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Ultraviolet B Radiation-Induced Cell Death: Critical Role of Ultraviolet Dose in Inflammation and Lupus Autoantigen Redistribution

Roberto Caricchio, Lenese McPhie, and Philip L. Cohen

The nuclear self-Ags targeted in systemic lupus erythematosus translocate to the cell membrane of UV-irradiated apoptotic keratinocytes and may represent an important source of self-immunization. It is hard to understand how the noninflammatory milieu accompanying most apoptosis might provoke an immunogenic response leading to autoantibodies. We have found that the precise amount of keratinocyte UV exposure is crucial in determining the rate of apoptosis, the amount of inflammatory cytokine production, and the degree of autoantigen translocation. Low doses of UVB (≤15 mJ/cm²) promptly induced a normal, caspase-dependent apoptosis, while intermediate doses of UV-B (35 mJ/cm²) caused apoptosis with altered morphology, slower DNA fragmentation, and poly(ADP-ribose) polymerase degradation accompanied by increased Bcl-2. High doses of UVB (80 mJ/cm²) induced instead necrosis. We observed IL-1 production upon intermediate and high UVB doses. Nuclear Ag redistribution was also markedly UV dose dependent: at low doses, Sm, Ku, and DNA translocated to the surfaces of early apoptotic cells. At intermediate doses, these Ags concentrated on the cell membrane when the nucleus was still visible. At high doses, these autoantigens diffused into the cytoplasm and were released into the supernatant. Taken together, the results show that low-dose UVB induces prompt noninflammatory apoptosis. In contrast, intermediate and high doses of UVB induce proinflammatory apoptosis and necrosis, where the production of inflammatory cytokines is accompanied by exposure and release of autoantigens. The key importance of the UV dose on the fate of apoptotic keratinocytes and on their potential immunogenicity should help clarify the role of UVB in inducing systemic lupus erythematosus autoimmunity.


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ystemic lupus erythematosus (SLE) is characterized by autoantibodies mainly directed at ubiquitous nuclear targets such as DNA, histones, La, poly(ADP-ribose) polymerase (PARP), and Ku (1, 2). In the past few years, it has become clear that the production of these autoantibodies is Ag driven (3–5) and T cell dependent (6). Nevertheless, the reason why the immune response in SLE targets only certain proteins or DNA/RNA-protein complexes and the source itself of these Ags are still unclear.

Recently, the process of cell death has emerged as a possible source of SLE autoantigens (7–9). Numerous autoantigens are cleaved during both apoptotic and necrotic cell death (10–13), thereby generating a new set of heretofore “unseen” neoantigens. Apoptotic cell redistribution on their membrane specific SLE autoantigens such as Ro/SSA, La/SSB, small nuclear ribonucleoprotein, and DNA-histone complexes (14). This redistribution could potentially expose modified autoantigens to autoantibodies and trigger a proinflammatory phagocytosis through the FcR (15). Impaired clearance of apoptotic debris may also contribute to the process of self-immunization in SLE (16–18).

Cell death can be either apoptotic or necrotic (19). Apoptosis is a tightly controlled mechanism, vital to multiple functions in a living organism, such as the morphological shaping of tissues during embryogenesis (20), elimination of damaged cells (21, 22), and the homeostasis of the immune system (23). During the apoptotic process, the cell undergoes a sequence of dramatic morphological and biochemical changes: in particular, epithelial cells initially detach from the extracellular matrix and “round up.” This phase is characterized by blebbing that is generated by dynamic plasma membrane protrusions and retractions (24). Subsequently, the executioner phase takes place, chromatin and nuclear fragmentation occur, and the cell condenses into varying numbers of small apoptotic bodies (24, 25). The prompt disposal of apoptotic cells is ensured by professional and nonprofessional phagocytic cells, and in vitro and in vivo studies have suggested that apoptotic cells induce no inflammation and exert an anti-inflammatory effect on the phagocytic cells (26–28). Necrosis, in contrast, is induced by extreme damage; the necrotic cell undergoes different morphological and biochemical changes (19) recently recognized to be well regulated under certain conditions (29). Necrotic cells have been shown to evoke an inflammatory response by the phagocytic cells (30), to activate professional APCs, and to initiate an immune response (31).

To understand SLE pathogenesis, it is essential to know how self-Ags are provided to the immune system and to identify the causes of the proinflammatory milieu that might favor effective activation of professional APCs and Ag presentation. UVB is a well-known stimulus capable of inducing apoptosis in vitro and in vivo, and it has been associated with lupus flares (32, 33). How can...
the same trigger induce an anti-inflammatory response by inducing apoptosis and yet a proinflammatory response by inducing flares in SLE? To resolve the apparent paradox, we hypothesized that UVB can induce either anti-inflammatory apoptosis or a proinflammatory cell death depending on dose. To test our hypothesis, we examined the Ag redistribution and the inflammatory consequences of graded doses of UVB. We found a crucial and previously unappreciated dependence of both inflammatory response and autoantigen redistribution on the dose of UVB exposure. Indeed, we found that, depending on dose, UVB can induce sharply different types of cell death, ranging from noninflammatory apoptosis, induced by low doses of UVB, to proinflammatory apoptosis with cytokine production, induced by intermediate doses, and finally to proinflammatory necrosis, induced by high doses of UVB. Finally, the different outcomes correlate with distinct autoantibody translocation patterns, namely, membrane exposure and release which could, in turn, allow pre-existing autoantibodies to bind their targets and activate proinflammatory Fc-mediated phagocytosis (15), augmenting the inflammation triggered by proinflammatory cytokines. Our data suggest a tight relationship between proinflammatory doses of UVB and availability of modified autoantigens, which in turn would constitute a constant danger for a genetically lupus-prone individual.

Materials and Methods

Cell lines and culture conditions

The human epidermoid carcinoma cell line A431 was used as source of keratinocytes. Cells were cultured in complete DMEM (l-glutamine, pyridoxine hydrochloride, sodium pyruvate, and FCS) at 37°C in 5% CO2. Cells were washed with IBS and replaced with 50°C for 30 min in 96-well microtiter plates. IBS was incubated at 4°C for 3 h postirradiation, with low doses of UVB (15 mJ/cm2), cells did not undergo any of the apoptotic phases, but induction of the late apoptotic phases. Finally, with high doses (80 mJ/cm2), cells were blebbing phase. In contrast, with intermediate doses (35 mJ/cm2), we found that, depending on dose, UVB induces sharply different DNA peak identified by PI immunofluorescence. Apoptosis was also detected by annexin V and PI staining to discriminate among early, late, and necrotic cells as described previously (36). To detect autoantigen release by dying cells, supernatants from different time points and doses were collected and DNA was extracted by using a DNA isolation kit (Qiagen, Valencia, CA) and analyzed by pulse-field electrophoresis on a 1.5% agarose gel containing 0.5 μg/ml of ethidium bromide. In some cases, prior to the induction of apoptosis, cells were incubated with z-DEVD-fmk (Enzyme System Products, Livermore, CA) to inhibit caspase 3 activation (37). Real-time light microscopy (Olympus CK2; Olympus, Tokyo, Japan) with ×40 objectives was used to image cell death induced by different UVB doses. Images were collected with a charge-coupled device camera (Sensys; Photometric, Tucson AZ) and processed with IPLab software (Scanalytic, Fairfax, VA).

Western blot

Samples of 4 × 10⁶ cells were washed in PBS and lysed for 20 min on ice in 50 mM HEPES, 150 mM NaCl, 1% Triton X-100, 10% glycerol, 10 mM NaF, 1 mM EDTA, 2 mM sodium orthovanadate, 1 mM DTT, and 1 mM PMSF. After centrifugation for 15 min at 14,000 rpm, supernatant protein was determined by BCA* Protein Assay Reagent (Pierce, Rockford, IL) using BSA standards. Thirty micrograms of proteins in Laemmli buffer was separated per lane on 12% SDS-PAGE. Western blots were probed with mouse monoclonal anti-bcl-2 (BD Pharmingen), mouse monoclonal anti-PARP (Enzyme Systems Products), and rabbit anti-active-c-Jun N-terminal kinase (JNK)/stress-activated protein kinases (SAPKs) (Promega, Madison, WI). Bound Abs were detected with goat anti-mouse and anti-rabbit HRP conjugate using an ECL system (Renaissance; NEN Life Science Products, Boston, MA).

Immunofluorescence

A431 cells were grown on glass chamber slides (Nunc, Napa, IL). Cells were washed once with PBS at 4°C and fixed with 100% methanol at 20°C for 15 min. Chamber slides were then washed with PBS and incubated with blocking buffer (PBS with 3% FCS) for 15 min. Cells were incubated in blocking buffer for 1 h with mouse monoclonal 111 anti-p80 (Ku; IgG2a) (kindly provided by Dr. W. Reeves, University of Florida, Gainesville, FL) and Y2 anti-Sm (mouse IgG2a) as primary Abs. Secondary Abs were added for 30 min with two washes between each step. For DNA staining, cells were first incubated with bovine RNase I (1 mg/ml; Sigma-Aldrich) for 5 min., then stained with PI (100 μg/ml) for 5 min. After the final step, the medium chamber was removed and the slides were mounted with Fluoromount G (Electronic Microscopy Sciences, Fort Washington, PA). Cells were examined with an immunofluorescence microscope (Olympus BX60; Olympus) with ×40 objectives. Images were collected with a charge-coupled device camera (Sensys; Photometric) and processed with IPLab software (Scanalytic).

Results

Low, intermediate, and high doses of UVB induce different morphological and biological apoptotic patterns in keratinocytes

Following the apoptotic insult, keratinocytes undergo the so-called round up phase, which is followed by the nuclear fragmentation and formation of blebs and apoptotic bodies, the “blebbing phase” (24). In Fig. 1 (top panels, phase-contrast pictures), we show that at 3 h postirradiation, with low doses of UVB (15 mJ/cm²), cells have already ended the round up phase and proceeded into the blebbing phase. In contrast, with intermediate doses (35 mJ/cm²), cells were “locked” into the round up phase, suggesting a delayed induction of the late apoptotic phases. Finally, with high doses (80 mJ/cm²), cells did not undergo any of the apoptotic phases, but rather showed a necrotic morphology, characterized by swelling of the cytoplasm and pyknotic nucleus (19). To further demonstrate the clear differences induced by low, intermediate, and high UVB doses, cells were stained with annexin V and PI to differentiate between apoptotic and necrotic cells. As shown in Fig. 1 (bottom panels, dot plots), low doses induced progressive filling of the membrane phospholipids (revealed by the increase of annexin V positivity), without loss of membrane integrity (revealed by the PI negativity). Intermediate doses induced a faster exposure of phospholipids, indicating that cells locked in the early round up phase...
were ongoing apoptosis (annexin V positive). In both cases, phospholipids exposure was halted by inhibition of caspase 3 activation, showing that both doses of UVB are inducing a caspase-dependent apoptosis. In contrast, high doses of UVB quickly induced necrosis (revealed by annexin V/PI positivity) and could not be affected by the inhibition of caspase 3.

Intermediate doses of UVB irradiation, in contrast to low doses, delay PARP cleavage and c-Jun N-terminal kinase (JNK)/SAPKs activation.

Keratinocytes were exposed to graded doses of UVB irradiation; PARP cleavage and JNK activation were then measured by Western blot (Fig. 2). PARP cleavage, hallmark of the “execution phase” of apoptosis, is an extremely sensitive indicator of DNA damage, and this enzyme is rapidly cleaved during the execution phase of apoptosis, playing an important role in the apoptotic pathway. JNK/SAPKs has been shown to be activated by UVB irradiation and its activation plays an important role in UVB-induced apoptosis. A431 cells were UVB irradiated (1, 4.5, 15 and 35 mJ/cm²). At different time points, cells were harvested and PARP cleavage and JNK/SAPKs were detected by Western blot. With 1-, 4.5-, and 15-mJ/cm² UV-B doses, both PARP and JNK/SAPKs were progressively cleaved and activated, respectively (1-, 3-, and 6-time points). In contrast, the 35-mJ/cm² dose induced not only delayed PARP cleavage and JNK/SAPK activation (6-time point), but more reduced PARP cleaved product and JNK/SAPK activated expression compared with lower doses (3-time point). These data show the nonlinear relation between UVB radiation and the apoptotic process. Similar results were obtained from three different experiments Rx, Treatment.
apoptosis, is an extremely sensitive indicator of DNA damage. Following UVB exposure, this enzyme is cleaved into two products, 89 and 24 kDa (36). As shown in Fig. 2, at doses ranging from 1 to 15 mJ/cm² UVB irradiation, PARP cleavage increased in a linear fashion at 1-, 3-, and 6-h time points. Surprisingly, with intermediate-high doses (35 mJ/cm²), at 3 h postirradiation, PARP was only minimally cleaved, suggesting a delayed induction of the final apoptotic phase (see also Fig. 1). However at 6 h, PARP was completely cleaved at mid-range UVB doses, confirming that the apoptotic process was eventually completed. Little PARP cleavage was detectable when cells were irradiated with 80 mJ/cm², suggesting that necrosis rather than apoptosis occurred (data not shown and Fig. 1). To ask whether another marker of apoptosis exhibited such marked UVB dose dependence, we tested the activation of JNK/SAPKs. As shown in Fig. 2, at the low 15-mJ/cm² dose, we detected a linear increase of JNK/SAPKs activation in parallel to the PARP cleavage data. In contrast, with 35 mJ/cm² at 1- and 3-h time points, little activation was induced, while a high level of activation was detectable after 6 h. These data fit the pattern observed for UV-induced PARP cleavage, confirming that the apoptotic machinery is markedly dependent on the degree of UV exposure and that an increase of the UVB dose causes a delay, and possibly an alteration, of the apoptotic process.

DNA fragmentation is delayed with intermediate and absent with high doses of UVB irradiation

In contrast to necrosis, the final steps of apoptosis induced by the majority of stimuli are marked by DNA fragmentation (38). Such degradation can be detected by staining the DNA with dyes such as PI (39). Fragmented DNA is identified and measured as the subdiploid peak (<2 N) (22). As shown in Fig. 3, before UVB irradiation, only 3% of cells had subdiploid DNA, while 3 h after a 15-mJ/cm² dose, those cells increased to 40%. In contrast, with a 35-mJ/cm² dose, only 20% of the cells fragmented their DNA, once again showing a delay in the execution phase of apoptosis. Finally, with 80 mJ/cm², little DNA degradation was observed (5%), suggesting that necrosis rather than apoptosis occurred.

Bcl-2 persists during the delay of the executioner phase of apoptosis induced by intermediate doses of UVB

To define the mechanism underlying the UVB dose-effect response on keratinocytes, i.e., linear apoptosis for low doses and delayed apoptosis for intermediate doses, we next tested bcl-2 expression by Western blot. The anti-apoptotic bcl-2 protein is known to decrease after UVB irradiation, and its decrease has been correlated the induction of UVB-induced apoptosis (40). A431 cells were therefore UVB irradiated with low and intermediate doses and at 1, 2, and 3 h after irradiation cell lysates were tested for bcl-2 expression. As shown in Fig. 4, as expected low doses (1, 4.5, and 15 mJ/cm²) decreased the expression of bcl-2. In contrast, intermediate doses (35 mJ/cm²) of UVB irradiation failed to decrease bcl-2 expression, suggesting an active role for this gene in delaying apoptosis after intermediate-high doses of UVB.

Intermediate-high doses of UVB, but not low doses, induce IL-1α expression

Keratinocyte-derived cytokines play an important role in the pathogenesis of UVB-induced immunological and inflammatory reactions (41). Among the many cytokines produced by keratinocytes, IL-1α plays an important role in the mediation of such reactions (42). We therefore investigated whether IL-1α expression was differentially affected by different doses of UVB irradiation. We studied IL-1α expression after three different doses of UVB irradiation and compared its expression with that after PMA treatment, a potent IL-1α inducer (43) (Fig. 5). Low doses (15 mJ/cm²) of UVB, after a 1.5- and 3-h incubation, induced little expression of IL-1α, suggesting that at this dose, UVB induced “normal” apoptosis, with little or no inflammation. In contrast, at 3 h postirradiation with the 35-mJ/cm² dose, IL-1α expression increased dramatically, suggesting that, at this dose, UVB induced delayed abnormal apoptosis along with the generation of an inflammatory context.

Finally, with the 80-mJ/cm² dose, which induced necrosis rather than apoptosis, IL-1α production dramatically increased at the first time point (1.5 h), indicating that this UVB dose was able to induce first an inflammatory response and then necrosis.

Redistribution and release of nuclear Ags during cell death depends on the UVB dose used

We have shown so far that the nature of the cellular response to UVB depends on the dose of irradiation used. Low UVB doses induce mainly apoptosis, while high doses usually lead to necrosis (see Figs. 1–3). Intermediate doses generate a delayed apoptosis that may create, through production of IL-1α, a proinflammatory environment. Because of these remarkable differences, we next investigated whether SLE autoantigen translocation from the nucleus to the cytoplasmic membrane and into the supernatant is dependent on the UVB dose used (Table I).

A431 cells were irradiated with three different UVB doses (Fig. 6): 15, 35, and 80 mJ/cm². Three hours after UV irradiation, cells were incubated with PI, anti-Sm, and anti-Ku, and cell morphology and translocation patterns were investigated by fluorescent microscopy. As shown in Fig. 6, with a low UVB dose (15 mJ/cm²), cells were in the apoptotic blebbing phase and apoptotic bodies were already forming (white arrows). At this dose, DNA, Sm, and Ku Ags concentrated within the forming apoptotic bodies as previously shown (Ref. 14 and Fig. 6, white arrows). The incipient apoptotic bodies demonstrated that cells had completed apoptosis (19). With 35 mJ/cm² (intermediate-dose range), the majority of the cells appeared in the “rounding-up” apoptotic phase (24) (see
During this early apoptotic phase, it is still possible to discriminate the nucleus from the cytoplasm and as shown in Fig. 6, all three autoantigens showed a clear translocation from the nucleus (still visible) to the membrane (yellow arrows), while the cytoplasm appeared negative, suggesting that translocation occurred through an active mechanism. This morphological pattern indicates that at this UV dose, cells are temporarily blocked at an early apoptotic phase; this is in agreement with our findings of delayed PARP cleavage and increased bcl-2 expression. Finally, with the 80-mJ/cm² dose (high-dose range), cells were clearly necrotic. Cells appeared swollen with loss of membrane integrity. All three SLE autoantigens redistributed throughout the cell, suggesting that, at this dose, the translocation occurred by diffusion due to loss of cellular integrity. Interestingly, before the final steps toward necrosis, at this dose keratinocytes were able to produce IL-1α (Table I).

**FIGURE 4.** Bcl-2 delays the executioner phase of apoptosis when intermediate doses of UVB are used. A, A431 cells were UVB irradiated (1, 4.5, 15, and 35 mJ/cm²) and mock-irradiated cells were used as a negative control. After different time points, cells were harvested and lysed and bcl-2 was detected by Western blot. Lysates from the Jurkat T cell line were used as positive control (PC). bcl-2 expression was detectable in all of the samples but with remarkable differences. With 1-, 4.5-, and 15-mJ/cm² doses at the 1-h time point, bcl-2 expression seemed to increase compared with untreated cells, while after 2 and 3 h, bcl-2 expression decreased. In contrast, with higher doses (35 mJ/cm²), an increase of bcl-2 expression was detectable after 2 and 3 h, suggesting an initial survival response to high doses of UVB irradiation. Ponceau S staining is shown as loading control. B, Expression of bcl-2 was normalized with the intensity of known bands obtained with the Ponceau S staining. Values are expressed in arbitrary units obtained by analyzing the ratio of bcl-2:Ponceau band intensity with NIH Image software (National Institutes of Health, Bethesda, MD). At the 2-h time point, bcl-2 expression increased proportionally to the UVB dose, suggesting a role of this gene for the “delayed apoptosis” that occurs with higher doses. Rx, Treatment.

**FIGURE 5.** Intermediate-high doses of UVB, but not low doses, induce IL-1α expression. To study IL-1α production after different UVB doses, A431 cells were irradiated with 15, 35, and 80 mJ/cm² UVB, and IL-1α production was detected by intracellular staining at 1.5 and 3 h after irradiation. Histograms with PE-conjugated anti-IL-1α and isotype-matched control Ab are shown. As a positive control, PMA-stimulated A431 cells were used. With the lowest dose (15 mJ/cm²), there was little increase of IL-1α production. In contrast, with the 35-mJ/cm² dose, in which we showed delayed apoptotic features, at the 3-h time point the majority of A431 were producing IL-1α. Surprisingly, with the highest dose (80 mJ/cm²) and within the first time point, A431 cells dramatically increased IL-1α production, suggesting that UVB-induced necrosis can trigger a rapid proinflammatory cytokine production. Rx, Treatment.
To investigate the fate of autoantigens beyond the cellular membrane and in the supernatant, cells were treated with different UVB doses and the presence of DNA was studied in the supernatant collected at 3 h postirradiation. As shown in Fig. 7, no DNA was detectable with low doses and only very little DNA was present with medium doses. In contrast, with high doses, although very little fragmentation was induced (see Fig. 3), nucleosomal DNA was clearly visible, suggesting that necrotic cells are mainly responsible for the release of autoantigens. Interestingly, release of nucleosomal DNA was caspase 3 dependent, suggesting that, when high doses of UVB are delivered, apoptosis and necrosis coexist.

Discussion

Compelling evidence has recently linked cell death to SLE. We and others have shown that lupus-like disease develops in mouse models in which phagocytosis of apoptotic cells (18) or clearance of DNA is impaired (44). These studies suggest that apoptotic cells may well be a reservoir of autoantigens. This idea is supported by the fact that Ags derived from phagocytosed apoptotic and necrotic cells can be presented by dendritic cells, the professional APCs able to initiate a primary immune response (31, 45, 46).

Exposure to UV light has long been associated with exacerbation of SLE and photosensitivity remains a diagnostic criterion for this disease (47), yet little is known about the intrinsic mechanisms of lupus photosensitivity. UVB irradiation is known to trigger apoptosis (22), which is thought to elicit no inflammation or even an anti-inflammatory response (27). Paradoxically, UVB is expected to elicit opposing effects (i.e., inflammatory) in lupus flares (48). In the present study, we sought to resolve the apparent dichotomy of UVB response by defining whether such dual effects, inflammatory vs anti-inflammatory, was based on the dose of irradiation implied.

It is noteworthy that, although our experiments were in vitro studies and used a keratinocyte cell line, the UV doses we have used in this report are based on the 1995 guidelines of the World Health Organization (34) and on the standardized method to measure sun exposure or SEDs (35). Moreover, since 90% of UVB is normally absorbed by the stratum corneum of the skin (49), the UVB doses that we chose were equivalent to 10% of the total dose that is absorbed by the skin. Therefore, the three levels of UVB used were equivalent to low, moderate, and excessive sunshine exposure, which are within the spectrum of exposure during a sunny day at the beach.

In our experiments, we have used somewhat lower doses than those used in the seminal work of Casciola-Rosen and colleagues (14), and yet obtained not only the same apoptotic results but also

![FIGURE 6](http://www.jimmunol.org/)

Redistribution of nuclear Ags during cell death depends on the UVB dose used. A431 cells irradiated with different UVB doses, stained with PI, anti-Ku, and anti-Sm after 3 h postirradiation are shown. With no treatment (no Rx), PI, Ku, and Sm staining were exclusively nuclear and no apoptotic cells were present. With low-dose (15 mJ/cm²), apoptosis occurred and consequently apoptotic bodies resulted with higher fluorescence intensity, suggesting a concentration of autoantigens such as native DNA, Ku, and Sm (white arrows). With the intermediate dose (35 mJ/cm²), A431 cells appeared rounded, with reduced cytoplasm. Although the nucleus was still relatively intact, native DNA, Ku, and Sm were detectable on the cytoplasmic membrane, suggesting an early translocation from the nucleus to the membrane compartment and therefore exposure of autoantigens to the extracellular compartment (yellow arrows). The highest dose (80 mJ/cm²), not only did not induce any of the classic apoptotic features tested, but the staining of autoantigens (DNA, Ku, and Sm) appeared diffuse throughout all of the cell compartments, suggesting a passive mechanism of autoantigen translocation.
necrosis, even if we delivered lower UVB doses. Plausible explanations for the different results are that Casciola-Rosen and colleagues (14) have used primary human keratinocytes, while we have used a human keratinocyte cell line, which was cycling and therefore more susceptible to UVB irradiation (50), and that the authors did not further increase the UVB dose to evaluate the occurrence of necrosis (14).

We used keratinocytes because they are the major targets of UVB irradiation (51). We found that the apoptotic response to UVB showed a marked triphasic pattern. Low doses of UVB triggered several classic steps of apoptosis, including flipping of the membrane, cell shrinkage, PARP cleavage, and DNA fragmentation, with little, if any, IL-1α production. With intermediate doses, keratinocytes showed flipping of the membrane but delayed PARP cleavage and DNA fragmentation, coincident with delayed loss of bcl-2 expression and delayed activation of the JNK/SAPKs pathway. It was noteworthy that these intermediate doses of UVB induced production of IL1-α and therefore proinflammatory apoptosis. Finally, with the highest doses, necrosis and production of IL1-α was induced along with release of autoantigen.

This result is in apparent contrast with the general thinking that apoptosis is a noninflammatory process (52) required for normal tissue homeostasis (25). We propose that for UVB irradiation, the same insult, at different doses, can generate qualitatively different apoptotic responses. At low doses, UVB induces a type of apoptosis resembling the physiological noninflammatory process used to regulate tissue homeostasis. At intermediate doses, UVB induces delayed apoptosis, where before death, cells produce proinflammatory cytokines to alert the immune system that damage occurred and exposed autoantigens. At the highest doses, UVB induces proinflammatory necrosis, where the production of inflammatory cytokines is accompanied by release of autoantigens, as shown by the presence of nucleosomal DNA in the supernatant (see Fig. 7), and danger signals (53). The autoantigen release, mainly observed with necrotic cells, was blocked by z-DEVD-fmk, yet necrotic cells were only partially rescued by the inhibition of caspase 3. These results suggest that with the highest doses of UVB, simultaneous activation of both apoptotic and necrotic pathways occurs and that the necrotic pathway, because of the loss of cellular integrity, allows autoantigen release.

Moreover, keratinocytes have been shown to up-regulate MHC class II upon stimulation with IFN-γ (54), and, under certain circumstances, can also present Ags to immune competent cells (55). It is therefore likely that prolonged exposure to UVB in vivo might create the conditions for a proinflammatory cell death, which in turn would stimulate the immune system.

One possible explanation for the different effects induced by UVB is the extent of the DNA damage inflicted to the cells and the concurrent activation of proinflammatory pathways. At low doses, DNA damage overcomes the protective effects of the DNA repair enzyme PARP, and the cells undergo apoptosis without activation of any other pathway (56). With intermediate doses, the DNA damage increases and PARP is activated but yet apoptosis is delayed because of heat shock proteins (HSPs). HSPs protect from UVB-induced apoptosis and prevent caspase 3 cleavage and PARP degradation, suggesting a direct role in the executioner phase of apoptosis (57). The hyperthermia induced by the UVB might induce HSPs, protecting cells from the executioner phase of apoptosis. Concurrently, a proinflammatory pathway is triggered and the production of IL-1α is the result. Finally, with the highest doses, IL-1α is produced, but the DNA damage is overwhelming, PARP is overactivated which in turn depletes cellular energy, therefore necrosis is the final outcome (58).

In recent years, it has become clear that necrosis is not just a mere destruction of cells due to a sudden and overwhelming insult, but rather that specific pathways distinct from those triggered during apoptosis are involved (13, 59, 60). Necrosis, in contrast to apoptosis, induces a proinflammatory response by activating professional APCs in vitro and acting as an adjuvant in vivo (31). Moreover, necrotic cells upon phagocytosis induce NF-κB activation (61) and production of proinflammatory cytokines by phagocytic cells, such as IL-8, TNF-α, and IL-10 (30). Our results showed indeed that with high doses of UVB, keratinocytes underwent necrosis; moreover, we also showed that necrotic cells produced IL-1α, demonstrating that necrosis induced by UVB can activate specific pathways within the dying cell and possibly initiate a proinflammatory cascade. Overall, our results and previous work suggest that necrotic cell death might be an ideal adjuvant for self-immunization in a genetically predisposed individual.

It is well established that lupus immune response is Ag driven (4, 6); therefore, a constant supply of self-Ag is needed. Cell death has been proposed as the possible source (62–65). Our results confirm that lupus autoantigens translocate to the membrane blebs and bodies during apoptosis and that such Ags are accessible to autoantibodies (data not shown). Nevertheless, we found remarkable differences depending on the dose. In particular, with intermediate doses, autoantigen redistribution was detectable throughout the membrane blebs while the nucleus was still intact,
suggesting first that autoantigen translocation might be an active phenomenon, and second that there might be an increased exposure to the surrounding milieu, which in turn could directly provide self-Ag to lupus-specific B cells and contribute to the generation of autoreactive plasma cells. Experiments are underway to investigate whether autoantigens, exposed by the membrane blebs, are actually capable of stimulating autoreactive B cells to mature into autoantibody-producing cells and whether proinflammatory cytokines have an additive effect when the highest doses of UVB are used.

When necrosis was induced by high doses of UVB, the distribution of autoantigens not only was diffuse, importantly, nucleosomal DNA autoantigen was found in the supernatant, suggesting loss from the nuclear compartment into the extracellular milieu and availability to form pathogenic immune complexes with pre-existing circulating anti-DNA autoantibodies. Consistent with this notion, protein extracts from necrotic keratinocytes blotted with human lupus sera revealed fewer autoantigens than apoptotic extracts, suggesting that release of lupus autoantigens occurs during necrosis (R.C., unpublished results).

In conclusion, based on our results, we propose the following scenario: during the normal exposure to UVB, the damage is contained and non-inflammatory apoptosis is the main result. When the UVB exposure is excessive, the cellular damage ranges from proinflammatory apoptotic to necrotic cell death. In a normal individual there is a flawless recovery from a transient autoreactive response, but in a genetically predisposed individual, the exposure and release of autoantigens and the release of proinflammatory cytokines could ignite the autoimmune response by directly stimulating autoreactive B cells or by providing lupus Ags to pre-existing autoantibodies. This would be an ideal condition in which two key elements for an optimal, genetically predisposed, autoimmune response are in place: 1) cell death, which would provide the autoantigens and 2) proinflammatory cytokines, induced by the UVB damage, which would provide the milieu for the autoimmune response. A better understanding of these two components will help to devise more effective treatments of SLE and its deleterious consequences after UVB exposure.

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