Photochemistry and Photobiology, Vol. 62, No. 1, pp. 176–183, 1995
Printed in the United States.

0031-8655/95 $05.00+0.00

HYDROGEN PEROXIDE MEDIATES UV-INDUCED IMPAIRMENT OF ANTIGEN PRESENTATION IN A MURINE EPIDERMAL-DERIVED DENDRITIC CELL LINE

GISELA CACERES-DITTMAR[1,2], KIYOSHI ARIIZUMI[1], SHAN XU[1], FELIX J. TAPIA[2], PAUL R. BERGSTRESSER[1] and AKIRA TAKASHIMA*[,1]

[1]Department of Dermatology, University of Texas Southwestern Medical Center, Dallas, TX 75235, USA and [2]Instituto De Biomedicina, Caracas, Venezuela

(*Received* 12 *December* 1994; *accepted* 8 *March* 1995)

Abstract—Ultraviolet-B (290–320 nm) radiation is known to impair the antigen-presenting cell (APC) function of Langerhans cells (LC), skin-specific members of the dendritic cell (DC) family. We sought to address mechanisms of this effect, focusing on the role played by hydrogen peroxide. For this purpose, we used a newly established murine DC line, XS52, which resembles epidermal LC in several respects. The APC capacity of XS52 cells, using two different $CD4^+$ T cell clones as responders, was inhibited significantly (>50%) by exposure to UV radiation (unfiltered FS20 sunlamps) at relatively small fluences (50–100 J/m^2). Ultraviolet radiation also inhibited growth factor-dependent proliferation of XS52 cells. On the other hand, cell surface phenotype was relatively well preserved after irradiation; expression levels of B7-1 and B7-2 were reduced slightly, while other molecules (*e.g.* Ia, CD54, CD11a and CD18) were not affected. With respect to the role played by hydrogen peroxide, pretreatment with purified catalase (900 U/mL) prevented UV-induced inhibition of APC function. Short-term exposure to 3 m*M* H_2O_2 or *t*-butyl H_2O_2 mimicked UV radiation by inhibiting APC function. Finally, intrinsic catalase activity was substantially lower in XS52 cells compared with Pam 212 keratinocytes. These results indicate that the generation of hydrogen peroxide alone is sufficient to produce some, but not all, of the deleterious effects of UV radiation on DC derived from the skin.

INTRODUCTION

The immunologic impact of cutaneous exposure to UVB (290–320 nm)† radiation has been the subject of extensive investigation, leading to several different hypotheses concerning the identity of photoreceptors and mechanisms of action (reviewed in Simon *et al.*,[1] Hruza and Pentland[2] and Muller *et al.*[3]). Recent studies have examined (a) relevant chromophores, *e.g.* plasma membrane-associated molecules, *trans*-urocanic acid, or DNA,[4–6] (b) initial molecular events, *e.g.* generation of reactive oxygen species (ROS), activation of transcription factors, photoisomerization of urocanic acid or DNA damage[5–8] and (c) subsequent alterations in cellular function, *e.g.* altered production of cytokines or modulated expression of adhesion molecules.[9–13] With respect to mechanisms by which UVB radiation affects the antigen-presenting cell (APC) function of Langerhans cells (LC), which are skin-specific members of the dendritic cell (DC) family (reviewed in Steinman[14] and Stingl *et al.*[15]), two different epidermal cell subpopulations have been implicated as relevant targets. Ultraviolet radiation may alter LC function directly,[16–18] or it may act primarily on keratinocytes to modulate their function in a fashion that influences LC indirectly.[19,20] The purpose of this study was to determine whether UV radiation would alter APC function of DC in the complete absence of keratinocytes, and if so, to examine the mechanisms for this effect.

Until recently, the unavailability of long-term DC lines has prevented studying the direct impact of UV radiation on DC alone. Because of this limitation, earlier studies have been conducted with conventional LC preparations that unavoidably contained small to large numbers of contaminating keratinocytes. Thus, variations in the number of keratinocytes may have contributed to discrepancies among different laboratories. For this reason, we have chosen to use a long-term DC line (XS52) established recently in our laboratories. This line, which was derived from the epidermis of newborn BALB/c mice, resembles resident epidermal LC in several respects, including (a) surface phenotype (Ia^+/$CD45^+$/$CD3^-$/$CD11b^+$/$CD16^+$),[21] (b) antigen-presenting profile (modest capacity to activate naive T cells and profound ability to activate primed T cells),[21] and (c) cytokine mRNA profile (constitutive expression of interleukin [IL]-1β and macrophage inflammatory protein [MIP]-1α mRNA and lack of IL-6 mRNA expression).[22] With the availability of the XS52 DC line, we took the unique opportunity to study the impact of UV radiation on this skin-derived DC, including the causative roles played by ROS, in the absence of keratinocytes.

MATERIALS AND METHODS

Cells. The phenotypic and functional characteristics of XS lines have been described elsewhere.[21,22] Briefly, they were established

*To whom correspondence should be addressed.

†*Abbreviations:* APC, antigen-presenting cells; CSF-1, colony-stimulating factor-1; DC, dendritic cells; DETC, dendritic epidermal T cells; DNBS, dintrobenzene sulfonate; DNFB, dinitrofluorobenzene; FACS, fluorescence activated cell sorter; FCS, fetal calf serum; GM-CSF, granulocyte/macrophage colony-stimulating factor; IL, interleukin; LC, Langerhans cells; mAb, monoclonal antibody; MIP, macrophage inflammatory protein; NAC, *N*-acetylcysteine; PBS, phosphate-buffered saline; R, receptors; ROS, reactive oxygen species; SOD, superoxide dismutase; UVA radiation, 320–400 nm radiation; UVB radiation, 290–320 nm radiation; UVC radiation, 200–290 radiation.

from epidermal cells of newborn BALB/c mice and expanded thereafter in complete RPMI[23] supplemented with 1 ng/mL murine recombinant granulocyte/macrophage colony-stimulating factor (rGM-CSF; Gibco BRL, Grand Island, NY) and 10% culture supernatants from NS01 cells, a stromal cell line that was established in parallel.[21] Line XS52 was chosen for this study, because large numbers of cells were available and because it exhibits a potent APC capacity.

As responder T cells, we used a dinitrobenzene sulfonate (DNBS)-specific CD4+ T cell clone, 5S8. This clone was established by limiting dilution microculture from T cells isolated from dinitrofluorobenzene (DNFB)-painted BALB/c mice.[24] Cells were maintained in complete RPMI by feeding with 20 U/mL IL-2 and by repeated stimulation with DNBS and γ-irradiated BALB/c spleen cells. We also used the KLH-specific Th1 clone, HDK-1.[16,17]

Other cell lines included (a) NS01 and NS47, stromal cell lines established from epidermal sheet preparations of newborn BALB/c mouse skin,[21] (b) DFB, a fibroblast line established from dermal portions of newborn BALB/c mouse skin, (c) the Pam 212 keratinocyte line derived from BALB/c mice,[25] (d) 7-17, a dendritic epidermal T cell (DETC) line derived from AKR mice[23,26] and (e) 3T3, a fibroblast line (ATCC). These lines were maintained as described previously.[21,27-29]

Splenic DC were prepared from BALB/c mice using a standard overnight-culture method.[30] Briefly, spleen cell suspensions were first cultured in tissue culture dishes for 2 h in complete RPMI. After removal of nonadherent cells, adherent cells were cultured overnight in complete RPMI. Cells that were released spontaneously during the overnight culture period were used as splenic DC without further purification. This preparation contained 40–60% DC as assessed by staining with a monoclonal antibody (mAb), N418, which was kindly provided by Dr. R. Steinman, Rockefeller University, NY.

UV radiation. The XS52 cells were harvested by pipeting and washed extensively with phosphate-buffered saline (PBS). Cells (1×10^6 cells/mL/dish) were plated over 35 mm tissue culture dishes and then exposed to UV radiation. Because irradiation was completed in relatively short periods (*e.g.* 90 s for 100 J/m²), no special device was employed to agitate cells during irradiation. Dishes were precoated with 1% heat-denatured bovine serum albumin,[31] because the cells would otherwise adhere firmly to the dishes during UVB exposure. Radiation was delivered by a bank of four unfiltered FS20 sunlamps (Westinghouse, Pittsburgh, PA), and the irradiance was measured by an IL 700 Research radiometer equipped with a SEE 240 photodetector (Newburyport, MA).[16,17,32] Immediately after irradiation, cells were harvested by pipeting and washed twice with PBS.

T cell activation assays. The KLH-specific HDK-1 and the DNBS-specific 5S8 T cell clones were used to test antigen presentation by XS cells as described previously.[16,17,21,24] Briefly, XS52 cells were γ-irradiated (1500 rad) and then co-cultured with T cells (4×10^4 cells/well) in the presence of KLH (100 μg/mL) or DNBS (125 μg/mL) and ^{3}H-thymidine uptake was measured on day 3.

Surface phenotype analyses. After exposed to UV radiation (100 J/m²), XS52 cells were incubated for 24 h in a hanging-drop culture in Terasaki well plates (2×10^4 cells/25 μL/well), so that the majority of cells could be harvested for fluorescence activated cell sorter (FACS) analyses. The regular XS cell growth medium containing 1 ng/mL GM-CSF and 10% NS01 supernatant was used for this 24 h incubation. Cells were then stained with a panel of mAb and analyzed with the FACScan as before.[23,30] These mAb were purchased from Pharmingen (San Diego, CA), Sigma (St. Louis, MO) or Becton Dickinson (San Jose, CA).

Proliferation assays. The XS52 cells (1×10^4 cells/well) were cultured in round-bottom, 96 well plates in the presence or absence of murine rGM-CSF (10 ng/mL) or recombinant colony-stimulating factor-1 (rCSF-1; Upstate Biotechnology, Lake Placid, NY). Cultures were pulsed on day 2 with ^{3}H-thymidine and harvested on day 3 as before.[27,33]

Treatment with antioxidants. The XS52 cells or splenic DC were incubated for 1 h at 37°C in 2% fetal calf serum (FCS) in DPBS(−) in the presence or absence of 900 U/mL bovine liver catalase, 40 m*M* *N*-acetylcysteine (NAC) or 300 U/mL superoxide dismutase (SOD) (all purchased from Sigma). In some experiments, the catalase was heat-inactivated by a 10 min incubation in boiling water. Cells were harvested by pipeting, resuspended in DPBS containing fresh catalase (900 U/mL) and then exposed to the UV radiation. After extensive washing with DPBS, cells were examined for their phenotypic and functional properties.

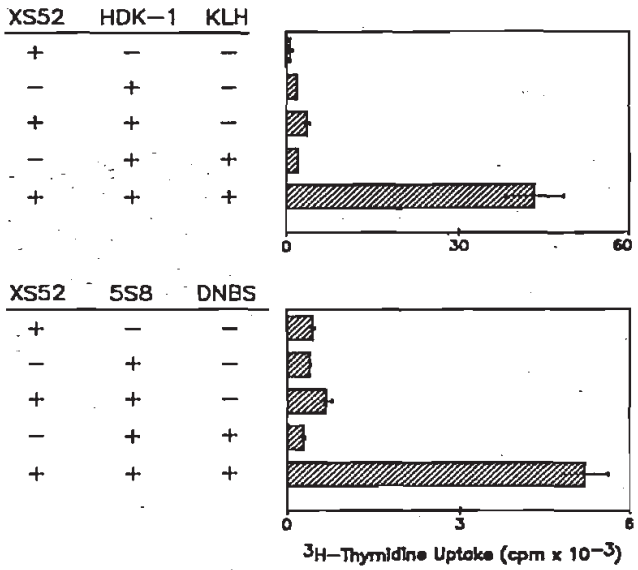

Figure 1. Antigen-presenting capacity of XS52 cells. The XS52 cells were γ-irradiated (1500 rad) and then examined for their capacity to activate the HDK-1 T cell clone (top) and the 5S8 T cell clone (bottom). The HDK-1 cells (4×10^4 cells) were cultured in the presence of XS52 cells (3×10^3 cells) and/or KLH (100 μg/mL), and 5S8 cells (4×10^4 cells/well) were cultured in the presence of XS52 cells (1×10^4 cells/well) and/or DNBS (125 μg/mL). Data shown are the mean ± SEM (n = 3) of the ^{3}H-thymidine uptake on day 3.

Treatment with H_2O_2. The XS52 cells were exposed to H_2O_2 or *t*-butyl H_2O_2 in 0.5% FCS in DPBS(−) at 37°C. After 15 min, cells were washed extensively with DPBS(−) and then examined for their immunological properties.

Assays for intrinsic catalase activity. After extensive washing with DPBS(−), cells were lysed in 1% Triton X-100, followed by 30 s sonication, and soluble fractions were examined for intrinsic catalase activity as described by others.[34] Briefly, cell extracts (200 μL) were incubated at room temperature with 19 m*M* H_2O_2 in 50 m*M* PBS (pH 7.0), and the absorbance at 240 nm was monitored for 180 s. Catalase activity (U/mg) was then calculated using bovine liver catalase as a standard, in which the protein concentration was measured by using the Bio-Rad Protein Assay (Hercules, CA).

RESULTS

UV radiation impairs the APC function of XS52 cells

To examine the impact of UV radiation on antigen presentation, we employed two different CD4+ T cell clones, HDK-1 which is specific for the protein antigen, KLH, and 5S8, which recognizes the chemical hapten, DNBS. Nonirradiated XS52 cells, in the presence of the appropriate antigen, induced significant proliferation by the respective T cell clone (Fig. 1). Only minimal, if any, proliferation was observed in the absence of the relevant antigen or the XS52 cells, indicating both antigen specificity and the APC requirement for this response. In addition, significant activation of the HDK-1 (40000 cells/well) was achieved with only 1000 APC/well, indicating their potency (Fig. 2).

We have reported previously that FACS-purified LC are capable of activating HDK-1 cells and that UV radiation abrogates this capacity.[16,17] In the current experiments, UV

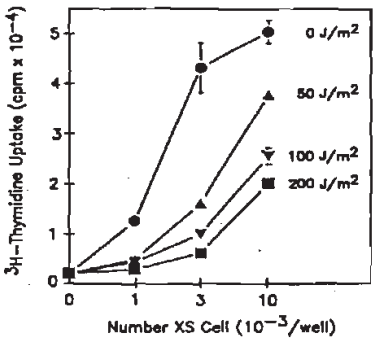

Figure 2. Ultraviolet radiation impairs the capacity of XS52 cells to present the protein antigen, KLH. Immediately after exposure to the indicated fluences of UV radiation, XS52 cells were γ-irradiated and then examined for their capacity to present KLH to the HDK-1 cells. Data shown are the mean ± SEM (n = 3) of the ^{3}H-thymidine uptake at the indicated number of XS52 cells per well. Other experimental conditions are described in Fig. 1.

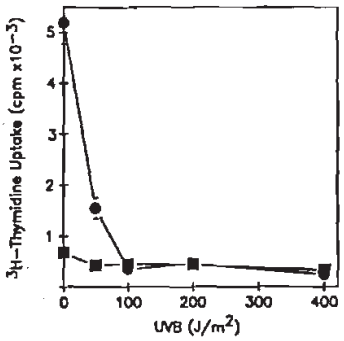

Figure 3. Ultraviolet radiation impairs the capacity of XS52 cells to present the chemical antigen, DNBS. After exposure to the indicated fluences of UV radiation, XS52 cells (1×10^4 cells/well) were examined for their capacity to present DNBS to the 5S8 cells. Data shown are the mean ± SEM (n = 3) of the ^{3}H-thymidine uptake (day 3) in the presence (circles) or absence (squares) of DNBS. Other experimental conditions are described in Fig. 1.

exposure decreased substantially the capacity of XS52 cells to activate HDK-1 cells, thus validating this experimental system (Fig. 2). Interestingly, the degree of inhibition varied substantially, depending on the numbers of XS52 cells added per well. At 1000 cells/well, for instance, 50 J/m^2 was sufficient to inhibit presentation almost completely, whereas the same UV fluence produced 60% inhibition at 3000 cells/well and only 25% inhibition at 10000 cells/well. Similarly, 100 J/m^2 produced complete inhibition at 1000 cells/well, 80% inhibition at 3000 cells/well and 50% inhibition at 10000 cells/well. In other words, UV-induced inhibition was overcome by the addition of similarly irradiated XS52 cells in excess, suggesting that a fraction of XS52 cells may have been resistant to or be protected from the irradiation. Alternatively, it may suggest that the effect of irradiation is to lessen, but not to abolish completely, the efficacy of an individual cell to present this antigen to T cells. Ultraviolet irradiation also inhibited in a dose-dependent fashion the ability of XS52 to present DNBS to T cells (Fig. 3). Again, relatively small fluences were sufficient to produce a significant inhibition of this capacity. Thus, we concluded that XS52 cells are sensitive to UV radiation; a single exposure to low doses (50–100 J/m^2) abrogates their capacity to present either protein or chemical antigens to CD4$^+$ T cells.

A crucial question concerned whether inhibition occurred merely through the cytotoxic effects of UV radiation. To address this question, XS52 cells were exposed to several different fluences of UV and then cultured in the absence of added growth factors for up to 3 days, the period employed for the APC assays (Fig. 4). Exposure to 50 J/m^2 of UV radiation produced minimal effect on cell viability during the next 3 days, as compared with the UV(−) control. Cytotoxic effects were also not evident even at 100 J/m^2 for at least 24 h after irradiation. By contrast, UV fluences above 200 J/m^2 caused acute cell death, with viability decreasing significantly within 24 h after exposure. Thus, it appears that UV radiation impairs APC function in fluences that do not cause a substantial decrease in cell viability, as measured by trypan blue exclusion. It is to be noted, however, that this assay is not sensitive enough to measure metabolic cell death.

Influences of UV radiation on the expression of adhesion molecules

It has been reported previously that UVB radiation affects the surface expression of CD54 (intercellular adhesion molecule-1) by LC[13] and of B7-1 monocytes.[35] Experiments were then conducted to study the impact of UV radiation on the expression of adhesion molecules by the XS52 cells. As noted in Fig. 5, irradiation with 100 J/m^2 decreased the expression of B7-1 and B7-2 modestly, and it failed to affect the expression of Ia, CD54, CD11a, CD11b or CD18 (Fig. 5). These results suggest that XS52 cells may not be an ideal model to study UV-dependent modulation of adhesion molecules. On the other hand, these results document that the altered expression of adhesion molecules is one, but not the only, mechanism by which UV radiation inhibits APC function.

XS52 cells are more susceptible than keratinocytes to the growth-inhibitory effects of UV radiation

To determine whether XS52 cells are more sensitive to UV radiation than other cells in skin, we compared XS52 cells with a panel of skin-derived lines for their sensitivities to the growth-inhibitory effects of UV radiation. As ob-

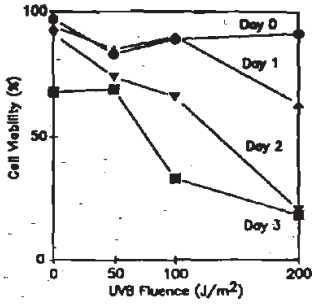

Figure 4. Cytotoxic effect of UV radiation on XS52 cells. The XS52 cells were exposed to the indicated fluences of UV radiation, cultured in the absence of added growth factors in a hanging-drop culture in Terasaki well plates. After 1–3 days, cells were harvested and then examined for cell viability by trypan blue exclusion. Data shown represent a result out of three independent experiments.

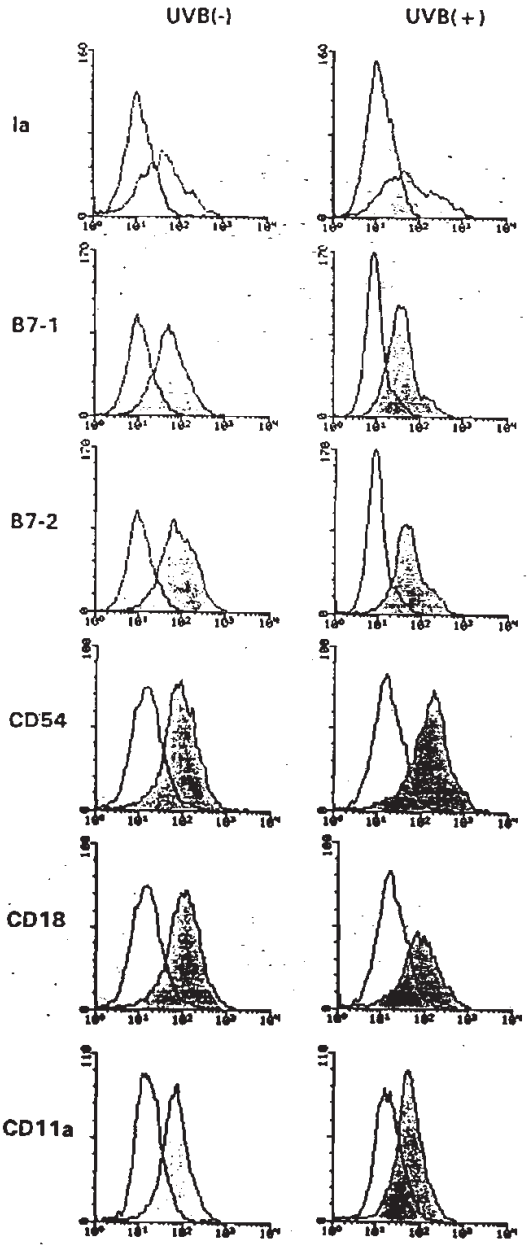

Figure 5. Effects of UV radiation on the expression of surface molecules. After exposure to 100 J/m² of UV radiation, XS52 cells were cultured in the absence of added growth factors in a hanging-drop culture in Terasaki well plates. Cells were harvested after 24 h and then stained with mAb against the indicated surface molecule (filled histogram) or an isotype-matched control IgG. B7-1 expression was tested in XS52 cells that had been cultured for 3 weeks with GM-CSF (10 ng/mL) alone. Data shown represent one set of results out of three independent experiments.

served previously,[21,22] XS52 cells proliferated vigorously in response to GM-CSF or CSF-1. Ultraviolet radiation inhibited in a dose-dependent fashion the proliferative responses to these growth factors: (a) 50 J/m² produced a 95% inhibition of GM-CSF responses and a 40% inhibition of CSF-1 responses (Fig. 6). Importantly, not all of the cell lines responded equally to the radiation: (a) bone marrow-derived epidermal leukocyte lines (*i.e.* the XS52 and XS20 lines and the 7-17 DETC lines) were quite susceptible; (b) Pam 212

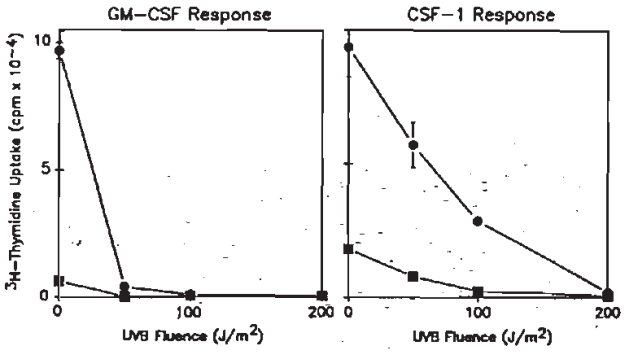

Figure 6. Ultraviolet radiation inhibits cytokine-dependent growth of XS52 cells. After exposure to the indicated fluences of UV radiation, XS52 cells (1×10^4 cells/well) were examined for their proliferative responses to GM-CSF (10 ng/mL) (left) and CSF-1 (10 ng/mL) (right). Data shown are the mean ± SEM (n = 3) of the ^{3}H-thymidine uptake on day 5 in the presence (circles) or absence (squares) of the relevant cytokine.

keratinocytes were completely resistant up to 400 J/m² and (c) fibroblasts (*i.e.* NS01 and NS47 lines, DFB dermal fibroblasts and 3T3 line) were intermediate (Fig. 7). Thus, XS52 cells were most sensitive to UV radiation among the cell lines tested.

The role of hydrogen peroxide in UV-induced alteration of XS cell function

Considering the recent concept that ROS may play a critical role in the UV-dependent signaling cascade,[8] we sought to determine whether we could modulate the deleterious effect of UV radiation on XS cells with exogenous antioxidants (Table 1). In their absence, UV radiation again caused substantial (81%) inhibition of the APC capacity. Pretreat-

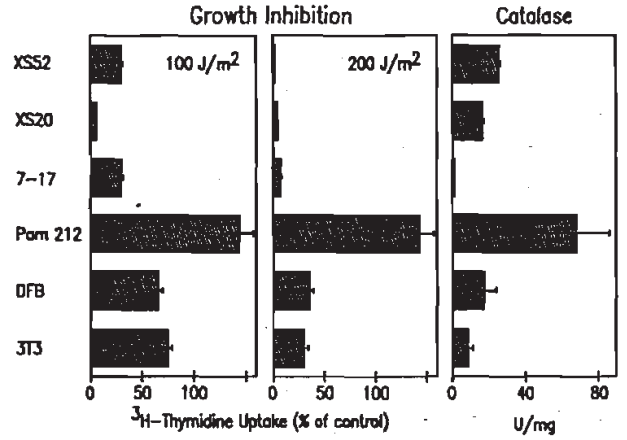

Figure 7. Comparison of different skin-derived cell lines for their susceptibility to UV-mediated growth inhibition and for their intrinsic catalase activity. Six different cell lines were exposed to UV radiation and then examined for their proliferative capacity. As growth factors, GM-CSF (10 ng/mL) or IL-2 (10 ng/mL) was added to XS cell lines or 7-17 DETC line, respectively. Data shown are the % of ^{3}H-thymidine uptake compared to the nonirradiated controls (mean ± SEM, n = 3). In a different set of experiments, the same cell lines were extracted in 1% Triton X-100, and soluble fractions were then examined for intrinsic catalase activity. Data shown are the catalase activity in units (U) per 1 mg of protein (mean ± SEM, n = 3).

Table 1. Effects of antioxidants on UV-induced impairment of APC capacity in XS52

Antioxidants	APC capacity (−) UV	APC capacity (±) UV*	% inhibition†
None	33 300 ± 3500§	6290 ± 950	81.1
NAC‡	20 200 ± 1300	13 500 ± 1500	33.2
SOD‡	35 300 ± 1700	9080 ± 1130	74.3
Catalase‡	37 900 ± 1600	25 700 ± 410	32.2

*The XS52 cells were irradiated with 100 J/m² UV.
†Percent inhibition induced by UV radiation.
‡The XS cells were pretreated for 60 min at 37°C with NAC (40 m*M*), SOD (300 U/mL) or catalase (900 U/mL) and exposed to UV in the continuous presence of each agent. Cells were then washed extensively and examined for their APC capacity.
§Proliferative responses of 5S8 T cells measured by ^{3}H-thymidine uptake on day 3 (mean ± SEM, n = 3). Background proliferation in the absence of DNBS was below 1000 cpm.

ment of XS52 cells with catalase or NAC reduced this inhibition to 32% or 33%, respectively. By contrast, pretreatment with SOD had no effect under the conditions employed. With respect to the influences of added antioxidants on unirradiated XS cells, NAC caused a substantial reduction in their APC function (20 200 ± 1300 cpm compared to 33 300 ± 3500 cpm). Because similar effects were observed in three experiments, it is likely that NAC at the tested concentration (40 m*M*) has some inhibitory effect on their APC function, in addition to the preventative effect against UV radiation. With respect to the influences of the antioxidants on different APC populations, treatment of splenic DC with catalase decreased their susceptibility to UV radiation: 80% inhibition without catalase *versus* 43% inhibition with catalase (Table 2). Importantly, heat-inactivated catalase preparations were no longer effective (73% inhibition), indicating the requirement for intact enzymatic activity.

We also observed that pretreatment with exogenous hydrogen peroxide would mimic UV radiation in its effects on APC function. A short-term (15 min) exposure to H_2O_2 or *t*-butyl H_2O_2 produced a significant inhibition of APC function of XS52 cells, with 3 m*M* *t*-butyl H_2O_2 causing almost complete inhibition to the level (80% inhibition) seen in UV-irradiated cells (83% inhibition) (Fig. 8). Hydrogen peroxide treatment also inhibited their proliferative response to CSF-1 (up to 62% inhibition), but this inhibition was incomplete compared with the 99% inhibition by UV radiation. Thus, it appears that UV-induced generation of hydrogen peroxide alone is essential to impair the APC function in XS52 cells, whereas additional mechanisms are required to inhibit their mitotic activities. Consistent with this notion is our observation that catalase failed to protect XS52 cells from UV-induced inhibition of their proliferative responses. In those experiments, UV radiation (100 J/m²) caused 92 ± 3% or 76 ± 7% inhibition of their proliferative responses to GM-CSF (10 ng/mL) or to CSF-1 (10 ng/mL), respectively. Even in the presence of catalase treatment (900 U/mL), UV radiation induced 87 ± 7% inhibition of GM-CSF responses and 75 ± 3% inhibition of the CSF-1 responses.

Table 2. Effects of catalase on UV-induced impairment of APC capacity in splenic DC

Antioxidants	APC capacity (−) UV	APC capacity (±) UV	% inhibition
None	15 000 ± 1800	3070 ± 510	79.5
Catalase	11 400 ± 1950	6530 ± 1180	42.7
Inactivated catalase*	13 200 ± 350	3590 ± 630	72.8

*Heat-inactivated catalase. Other experimental conditions are described in Table 1.

Intrinsic catalase activity is modest in XS52 cells compared to keratinocytes

Because of their high sensitivity to hydrogen peroxide, we reasoned that XS52 cells might not possess high intrinsic catalase activity. Crude cell extracts prepared from XS52 cells contained catalase activity at a detectable level (25 U/mg), but it was substantially lower than that (68 U/mg) detected in Pam 212 keratinocytes (Fig. 7). The XS20 and 7-17 DETC also exhibited relatively low catalase activities. It is important to note, however, that catalase activity is not the only factor that determines the susceptibility to UV-induced growth inhibition. The 3T3 fibroblast line, for instance, which contained even lower catalase activity than XS52 cells, was more resistant to UV radiation than was XS52. This, again, suggests that the generation of hydrogen peroxide is not the sole mechanism for UV-induced growth inhibition. Nevertheless, we interpret our data to mean that bone marrow-derived leukocytes that home to epidermis (*i.e.* LC and DETC) possess relatively limited catalase activities, whereas keratinocytes that are committed to form the epidermal structure are more fully protected with higher intrinsic catalase activities.

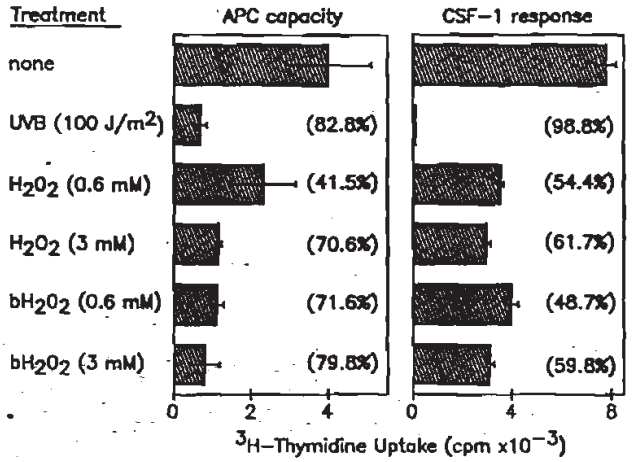

Figure 8. Hydrogen peroxide mimics UV radiation in its effects on APC function. After a 15 min exposure to H_2O_2 or *t*-butyl H_2O_2 (bH_2O_2), XS52 cells were washed extensively and then examined for their capacity to present DNBS to the 5S8 T cell clone and to proliferate in response to CSF-1 (10 ng/mL). Numbers in the parentheses indicate the % of the ^{3}H-thymidine uptake compared to the nontreated controls. Other experimental conditions are described in Figs. 1 and 6.

DISCUSSION

We have demonstrated in this study that UV radiation impairs the APC function in XS52 cells, an epidermal-derived DC line. Importantly, this effect was prevented by purified catalase. Because macromolecules, such as catalase, are not readily incorporated into cells, it is likely that added catalase manifested its activity outside the cells, presumably at the plasma membrane. If so, our results may suggest that hydrogen peroxide generated at this location mediates at least some of the deleterious effects of UV radiation on DC in skin. This hypothesis is supported by the observation that APC function of XS52 cells was impaired significantly by brief exposure to hydrogen peroxide.

The ROS have been considered to serve as important mediators in UV-induced alterations in skin. It was reported a decade ago that UVB-induced keratinocyte injury, *i.e.* sunburn cell formation, may be prevented by local injections of SOD, but not catalase.[36] Interestingly, injections of SOD failed to prevent UVB-induced cutaneous inflammation, suggesting that keratinocyte injury and inflammation occur by different mechanisms. The ROS also appear to be partially responsible for UVB-induced depletion of epidermal LC; LC densities were preserved in UVB-irradiated guinea pig skin when SOD, but not catalase, was injected locally before or immediately after UVB irradiation.[7] By contrast, indomethacin, which inhibits UVB-induced skin inflammation, failed to prevent UVB-induced reduction in LC numbers.[37] These observations illustrate the complexity of mechanisms by which UVB radiation affects skin biology: (a) ROS play causative roles in UVB-induced injury of epidermal keratinocytes and LC, whereas other chemical mediators (*e.g.* prostaglandins) are more important in UVB-mediated cutaneous inflammation and (b) SOD, which converts superoxide into oxygen and hydrogen peroxide, is more effective than catalase, which converts hydrogen peroxide into oxygen and water, in preventing cellular injury in animal experiments.

With respect to mechanisms for action of catalase, one argument would be that catalase may have simply acted as a sunscreen. The following observations, however, suggest this possibility to be trivial. First, heat-denatured catalase failed to prevent UV-induced alteration of the APC function. Second, catalase was added at a relatively low protein concentration (*i.e.* <20 μg/mL), and proteins at this concentration would exhibit only negligible sunscreen effects, as indicated by the observation that SOD at 50 μg/mL showed no preventative effects. Therefore, it is more likely that catalase exhibits its biological effects through conversion of hydrogen peroxide that is generated during UV exposure.

Recent studies by Karin and his co-workers have established a new concept that ROS play a critical role in UV-dependent signaling cascade. To summarize their observations, (a) UVC (200–290 nm) irradiation of fibroblasts induces a rapid activation of c-jun, c-fos and NFkB, prototypic transcription factors[4,8,38]; (b) UV-dependent NFkB activation is inducible even in enucleated cells, suggesting that the initial signal occurs at or near the plasma membrane,[4] (c) Ha-Ras, Src tyrosine kinases and JNK1, a serine/threonine kinase, mediate UV-dependent c-jun activation[39,40] and, most importantly, (d) hydrogen peroxide mimics UV radiation by its ability to activate c-jun, and UV-dependent c-jun activation is inhibited by NAC, an intracellular free radical scavenger.[8,38,41] Our study has extended this concept by showing that ROS mediate UV-dependent impairment of APC function in a DC line.

It has been well established that skin exposure to UVB radiation alters the APC function of LC, leading to immunosuppression (reviewed in Simon *et al.*[1]). The present study, together with our previous observations made using FACS-purified LC,[16–18] demonstrate that UV radiation can act directly on LC and modulate directly their APC function. Our data also provide a conceptual basis for the future application of XS52 cells to photobiological studies. For instance, the present study, being conducted with an unfiltered FS20 sunlamp as the light source, has failed to address the wavelengths that are responsible for the observed biological influences of irradiation. Wavelengths within the UVB range are generally believed to be responsible for deleterious influences of UV radiation on cutaneous immunity. In this scenario one may argue that UVB is more accountable than UVA (320–400 nm) radiation. On the other hand, UVA, which is much more predominant in this light source, is generally believed to be more potent in producing ROS. Thus, it is possible that a major part of the observed influences is due to the wavelengths in the UVA range. Obviously, further experiments are required to clarify this important issue, and the XS52 cells will provide a useful opportunity for this line of investigation.

Another intriguing observation was that our bone marrow-derived, epidermal leukocyte populations (*i.e.* XS lines and a DETC line) contained substantially lower catalase activities compared to Pam 212 keratinocytes. This complements the earlier observations that catalase activity is higher in keratinocytes than in melanocytes[42] and in the epidermis than in the dermis.[43] Relative deficiency of catalase activities in the epidermal leukocytes may explain one mechanism by which skin exposure to UV radiation depletes LC and DETC without causing severe damage in neighboring keratinocytes.[44] This hypothesis is supported further by observations made by others that surface densities of LC and DETC are increased by topical application of antioxidants.[45,46] Therefore, it will be interesting to determine whether one can prevent deleterious effects of UV radiation on epidermal leukocytes *in vivo* by compensating for this intrinsic deficiency with exogenous catalase.

Finally, we are fully aware of the limitations associated with experiments conducted by using cell lines. For instance, it still remains unclear whether epidermal LC respond to UV radiation in the same manner as did the XS52 line. On the other hand, it is also true that the application of long-term cell lines has several advantages. Our earlier attempt to conduct similar experiments using FACS-purified LC has been unsuccessful because of several technical limitations: (a) trypsin treatments used for procurement of epidermal cells produced unavoidable experimental artifacts on surface phenotype, (b) the numbers of LC obtained after several purification steps (*e.g.* 5–20 000 cells per mouse after FACS purification) were not sufficient and (c) contaminating keratinocytes, which possess a high intrinsic catalase activity, made it difficult to assess the role of hydrogen peroxide. Therefore, we believe that XS52 cells will provide a unique opportunity

for many investigators in photoimmunology to examine hypotheses in a cost- and time-efficient manner.

Acknowledgements—We thank Dale Edelbaum for his technical assistance and Mrs. Betty Janes for her superb secretarial assistance. This work was supported by National Institutes of Health grants R01 AR35068, R01 AR40042, R01 AR 41150, 1P30 AR41940 and by Mary Kay Cosmetics, Inc., the Dermatology Foundation, the Carl J. Herzog Foundation, the Lester I. Conrad Foundation and The Perry R. and Nancy Lee Bass Research Fund in the name of Thomas L. Shields. Dr. Caceres-Dittmar was on leave from the Instituto de Biomedicina, Universidad Central de Venezuela, and was supported by a fellowship grant, F05 TW 05028, from the Fogarty International Center.

REFERENCES

1. Simon, J. C., J. Krutmann, C. A. Elmets, P. R. Bergstresser and P. D. Cruz, Jr. (1992) Ultraviiolet B-irradiated antigen-presenting cells display accessory signaling for T-cell activation: relevance to immune responses initiated in skin. *J. Invest. Dermatol.* **98**, 66S–69S.
2. Hruza, L. L. and A. P. Pentland (1993) Mechanisms of UV-induced inflammation. *J. Invest. Dermatol.* **100**, 35s–41s.
3. Muller, H. K., C. Bucana and M. L. Kripke (1992) Antigen presentation in the skin: modulation by u.v. radiation and chemical carcinogens (Review). *Semin. Immunol.* **4**, 205–215.
4. Devary, Y., C. Rosett, J. A. DiDonato and M. Karin (1993) NF-kappa B activation by ultraviolet light not dependent on a nuclear signal. *Science* **261**, 1442–1445.
5. Noonan, F. P. and E. C. DeFabo (1992) Immunosuppression by ultraviolet B radiation: initiation by urocanic acid. *Immunol. Today* **13**, 250–254.
6. Kripke, M. L., P. A. Cox, L. G. Alas and D. B. Yarosh (1992) Pyrimidine dimers in DNA initiate systemic immunosuppression in UV-irradiated mice. *Proc. Natl. Acad. Sci. USA* **89**, 7516–7520.
7. Horio, T. and H. Okamoto (1987) Oxygen intermediates are involved in ultraviolet radiation-induced damage of Langerhans cells. *J. Invest. Dermatol.* **88**, 699–702.
8. Devary, Y., R. A. Gottlieb, T. Smeal and M. Karin (1992) The mammalian ultraviolet response is triggered by activation of Src tyrosine kinases. *Cell* **71**, 1081–1091.
9. Ullrich, S. E., B. W. McIntyre and J. M. Rivas (1990) Suppression of the immune response to alloantigen by factors released from ultraviolet-irradiated keratinocytes. *J. Immunol.* **145**, 489–498.
10. Matsue, H., P. D. Cruz, Jr., P. R. Bergstresser and A. Takashima (1992) Cytokine expression by epidermal cell subpopulations. *J. Invest. Dermatol.* **99**, 42S–45S.
11. Krutmann, J., I. U. Khan, R. S. Wallis, *et al.* (1990) Cell membrane is a major locus for ultraviolet B-induced alterations in accessory cells. *J. Clin. Invest.* **85**, 1529–1536.
12. Norris, D. A., M. B. Lyons, M. H. Middleton, J. J. Yohn and M. Kashihara-Sawami (1990) Ultraviolet radiation can either suppress or induce expression of intercellular adhesion molecule 1 (ICAM-1) on the surface of cultured human keratinocytes. *J. Invest. Dermatol.* **95**, 132–138.
13. Tang, A. and M. C. Udey (1991) Inhibition of epidermal Langerhans cell function by low dose ultraviolet B radiation. Ultraviolet B radiation selectively modulates ICAM-1 (CD 54) expression by murine Langerhans cells. *J. Immunol.* **146**, 3347–3355.
14. Steinman, R. M. (1991) The dendritic cell system and its role in immunogenicity. *Annu. Rev. Immunol.* **9**, 271–296.
15. Stingl, G., C. Hauser and K. Wolff (1993) The epidermis: an immunologic microenvironment. In: *Dermatology in General Medicine* (Edited by T. B. Fitzpatrick), pp. 172–197. McGraw Hill, New York.
16. Simon, J. C., P. D. Cruz, P. R. Bergstresser and R. E. Tigelaar (1990) Low dose ultraviolet B-irradiated Langerhans cells preferentially activate CD4+ cells of the T helper 2 subset. *J. Immunol.* **145**, 2087–2091.
17. Simon, J. C., R. E. Tigelaar, P. R. Bergstresser, D. Edelbaum and P. D. Cruz (1991) UVB radiation converts Langerhans cells from immunogenic to tolerogenic antigen presenting cells. Induction of specific clonal anergy in CD4+ T helper 1 cells. *J. Immunol.* **146**, 485–491.
18. Cruz, P. D., Jr. J. Nixon-Fulton, R. E. Tigelaar and P. R. Bergstresser (1989) Disparate effects of *in vitro* UVB irradiation on intravenous immunization with epidermal cell subpopulations for the induction of contact hypersensitivity. *J. Invest. Dermatol.* **92**, 160–165.
19. Enk, A. H. and S. I. Katz (1992) Identification and induction of keratinocyte-derived IL-10. *J. Immunol.* **149**, 92–95.
20. Enk, A. H., V. L. Angeloni, M. C. Udey and S. I. Katz (1993) Inhibition of Langerhans cell antigen-presenting function by IL-10. A role for IL-10 in induction of tolerance. *J. Immunol.* **151**, 2390–2398.
21. Xu, S., K. Ariizumi, G. Caceres-Dittmar, *et al.* (1995) Successive generation of antigen-presenting, dendritic cell lines from murine epidermis. *J. Immunol.* (In press)
22. Xu, S., K. Ariizumi, D. Edelbaum, P. R. Bergstresser and A. Takashima (1995) Cytokine-dependent regulation of growth and maturation in murine epidermal dendritic cell lines. *Eur. J. Immunol.* **154**, 2697–2705.
23. Takashima, A., J. L. Nixon-Fulton, P. R. Bergstresser and R. E. Tigelaar (1988) Thy-1+ dendritic epidermal cells in mice: precursor frequency analysis and cloning of concanavalin A-reactive cells. *J. Invest. Dermatol.* **90**, 671–678.
24. Fehr, B., A. Takashima, H. Matsue, J. Gerometta, P. Bergstresser and P. D. Cruz, Jr. (1994) Contact sensitization induces proliferation of heterogeneous populations of hapten-specific T cells. *Exp. Dermatol.* **3**, 189–197.
25. Yuspa, S. H., P. Hawley-Nelson, B. Koehler and J. R. Stanley (1980) A survey of transformation markers in differentiating epidermal cells in culture. *Cancer Res.* **40**, 4694–4703.
26. Kuziel, W. A., A. Takashima, M. Bonyhadi, *et al.* (1987) Regulation of T-cell receptor gamma chain RNA expression in murine Thy-1+ dendritic epidermal cells. *Nature* **328**, 263–266.
27. Matsue, H., P. R. Bergstresser and A. Takashima (1993) Keratinocyte-derived IL-7 serves as a growth factor for dendritic epidermal T cells in mouse skin. *J. Immunol.* **151**, 6012–6019.
28. Matsue, H., K. G. Rothberg, A. Takashima, B. A. Kamen, R. G. Anderson and S. W. Lacey (1992) Folate receptor allows cells to grow in low concentrations of 5-methyltetrahydrofolate. *Proc. Natl. Acad. Sci. USA* **89**, 6006–6009.
29. Chung, B. S., P. R. Bergstresser and A. Takashima (1993) Mouse dendritic epidermal T cells exhibit chemotactic migration toward PAM 212 keratinocyte cultured supernatants. *J. Invest. Dermatol.* **101**, 371–376.
30. Girolomoni, G., J. C. Simon, P. R. Bergstresser and P. D. Cruz, Jr. (1990) Freshly isolated spleen dendritic cells and epidermal Langerhans cells undergo similar phenotypic and functional changes during short term culture. *J. Immunol.* **145**, 2820–2826.
31. Takashima, A. and F. Grinnell (1984) Human keratinocyte adhesion and phagocytosis promoted by fibronectic. *J. Invest. Dermatol.* **83**, 352–358.
32. Toews, G. B., P. R. Bergstresser and J. W. Streilein (1980) Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. *J. Immunol.* **134**, 445–453.
33. Matsue, H., P. R. Bergstresser and A. Takashima (1993) Reciprocal cytokine-mediated cellular interactions in mouse epidermis: promotion of gamma delta T cell growth by IL-7 and TNF alpha and inhibition of keratinocyte growth by gamma IFN. *J. Invest. Dermatol.* **101**, 543–548.
34. Aebi, H. (1974) Catalase. In *Methods of Enzymatic Analysis* (Edited by H. U. Bergmeyer), pp. 673–684. Academic Press, New York.
35. Young, J. W., J. Baggers and S. A. Soergel (1993) High-dose UV-B radiation alters human dendritic cell costimulatory activity but does not allow dendritic cells to tolerize T lymphocytes to alloantigen *in vitro*. *Blood* **81**, 2987–2997.
36. Danno, K., T. Horio, M. Takigawa and S. Imamura (1984) Role of oxygen intermediates in UV-induced epidermal cell injury. *J. Invest. Dermatol.* **83**, 166–168.
37. Lim, H. W., G. J. Thorbecke, R. L. Baer and I. Gigli (1983)

Effects of indomethacin on alteration of TPase-positive Langerhans cell density and cutaneous sunburn reaction induced by ultraviolet-B radiation. *J. Invest. Dermatol.* **81**, 455–458.

38. Devary, Y., R. A. Gottlieb, L. F. Lau and M. Karin (1991) Rapid and preferential activation of the c-jun gene during the mammalian UV response. *Mol. Cell. Biol.* **11**, 2804–2811.
39. Devary, Y., D. Engelberg, C. Klein, H. Martinetto, K. Struhl and M. Karin (1994) The UV response involving the Ras signaling pathway and AP-1 transcription factors is conserved between yeast and mammals. *Cell* **77**, 381–390.
40. Derijard, B., M. Hibi, I. H. Wu, *et al.* (1994) JNK1; a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* **76**, 1025–1037.
41. Nabavi, N., G. J. Freeman, A. Gault, D. Godfrey, L. M. Nadler and L. H. Glimcher (1992) Signalling through the MHC class II cytoplasmic domain is required for antigen presentation and induces B7 expression. *Nature* **360**, 266–268.
42. Yohn, J. J., D. A. Norris, D. G. Yrastorza, *et al.* (1991) Disparate antioxidant enzyme activities in cultured cutaneous fibroblasts, keratinocytes and melanocytes. *J. Invest. Dermatol.* **97**, 405–409.
43. Shindo, Y., E. Witt and L. Packer (1993) Antioxidant defense mechanisms in murine epidermis and dermis and their responses to ultraviolet light. *J. Invest. Dermatol.* **100**, 260–265.
44. Aberer, W., N. Romani, A. Elbe and G. Stingl (1986) Effects of physicochemical agents on murine epidermal Langerhans cells and Thy-1-positive dendritic epidermal cells. *J. Immunol.* **136**, 1210–1216.
45. Rheins, L. A., E. M. Young, M. L. Nordlund, R. B. Berning and J. J. Nordlund (1987) Rapid induction of Thy-1 antigenic markers on keratinocytes and epidermal immune cells in the skin of mice following topical treatment with common preservatives used in topical medications and in foods. *J. Invest. Dermatol.* **89**, 489–494.
46. Rheins, L. A. and J. J. Nordlund (1986) Modulation of the population densities of identifiable epidermal Langerhans cells associated with enhancement or suppression of cutaneous immune reactivity. *J. Immunol.* **136**, 867–876.