosis can be distinguished either by the time displacement of mitochondrial transmembrane depolarization and/or by shielding the megapores "S" site using a blocker, like CsA (Godar, 1999a). Both UVA1 radiation and PDT generate singlet oxygen, which immediately depolarize mitochondrial membranes and causes immediate apoptosis unless inhibited by CsA. Though not conclusively proven for singlet oxygen-triggered immediate apoptosis, megapore opening has been shown to release AIF unless either CsA or bongkrekic acid are present (Marchetti *et al*, 1996). CsA can distinguish between the two final apoptotic pathways by binding to cyclophilin D and shielding the release of AIF (Kroemer *et al*, 1997). Once AIF is released, it cleaves procaspase 3 to form active caspase 3 that triggers rapid apoptotic morphology (Susin *et al*, 1996; Kroemer *et al*, 1997; Marzo *et al*, 1998). UVA1 radiation is similar to this mechanism because it also activates a caspase 3-like activity (Godar, unpublished results). Unlike the "S" site, all but one signal transduction pathway utilizes the "P" site where the mitochondrial transmembrane potential takes 3 h or more to depolarize (Bossy-Wetzel *et al*, 1998; Godar, 1999a). Bax or Bid form a pore at the "P" site, which somehow allows cyto c to translocate to the cytosol where it associates with Apaf-1 (and dATP or ATP) recruiting procaspase 9 to become active caspase 9. This postmitochondrial initiator caspase then activates an executioner caspase cascade involving caspase 3 and eventually leads to mitochondrial transmembrane depolarization, perhaps by cleaving Bcl-2 (Cheng *et al*, 1997). Contrasting the "S" site mechanism, the "P" site mechanism is not inhibited by CsA because it does not prevent the release of cyto c if Bax or Bid form a pore. Thus, the two points-of-no-return and their corresponding final apoptotic pathways can be distinguished by monitoring the mitochondrial transmembrane potential over time in the absence or presence of CsA (Godar, 1999a).

SUMMARY

In light of these recent findings, one might ask what practical application knowledge about the different apoptotic mechanisms might have in the therapeutic setting. Different therapies take advantage of initiating different apoptotic mechanisms and knowledge of how these mechanisms work will enable clinicians to choose the best therapeutic approach when treating different diseases and patients. Several factors, such as the dose and wavelength of radiation, the concentration and type of photosensitizer (if any), the drugs taken by or dietary habits of a patient, and the redox potential and pyrimidine nucleotide status of the cells along with genetic mutations, are all important for determining which apoptotic mechanism will ultimately prevail.

These points are illustrated in the following scenarios. First, low to moderate doses (<99.9% reproductive death) of the shorter UV wavelengths (UVB/C) initiate delayed apoptosis via DNA damage, while high doses (>99.9% reproductive death) initiate the intermediate mechanism via receptor cross-linking, which short-circuits the delayed apoptotic mechanism. Unlike the shorter wavelengths (UVB/C), even very high therapeutic doses of the longer UV wavelengths (UVA) triggers immediate apoptosis via mitochondrial damage, which short-circuits both the intermediate and delayed mechanisms. Second, high concentrations and/or certain types of photosensitizing agents can go beyond the mitochondrial damage that causes immediate apoptosis and result in necrosis. Third, drugs or dietary habits that inhibit or promote an apoptotic mechanism can influence the effectiveness of a therapy. For example, CsA is used on transplant patients and it inhibits immediate apoptosis triggered by either PDT or UVA1-mediated singlet-oxygen production by interfering with AIF release from the "S" site. Because X-rays, PUVA, or narrow-band UVB phototherapies cause DNA damage that induces delayed apoptosis through the "P" site of the megapore, these therapies would not be affected by CsA. Similarly, antioxidant vitamins (especially vitamin E) and dietary habits that increase antioxidant levels interfere with PDT- or UVA1-triggered immediate apoptosis by scavenging singlet oxygen before it causes the megapore to open at the "S" site. Besides using alternate phototherapies like UVB, PUVA, or X-rays, these dietary habits could be reduced and/or eliminated during the course of PDT or UVA1 phototherapies. In addition, some phototherapies might benefit if a vehicle other than ethanol were chosen for skin applications of photosensitizers, drugs, or other agents. On the other hand, promoters of the different apoptotic mechanisms may improve some phototherapeutic approaches, such as vitamin K_3 or pyruvate with UVA1 phototherapy. Finally, some diseased cells have mutations in critical genes required for a particular apoptotic mechanism and consequently do not die. For example, lupus autoimmune cells do not undergo negative selection in the thymus due to mutations in the FasR/FasL pathway. UVA1 phototherapy, unlike UVB or PUVA, which aggravate lupus, may help these patients by triggering immediate apoptosis of autoimmune cells at the mitochondrial level, downstream of the defective FasR/FasL pathway. Furthermore, using only one therapeutic approach at a time to treat certain diseases, like cancer, will select for mutant populations resistant to undergoing that particular apoptotic mechanism, which will lead to eventual failure of that approach. Altering or combining therapeutic approaches that induce p53-dependent (e.g., PUVA, UVB, and X-ray) with those that trigger p53-independent (e.g., UVA1 and PDT)

apoptotic mechanisms may prevent selection for p53 mutants or overexpressors of Bcl-2, respectively. Future clinical research will determine which phototherapeutic approaches are most effective for each patient and disease.

The author recognizes that Dr. Farrington Daniels Jr. was the first to characterize and coin the term "sunburn cells". This paper is dedicated to him on his 80th birthday (born on September 29, 1918). The author is grateful to Drs Francis P. Gasparro and Anne D. Lucas for excellent critical reviews of this manuscript. The author also thanks Drs Douglas R. Green and Nigel J. Waterhouse for helpful scientific discussions. In addition, apologies are extended to those many investigators whose important contributions could not be described in detail or cited at all due to space limitations.

REFERENCES

Adams JM, Cory S: The Bcl-2 protein family: arbiters of cell survival. *Science* 281:1322–1326, 1998

Aragane Y, Kulms D, Metze D, Wilkes G, Poppelmann B, Luger TA, Schwarz T: Ultraviolet light induces apoptosis via direct activation of CD95 (Fas/APO-1) independently of its ligand CD95L. *J Cell Biol* 140:171–182, 1998

Ashkenazi A, Dixit M: Death receptors: signaling and modulation. *Science* 281: 1305–1308, 1998

Ball DJ, Luo Y, Kessel D, Griffiths J, Brown SB, Vernon DI: Induction of apoptosis by a positively charged methylene blue derivative. *J Photobiol Photochem* 42: 159–163, 1998

Belka C, Marini P, Budach W, Schulze-Osthoff K, Lang F, Gulbins E, Bamberg M: Radiation-induced apoptosis in human lymphocytes and lymphoma cells critically relies on the up-regulation of CD95/Fas/Apo-1 ligand. *Radiat Res* 149:588–595, 1998

Bennett M, Macdonald K, Chan S-W, Luzio JP, Simari R, Weissberg P: Cell surface trafficking of Fas: a rapid mechanism of p53-mediated apoptosis. *Science* 282:290–293, 1998

Bossy-Wetzel E, Newmeyer DD, Green DR: Mitochondrial cytochrome c release in apoptosis occurs upstream of DEVD-specific caspase activation and independently of mitochondrial transmembrane depolarization. *EMBO* 17:37–49, 1998

Brash DE, Ziegler A, Jonason AS, Simon JA, Kunala S, Leffell DJ: Sunlight and sunburn in human skin cancer: p53, apoptosis, and tumor promotion. *J Invest Dermatol Symp Proc* 1:136–142, 1996

Bruls WAG, Slaper H, van der Leun JC, Berrens L: Transmission of human epidermis and stratum corneum as a function of thickness in the ultraviolet and visible wavelengths. *Photochem Photobiol* 40:485–494, 1984

Cheng EHY, Kirsch DG, Clem RJ, *et al*: Conversion of Bcl-2 to a Bax-like death effector by caspases. *Science* 278:1966–1968, 1997

Clarke AR, Purdie CA, Harrison DJ, Morris RG, Bird DC, Hooper ML, Wyllie AH: Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature* 362:849–852, 1993

Costantini P, Chernyak BV, Petronilli V, Bernardi P: Modulation of the mitochondrial permeability transition pore by pyrimidine nucleotides and dithiol oxidation at two separate sites. *J Biol Chem* 271:6746–6751, 1996

Cotter TG, Martin SJ (eds). *Techniques in Apoptosis: A Users Guide*, London: Portland Press, 1996

Daniels F, Jr, Brophy D, Lobitz WC Jr: Histochemical responses of human skin following ultraviolet irradiation. *J Invest Derm* 37:351–356, 1961

Dive C, Gregory CD, Phipps DJ, Evans DL, Milner AE, Wyllie AH: Analysis and discrimination of necrosis and apoptosis (programmed cell death) by multiparameter flow cytometry. *Biochim Biophys Acta* 1133:275–285, 1992

El-Ghorr AA, Norval M: Biological effects of narrow-band (311 ηm TL01) UVB irradiation: a review. *J Photochem Photobiol* 38:99–106, 1997

Evan G, Littlewood T: A matter of life and cell death. *Science* 281:1317–1322, 1998

Fisher DE: Apoptosis in cancer therapy: crossing the threshold. *Cell* 78:539–542, 1994

Fritsch C, Goerz G, Ruzicka T: Photodynamic therapy in dermatology. *Arch Dermatol* 134:207–214, 1998

Gasparro FP, Liao B, Foley PJ, Wang XM, McNiff JM: Psoralen chemotherapy, clinical efficacy, and photomutagenicity: the role of molecular epidemiology in minimizing risks. *Environ Mol Mutagen* 31:105–112, 1998

Giordano C, Stassi G, DeMaria R, *et al*: Potential involvement of Fas and its ligand in the pathogenesis of Hashimoto's thyroiditis. *Science* 275:960–963, 1997

Godar DE: Preprogrammed and programmed cell death mechanisms of apoptosis: UV-induced immediate and delayed apoptosis. *Photochem Photobiol* 63:825–830, 1996

Godar DE: UVA1 radiation triggers two different final apoptotic pathways. *J Invest Dermatol* 112:101–111, 1999a

Godar DE: Singlet oxygen-triggered immediate preprogrammed apoptosis. *Meth Enzymol*, in press, 1999b

Godar DE, Lucas AD: Spectral dependence of UV-induced immediate and delayed apoptosis: the role of membrane and DNA damage. *Photochem Photobiol* 62: 108–113, 1995

Godar DE, Miller SA, Thomas DP: Immediate and delayed apoptotic cell death mechanisms: UVA versus UVB and UVC radiation. *Cell Death Different* 1:59–66, 1994

Gorman A, McGowan A, Cotter TG: Role of peroxide and superoxide anion during tumour cell apoptosis. *FEBS Lett* 404:27–33, 1997

Green DR, Reed JC: Mitochondria and apoptosis. *Science* 281:1309–1312, 1998

Green DR, Ware CF: Fas-ligand: privilege and peril. *Proc Natl Acad Sci USA* 94: 5986–5990, 1997

He J, Agarwal ML, Larkin HE, Friedman LR, Xue LY, Oleinick NL: The induction of partial resistance to photodynamic therapy by the protooncogene bcl-2. *Photochem Photobiol* 64:845–852, 1996

Johnson R, Staiano-Coico L, Austin L, Cardinale I, Nabeya-Tsukifuji R, Krueger JG: PUVA treatment selectively induces a cell cycle block and subsequent apoptosis in human T-lymphocytes. *Photochem Photobiol* 63:5666–5571, 1996

Jones CA, Huberman E, Cunningham ML, Peak MJ: Mutagenesis and cytotoxicity in human epithelial cells by far- and near-ultraviolet radiations: action spectra. *Radiat Res* 110:244–254, 1987

Jurgensmeier JM, Xie Z, Deveraux Q, Ellerby L, Bredesen D, Reed JC: Bax directly induces release of cytochrome c from isolated mitochondria. *Proc Natl Acad Sci USA* 95:4997–5002, 1998

Kasibhatla S, Brunner T, Genestier L, Echeveri F, Mahboubi A, Green DR: DNA damaging agents induce expression of Fas ligand and subsequent apoptosis in T lymphocytes via the activation of NF-κB and AP-1. *Mol Cell* 1:543–551, 1998

Kato H: Photodynamic therapy for lung cancer – a review of 19 years' experience. *J Photobiol Photochem* 42:96–99, 1998

Kerr JFR, Wyllie AH, Currie AR: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26:239–257, 1972

Kessel D, Luo Y: Mitochondrial photodamage and PDT-induced apoptosis. *J Photobiol Photochem* 42:89–95, 1998

Kimura C, Zhao Q-L, Kondo T, Amatsu M, Fujiwara Y: Mechanism of UV-Induced apoptosis in human leukemia cells: Roles of Ca^{2+}/Mg^{2+}-dependent endonuclease, caspase-3, and stress-activated protein kinases. *Exp Cell Res* 239:411–422, 1998

Kluck RM, Bossy-Wetzel E, Green DR, Newmeyer DD: The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science* 275:1132–1136, 1997

Kroemer G, Zamzami N, Susin SA: Mitochondrial control of apoptosis. *Immunol Today* 18:44–51, 1997

Krutmann J: Phototherapy for atopic dermatitis. *Dermatol Therapy* 1:24–31, 1996

Krutmann J: High-dose ultraviolet A1 phototherapy for inflammatory skin diseases. *Dermatol Therapy* 4:123–128, 1997

Lauharanta J: Photochemotherapy. *Clin Dermatol* 15:769–780, 1997

Ley RD, Applegate LA: Ultraviolet radiation-induced histopathological changes in the skin of the marsupial *Monodelphis domestica*. II. Quantitative studies of the photoreactivation of induced hyperplasia and sunburn cell formation. *J Invest Dermatol* 85:365–367, 1985

Li H, Zhu H, Xu CJ, Yuan J: Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell* 94:491–501, 1998

Lowe SW, Schmitt EM, Smith SW, Osborne BA, Jacks T: p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature* 362:847–849, 1993

Marchetti P, Hirsch T, Zamzami N, *et al*: Mitochondrial permeability transition triggers lymphocyte apoptosis. *J Immunol* 157:4830–4836, 1996

Marchetti P, Decaudin D, Macho A, Zamzami N, Hirsch T, Susin SA, Kroemer G: Redox regulation of apoptosis: impact of thiol oxidation status on mitochondrial function. *Eur J Immunol* 27:289–296, 1997

Marks DI, Fox RM: Mechanisms of photochemotherapy-induced apoptotic cell death in lymphoid cells. *Cell Biol* 69:754–760, 1991

Martin SJ, Newmeyer DD, Mathias S *et al*: Cell-free reconstitution of Fas-, UV radiation- and ceramide-induced apoptosis. EMBO 14:5191–5200, 1995

Marzo I, Brenner C, Zamzami N, *et al*: The permeability transition pore complex: a target for apoptosis regulation by caspases and Bcl-2-related proteins. *J Exp Med* 187:1261–1271, 1998

McGrath H Jr: Ultraviolet-A1 phototherapy for lupus erythematosus. *Dermatol Therapy* 4:109–117, 1997

Morita A, Werfel T, Stege H, *et al*: Evidence that singlet oxygen-induced human T-helper cell apoptosis is the basic mechanism of ultraviolet-A radiation phototherapy. *J Exp Med* 186:1763–1768, 1997

Nagata S: Human autoimmune lymphoproliferative syndrome, a defect in the apoptosis-inducing Fas receptor: a lesson from the mouse model. *J Hum Genet* 43:2–8, 1998

Nestle FO, Haffner AC, Schmid MH, Dummer R, Burg G: Current therapy concepts in cutaneous T-cell lymphomas. *Schweiz Med Wochenschr* 127:311–320, 1997

Norris DA, Whang K, David-Bajar K, Bennion SC: The influence of ultraviolet light on immunological cytotoxicity in the skin. *Photochem Photobiol* 65:636–646, 1997

O'Grady A, Kay EW, McKenna DB, Bennett MA, Murphy GM, Leader MB: Altered expression of the p53-regulated proteins, p21Waf1/Cip1, MDM2, and Bax in ultraviolet-irradiated human skin. *Hum Pathol* 29:559–564, 1998

Olive PL, Durand RE: Apoptosis: an indicator of radiosensitivity in vitro? *Int J Radiat Biol* 71:695–707, 1997

Peak JG, Peak MJ: Comparison of initial yields of DNA-to-protein crosslinks and single-strand breaks induced in cultured human cells by far- and near-ultraviolet light, blue light and X-rays. *Mutat Res* 246:187–191, 1991

Pourzand C, Rossier G, Reelfs O, Borner C, Tyrrell RM: The overexpression of Bcl-2 inhibits UVA-mediated immediate apoptosis in rat 6 fibroblasts: evidence for the involvement of Bcl-2 as an antioxidant. *Cancer Res* 57:1405–1411, 1997

Rehemtulla A, Hamilton CA, Chinnaiyan AM, Dixit VM: Ultraviolet radiation-induced apoptosis is mediated by activation of CD-95 (Fas/APO-1). *J Biol Chem* 272:25783–25786, 1997

Reinke V, Lozano G: Differential activation of p53 targets in cells treated with ultraviolet radiation that undergo both apoptosis and growth arrest. *Radiat Res* 148:115–122, 1997

Salvioli S, Ardizzoni A, Franceschi C, Cossarizza A: JC-1, but not $DiOC_6$ (3) or rhodamine 123, is a reliable probe to assess changes in intact cells: implications for studies on mitochondrial functionality during apoptosis. *FEBS letters* 411:77–82, 1997

Schuitmaker JJ, Bass P, van Leengoed HLLM, van der Meulen FW, Star WM, van Zandwijk N: Photodynamic therapy: a promising new modality for the treatment of cancer. *J Photobiol Photochem* 34:3–12, 1996

Schwarz T: UV light affects cell membrane and cytoplasmic targets. *J Photobiol Photochem B* 44:91–96, 1998

Schwarz A, Bhardwaj R, Aragane Y, *et al*: Ultraviolet-B-induced apoptosis of keratinocytes: evidence for partial involvement of tumor necrosis factor-alpha in the formation of sunburn cells. *J Invest Dermatol* 104:922–927, 1995

Selvakumaran M, Lin K-H, Miyashita T, *et al*: Immediate early up-regulation of bax expression by p53 but not TGFβ1: a pardigm for distinct apoptotic pathways. *Oncogene* 9:1791–1798, 1994

Sheikh MS, Antinore MJ, Huang Y, Fornace AJ Jr: Ultraviolet–irradiation-induced apoptosis is mediated via ligand independent activation of tumor necrosis factor receptor 1. *Oncogene* 19:2555–2563, 1998

Stennicke HR, Jurgensmeier JM, Shin H, *et al*: Pro-caspase-3 is a major physiologic target of caspase-8. *J Biol Chem* 273:27084–27090, 1998

Susin SA, Zamzami N, Castedo M, *et al*: Bcl-2 inhibits the mitochondrial release of an apoptogenic protease. *J Exp Med* 184:1331–1341, 1996

Susin SA, Lorenzo HK, Zamzami N *et al*: Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* 397:441–446, 1999

Suzuki N, Ichino M, Mihara S, Kaneko S, Sakane T: Inhibition of Fas/Fas ligand-mediated apoptotic cell death of lymphocytes in vitro by patients with systemic lupus erythematosus. *Arthritis Rheum* 41:344–353, 1998

Thornberry NA, Lazebnik Y: Caspases: enemies within. *Science* 281:1312–1316, 1998

Vowels BR, Yoo EK, Gasparro FP: Kinetic analysis of apoptosis induction in human cell lines by UVA and 8-MOP. *Photochem Photobiol* 63:572–576, 1996

Wang B, Fujita K, Watanabe K, Mitani H, Yamada T, Shima A: Induction of apoptosis in cultured midbrain cells from embryonic mice. *Radiat Res* 147:304–308, 1997

Wang Y, Rosenstein B, Goldwyn S, Zhang X, Lebwohl M, Wei H: Differential regulation of p53 and Bcl-2 expression by ultraviolet A and B. *J Invest Dermatol* 111:380–384, 1998

Yoo EK, Rook AH, Elenitsas R, Gasparro FP, Vowels BR: Apoptosis induction by ultraviolet light A and photochemotherapy in cutaneous T-cell lymphoma: relevance to mechanism of therapeutic action. *J Invest Dermatol* 107:235–242, 1996

Young AR: The sunburn cell. *Photodermatol* 4:127–134, 1987

Zeigler A, Jonason AS, Leffell DJ, *et al*: Sunburn and p53 in the onset of skin cancer. *Nature* 372:773–730, 1994

Zhan Q, Fan S, Bae I, Guillouf C, Liebermann DA, O'Connor PM, Fornace AJ Jr: Induction of bax by genotoxic stress in human cells correlates with normal p53 status and apoptosis. *Oncogene* 9:3743–3751, 1994