Rapid lupus autoantigen relocalization and reactive oxygen species accumulation following ultraviolet irradiation of human keratinocytes

W. Lawley, A. Doherty, S. Denniss, D. Chauhan, G. Pruijn1, W. J. van Venrooij1, J. Lunec and K. Herbert

Division of Chemical Pathology, Centre for Mechanisms of Human Toxicity, Hodgkin Building, University of Leicester, Lancaster Road, Leicester LE1 9HN, UK and 1Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands

Abstract

Objective. In vitro treatment with ultraviolet B (UVB) induces relocalization of lupus autoantigens to the cell surface. We have addressed the relationship between autoantigen relocalization, accumulation of intracellular reactive oxygen species (ROS) and the induction of apoptosis following UVA and UVB exposure.

Methods. Human primary keratinocytes were exposed in vitro to doses of UVA and UVB equivalent to 0.01–4 times the minimal erythemal dose. The cellular locations of Ro60, Ro52, Sm, U2-B and La were determined using monoclonal antibodies. ROS accumulation and apoptosis induction were assessed using the intracellular ROS probe 2′-7′-dichlorodihydrofluorescein diacetate, and the viability stains Hoechst 33342 and propidium iodide.

Results. UV treatment induced the relocalization of all five autoantigens investigated and an accumulation of ROS. UVA and UVB induced necrosis and apoptosis, respectively.

Conclusion. These data suggest that both UVA and UVB induce ROS within keratinocytes but have significantly different effects upon autoantigen relocalization and cell viability.

Key Words: Systemic lupus erythematosus, Reactive oxygen species, Ultraviolet light, Apoptosis, Autoantigen, Necrosis.

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease often characterized by severe sensitivity to sunlight and the production of high titres of autoantibodies, which are believed to have a pathogenic role. The production of autoantibodies isolated from SLE patients target a range of cellular components including DNA, nucleosomes and the protein constituents of ribonucleoprotein complexes (RNP), resulting in a sustained and potentially fatal condition. The RoRNP complex comprises the La protein as well as two Ro proteins, Ro52 and Ro60. Under normal conditions, the RoRNP complex is located within the cytoplasm, while free Ro and La protein are detected predominantly in the nucleus [1, 2]. Following cell stress, however, Ro and La have been detected at the cell surface in primary keratinocytes. This protein relocalization occurs following in vitro exposure to ultraviolet B (UVB) [3–7]. UVA did not induce cell surface expression of Ro in two cases [4, 7] but induced nuclear to cytoplasmic shuttling of La [5]. Cell surface expression has also been induced by virus infection and cytokine treatment [8–10].

Further investigation of this phenomenon concluded that Ro and La may accumulate within apoptotic bodies located at the cell surface [11]. UV induces apoptosis in a range of cell types [12–14], including keratinocytes which are the cells most likely to be exposed to physiological UV and therefore the most relevant for in vitro study.

UV-induced cell death may be inhibited in vitro by antioxidants such as ascorbic acid 2-O-α-glucoside [15], β-carotene [16], α-tocopherol [17] and superoxide dismutase (SOD) [18]. The accumulation of reactive oxygen species (ROS) within UV-treated keratinocytes may promote cell death and autoantigen relocalization as well as altering the structure and antigenicity of autoantigens, exacerbating the pathogenesis of SLE.

There is an extensive literature addressing the effect of UVB upon autoantigen relocalization and its relationship to the induction of apoptosis. It is currently unclear, however, whether UVA may induce a similar effect under the correct conditions. The role of UV-induced...
ROS, the time course of antigen relocalization and the requirement for apoptotic cell death also require further investigation.

The aim of the current investigation was to compare in greater detail the induction of autoantigen cell surface expression following in vitro exposure to UVA and UVB. In addition, an intracellular fluorescent marker of ROS was used to determine the increase in ROS within keratinocytes treated with UV and to correlate UV exposure with ROS accumulation, autoantigen relocalization and apoptosis induction.

Materials and methods

Antibodies and cell stains

Mouse monoclonal antibodies (mAb) specific for lupus autoantigens have been previously described [19–24]. The antibodies and their targets are shown in Table 1. FITC-conjugated goat anti-mouse IgG (Fab-specific) (Sigma, Poole, UK) was used to detect primary staining. 2′,7′-dichlorodihydrofluorescein diacetate (H$_2$DCFDA) and Hoechst 33342 were obtained from Molecular Probes (Cambridge Bioscience, Cambridge, UK).

Cell culture

All media were obtained from Gibco BRL (Paisley, UK) unless otherwise stated. Primary human keratinocytes were isolated from foreskin, and maintained in complete keratinocyte culture medium (KCM) as described previously [25]. 3T3 feeder layer cells were irradiated with 6000 rads using a Vickrad Cobalt-60 gamma source. The keratinocytes used in irradiation experiments were subconfluent and in an exponential growth phase. Keratinocytes which had been cultured for more than 5 weeks were not used as they were observed to undergo changes after this time.

UV irradiation

All UV irradiation was carried out in phosphate-buffered saline (PBS), and on ice to prevent overheating. UVB doses in the range 0.4–147.6 mJ/cm$^2$ were produced with a UVM-57 Chromato Vue lamp (Knight Optical Technologies, Leatherhead, UK). UVA doses in the range 400–146 880 mJ/cm$^2$ were produced using a UVL 56 Blak Ray lamp (Ultraviolet Products Inc., Knight Optical Technologies, Leatherhead UK). UVC doses of 175.02 mJ/cm$^2$ and 525.06 mJ/cm$^2$ were produced with a UVGL-58 multiband source (UVGL-58, Knight Optical Technologies). Doses were measured using an optical radiometer (Micropulse Technology, Knight Optical Technologies, UK). Following irradiation with the required UV dose, cells were either used immediately or incubated in KCM for the required period of time. Mock-irradiated control cells were incubated on ice in PBS for the same period of time as UV-treated cells but were not exposed to doses of UV.

Confocal microscopy

Keratinocytes were trypsinized and resuspended in PBS at a concentration of $1 \times 10^6$/ml. Cells were cultured on chambered slides in KCM until subconfluent and irradiated with UV as required. Cells were fixed in 1% paraformaldehyde for 5 min at 4°C then permeabilized in acetone for 5 min at 4°C. Following rehydration in PBS for 10 min at 4°C, the cells were incubated with primary mAb at the correct concentration for 1 h (see Table 1). The cells were then washed with PBS and incubated with goat anti-mouse FITC secondary antibody diluted 1:50 in PBS for 1 h. The cells were washed once more in PBS, mounted in DAPI Vectashield (Vector Labs, Peterborough, UK) and analysed using a Leica TCS4D (Milton Keynes, UK) true confocal scanning microscope.

Flow cytometry with H$_2$DCFDA

Keratinocytes were grown to subconfluence, trypsinized and washed twice in PBS. The cells were pelleted, resuspended in 2 μM H$_2$DCFDA and incubated at room temperature for 30 min. The cells were washed twice more in PBS to remove any H$_2$DCFDA which had not been internalized, and irradiated with UV for the required time. The percentage of positive cells was analysed by flow cytometry (Becton Dickinson FACScan, Oxford, UK).

Fluorescence microscopy with Hoechst 33342 and propidium iodide (PI)

Glass no. 0 coverslips were coated with rat tail collagen type I (Sigma). Once coated, the coverslips were washed with PBS and placed in wells of a 24-well tissue culture plate (Nunc, Gibco BRL, Paisley, UK). The keratinocytes were trypsinized, added to the wells and cultured in KCM until adhered and growing on the coverslips. At this stage the coverslips were washed twice in PBS and then covered with 1 ml of PBS while treated with the required dose of UV. Once irradiated, Hoechst 33342 at a final concentration of 1.5 μg/ml and PI at a final concentration of 5 μg/ml were added and the cells incubated for 15 min. Once incubation was complete, the cells were washed with PBS and the numbers of viable, early apoptotic and necrotic cells quantified by fluorescence microscopy using an Axiovert 135 microscope (Zeiss, Thornwood, USA). Cells were quantified by the differential abilities of viable and non-viable nuclei to take up Hoechst 33342 and PI. Specifically, the criteria were: cells were considered viable if the nucleus was stained blue by Hoechst 33342. Cells were considered to be apoptotic if blue staining could be observed throughout the cell, an indication of DNA damage.

Table 1. Mouse mAb used to locate intracellular autoantigens by confocal microscopy

<table>
<thead>
<tr>
<th>Antibody name</th>
<th>Specificity</th>
<th>IgG isotype</th>
<th>Reference number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y12</td>
<td>Anti-Sm</td>
<td>IgG3</td>
<td>19</td>
</tr>
<tr>
<td>2E7</td>
<td>Anti-Ro52</td>
<td>IgG1</td>
<td>24</td>
</tr>
<tr>
<td>2G10</td>
<td>Anti-Ro60</td>
<td>IgG1</td>
<td>22</td>
</tr>
<tr>
<td>4G3</td>
<td>Anti-U2-B*</td>
<td>IgG1</td>
<td>21</td>
</tr>
<tr>
<td>SW5</td>
<td>Anti-La</td>
<td>IgG2b</td>
<td>20, 23</td>
</tr>
</tbody>
</table>
fragmentation. Chromatids within apoptotic cells were condensed and blebs could be observed around the cell. Cells were designated necrotic if the nucleus was stained red by PI. Cells with blue and red staining were also classified as necrotic. Cells which were no longer adhered to the slide were not counted. All cell stage determinations were carried out by the same ‘blinded’ observer to ensure that the same criteria were applied to all samples.

Results

Lupus autoantigens relocalize in response to UVA, UVB and UVC treatments

Mouse mAb specific for the lupus autoantigens Sm, Ro52, Ro60, U2snRNP B² protein (U2-B²) and La were used to identify the location of these autoantigens in primary human keratinocytes which had been exposed to UV. Keratinocytes were treated with UVA (146.880 mJ/cm²), UVB (147.6 mJ/cm²) or UVC (175.02 mJ/cm²) and incubated with the required primary antibody followed by fluorescein isothiocyanate-conjugated goat anti-mouse secondary antibody. Autoantigen localization was determined by confocal microscopy. Treatment with UVC was included as a positive control, as it has been shown in our laboratory that one dose of 175.02 mJ/cm² rapidly produces a homogenous culture of apoptotic keratinocytes (data not shown).

Figure 1 shows the relocalization of Sm in keratinocytes treated with UVA (Fig. 1B), or UVB (Fig. 1C), and stained with the mAb Y12. Untreated (mock-irradiated) controls are shown in Fig. 1A. In untreated cells, Sm was exclusively nuclear, staining with a speckled appearance. Following UVA exposure, Sm nuclear staining was minimal, but there was extensive cytoplasmic and membrane staining. UVB induced nuclear and cytoplasmic staining, but Sm was not detected at the cell membrane.

Table 2 details the effect of UVC on Sm, Ro52, Ro60, U2-B² and La. In the absence of UV, Sm, Ro60, La and U2-B² were all located primarily in the nucleus, while Ro52 was found in the cytoplasm. UVA induced Sm, Ro60 and La to move into the cytoplasm. La and U2-B² were also detected in blebs. UVB caused Sm and Ro52 to be detected in both the cytoplasm and nucleus, while Ro60, U2-B² and La relocated almost exclusively to the cytoplasm. Ro52 and Ro60 were also detected within blebs following UVB. UVC-induced apoptosis resulted in the relocalization of all five autoantigens into cell blebs.

UVA- and UVB-induced relocalization is accompanied by an increase in ROS

The production of ROS within keratinocytes following UV exposure was measured using the intracellular fluorophore H₂DCFDA, which becomes fluorescent only upon activation by ROS. Following 30 min incubation with H₂DCFDA, keratinocytes were exposed to 800–20 000 mJ/cm² UVA (Fig. 2A) or 0.4–40 mJ/cm² UVB (Fig. 2B). Immediately following UV exposure, the percentage of cells stained positive for fluorescent H₂DCFDA was determined by flow cytometry. A dose of 8000 mJ/cm² UVA resulted in a two-fold increase in the number of cells staining positive for H₂DCFDA when compared with mock-irradiated control cells. A small number of control cells were fluorescent; this accounts for H₂DCFDA activation by ROS already present within cells. Any subsequent increase in fluorescence is attributed to the generation of ROS following UV exposure. ROS was also detected in keratinocytes treated with UVB, with a two-fold increase in H₂DCFDA activation occurring in keratinocytes exposed to doses of 10 mJ/cm².

Keratinocyte viability is reduced immediately following UVB exposure

Keratinocytes were treated with UVB (40–80 mJ/cm²) and incubated for 0–20 h before staining with the viability stains Hoechst 33342 and PI. The cells were categorized visually as either viable, early apoptotic or necrotic in 10 fields of view from duplicate experiments. The data are presented in Fig. 3. Immediately following UVB exposure, there was a small but insignificant decrease in viability in UV-treated cells compared with mock-irradiated controls (Fig. 3A). The number of viable cells decreased slightly 3 h after UV treatment (Fig. 3B). A decrease in viability was observed in cells treated with 20 000 mJ/cm² UVB 6 h after irradiation (Fig. 3C). Twenty hours after UV treatment (Fig. 3D), cell viability was reduced in both mock-irradiated and UV-irradiated cells, but with a clear increase in both apoptotic and necrotic cells in UV-treated samples when compared with mock-irradiated samples. Table 3 shows the overall percentage decrease in keratinocyte viability observed 20 h after treatment with no UVB (mock-irradiated), 40 mJ/cm² UVB and 80 mJ/cm² UVB. Prior to treatment with UVB, keratinocyte viability was greater than 96%, decreasing by 7.2% in mock-irradiated cells after 20 h, compared with a decrease in viability of 29.9% and 24.2% in cells treated with 40 mJ/cm² UVB and 80 mJ/cm² UVB, respectively. In mock-irradiated cells, there was an increase in both apoptotic and necrotic cells after 20 h incubation, but keratinocytes were not preferentially undergoing apoptotic cell death. In UVB-treated cells, however, there was a significant increase in apoptotic cell death (P ≤ 0.05, Chi-squared analysis) with a small but statistically insignificant increase in necrosis (Table 3). These data confirm that UVB induces apoptotic cell death over and above the gradual decrease in cell viability observed following mock irradiation.

UVA induces a significant increase in necrosis

Keratinocytes were irradiated with 20 000 mJ/cm² UVA and incubated for 0, 6 or 20 h before staining with Hoechst 33342 and PI. The cells were categorized as viable, early apoptotic or necrotic. The data are presented in Fig. 4. There was little immediate effect upon cell viability (Fig. 4A). A decrease in viability was observed in cells treated with 20 000 mJ/m² UVA 6 h
Intracellular detection of Sm by confocal microscopy. The intracellular location of Sm was detected using mAb Y12 and visualized with goat anti-mouse IgG–FITC (blue). Counterstaining was applied by using 4,6-diamidino-2-phenylindole (DAPI) mounting agent (red). Primary keratinocytes were exposed to UV mock irradiation (A), 146.880 mJ/cm² UVA (B) or 147.6 mJ/cm² UVB (C). Following UV irradiation, the cells were immediately stained for confocal microscopy.

Fig. 1. Intracellular detection of Sm by confocal microscopy. The intracellular location of Sm was detected using mAb Y12 and visualized with goat anti-mouse IgG–FITC (blue). Counterstaining was applied by using 4,6-diamidino-2-phenylindole (DAPI) mounting agent (red). Primary keratinocytes were exposed to UV mock irradiation (A), 146.880 mJ/cm² UVA (B) or 147.6 mJ/cm² UVB (C). Following UV irradiation, the cells were immediately stained for confocal microscopy.

after irradiation (Fig. 4B). Twenty hours after UVA exposure, there was a significant increase in the number of necrotic cells present in UVA-treated samples when compared with mock-irradiated controls (Fig. 4C). The percentages of viable, apoptotic and necrotic cells present in 10 fields of view from duplicate experiments are shown in Table 3. Prior to treatment with UVA, keratinocyte viability was greater than 95%, (Table 3). There was a significant decrease in cell viability 20 h after UVA treatment, compared with the reduction in viability observed for mock-irradiated cells (17.8% and 3.3%, respectively). There was a significant preferential
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Table 2. Autoantigen relocalization following exposure to UV light. Primary human keratinocytes were stained with mAb specific for lupus autoantigens and the location of autoantigens within the cells was visualized by confocal microscopy. Data are the summarized findings of several experiments.

<table>
<thead>
<tr>
<th>Antibody (specificity)</th>
<th>No UV</th>
<th>UVA 146 680 mJ/cm²</th>
<th>UVB 147.6 mJ/cm²</th>
<th>UVC 175.02 mJ/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y12 (Sm)</td>
<td>N</td>
<td>C and M</td>
<td>C and N</td>
<td>N and B</td>
</tr>
<tr>
<td>2E7 (Ro52)</td>
<td>C</td>
<td>C</td>
<td>C, N and B</td>
<td>C and B</td>
</tr>
<tr>
<td>2G10 (Ro60)</td>
<td>N</td>
<td>C</td>
<td>C and B</td>
<td>C and B</td>
</tr>
<tr>
<td>4G3 (U2-B*)</td>
<td>N</td>
<td>N and B</td>
<td>C</td>
<td>N and B</td>
</tr>
<tr>
<td>SW5 (La)</td>
<td>N</td>
<td>C, M and B</td>
<td>C</td>
<td>M and B</td>
</tr>
</tbody>
</table>

C, cytoplasm; N, nucleus; M, membrane; B, blebbing.

Discussion

It has previously been shown that UVB induces keratinocyte apoptosis following a 6-h incubation, and that apoptotic blebs contain Ro60 and La [11]. In addition, UVA has not been shown to induce surface expression of Ro60 [4, 7] but can induce the translocation of La from nucleus to cytoplasm and cell surface [5]. Previous research has used autoantibodies derived from the sera of autoimmune individuals. We have confirmed and extended the findings of previously published work using mAb directed specifically at the autoantigen of interest.

We confirm that there is a clear difference between UVA- and UVB-mediated autoantigen translocation. In our investigations, Ro60 was not present as membrane blebs following exposure to UVA, but was present following treatment with UVB and UVC. UVA did, however, induce a nuclear to cytoplasmic translocation similar to that reported previously for La [5]. Ro52 was found initially in the cytoplasm, relocating in response to UVB and UVC but not UVA.

Our data confirm previous findings, that Ro and La respond differently to UV [3–11]. La was present in membrane blebs following exposure to UVA and UVC but not UVB. UVB did, however, induce the movement of La from nucleus to cytoplasm. These data do not entirely concur with the findings of Casciola-Rosen et al. [11], who showed that La was present on the cell surface of UVB-irradiated keratinocytes 6 h after irradiation. The most likely reason for this difference is that all of our localization work was performed immediately after UV exposure, whereas Ro60 and Ro52 may relocate much sooner.

It has been clearly shown that UVB induces apoptosis in keratinocytes [11–13]. Our data support the induction of apoptosis by UVB, although in our experiments, membrane expression of autoantigens occurred before a significant increase in apoptotic cells could be detected by visual categorization alone. This confirms the findings of Norris et al. [26] who showed that translocation of Ro60 to the cell surface could be induced by certain
The effect of UVB on keratinocyte viability. Primary keratinocytes were cultured on glass coverslips, treated with 40 mJ/cm² or 80 mJ/cm² UVB on ice and incubated for 0 h (A), 3 h (B), 6 h (C) or 20 h (D). Following incubation, the cells were stained with Hoechst 33342 and PI, and categorized visually as viable, apoptotic or necrotic. Total numbers of cells in 10 fields of view were counted and categorized in duplicate experiments. The keratinocytes used in each experiment were derived from independent cultures, with the exception of the cells incubated for 20 h, which were derived from the same culture but were irradiated in independent experiments with appropriate mock-irradiated controls. The number of viable (black circles), apoptotic (open triangles) and necrotic cells (open squares) counted in each field are plotted individually. Where the number of cells in a category was zero, data are not shown.

The effect of UVA and UVB on keratinocyte viability. Primary keratinocytes were cultured on glass coverslips, treated with the required dose of UV on ice and incubated for 0–20 h in KCM. The cells were stained with Hoechst 33342 and PI and categorized visually as viable, apoptotic or necrotic. The total number of cells in 10 fields of view was counted and categorized in duplicate experiments. The percentages of viable, apoptotic or necrotic cells resulting from each treatment 0 and 20 h after treatment are shown. The overall increase or decrease in percentage viability after 20 h is shown in parentheses for each treatment condition.

<table>
<thead>
<tr>
<th>UV treatment</th>
<th>Incubation time (h)</th>
<th>UV dose (mJ/cm²)</th>
<th>Viable</th>
<th>Apoptotic</th>
<th>Necrotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>UVB</td>
<td>0</td>
<td>0</td>
<td>98.6</td>
<td>0.2</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>96.1</td>
<td>0.8</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>96.9</td>
<td>0.8</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0</td>
<td>91.4 (−7.2)</td>
<td>3.7 (+3.4)</td>
<td>4.9 (+3.7)</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>66.2 (−29.9)</td>
<td>23.7 (+22.9)</td>
<td>10.1 (+7.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>72.7 (−24.2)</td>
<td>15.1 (+14.3)</td>
<td>12.1 (+9.9)</td>
<td></td>
</tr>
<tr>
<td>UVA</td>
<td>0</td>
<td>0</td>
<td>97.5</td>
<td>1.6</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0</td>
<td>95.4</td>
<td>2.5</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>20 000</td>
<td>0</td>
<td>94.2 (−3.3)</td>
<td>2.1 (+0.5)</td>
<td>3.7 (+2.9)</td>
</tr>
<tr>
<td></td>
<td>20 000</td>
<td>0</td>
<td>77.6 (−17.8)</td>
<td>6.0 (+3.5)</td>
<td>16.4 (+14.3)</td>
</tr>
</tbody>
</table>

Stimuli in the absence of apoptosis. It is clear, however, that cells exposed to UVB eventually become visibly apoptotic, suggesting that very early apoptotic events may have a role in autoantigen relocalization. We did not detect a significant increase in apoptosis following UVA irradiation. Twenty hours after UVA irradiation there was a significant increase in necrotic cells, but a much smaller increase in apoptosis. It is not possible using visual categorization alone to distinguish between late apoptotic and necrotic cells. It is plausible, therefore, that UVA does induce apoptosis but at a faster rate than UVB. However, the degree of apoptosis...
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Fig. 4. The effect of UVA on keratinocyte viability. Primary keratinocytes were cultured on glass coverslips, treated with 20,000 mJ/cm² UVA on ice and incubated for 0 h (A), 6 h (B) or 20 h (C). Following incubation, the cells were stained with Hoechst 33342 and PI, and categorized visually as viable, apoptotic or necrotic. Total numbers of cells in 10 fields of view were counted and categorized in duplicate experiments. The keratinocytes used in each experiment were derived from independent cultures, with the exception of the cells incubated for 6 h, which were derived from the same culture but were irradiated in independent experiments with appropriate mock-irradiated controls. The number of viable (black circles), apoptotic (open triangles) and necrotic cells (open squares) counted in each field are plotted individually. Where the number of cells in a category was zero, data are not shown.

and necrosis in UVA- and UVB-treated keratinocytes 6 h after irradiation was very similar (Figs 3C, 4B). If UVA induced rapid apoptosis, it may be expected to observe significant apoptosis 6 h after UVA treatment, followed by an increase in necrosis 20 h after UVA treatment. The lack of a significant increase in apoptosis at any of the time points investigated suggests that UVA-induced cell death does not occur by the induction of apoptosis.

Apoptosis has been postulated as a mechanism by which certain nuclear proteins may be cleaved, producing novel and immunogenic fragments [11]. Casiano et al. [27], however, suggested that the majority of autoantigens involved in systemic autoimmunity are not detectably cleaved during apoptosis, and that cleavage during necrotic cell death may also be relevant. It is also possible that cleavage or modification of other autoantigens does occur but is difficult to detect.

UVA and UVB irradiation also induced keratinocytes to produce ROS, as detected by the conversion of H₂DCFDA from the non-fluorescent to the fluorescent form. It is not clear from these data what role, if any, ROS may play in autoantigen relocalization. We hypothesize, however, that the presence of ROS so early after UV irradiation may result in damage to many cellular macromolecules and cause alterations in the structure of autoantigens and consequently, the revelation of cryptic or neo-epitopes. The revelation of such epitopes may exacerbate the immune response in SLE.

It is important to relate in vitro experiments to potential environmental UV exposure. The dose of UV required to induce subsequent erythema (minimal erythemal dose; MED) varies depending upon skin type. One MED for an individual with skin type I (burns easily but never tans) will be much lower than that for an individual with skin type IV (tans easily without burning) [28]. MED differs, therefore, for each skin type. One MED of UVB is in the range of 10–60 mJ/cm², while 1 MED of UVA ranges from 10,000 to 100,000 mJ/cm² [29]. The doses of UV used in our investigations would be expected to induce a mild sunburn reaction in most individuals and may be considered environmentally relevant.

We have demonstrated that relocalization of Ro60 and Ro52 occurs immediately following UVB irradiation. We have additionally shown that the same effect
may be induced with UVA with regard to Sm, U2-B’ and La. We have demonstrated also that apoptosis may not be an absolute requirement for the induction of autoantigen cell surface expression, but that it remains an important feature of the relocalization response. Finally, we have provided evidence that cellular levels of ROS increase immediately following both UVA and UVB irradiation in keratinocytes, suggesting a potential role for ROS in the generation of altered and potentially immunogenic autoantigens.

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