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Ultraviolet B irradiation and cytomegalovirus infection synergize to induce the cell surface expression of 52-kD/Ro antigen

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SUMMARY

Cultured human fibroblasts (MRC-5) have been previously demonstrated to express calreticulin, but not Ro autoantigen, on their surface after human cytomegalovirus (CMV) infection. The present study addresses the question of whether other stimuli, alone or in combination with CMV, can induce the surface expression of Ro autoantigens on human fibroblasts. Using a fixed-cell ELISA to detect autoantigen expression, a synergistic effect between ultraviolet B (UVB) exposure and CMV infection on the surface expression of 52-kD/Ro antigen, but not 60-kD/Ro or calreticulin, was observed. The enhanced expression of 52-kD/Ro antigen was significant and specific, compared with untreated cells, cells infected with CMV alone or irradiated with UVB only, and cells subjected to other treatments, such as low pH. Immunofluorescence studies confirmed these findings and indicated that cells expressed 52-kD/Ro protein on their surface at 24 h after a combined UVB and CMV treatment. These studies provide evidence that synergy between UVB irradiation and CMV infection may play a role in the induction of cell surface expression of the human autoantigen, 52-kD/Ro.

Keywords Ro autoantigens calreticulin fibroblasts cytomegalovirus ultraviolet B irradiation

INTRODUCTION

Ro (60 kD and 52 kD) and calreticulin (46 kD) autoantigens are small intracellular ribonucleoproteins, which have been isolated and cloned [1–4], but their biological functions remain unclear. Antibodies directed against Ro and calreticulin antigens are of clinical interest, as they are found in $\approx$50% of patients with systemic lupus erythematosus (SLE) and associated with lupus skin lesions [5–7]. However, the mechanisms by which autoantibodies to these intracellular proteins are induced are not fully understood.

In previous studies, ultraviolet B (UVB) irradiation has been demonstrated to induce the expression of Ro antigens on the surface of cultured human keratinocytes [8–11]. This expression appeared to be an active process rather than a passive leakage of antigens to the cell surface. Estradiol has also been reported to augment the binding of IgG from anti-Ro antibody-containing sera to the surface of cultured keratinocytes [12]. This augmentation was not induced by other hormones, such as dihydrotestosterone, testosterone and progesterone, and was partially inhibited by the anti-oestrogen, nafoxidine [12].

In addition to UVB and estradiol, viruses have been proposed as important factors in disturbing normal cellular levels of autoantigens [13,14]. Human cytomegalovirus (CMV) is a potential candidate in the induction of Ro antigen expression. CMV is a common viral agent worldwide, which infects 50–100% of the population, targets all organ systems of the host, and persists in the host for life [15,16]. Elevated anti-CMV antibodies [17,18], as well as CMV-induced flares [19,20], have been reported in SLE patients, suggesting that patients with SLE may have a different immune response to CMV infection than normal individuals. In addition, CMV RNA, including the abundant 2·8-kb RNA as well as the much less abundant 2·5, 1·9, 1·45 and 1·3-kb RNAs, has been immunoprecipitated from infected fibroblasts by SLE sera containing anti-Ro antibodies [21], suggesting that viral RNA may interact with cellular Ro proteins, resulting in 'altered self' proteins.

In a previous study [22], we reported the enhanced expression of calreticulin, but not Ro antigen, on the surface of cultured human fibroblasts infected with CMV. The purpose of the present study was to investigate whether other stimuli (in particular, UVB irradiation) in combination with CMV infection can modulate the expression of Ro antigens in cultured human fibroblasts, which are the only cells that are permissive for a full cycle of human CMV replication *in vitro* [23,24]. The demonstration of Ro antigen expression on the surface of human fibroblasts, after combined UVB and CMV treatment but not either treatment alone, suggests that multiple factors may synergize in the induction of cell surface expression of the human autoantigen, Ro.

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MATERIALS AND METHODS

Cell culture and CMV infection

The human embryo lung fibroblast cell line, MRC-5, and human CMV (Davis strain) were both obtained from American Type Culture Collection (ATCC, Rockville, MD). MRC-5 cells were expanded and passaged in Dulbecco's minimal essential medium (DMEM; GIBCO Labs, Grand Island, NY), supplemented with 10% heat-inactivated calf serum (HICS; GIBCO) and 0·01% (w/v) gentamycin sulphate (USB, Cleveland, OH), and grown at 37°C with 5% CO_2. When MRC-5 monolayers were grown to ≈90% confluency, cells were infected with 10^3 plaque-forming units (PFU) of CMV, as previously described [22]. Cell viability following CMV infection was assessed by trypan blue dye exclusion. The mean viability (± s.e.m.) of the infected cells was 96·7 ± 0·5% at 24 h, 95·5 ± 0·7% at 48 h, and 90·0 ± 2·3% at 72 h of CMV infection, compared with uninfected controls (95·8 ± 1·2% at 24 h, 96·0 ± 0·4% at 48 h, and 94·8 ± 0·9% at 72 h, respectively).

Effects of UVB irradiation and low pH treatment on cells

MRC-5 cells (2×10^5/ml) were grown for 3 days in 56·7 cm^2 plastic Nunclon dishes (GIBCO) or in 96-well flat-bottomed plates (Falcon, Becton Dickinson, Lincoln Park, NJ). For UVB exposure, cells were irradiated for 5 s with UVB (2·0 mJ/cm^2, 302 nm) emitted from a transilluminator (Spectroline; Fisher, St Laurent, Quebec, Canada). This source of irradiation does not contain any UVA or UVC component. The dose of 2·0 mJ/cm^2 was chosen since this amount of UVB irradiation achieves cell injury without killing the cells [8]. For low pH treatment, cells were incubated with DMEM medium at pH 5·0 for 5 s. Five-second exposures were used to be consistent with the UVB experiments. Cell viability was assessed after each treatment by trypan blue dye exclusion (mean cell viability ± s.e.m. was 95·2 ± 1·3% after UVB irradiation and 94·4 ± 0·6% after low pH treatment, compared with 96·2 ± 2·1% for untreated control cells). After treatment, the medium was replaced with fresh DMEM medium at 37°C. Cells were returned to the 37°C incubator, and grown in the presence and absence of CMV for up to 3 days.

Antibodies

Rabbit anti-peptide antibodies, specific for the amino terminal region (amino acids 7–23) of calreticulin (KEQFLDGDG-WTSRWIES), the carboxyl terminal region (amino acids 489–499) of 60-kD/Ro (RKKMDIPAKLIVC), and the amino acid region (amino acids 129–143) of 52-kD/Ro (EEAAQEYQEKLQ-VAL) were kindly provided by Dr Marianna Newkirk (The Montreal General Hospital Research Institute, Montreal, Quebec, Canada) and have been described previously [22]. These anti-peptide antibodies are capable of binding to the native antigens, and such binding can be inhibited by the respective peptides or by anti-Ro-positive SLE serum. IgG $F(ab')_2$ fragments from the anti-peptide antibodies were prepared as previously described [22]. The MoAb W6/32 (ATCC), directed against a non-polymorphic determinant on HLA-ABC, was used to study the surface expression of MHC class I antigens.

Fixed-cell ELISA

Fibroblast cells (2×10^5/ml, 100 μl per well) were cultured in 96-well flat-bottomed plates and treated with CMV and/or UVB, as described above. For the detection of surface antigens, cells were washed with PBS and fixed to the plates by adding 100 μl of 0·07% glutaraldehyde per well and incubating for 5 min at 25°C [22]. For the detection of cytoplasmic and nuclear antigens, cells were washed with PBS, and permeabilized and fixed with 0·005% digitonin in 2% paraformaldehyde (100 μl/well) for 5 min at 37°C, followed by 0·07% glutaraldehyde (100 μl/well) for 5 min at 25°C. Under these conditions, cytoplasmic, nuclear and surface antigens could be detected (referred to as 'total cellular' antigen expression). After three washes with PBS, the fixed cells were incubated with anti-peptide IgG $F(ab')_2$ fragments (20 μg/ml in PBS), control pre-immunized rabbit IgG $F(ab')_2$ fragments, or PBS for 30 min at 4°C. Following three washes with PBS, the cells were incubated with biotin-conjugated anti-rabbit IgG (Vector, Mississauga, ON). Alkaline phosphatase-conjugated Avidin D (Vector) was used to detect the bound antibodies. To correct for differences in cell numbers between individual wells, the cell number in each well was determined by staining with crystal violet [22]. At the termination of the ELISA, plates were stained with 0·2% crystal violet for 1 min at 25°C. After PBS washing, 100 μl/well of PBS were added and the OD at 550 nm was read on an ELISA reader. A standard curve was established by plating known concentrations of cells and staining with crystal violet. Total antigen expression was corrected for the number of cells in the same wells, as detected by crystal violet staining, and was expressed as OD_{405} units per cell, as described previously [22]. In each experiment, wells coated with positive control peptides (2·5 μg/ml) and incubated with the appropriate anti-peptide antibody served as positive controls. Each sample was run in duplicate in three separate experiments. The results were analysed for variance (s.e.m.) between the three experiments for control and test samples.

Immunofluorescence

MRC-5 cells, grown on coverslips in DMEM medium, were stained as either fixed or living cells. Staining of fixed cells was performed as described previously [22]. Living cells were washed with cold (4°C) PBS and then incubated with antibodies for 1 h at 4°C. $F(ab')_2$ of affinity-purified rabbit anti-peptide antibodies (anti-calreticulin, anti-52-kD/Ro and anti-60-kD/Ro) were used to detect antigen expression (20 μg/ml in PBS). A MoAb to HLA-ABC was used to detect cell surface expression of HLA-ABC antigens under the different conditions. Cells were washed with cold PBS and immediately fixed by incubation with 0·005% digitonin in 2% paraformaldehyde for 5 min at 37°C. Surface binding of rabbit IgG $F(ab')_2$ fragments was detected with FITC-conjugated anti-rabbit IgG (1 : 50 dilution) for 30 min at 37°C, while binding of the murine anti-HLA MoAb was detected with a rhodamine-conjugated anti-mouse IgG [22]. After three washes in PBS, the slides were mounted in a 90% glycerol/PBS solution containing paraphenylenediamine (0·1 mg/ml), and viewed through a Zeiss fluorescent microscope (Zeiss, Oberkochen, Germany), using oil immersion and a × 40 objective. In each experiment, negative controls included untreated cells that were stained with the same anti-peptide antibody and treated cells that were incubated with pre-immunized rabbit IgG $F(ab')_2$ fragments or PBS, and the second antibody. Each sample was run in duplicate and experiments were repeated three times.

Immunoblotting

This method has been described previously [22] and is briefly summarized as follows. The proteins in MRC-5 cell extracts were separated using standard 12% SDS–PAGE and blotted to

nitrocellulose membrane (0·45 μm) (BioRad, Richmond, CA). After blocking with PBS containing 0·4% bovine serum albumin (BSA) for 16 h at 4°C, blots were incubated with antiserum 1 : 50 diluted in PBS for 2 h at 37°C. The nitrocellulose filters were then washed three times with PBS containing 0·05% Tween 20 (PBS–T) and incubated with biotinylated anti-rabbit IgG (1 : 2000 dilution) for 1 h at 37°C. After three washes with PBS–T, the bound antibody was detected by incubation with horseradish peroxidase (HRP)-labelled Avidin D (Vector) (1 : 3000 dilution) and 3,3′-diaminobenzidine tetrahydrochloride substrate (Sigma, St Louis, MO). The positions of the bands were compared with standard molecular weight markers.

Statistical analysis

Data were analysed by the paired *t*-test using the Instat program (GraphPAD Software, San Diego, CA). *P* values (one-tailed) $< 0{\cdot}05$ were considered significant.

RESULTS

Effects of UVB and CMV stimuli on cell surface expression of antigens

The analysis of Ro and calreticulin autoantigen expression was performed using a fixed-cell ELISA, and the results are shown in Figs 1 and 2. As shown in Fig. 1, CMV infection did not significantly increase 52-kD/Ro or 60-kD/Ro antigen expression on the fibroblast surface at 24 h of infection, while cell surface expression of calreticulin was enhanced, as in our previous report [22]. When cells were exposed to UVB alone, there was also an increase in the cell surface expression of calreticulin, but not 52-kD/Ro or 60-kD/Ro antigen. This surface expression was similar to that observed with CMV infection alone. However, when fibroblast cells were treated with a combination of UVB irradiation and CMV infection, 52-kD/Ro antigen expression on the cell surface was significantly increased at 24 h of treatment, compared with untreated cells, CMV-infected cells and UVB-irradiated cells ($P < 0{\cdot}05$). A specific increase of 52-kD/Ro antigen expression for UVB plus CMV-treated cells, compared with cells treated with UVB or CMV alone, was also observed when the results were expressed as paired comparisons of the test *versus* control cells, where the control cells were normalized to 1·0. The mean value ± s.e.m. of the ratio of this antigen expression for UVB plus CMV-infected cells to untreated control cells was $1{\cdot}40 \pm 0{\cdot}23$, compared with $0{\cdot}86 \pm 0{\cdot}14$ for UVB-treated cells and $1{\cdot}00 \pm 0{\cdot}05$ for CMV-infected cells.

In contrast to the observation that 52-kD/Ro expression was increased only after combined UVB and CMV treatment, cell surface expression of 60-kD/Ro antigen was not significantly induced by this treatment. Although cell surface expression of calreticulin was increased by either UVB irradiation or CMV infection, no further increase was caused by these two treatments together. In addition, cell surface expression of MHC class I was increased by CMV infection, with or without UVB irradiation (data not shown).

Effects of UVB and CMV stimuli on total cellular expression of antigens

The total cellular (surface plus nuclear and cytoplasmic) antigen expression, obtained by fixed-cell ELISA, is shown in Fig. 2. CMV infection did not cause an increase of 52-kD/Ro antigen expression in cells, while UVB exposure alone or UVB plus CMV treatment

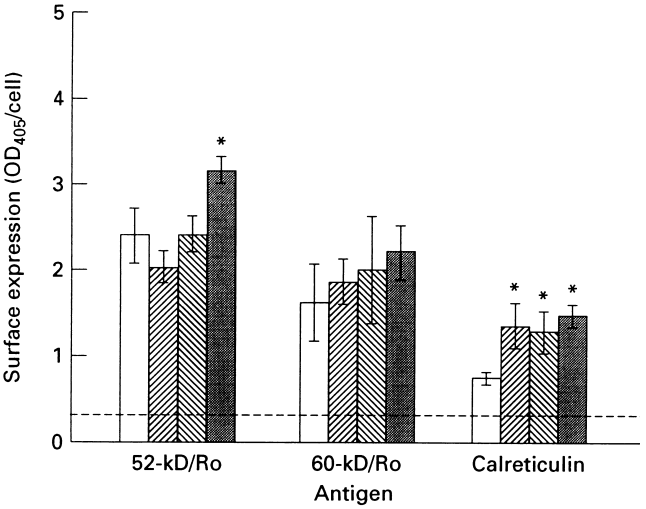

Fig. 1. Cell surface expression of 52-kD/Ro, 60-kD/Ro and calreticulin antigens. The binding of anti-Ro or anti-calreticulin peptide antibodies to human fibroblasts untreated (control) or treated with UVB, cytomegalovirus (CMV) or UVB plus CMV was assessed by fixed-cell ELISA. Results shown are the mean binding (OD_{405}/cell ± s.e.m.) of three independent experiments. The dashed line represents the mean binding (OD_{405}/cell) of pre-immunized rabbit serum for all experiments. *Significant differences in paired comparisons with untreated control cells ($P < 0{\cdot}05$). □ , UVB–, CMV–; ▨, UVB+, CMV–; ▧, UVB–, CMV+; ■, UVB+, CMV+.

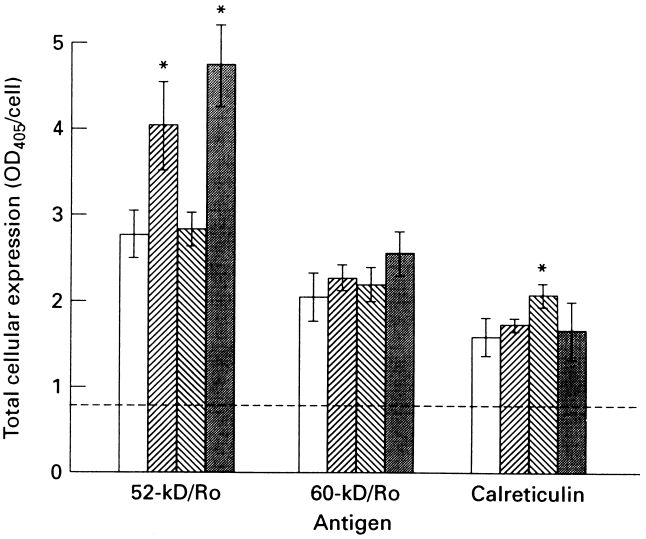

Fig. 2. Total cellular (surface, nuclear and cytoplasm) expression of 52-kD/Ro, 60-kD/Ro, and calreticulin antigens. The binding of anti-Ro or anti-calreticulin peptide antibodies to human fibroblasts untreated (control) or treated with UVB, cytomegalovirus (CMV), or UVB plus CMV was assessed by fixed-cell ELISA. Results shown are the mean binding (OD_{405}/cell ± s.e.m.) of three independent experiments. The dashed line represents the mean binding (OD_{405}/cell) of pre-immunized rabbit serum for all experiments. *Significant differences in paired comparisons with untreated control cells ($P < 0{\cdot}05$). □, UVB–, CMV–; ▨, UVB+, CMV–; ▧, UVB–, CMV+; ■, UVB+, CMV+.

showed an augmentation in total cellular 52-kD/Ro antigen ($P < 0{\cdot}05$), compared with untreated cells. The cellular expression of 60-kD/Ro antigen did not change significantly with any stimulation, including CMV infection, UVB irradiation and UVB plus CMV treatment. Although cellular calreticulin expression was increased in CMV-infected cells ($P < 0{\cdot}05$), there was no marked increase in calreticulin in cells after UVB irradiation or UVB plus CMV treatment, compared with untreated cells.

Effects of low pH treatment on surface and total cellular expression of antigens

Experiments using other stimuli, such as low pH treatment, were performed to determine whether the synergistic effect between UVB exposure and CMV infection on the cell surface expression of 52-kD/Ro antigen was specific. Cells were pre-incubated at low pH (pH 5·0) before CMV infection, and compared with incubation at pH 7·2. For surface expression of cells incubated at low pH, the OD_{405}/cell values (mean ± s.e.m.) of uninfected and CMV-infected cells, respectively, were $2{\cdot}36 \pm 0{\cdot}35$ and $2{\cdot}46 \pm 0{\cdot}29$ (compared with $2{\cdot}41 \pm 0{\cdot}07$ and $2{\cdot}42 \pm 0{\cdot}50$ at pH 7·2) for 52-kD/Ro; $1{\cdot}67 \pm 0{\cdot}47$ and $2{\cdot}10 \pm 0{\cdot}45$ (compared with $1{\cdot}61 \pm 0{\cdot}46$ and $2{\cdot}11 \pm 0{\cdot}63$) for 60-kD/Ro; and $1{\cdot}04 \pm 0{\cdot}10$ and $1{\cdot}06 \pm 0{\cdot}17$ (compared with $0{\cdot}59 \pm 0{\cdot}09$ and $1{\cdot}18 \pm 0{\cdot}28$) for calreticulin. For total cellular expression of cells incubated at low pH, the mean OD_{405}/cell values for uninfected cells and CMV-infected cells, respectively, were $2{\cdot}83 \pm 0{\cdot}27$ and $2{\cdot}52 \pm 0{\cdot}11$ (compared with $2{\cdot}60 \pm 0{\cdot}28$ and $2{\cdot}82 \pm 0{\cdot}17$ at pH 7·2) for 52-kD/Ro; $2{\cdot}20 \pm 0{\cdot}22$ and $2{\cdot}17 \pm 0{\cdot}35$ (compared with $2{\cdot}02 \pm 0{\cdot}27$ and $2{\cdot}16 \pm 0{\cdot}20$) for 60-kD/Ro; and $1{\cdot}89 \pm 0{\cdot}49$ and $1{\cdot}75 \pm 0{\cdot}34$ (compared with $1{\cdot}54 \pm 0{\cdot}22$ and $1{\cdot}83 \pm 0{\cdot}30$) for calreticulin. Treatment with low pH did not induce a consistent increase in either the cell surface or total cellular expression of the 52-kD/Ro antigen or 60-kD/Ro antigen. In contrast, calreticulin surface expression, but not total cellular expression, was induced by low pH and was similar to that observed following treatment with CMV, UVB or UVB plus CMV.

Immunofluorescence localization of 52-kD/Ro antigen in UVB and CMV stimulated cells

The synergy between UVB irradiation and CMV infection in the induction of surface expression of the 52-kD/Ro antigen on fibroblast cells suggested by the ELISA results was confirmed by immunoflorescence, which is shown in Fig. 3. Untreated cells showed no cell surface staining with anti-52-kD/Ro antibody (Fig. 3A). However, dense granular surface staining with anti-52-kD/Ro antibody was seen on UVB-irradiated CMV-infected cells at 24 h (Fig. 3B), and at 72 h of treatment (Fig. 3C). When cells were infected with CMV only, or irradiated with UVB alone, cell surface staining for 52-kD/Ro antigen was also evident, but much weaker than that observed with UVB plus CMV treatments (data not shown). In contrast, there was no evidence of surface fluorescence staining on CMV- and/or UVB-treated cells incubated with anti-60-kD/Ro antibody, pre-immunized rabbit IgG or PBS, compared with untreated cells (data not shown). Anti-calreticulin antibody showed weak surface staining on CMV- and/or UVB-treated cells (data not shown).

When cells were fixed before reaction with the primary antibody, typical nuclear or cytoplasmic staining was clearly distinguishable from the cell surface staining (Fig. 3D–F). After 24 h of treatment, nuclear 52-kD/Ro staining was increased in CMV-infected cells and UVB-irradiated cells (data not shown) and UVB plus CMV-treated cells (Fig. 3E), compared with untreated cells (Fig. 3D). This staining appeared to shift from the nucleus to the cytoplasm after 72 h of treatment (Fig. 3F). However, no differences were observed between the fluorescence patterns of untreated cells and treated cells incubated with anti-60-kD/Ro antibody (data not shown). Although anti-calreticulin antibody showed increased nuclear fluorescence staining in CMV-infected cells, no increased nuclear or cytoplasmic staining was found in UVB-irradiated cells or untreated cells (data not shown).

Analysis of total cellular 52-kD/Ro antigen in fibroblast cell extracts

To examine whether the changes in the cellular distribution of 52-kD/Ro antigen observed by the fixed-cell ELISA or immunofluorescence were associated with changes in the total cellular content of Ro, cellular extracts were made from MRC-5 cells that had undergone different treatments. By Western blotting, there was a slight increase in the 52-kD/Ro protein in the extracts of treated cells, compared with untreated cells (data not shown), but there was no obvious quantitative difference between the extracts of cells treated with either CMV, UVB or CMV plus UVB (data not shown).

DISCUSSION

Our results demonstrate a significant synergy between UVB exposure and CMV infection on the surface expression of 52-kD/Ro antigen in human fibroblasts (MRC-5). In a fixed-cell ELISA, this phenomenon was significant and specific, compared with untreated cells and cells treated with CMV or UVB alone, or subjected to other treatments, such as low pH. Immunofluorescence confirmed these findings and showed that cells expressed 52-kD/Ro protein on their surface after combined UVB and CMV treatment. These results extend our previous observations, which showed that MRC-5 cells treated with CMV alone demonstrated an increased expression of calreticulin on the surface, whereas no increase in Ro antigen surface expression was observed [22].

The specific induction of the surface expression of 52-kD/Ro antigen, but not 60-kD/Ro or calreticulin, by UVB plus CMV treatment is intriguing. Although this may be due to specific structural or functional features of the 52-kD/Ro protein, the limited data available make it difficult to correlate the antigenic properties with known characteristics of the protein. Surface expression of the 52-kD/Ro antigen occurred only in UVB-irradiated cells that had subsequently undergone CMV infection, while UVB irradiation caused an augmentation in total cellular 52-kD/Ro antigen. The 52-kD/Ro protein, which contains zinc finger motifs, may play a role in the DNA repair process after UVB irradiation of cells [3,25–27], as proteins sharing this motif have been found to be involved in DNA repair, regulation of gene expression, or protein transformation [27,28]. In addition, a recent study has shown that apoptotic skin keratinocytes contain surface blebs expressing autoantigens, such as Ro [29]. Apoptosis may occur in response to many different stimuli, including DNA damage [30,31], heat shock [32] and viral infection [33,34]. Although the mechanism responsible for the synergistic effect of UVB and CMV in the induction of 52-kD/Ro antigen surface expression is not clear, it is possible that apoptosis, induced by combined UVB and CMV treatment, results in the surface expression of 52-kD/Ro and other nuclear autoantigens.

In this study, calreticulin was the only one of the three antigens to show increased cell surface expression with all of the various

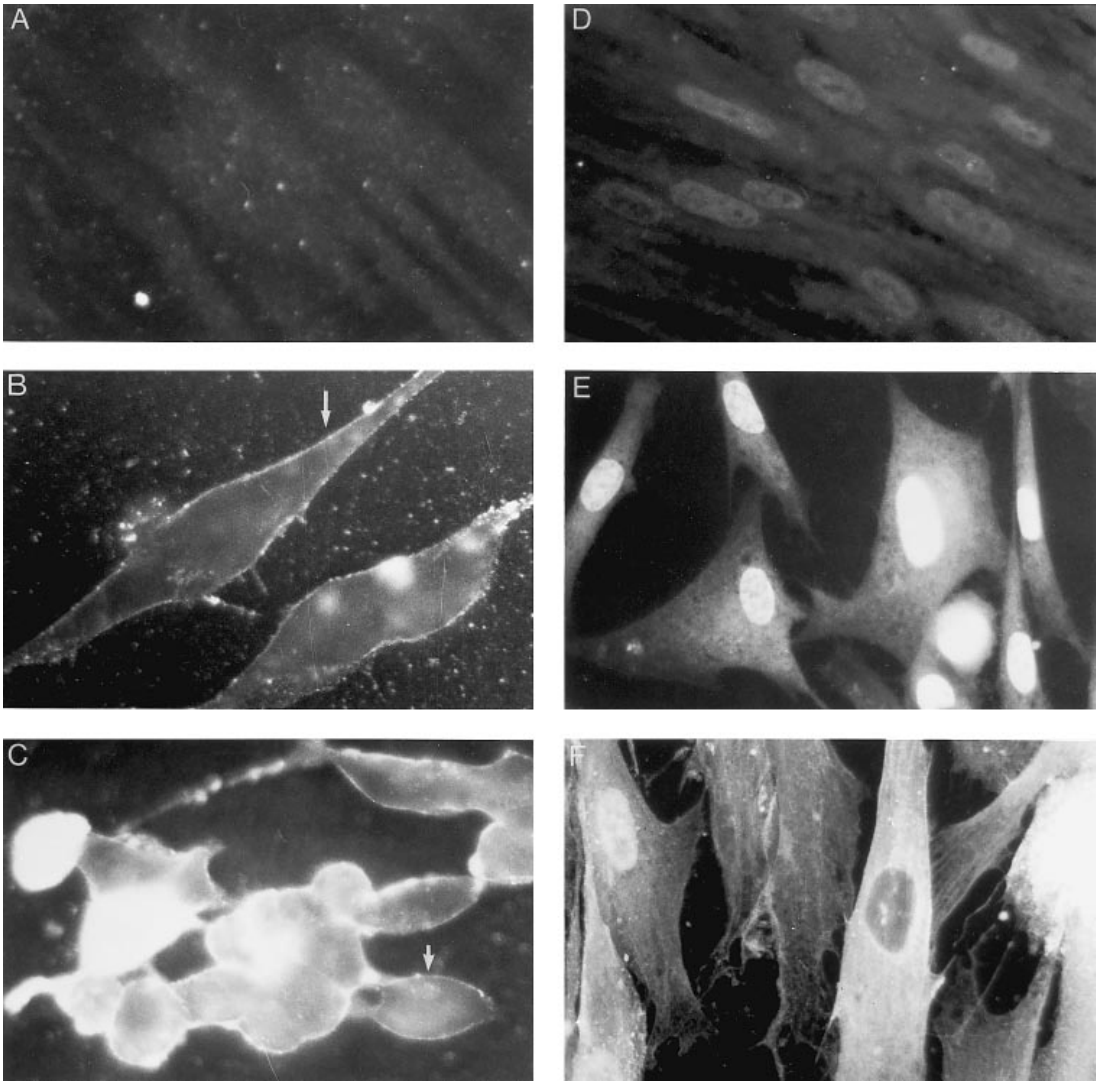

Fig. 3. Indirect immunofluorescent staining of human fibroblasts with anti-52-kD/Ro antibody. (A,D) Untreated cells. (B,E) Cells at 24 h after UVB plus cytomegalovirus (CMV) treatment. (C,F) Cells at 72 h after UVB plus CMV treatment. In A–C, cells were fixed after incubation with the primary antibody (surface staining). In D–F, cells were fixed before incubation with the primary antibody (cytoplasmic staining). Arrows indicate membrane fluorescence. Results shown are representative of three independent experiments.

stimuli, including CMV, UVB and low pH. This phenomenon may be explained by its cellular functions. Previous studies have suggested that calreticulin is a stress protein [35,36]. Homology between the promoter region of calreticulin and other related stress proteins has been reported [37], and a link between heat shock/stress proteins and autoimmunity has been described [38–40]. Recently, Erkeller-Yuksel *et al.* [41] demonstrated that a group of patients with SLE expressed heat shock protein 90 (hsp90) on the surface of their blood mononuclear cells, and this increased level correlated with high disease activity. As there was no difference in cytoplasmic expression of hsp90 in blood mononuclear cells from SLE patients and controls, these authors suggest that hsp may chaperone integral proteins and/or damaged intracellular proteins to the cell surface, or that hsp, released from stressed cells, may bind to the surface of viable cells [41].

Anti-52-kD/Ro antibody was found to bind to the surface of UVB plus CMV-treated cells by both the fixed-cell ELISA assay and immunofluorescence analysis. However, binding of the same anti-52-kD/Ro antibody to the surface of CMV-infected or UVB-irradiated cells was observed only by immunofluorescence analysis. These observations are consistent with the findings of Peek *et al.* [42], who reported that surface expression of the La autoantigen could be detected on adenovirus-infected cells by immunofluorescence, but not by other techniques. As there was no difference between the cellular content of 52-kD/Ro antigen in cells subjected to the different treatments, it is probably not the total cellular content of Ro protein that changes during stimulation, but the cellular distribution of the protein or its ability to be recognized by specific anti-Ro antibodies. However, it is also possible that Western blot analysis is a less sensitive quantitative measure of cellular protein expression than the ELISA [43].

The Ro antigen/antibody system is highly associated with cutaneous lupus syndromes [44–47]. The results described here demonstrate that synergy between UVB irradiation and CMV infection can induce cell surface expression of the 52-kD/Ro antigen in cultured human fibroblasts, and may help to explain

why environmental stimuli can promote the development of lupus skin lesions. Future studies are required to understand better the role of multifactorial induction of Ro in the skin. Human keratinocytes in culture should provide a useful model to study these effects *in vitro*.

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