Immunology 1984 **52** 299

Systemic suppression of contact hypersensitivity by UVB radiation is unrelated to the UVB-induced alterations in the morphology and number of Langerhans cells

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Accepted for publication 17 January 1984

Summary. Exposure of mice to UVB (280–320 nm) ultraviolet radiation reduces contact hypersensitivity (CHS) reactions to chemicals that are applied subsequently to unirradiated skin. It also decreases the number and alters the morphology of Langerhans cells at the site of irradiation. We addressed the question of whether the systemic suppression of CHS was related to these modifications of Langerhans cells by UVB radiation. In mice treated on the dorsum with UVB radiation, the number and morphology of Langerhans cells in the unexposed areas of skin used for inducing and eliciting CHS appeared normal. Therefore, the depression of CHS could not be attributed to a depletion of Langerhans cells at the sites of application of the sensitizing agent. We also examined the correlation between alterations in Langerhans cells and systemic suppression of CHS after treatment with various types of nonionizing radiation. Treatment of mice with UVA (320–400 nm) radiation eliminated detectable Langerhans cells from the exposed skin, based on ATPase staining and electron microscopy, but did not reduce CHS; in fact, CHS was enhanced in these animals. Neither rose bengal nor eosin, in combination with visible (>400 nm) radiation, affected the number or appearance of Langerhans cells, even though microscopic evidence of phototoxicity was present. However, rose bengal plus visible radiation depressed CHS reactions that were induced and elicited through unexposed skin. Depletion of Langerhans cells from dorsal skin by exposure to UVA radiation did not prevent suppression of CHS by subsequent exposure of the Langerhans cell-depleted skin to UVB radiation. We conclude that systemic suppression of CHS by UVB irradiation is not related to the numerical and morphological alterations in Langerhans cells that occur locally at the site of irradiation.

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Abbreviations: ATPase, adenosine triphosphatase; CHS, contact hypersensitivity; EDTA, ethylenediamine tetraacetic acid; TNCB, trinitrochlorobenzene; TNP, 1,3,5-trinitrophenyl; UV, ultraviolet; UVA, 320–400 nm radiation; UVB, 280–320 nm radiation.

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INTRODUCTION

Exposure of mice to UVB (280–320 nm) radiation interferes with the induction of delayed and contact hypersensitivity (CHS) reactions to haptens that are subsequently applied on or injected into unirradiated skin (Jessup *et al.*, 1978; Noonan, De Fabo & Kripke, 1981a; Noonan *et al.*, 1981b; Greene *et al.*, 1979). The systemic depression of these reactions is associated with the appearance of hapten-specific suppressor T lymphocytes (Noonan *et al.*, 1981a; Greene *et al.*, 1979). Induction of the suppressor cell pathway is thought to result from an alteration in antigen presen-

tation (Jessup *et al.*, 1978; Noonan *et al.*, 1981b; Greene *et al.*, 1979). An alteration of antigen presentation by adherent spleen cells (presumably macrophages) from UV-irradiated mice was found by Green *et al.* (1979) using an *in vivo* assay. Subsequent studies have shown that adherent cells from the spleens of UV-irradiated mice also are deficient in their ability to present antigen in a variety of *in vitro* assays (Letvin *et al.*, 1980; Jensen, 1983).

Because UVB radiation does not penetrate deeper than the skin of mice, it is not obvious how irradiation of dorsal skin brings about changes in the activity of splenic antigen-presenting cells and leads to systemic suppression of CHS. An attractive explanation for this phenomenon is derived from the study of Toews, Bergstresser & Streilein (1980) on the effects of UVB radiation on Langerhans cells, which are the antigen-presenting cells of the epidermis. These investigators found that following irradiation of mouse skin with very low doses of UVB radiation, the number of Langerhans cells was reduced and the remaining cells were altered morphologically. Application of a contact sensitizer to such irradiated skin resulted in the induction of specific unresponsiveness rather than in the development of CHS, again suggesting that a UV radiation-mediated alteration in antigen presentation had altered immunological responsiveness. These findings raised the question of whether systemic suppression of CHS by UVB radiation was related to these effects of UV radiation on epidermal Langerhans cells.

In studies presented here, two hypotheses are tested. Firstly, we consider the possibility that damage to Langerhans cells at the site of irradiation could cause a systemic redistribution of these cells, leading to depletion of Langerhans cells at the site of sensitization and/or elicitation of CHS, even though these sites are not exposed directly to UV radiation. Secondly, we test the hypothesis that the injury to Langerhans cells that is observable morphologically following their direct exposure to UVB radiation is the initiating event in the systemic suppression of CHS by UV radiation. For example, UV-irradiated Langerhans cells might migrate to the spleen and thus be responsible for the altered activity of antigen-presenting cells from this organ; alternatively, UV radiation-induced injury of these cells might provide a stimulus for the emigration of splenic macrophages, thereby depleting the spleen of its reservoir of antigen-presenting cells. This hypothesis was tested using two approaches. Firstly, mice were treated with various types of non-ionizing radiation to determine whether there was a consistent association between the morphological alteration of Langerhans cells and systemic suppression of CHS. Secondly, the skin was depleted of all identifiable Langerhans cells and then exposed to UVB radiation to see whether systemic suppression of CHS could be induced by UV radiation in the apparent absence of Langerhans cells.

MATERIALS AND METHODS

Mice

Specific pathogen-free female mice of the inbred strains C3H/HeNCR (mammary tumor virus negative; $C3H^-$) and BALB/cAnNCR were supplied by the NCI-Frederick Cancer Research Facility's animal production area. The animals were 10–14 weeks old at the start of an experiment, and within each experiment the age of the animals did not vary by more than 1 week. The mice had free access to Purina mouse chow and chlorinated water (5 p.p.m.) and were housed in rooms where ambient light was regulated automatically on a 12 hr light–dark cycle.

Exposure to radiation

Immediately before irradiation, the mice were anaesthetized lightly with metafane and their dorsal fur was plucked. Their ears were covered with black electrical tape prior to irradiation. UVA radiation was provided by a bank of six PUVA fluorescent bulbs (Sylvania, Danvers, MA) and filtered through a 0·05 mm sheet of Mylar to eliminate radiation < 312 nm. Animals were exposed to UVA radiation continuously for 72 hr at a distance of approximately 20 cm from the bulbs. During irradiation, the temperature in the animals' cages did not increase by more than 2° over the ambient room temperature. The dorsal fur of control animals was also plucked, their ears were taped, and they were kept in a room illuminated by Coolwhite fluorescent bulbs (Sylvania), at a distance of 2·4 m from the bulbs. UVB radiation was provided by a bank of six FS40 sunlamp bulbs (Westinghouse, Bloomfield, NJ) at a distance of approximately 20 cm. Mice received a single 3 hr exposure to UVB radiation unless stated otherwise. Visible radiation was used in combination with rose bengal or eosin, which are photosensitizing agents. Each compound was given at a dose of 2 mg in saline by intraperitoneal injection, 30 min before a 4 hr exposure to visible radiation. This

radiation was provided by a bank of 6 Coolwhite fluorescent bulbs and filtered through a 0·05 mm sheet of weathered Mylar to eliminate wavelengths < 390 nm, and the exposures were given at a distance of 20 cm from the source.

Radiometry

The irradiances of the sources of UVA and UVB radiation were measured with an IL 700 radiometer (International Light Inc., Newburyport, MA) using a WB350 filter and SEE400 detector for measuring UVA and a WB320 filter and an A127 quartz diffuser for measuring UVB. The average irradiance of the UVA source was 27 W/m^2, and that of the UVB source was 10 W/m^2. The irradiance of the visible source was measured with an Optronic model 742 spectroradiometer (Optronic Lab., Inc., Orlando, Fla.) interfaced with a Hewlett Packard HP 9815/s computer (Fort Collins, CO). The average irradiance was 19 W/m^2 over the 400–800 nm waveband. The actual irradiances at the level of the animals' backs was about 50% of these figures because of screening by the cage and its wire top.

Induction of contact hypersensitivity

The method of Asherson & Ptak (1968) was used to induce CHS 3 days after exposure to radiation. Briefly, the abdominal fur was removed from mice with electric clippers and the abdomen was then shaved with a razor blade to remove all traces of hair. The surface was painted with 50 μl of 1% trinitrochlorobenzene (TNCB) in acetone. The mice were tested for CHS 6 days later. TNCB in acetone (5 μl of a 1% solution) was applied to both surfaces of each ear. Ear thickness was measured with a spring-loaded micrometer (model 7309, Mitutoyo, Japan) before and 24 hr after application of the challenge dose, and the difference between the two readings was recorded as the ear swelling.

Histological and ultrastructural studies

Specimens of skin were obtained from the plucked dorsum and untreated ears of mice. Abdominal skin was depilated (Neet, thioglycollic acid) immediately before sampling. Some skin samples were fixed in Bouin's solution, processed for routine light microscopy, and stained with haematoxylin and eosin. Sheets of epidermis were obtained from other specimens of skin by incubation in ethylenediamine tetraacetic acid (EDTA) solution and stained histochemically for adenosine triphosphatase (ATPase) activity by the method of MacKenzie & Squier (1975). The epidermal sheets were examined by light microscopy, and the ATPase-positive cells were counted with the aid of a calibrated grid inserted in the eyepiece of the microscope. For these studies, two animals were examined in each group and three separate 1 mm^2 sites were evaluated on each of two pieces of skin from each mouse.

For ultrastructural examination, skin samples were fixed in a solution containing 3% glutaraldehyde and 2% paraformaldehyde in 0·1 M cacodylate buffer for 1 hr and then cut into 1 mm^3 pieces. These pieces were transferred to fresh fixative and allowed to stand overnight at 4°. The samples were rinsed in cacodylate buffer and postfixed for 1 hr at room temperature with cacodylate-buffered OsO_4. The samples were rinsed briefly with distilled water, fixed with 1% aqueous uranyl acetate for 1 hr, and then dehydrated with a graded series of ethanol. The samples were infiltrated and embedded in Emix resin (Ted Pella Co., Tustin, CA). The blocks were polymerized for 48 hr in a 60° oven, and thick sections were cut with a glass knife in a LKB Ultratome III. Thick sections were stained with azure B-methylene blue stain and examined for specimen orientation. Thin sections were cut with a diamond knife, stained with Reynold's lead citrate, and examined in a Hitachi HU-12A electron microscope at an accelerating voltage of 75 kV. At least three different areas in each block were examined. Langerhans cells were identified by their clear cytoplasm, absence of tonofilaments, and presence of Birbeck granules.

Statistical analysis

The statistical significance of the differences in ear swelling between groups was evaluated by using the analysis of variance (ANOVA) test.

RESULTS

Number and morphology of Langerhans cells in UV-irradiated and unirradiated skin

The first hypothesis we addressed was that systemic suppression of CHS by UVB radiation resulted from a depletion of Langerhans cells in the unirradiated skin at the site of sensitization or challenge. The plucked dorsal skin of C3H$^-$ mice was either exposed to UVB radiation for 3 hr or left untreated. Three days later, some of the mice in each group were sensitized with TNCB on the shaved abdomen, and along with

unsensitized mice, they were challenged on day 9 (6 days after sensitization) by painting the ears with TNCB. As expected from previous studies (Noonan *et al.*, 1981a, b; Kripke, Morison & Parrish, 1983), CHS was reduced in UVB-irradiated mice to 47% of the response observed in unirradiated animals. At the time of sensitization (day 3), the skin from other mice in the UV-irradiated and unirradiated groups was examined for the presence of Langerhans cells, both on the dorsal (UV-irradiated) and abdominal (unexposed) surfaces. On the day of challenge (day 9), ear skin of unsensitized mice from both treatment groups was examined for Langerhans cells. ATPase-stained cells with dendrites were counted under a light microscope, as described in Materials and Methods, and these results are presented in Table 1. As expected based on earlier studies by others (Toews *et al.*, 1980; Aberer *et al.*, 1981; Lynch, Gurish & Daynes, 1981), the number of Langerhans cells identified on the basis of ATPase staining and dendritic morphology was reduced at the site of exposure to UVB radiation. At 3 days after irradiation, the number of normal-appearing Langerhans cells in the dorsal skin was only 28% of the number found in unirradiated animals. In contrast, no significant reduction in the number of Langerhans cells was observed in abdominal skin from animals exposed on the dorsum to UVB radiation versus unirradiated animals ($P > 0.05$ by Student's t-test). The number of Langerhans cells in ear skin at the time of challenge also was unaffected by prior exposure of the dorsum to UVB radiation. Ultrastructural examination of skin from the unirradiated sites revealed no discernible changes after dorsal irradiation. Normal-appearing Langerhans cells were readily observed and could be identified by the presence of Birbeck granules. Dorsal skin exposed directly to UVB radiation had considerably fewer Langerhans cells, and many of these appeared to be damaged, as evidenced by extensive cytoplasmic vacuolation and absence of dendrites. Thus, the UVB radiation-induced reduction in CHS is not attributable to a decrease in the number of Langerhans cells at the sites of sensitization or challenge.

Table 1. Effect of dorsal UVB radiation on number of Langerhans cells in exposed and unexposed skin

Site of skin examined	Number of ATPase-positive dendritic cells/mm²*		Percent of control
	Control	Dorsal UVB†	
Dorsal	816±79	230±91	28
Ventral	715±62	652±96	91
Ear	576±69	604±58	105

* Mean ± standard deviation of 12 samples taken from two animals.

† Incident dose = 5.4×10^4 J/m² of 280–320 nm radiation, delivered in a single 3 hr exposure.

Correlations between the number and morphology of Langerhans cells and systemic suppression of CHS

Four agents were evaluated for their ability to alter the number and/or morphology of Langerhans cells at the site of treatment and for their ability to modify CHS to TNCB applied at an untreated site. UVA radiation was used because it was reported to reduce the number of Langerhans cells in mouse and human skin (Aberer *et al.*, 1981). Eosin and rose bengal are photoactive dyes that absorb visible radiation.

Groups of C3H⁻ mice were treated with UVB radiation, UVA radiation, eosin plus visible radiation, or rose bengal plus visible radiation as described in 'Materials and Methods'. The 3 hr exposure of mice to UVB radiation produced epidermal hyperplasia and a cellular infiltrate in the dermis at the site of irradiation, based on light microscopic examination of the skin at 24 hr after treatment. By 7 days after treatment, partial ulceration with crusting and scaling had occurred in the exposed area. The 72 hr exposure to UVA radiation resulted in marked thickening of the dorsal skin by the end of the treatment, and, on light microscopic examination, there was considerable hyperplasia of the epidermis and a cellular infiltrate in both the dermis and subcutaneous tissue. Subsequent ulceration of the skin did not occur. Rose bengal or eosin, in combination with visible radiation, produced no gross alterations in the skin. Examination of haematoxylin- and eosin-stained sections of the skin from animals treated with these agents 24 hr previously revealed minimal thickening of the epidermis and a marked cellular infiltrate that was limited to the subcutaneous fat and muscle directly beneath the area exposed to the visible radiation. At the doses used, the changes produced by eosin and rose bengal were similar in magnitude.

The number of ATPase-positive dendritic cells in the dorsal skin of mice from each treatment group was determined 3 days after irradiation (Table 2). As before, UVB radiation reduced the number of Langer-

Table 2. Effect of various types of nonionizing radiation on the number of ATPase-positive Langerhans cells in the dorsal skin 3 days after irradiation

Treatment	Number of ATPase-positive dendritic cells/mm^2	Percent of control
None	626±75	100
UVB radiation*	326±108	52
UVA radiation†	0	0
Rose bengal+ visible radiation	643±71	103
Eosin+visible radiation	706±115	113

* $5{\cdot}4 \times 10^4$ J/m^2 of incident 280–320 nm radiation.
† $3{\cdot}5 \times 10^6$ J/m^2 of incident 320–400 nm radiation.
‡ 2 mg rose bengal or eosin administered intraperitoneally, followed 30 min later by exposure of dorsum to $1{\cdot}4 \times 10^5$ J/m^2 of incident 400–800 nm radiation.

hans cells that could be identified in this manner. Some ATPase-positive cells with rounded morphology were present also (95±27 cells/mm^2), but not in sufficient numbers to account entirely for the observed reduction in the number of ATPase-positive dendritic cells. No ATPase-positive cells were found in the skin of mice treated with UVA radiation, which was examined immediately after the 72 hr irradiation and 3 days later. Scattered fragments of ATPase-positive material were present that might have been remnants of Langerhans cells. Ultrastructural examination of the UVA-irradiated skin revealed occasional cells that resembled Langerhans cells, but none of these contained Birbeck granules. Skin from mice exposed to visible radiation after intraperitoneal administration of eosin or rose bengal had a normal complement of ATPase-positive dendritic cells, and many normal Langerhans cells were observed upon ultrastructural examination of irradiated skin from these animals.

The ability of each of these treatments to modify CHS to TNCB applied 3 days later to abdominal (unirradiated) skin was assessed. The results of a single test with each agent is presented in detail in Table 3, and a summary of all experiments is given in Table 4. Exposure of mice to rose bengal plus visible radiation or to UVB radiation markedly reduced the CHS reaction. In contrast, exposure to UVA radiation increased the response, and treatment with eosin plus visible radiation had no effect. As depicted in Table 4, there was no correlation between the ability of a given treatment to alter the number and morphology of Langerhans cells and its ability to suppress a CHS

Table 3. Effect of various forms of nonionizing radiation on contact hypersensitivity in C3H$^-$ mice

Treatment	Ear swelling* ($cm \times 10^{-3}$) +TNCB	Ear swelling* ($cm \times 10^{-3}$) −TNCB	Δ ear swelling	Percent control response†	P‡
None	10·7±0·5	3·3±0·7	7·4	100	
Rose bengal	8·5±0·6	1·9±0·3	6·6	89	NS
Rose bengal+ visible radiation	5·1±0·4	4·5±0·8	0·6	8	<0·0001
None	13·7±0·2	0·9±0·2	12·8	100	
Eosin	13·4±0·4	0·8±0·2	12·6	98	
Eosin+visible radiation	11·6	1·1±0·2	10·5	82	NS
None	11·9±1·5	3·6±1·2	8·3	100	
UVB	7·5±1·2	3·1±2·1	4·4	53	<0·05
UVA	15·2±4·8	4·3±2·2	10·9	131	<0·05

* Mean±standard error of the mean of five or more mice challenged 24 hr earlier with TNCB on the ears.
† Percent control response = (Δ of test group/Δ of untreated group) × 100.
‡ Probability of no difference in ear swelling between treated and untreated mice that were sensitized to TNCB as determined by ANOVA test.
NS, not significant.

Table 4. Lack of correlation between effect of nonionizing radiation on Langerhans cells and on systemic suppression of CHS

Treatment	Effect on number of Langerhans cells	Effect on CHS at unexposed sites
UVB radiation	decrease	decrease
UVA radiation	decrease	increase (28%–105% increase in five experiments)
Rose bengal + visible radiation	none	decrease (45%–92% suppression in six experiments)
Eosin + visible radiation	none	none (2%–18% suppression in three experiments)

reaction at a distant site. Exposure to UVA radiation resulted in a dramatic decrease in the number of Langerhans cells without decreasing the CHS reaction, and, conversely, treatment with rose bengal plus visible radiation depressed CHS without altering the number or appearance of Langerhans cells.

Suppression of CHS by UVB radiation in the absence of detectable Langerhans cells

Exposure of mice to UVA radiation for 72 hr decreased the number of ATPase-positive cells in the epidermis to a level that was undetectable by light microscopy, and only a few cells resembling Langerhans cells were found after extensive examination of skin sections by transmission electron microscopy. We reasoned that if systemic suppression of CHS by UVB radiation were related to the UVB-induced alterations in the morphology and number of Langerhans cells, then prior depletion of normal Langerhans cells with UVA radiation should prevent UVB-induced suppression of CHS. One difficulty with this experiment stems from the epidermal hyperplasia that results from the exposure to UVA radiation. In an attempt to compensate for this thickening, which might screen a target cell or molecule from the UVB radiation, we arbitrarily increased the UVB irradiation time to 4 hr in the groups that were pretreated with UVA. As can be seen in Table 5, depletion of normal Langerhans cells from the skin by UVA radiation did not prevent a subsequent treatment of the same site with UVB radiation from decreasing the induction of CHS in unirradiated skin. UVB irradiation reduced the CHS reaction whether or not the mice had been pretreated with UVA radiation. In both strains of mice, the degree of suppression by UVB radiation was similar with and without UVA irradiation, provided allowance was made for the increased CHS reaction in UVA-irradiated mice. This implies that the decreased number and altered morphology of Langerhans cells that are induced by direct exposure to UVB radiation are neither sufficient nor required to bring about the systemic alterations in CHS.

DISCUSSION

Two conclusions can be drawn from these studies. Firstly, the decrease in CHS following exposure of mice to UVB radiation does not result from a decrease in the number of Langerhans cells at the sites of sensitization or elicitation. This study does not rule out the possibility that Langerhans cells at these sites are functionally altered, despite their normal morphological characteristics. However, this seems unlikely in view of the fact that a normal CHS reaction can be elicited in the ears of UVB-irradiated mice following passive administration of immune lymphocytes (Kripke *et al.*, 1983). This indicates that epidermal Langerhans cells in unirradiated skin can carry out at least some antigen-presenting functions in a normal fashion.

Secondly, the morphological alterations in Langerhans cells that result from direct exposure of the skin to UVB radiation do not appear to be responsible for the systemic suppression of CHS in UVB-irradiated mice. This conclusion is based, in part, on the lack of correlation between these two events following exposure to different wavebands of nonionizing radiation. This finding agrees with a preliminary report (Noonan *et al.*, 1982) that these two effects may be dependent on different wavelengths of UV radiation. In addition,

Table 5. Effect of prior UVA irradiation on UVB-induced suppression of CHS

Mouse strain	Treatment	Ear swelling* ($cm \times 10^{-3}$) TNCB-sensitized	Ear swelling* ($cm \times 10^{-3}$) Unsensitized	Δ ear swelling	Percent control response	P†
C3H$^-$	None	14·0±0·7	4·8±0·4	9·2	100	
	UVB‡	8·4±0·9	4·8±0·6	3·6	39	<0·01
	UVA§	22·2±1·2	3·3±0·3	18·9	100	
	UVA+UVB¶	11·7±0·6	5·4±0·5	6·3	33	<0·0001
BALB/c	None	14·7±2·1	5·1±0·6	9·6	100	
	UVB‡	8·8±1·1	4·2±0·9	4·6	48	<0·05
	UVA§	16·6±0·7	3·0±0·9	13·6	100	
	UVA+UVB¶	11·4±1·4	4·1±0·7	7·3	54	<0·05

* Mean ± standard error of mean of at least 10 mice challenged 24 hr earlier with TNCB ($cm \times 10^{-3}$).

† Probability of no difference in ear swelling between TNCB-sensitized, irradiated and unirradiated mice, as determined by the ANOVA test.

‡ $5{\cdot}4 \times 10^4$ J/m^2 of 280–320 nm radiation (3 hr exposure).

§ $3{\cdot}5 \times 10^6$ J/m^2 of 320–400 nm radiation (72 hr exposure).

¶ 72 hr of UVA followed by $7{\cdot}2 \times 10^4$ J/m^2 of 280–320 nm radiation (4 hr exposure).

systemic suppression of CHS could be induced by UVB irradiation of skin that contained no recognizable Langerhans cells. It is conceivable that Langerhans cells were still present in the skin following UVA irradiation but that they had lost the distinctive characteristics of Langerhans cells, i.e. Birbeck granules, membrane-associated ATPase, and dendritic morphology. Thus, we can conclude that the UVB radiation-induced morphological alterations in Langerhans cells are not sufficient to lead to systemic suppression of CHS, but we cannot rule out the possibility that suppression is initiated by some other effect of UVB radiation on the Langerhans cell that is not accompanied by a change in its morphology.

If systemic suppression of CHS by UVB radiation is due to altered antigen presentation, then pathways for this alteration other than migration of Langerhans cells or injury to Langerhans cells must be considered. Letvin *et al.* (1980) suggested that direct irradiation of circulating blood monocytes might be responsible for altered antigen presentation, but this seems unlikely based on a recent report by De Fabo & Noonan (1983). They provide evidence suggesting that the initial UV radiation-absorbing step in the suppression of CHS occurs at a very superficial level on or within the epidermis, a result that is incompatible with the model involving irradiation of monocytes within capillaries of the dermis. A more attractive hypothesis is that UVB radiation produces a photoproduct in skin that either modifies the activity of splenic antigen-presenting cells directly or serves as a stimulus for the egress of antigen-presenting cells out of the spleen. In either case, this implies that UVB radiation could interfere with antigen presentation in at least two different ways: (i) by direct interaction with the antigen-presenting cell, as in the case of UVB irradiation of Langerhans cells *in situ* (Toews *et al.*, 1980) or irradiation of splenic macrophages (Fox *et al.*, 1981) or Langerhans cells (Stingl *et al.*, 1981; Sauder *et al.*, 1981) *in vitro*; and (ii) by an indirect mechanism involving the formation or release of a chemical intermediate in the skin. In both instances, the UVB radiation-induced alteration in antigen presentation modifies the CHS reaction by leading to the formation of antigen-specific suppressor lymphocytes.

ACKNOWLEDGMENTS

The authors are grateful to Richard Pike whose careful work made this study possible. Mr Charles Riggs kindly performed the statistical analysis. This research was sponsored by the National Cancer Institute, DHHS, under contract No. NO1-CO-23909 with

Litton Bionetics Inc. The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

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