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Topical and oral retinoids protect Langerhans' cells and epidermal Thy-1$^+$ dendritic cells from being depleted by ultraviolet radiation

K. K.-L. HO, G. M. HALLIDAY & R. ST. C. BARNETSON *Department of Medicine (Dermatology), Royal Prince Alfred Hospital, University of Sydney, Australia*

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SUMMARY

Murine epidermis contains two types of bone marrow-derived cells of the immune system, Langerhans' cells (LC), which are dendritic antigen-presenting cells, and Thy-1$^+$ dendritic cells (Thy-1$^+$ DEC), which express the gamma/delta T-cell receptor for antigen and hence are probably T cells whose function in the epidermis is unknown. Ultraviolet (UV) light greatly reduces the density of both of these cell types, and hence this may be one of the mechanisms by which UV light induces immunosuppression. It is important to develop strategies for protecting these cells from the effects of UV light. In this study we show that topical all-trans-retinoic acid (RA) and an orally administered retinoid, temarotene, protect both LC and Thy-1$^+$ DEC from being depleted by UV light. However, neither retinoid inhibited the development of immunosuppression in response to application of a contact sensitizer. We also compared two congenic mouse strains, one albino, the other lightly pigmented and capable of tanning in response to UV light. There was no difference in the ability of UV light to deplete LC or Thy-1$^+$ DEC in these two strains or of retinoids to inhibit their depletion. These studies demonstrate that retinoids but not melanin are able to inhibit UV light from depleting LC and Thy-1$^+$ DEC; however, there are other immunosuppressive effects of UV light which are not protected by the retinoids.

INTRODUCTION

Ultraviolet (UV) radiation-induced murine skin tumours are highly immunogenic. When transplanted into syngeneic mice, many of these tumours are immunologically rejected unless the recipient mice are immunocompromised.[1] UV also causes immunosuppression,[2] which is likely to be an important factor for the growth of UV-induced tumours.

UV-induced immunosuppression has been experimentally divided into 'local' and 'systemic', where contact sensitizers applied either 'locally' to the irradiated skin or 'systemically' to a skin site distant to that which was irradiated both induce immunosuppression. The local effects of UV light include reductions in the densities of Langerhans' cells (LC) and Thy-1$^+$ dendritic epidermal cells (Thy-1$^+$ DEC), which are likely to be involved in the local immunosuppression.[3,4]

LC are epidermal antigen-presenting cells capable of activating antigen-specific effector T cells.[5] LC depletion may be partly responsible for escape of neoplastic cells from immune destruction.[6] Thy-1$^+$ DEC, another dendritic cell type present in the murine epidermis, expresses gamma/delta T-cell receptors for antigen on their cell surface and are hence probably a subpopulation of T lymphocytes.[7] The physiological function of these cells is presently unclear. Various factors produced in response to UV irradiation have been proposed to be responsible for systemic immunosuppression. These include urocanic acid,[8] prostaglandin E_2[9] and factors produced by keratinocytes.[10] It is likely that UV-induced immunosuppression results from a combination of these mechanisms.

Retinoids are vitamin A-related compounds with therapeutic and prophylactic effects against UV radiation-related skin damage.[11] They also cause immunopotentiation, which may partially explain their anti-carcinogenic effects.[12]

Chemical carcinogens and tumour promotors but not initiators deplete LC from murine skin, and suppressor cells are activated when contact sensitizers are applied to carcinogen or promotor-treated skin.[13,14] However, the effects of chemical carcinogens and UV light on the immune system differ as only local, not systemic, immunosuppression occurs during chemical carcinogenesis,[15] and this immunosuppression has been shown to result from the effects of the carcinogens on local antigen-presenting cells.[16] We have shown recently that all-trans-retinoic acid (RA) applied topically 24 hr and immediately prior to the tumour promotor 12-O-tetradecanoylphorbol 13-acetate (TPA)

Abbreviations: BCC, basal cell carcinoma; CS, contact sensitivity; LC, Langerhans' cells; RA, all-trans-retinoic acid; SCC, squamous cell carcinoma; Thy-1$^+$ DEC, Thy-1$^+$ dendritic epidermal cells; TNCB, 2,4,6-trinitrochlorobenzene; TPA, 12-O-tetradecanoylphorbol 13-acetate; UV, ultraviolet.

Correspondence: Dr G. Halliday, Dept. of Dermatology, University of Sydney, Gloucester House, Royal Prince Alfred Hospital, Camperdown, NSW 2050, Australia.

prevented the carcinogen from depleting LC, and inhibited the development of local immunosuppression in TPA-treated mice.[17]

In the present study, we investigated the effects of topical RA and an orally administered retinoid, temarotene, on UV-induced local immunosuppression and depletion of LC and Thy-1$^+$ DEC.

MATERIALS AND METHODS

Animals

Inbred albino HRA:Skh-1 and pigmented HRA:Skh-2 hairless mice were bred and housed in the animal house of the Department of Veterinary Pathology, University of Sydney, Australia. HRA:Skh-2 mice became congenic with HRA:Skh-1 mice after having been back-crossed with HRA:Skh-1 mice for more than 20 generations.[18]

Mice which were not on the defined diet were fed standard mouse pellets (Rat & Mouse Standard Ration; Lab-Feed, Northbridge, NSW, Australia). Water was provided *ad libitum*. Mice were between 8 and 12 weeks old at the commencement of experiments. Age of mice within any group did not differ by more than 2 weeks.

Ultraviolet irradiation

Simulated solar UV radiation was provided by a bank of six Sylvania F40BL UVA and a single Oliphant FL40SE UVB tube. Radiation was filtered through a layer of Kodacel (Eastman Chemical Products Inc., Kingsport, TN) cellulose acetate film (0·125 mm), which reduced radiation sharply below 295 nm, thus filtering out any UVC. The spectral properties of this light source have been described previously.[19] The integrated irradiance of this light source was $2{\cdot}7 \times 10^{-4}$ W/cm^2 for UVB (290–315 nm) and $5{\cdot}2 \times 10^{-3}$ W/cm^2 for UVA (315–400 nm). Except for the contact sensitization experiments, the mice were irradiated while unrestricted in their cages. The wire cage tops were removed and the cages placed at 45 cm from the light source. They were exposed daily at the minimal erythemal dose, previously determined to be 10 min. Mice were irradiated 5 days per week for 4 weeks. Exposure times were increased every week by 20% of the initial exposure time to overcome acquired tolerance so that the minimal erythema dose was maintained. The average cumulative doses were 4·2 J/cm^2 UVB and 81·1 J/cm^2 UVA.

Topical retinoic acid

All-trans-retinoic acid type XX (RA; Sigma, St Louis, MO) was dissolved in a solvent consisting of ethanol, dimethylsulphoxide and acetone in the ratio of 1:1:6 (v/v/v) at 0·5 mg/ml. Twenty microlitres containing 34 nmol, were applied to the dorsal trunk of mice 24 hr before irradiation to avoid photo-isomerization and photo-degradation. This procedure was repeated 5 days per week for 4 weeks.[17]

Oral temarotene

A defined mouse diet was prepared with ingredients recommended by the American Institute of Nutrition (Bethesda MD),[20] which enables normal mouse growth. This diet contained powdered soybean, sucrose, fat, fibre, a mineral and vitamin mixture with vitamin A omitted. Each mouse was fed once per day with an average of 3·7 g diet/day to maintain their body weight. The diet was served during the 12-hr dark cycle, thus minimizing exposure of the vitamins to light. For some mouse groups, the defined diet was supplemented with temarotone (kindly donated by Hoffmann-LaRoche, Basle, Switzerland). Each mouse received an average of 270 mg temarotene/kg body weight per day. The defined diets commenced 4 weeks prior to irradiation and continued until the mice were killed.

Preparation and staining of epidermal sheets

At the end of the treatment regimes, the mice were killed by cervical dislocation, the stratum corneum was removed by repeated applications of cellophane tape and the skin pieces were incubated in isotonic phosphate-buffered saline (PBS) containing 20 mM EDTA (pH 7·3) at 37 for 3 hr. The epidermis was then separated from the dermis using a dissecting microscope and fine forceps.[13]

Freshly prepared epidermal sheets were fixed in acetone for 20 min at room temperature. The epidermal sheets were then washed with three changes of PBS (pH 7·3) over 20 min and incubated with primary antibody overnight at room temperature. Hybridoma supernatants containing rat anti-Ia monoclonal antibodies (TIB 120; American Type Culture Collection, Rockville, MD)[21] were used as the primary antibody to stain LC. To stain Thy-1$^+$ DEC, hybridoma supernatants containing rat anti-Thy-1 monoclonal antibodies (T24.31.7; gift from I. McKenzie, Melbourne, Australia)[22] were used. The epidermal sheets were then washed, incubated with biotin-conjugated goat anti-rat IgG (Caltag Laboratories, San Francisco, CA) diluted in 1% bovine serum albumin (BSA) in PBS for 2 hr at room temperature, washed, incubated with streptavidin–alkaline phosphate conjugate (Caltag Laboratories) diluted in BSA-PBS solution for 1 hr, washed and finally incubated with alkaline phosphate substrate solution containing 5% New Fuchsin[23] for 20 min. The specimens were then placed onto glass microscope slides, air-dried and mounted under a glass cover slip with Histomount (National Diagnostics, Manville, NJ).

Cell quantification

LC and Thy-1$^+$ DEC stained by immunohistochemistry were enumerated manually with the assistance of an image analysis system attached to a light microscope (Chromatic Colour Image Analysis System, L. R. Jarvis, Leitz, Sydney, Australia). The area enumerated was calculated by the image analysis system. For each mouse, cells within six separate randomly selected regions, incorporating a total area of 0·3 mm^2, were counted.

Contact sensitization

For the contact sensitization experiments, mice were restrained by mesh wire during irradiation so that their ears could be sheilded from UV by tin foil. Mice were sensitized with 50 μl of 1% 2,4,6-trinitrochlorobenzene (Tokyo Kasei, Tokyo, Japan) (TNCB) in acetone/olive oil (4:1, v/v) applied topically to the dorsal trunk twice 24 hr apart on days 0 and 1. Mice were then challenged on Day 5 with 5 μl of 0·5% TNCB on each side of the right ear. Ear swelling was then assessed 24 hr following challenge by measuring the increase in ear thickness with an engineer's micrometer (Mercer, St Albans, Herts, U.K.). The local contact sensitivity (CS) response was calculated using the formula:

$$\frac{\text{right ear thickness} - \text{left ear thickness}}{\text{left ear thickness}} \times 100.$$

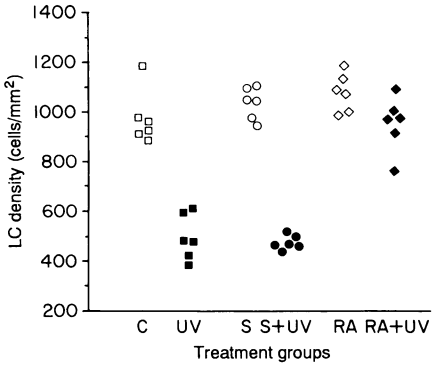

Figure 1. Effects of UV and topical retinoic acid on LC densities (HRA:Skh-1 mice). Treatment groups include: (□) mice fed on a normal diet, no other treatments (C); (■) mice irradiated daily with UV for 4 weeks (UV); (○) mice treated with topical solvent (of retinoic acid solution) (S); (●) mice treated with topical solvent 24 hr before irradiation (S + UV), (◇) mice treated with topical RA (RA); (◆) mice treated with topical RA 24 hr before irradiation (RA + UV). Each data point represents a single mouse.

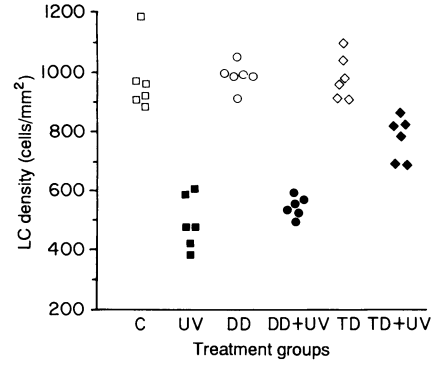

Figure 2. Effects of UV and oral temarotene on LC densities (HRA:Skh-1 mice). Treatment groups include: (□) mice fed on a normal diet, no other treatments (C); (■) mice fed on a normal diet and irradiated daily with UV for 4 weeks (UV); (○) mice fed on a defined diet to which no vitamin A or retinoid was added (DD); (●) mice fed on a defined diet to which no vitamin A or retinoid was added and irradiated for 4 weeks (DD + UV); (◇) mice fed on a defined diet to which temarotene was added (TD); (◆) mice fed on a defined diet to which temarotene was added and irradiated daily for 4 weeks (TD + UV). Each data point represents a single mouse.

Statistics

For statistical analysis, the analysis of variance (ANOVA) for factorial models was used to analyse cell densities. Contact sensitivity results were not normally distributed, and hence were analysed using the Mann–Whitney *U*-test. A P value of <0.01 was regarded as significant.

RESULTS

LC densities

LC were depleted from HRA:Skh-1 mouse epidermis after 4 weeks of daily simulated solar UV irradiation (Fig. 1). The mean LC density of the unirradiated mouse group was 955, while that of the irradiated group was significantly (ANOVA) lower, 475 cells/mm^2. After 4 weeks of daily UV, HRA:Skh-1 mice treated with the topical RA 24 hr before each UV exposure had a mean LC density not statistically different from that of the unirradiated controls (931 cells/mm^2) but significantly higher than mice which received UV either alone or with the solvent in which RA was dissolved. Unirradiated mice treated with RA had a mean LC density of 1059 cells/mm^2, which was not significantly different from that of either (1) the unirradiated controls, (2) mice treated with solvent only, or (3) the irradiated group treated with RA. Thus RA prevented UV depleting LC from the epidermis.

HRA:Skh-1 mice fed the defined diet supplemented with temarotene (T diet) also exhibited resistance to UV-induced LC depletion. Irradiated mice fed a T diet had a mean LC density of 759 cells/mm^2, which was not significantly different from that of the unirradiated controls fed with either a normal diet, the defined diet with no added retinoid (972 cells/mm^2) or the T diet (966 cells/mm^2; ANOVA; Fig. 2). The mean LC density of the irradiated mice fed the T diet was significantly higher than the irradiated group fed a normal diet or the defined diet (529 cells/mm^2).

RA similarly abrogated the UV-induced LC depletion in pigmented HRA:Skh-2 mice. Mice treated with UV 24 hr after RA had a mean LC density which was not significantly different from that of the unirradiated mice but significantly higher than that of the group which received UV in the absence of RA (Table 1). Irradiated HRA:Skh-2 mice fed a T diet had a mean LC density which was not significantly different from that of the unirradiated controls fed a normal diet, the defined diet with no added retinoid or the T diet (Table 1). The mean LC density of the irradiated mice fed the T diet was significantly higher than the irradiated group fed a normal diet or the defined diet without the retinoid.

Thy-1$^+$ DEC densities

Thy-1$^+$ DEC were significantly depleted (ANOVA) from the epidermis of HRA:Skh-1 mice after 4 weeks of daily UV, from 962 to 455 cells/mm^2 (Fig. 3). Mice treated with RA 24 hr before UV had a mean Thy-1$^+$ DEC density of 856 cells/mm^2, which was significantly higher than the irradiated groups treated with either no RA or solvent. It was not significantly different from the mean Thy-1$^+$ DEC densities of the unirradiated control group or the group treated with RA (871 cells/mm^2) or solvent only. Thus, RA maintained the Thy-1$^+$ DEC density at a level comparable with that of the unirradiated controls. When applied in the absence of UV, RA did not alter the Thy-1$^+$ DEC density.

Similar protection of Thy-1$^+$ DEC density from UV irradiation was provided by adding temarotene to the diet of HRA:Skh-1 mice (Fig. 4). Mice on the T diet which received UV had a mean Thy-1$^+$ DEC density of 930 cells/mm^2, which was not significantly different from that of the unirradiated mice on either the normal diet, the defined diet without retinoids (959 cells/mm^2) or the T diet (1006 cells/mm^2). It was significantly higher than the mean Thy-1$^+$ DEC density of irradiated mice fed a normal diet or the defined diet (426 cells/mm^2). Hence feeding of unirradiated mice with the defined diet with or without added retinoid did not affect Thy-1$^+$ DEC density.

HRA:Skh-2 mice treated with RA 24 hr before UV had a mean Thy-1$^+$ DEC density which was significantly higher than the irradiated groups with no RA application or treated with solvent (Table 2). It was not significantly different from the

Table 1. Effects of UV light and topical retinoic acid or oral temarotene on LC densities (HRA:Skh-2 mice)

Treatment groups	Mean cells/ mm^2 (SD)	n	P^1	P^2	P^3	P^4	P^5
1. No treatment	980 (122)	6	—	—	—	—	—
2. UV	369 (54)	6	<0·001	—	—	—	—
3. Solvent	1005 (104)	6	NS	<0·001	—	—	—
4. UV+solvent	379 (25)	6	<0·001	NS	<0·001	—	—
5. Top RA	984 (174)	6	NS	<0·001	NS	<0·001	—
6. UV+Top. RA	939 (79)	6	NS	<0·001	NS	<0·001	NS
7. Defined diet	942 (29)	6	NS	<0·001	—	—	—
8. UV+defin. diet	531 (98)	6	<0·001	NS	<0·001	—	—
9. T diet	925 (43)	6	NS	<0·001	NS	<0·001	—
10. UV+T diet	790 (87)	6	NS	<0·001	NS	<0·001	NS

ANOVA, F stat for equal means (RA), 53·405, df 5·35.

ANOVA, F stat for equal means (temarotene), 60·569, df 5·35.

Treatment groups: (1) mice fed on a normal diet, no other treatments; (2) mice irradiated daily with ultraviolet radiation (UV) for 4 weeks; (3) mice treated with topical solvent (of retinoic acid solution); (4) mice treated with topical solvent 24 hr before irradiation; (5) mice treated with topical RA solution; (6) mice treated with topical RA solution 24 hr before irradiation; (7) mice fed on a defined diet to which no vitamin A or retinoid was added; (8) mice fed on a defined diet to which no vitamin A or retinoid was added and irradiated daily for 4 weeks; (9) mice fed on a defined diet to which temarotene was added; (10) mice fed on a defined diet to which temarotene was added and irradiated daily for 4 weeks.

P^1, statistical comparison with treatment Group 1 (ANOVA); P^2, statistical comparison with treatment Group 2 (ANOVA); P^3, statistical comparison with treatment Group 3 or 7 (ANOVA); P^4, statistical comparison with treatment Group 4 or 8 (ANOVA); P^5, statistical comparison with treatment Group 5 or 9 (ANOVA).

n, number of mice per treatment group; SD, standard deviation; NS, not significant.

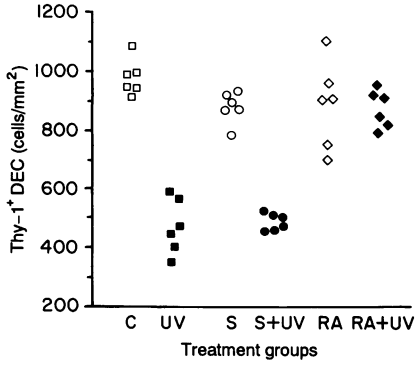

Figure 3. Effects of UV and topical retinoic acid on Thy-1$^+$ DEC densities (HRA:Skh-1 mice). Treatment groups include: (□) mice fed on a normal diet, no other treatments (C); (■) mice irradiated daily with UV for 4 weeks (UV); (○) mice treated with topical solvent (of retinoic acid solution) (S); (●) mice treated with topical solvent 24 hours before irradiation (S+UV); (◇) mice treated with topical RA (RA); (◆) mice treated with topical RA 24 hr before irradiation (RA+UV). Each data point represents a single mouse.

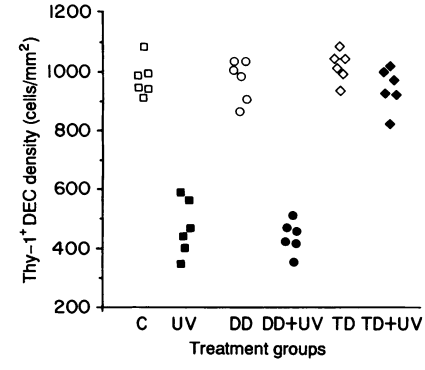

Figure 4. Effects of UV and oral temarotene on Thy-1$^+$ DEC densities (HRA:Skh-1 mice). Treatment groups include: (□) mice fed on a normal diet, no other treatments (C); (■) mice fed on a normal diet and irradiated daily with UV for 4 weeks (UV); (○) mice fed on a defined diet to which no vitamin A or retinoid was added (DD); (●) mice fed on a defined diet to which no vitamin A or retinoid was added and irradiated daily for 4 weeks (DD+UV); (◇) mice fed on a defined diet to which temarotene was added (TD); (◆) mice fed on a defined diet to which temarotene was added and irradiated daily for four weeks (TD+UV). Each data point represents a single mouse.

mean Thy-1$^+$ DEC densities of the unirradiated controls or the groups treated with RA or solvent in the absence of UV. Irradiated HRA:Skh-2 mice on the T diet also had a mean Thy-1$^+$ DEC density which did not differ significantly from that of the unirradiated mice on the normal, defined or T diets (Table 2). It was significantly higher than the mean Thy-1$^+$ DEC density of irradiated mice fed a normal diet or the defined diet.

Contact sensitivity

HRA:Skh-1 mice irradiated 5 days/week with UV for 4 weeks were locally immunosuppressed, as demonstrated by the reduced mean percentage increase in ear thickness after challenge with TNCB (Table 3). Topical application of RA prior to

Table 2. Effects of UV light and topical RA or oral temarotene on Thy-1+DEC densities (HRA:Skh-2 mice)

Treatment groups	Mean cells/ mm² (SD)	*n*	P^1	P^2	P^3	P^4	P^5
1. No treatment	959 (104)	6	—	—	—	—	—
2. UV	598 (65)	6	<0·001	—	—	—	—
3. Solvent	925 (65)	6	NS	<0·001	—	—	—
4. UV+solvent	357 (63)	6	<0·001	NS	<0·001	—	—
5. Top. RA	833 (112)	6	NS	NS	NS	<0·001	—
6. UV+Top. RA	919 (90)	6	NS	<0·001	NS	<0·001	NS
7. Defined diet	978 (75)	6	NS	<0·001	—	—	—
8. UV+defin. diet	410 (53)	6	<0·001	NS	<0·001	—	—
9. T diet	971 (45)	6	NS	<0·001	NS	<0·001	—
10. UV+T diet	953 (106)	6	NS	<0·001	NS	<0·001	NS

ANOVA, *F* stat for equal means (RA), 46·798, df 5·35.
ANOVA, *F* stat for equal means (temarotene), 58·825, df 5·35.
All other footnotes as for Table 1.

Table 3. Effects of UV light and topical retinoic acid or oral temarotene on local contact sensitivity reactions to TNCB (HRA:Skh-1 mice)

Treatment groups	Median (range)*	*n*	*P*
1. No treatment	84 (72–100)	6	—
2. UV	56 (47–67)	6	<0·01
3. Solvent	83 (67–94)	6	NS
4. UV+solvent	50 (47–50)	6	<0·01
5. Top. RA	52 (43–100)	6	NS
6. UV+Top. RA	23 (4–50)	6	<0·01
7. Defined diet	82 (75–94)	6	NS
8. UV+defin. diet	58 (33–75)	6	<0·01
9. T diet	83 (63–100)	6	NS
10. UV+T diet	50 (33–63)	6	<0·01
11. Control	9 (0–18)	6	<0·001

Groups 1 to 10 sensitized with 1% TNCB, Group 11 unsensitized but treated only with solvent of sensitizer.

* Percentage increase in ear thickness 24 hr after challenge with 0·5% TNCB.

n, number of mice in each treatment group.

P, statistical comparison with treatment Group 1, Mann–Whitney *U*-test.

NS, not significant.

Treatment groups: Groups 1–10, as Table 1; (11) unsensitized control; mice fed on a normal diet and unirradiated, treated only with solvent of the sensitizer then challenged with 0·5% TNCB.

UV did not abolish this immunosuppression, as the mean percentage increase in ear thickness after TNCB challenge remained significantly lower than that of the untreated controls. Application of either solvent or RA alone did not significantly affect the CS response. Local immunosuppression also occurred in the irradiated mice treated topically with solvent of RA.

Irradiated HRA:Skh-1 mice fed with the T diet also exhibited local immunosuppression. The CS response was significantly lower than that of the unirradiated control mice fed on a normal diet (Table 3). Irradiated mice fed on the defined diet had a mean percentage increase in ear thickness significantly lower than that of the unirradiated controls on a normal diet. Unirradiated mice fed either on the defined diet or the T diet had mean percentage increases in ear thickness which did not differ from the unirradiated controls on a normal diet.

DISCUSSION

In our experiments, we used daily low doses of UV irradiation which closely approximated the spectrum of the sun at ground level and which was filtered to remove any UVC, thus resembling the sunlight exposure experienced by many humans. Using this irradiation procedure both LC and Thy-1⁺ DEC were depleted to about 40% and 60%, respectively, of their initial values, and depletion of both of these cells was prevented by either topical or oral retinoids. However, retinoid treatment did not inhibit UV-induced local immunosuppression, as determined by CS.

The albino HRA:Skh-1 mice are genetically incapable of producing melanin. In contrast, the lightly pigmented HRA:Skh-2 mice possess melanocytes in the basal layer of the epidermis which are capable of producing melanin and they develop a light tan in response to UV irradiation. Therefore, compared with the albino mice, the HRA:Skh-2 mice are probably more representative of Caucasian human skin. Our observation that a similar reduction in both LC and Thy-1⁺ DEC occurred in both the albino and pigmented mice indicates that neither intrinsic nor UV-induced pigmentation are able to prevent UV light from reducing the density of these cells.

Both topical RA as well as an orally administered retinoid, temarotene, prevented UV radiation from depleting the number of epidermal LC and Thy-1⁺ DEC. This is comparable with our previous observations that topical RA at the same concentration used in the present experiments prevented the decrease in LC density induced by a single topical application of the tumour promotor TPA .[17] However, RA also inhibited the development of local imunosuppression through TPA-treated skin, whereas the retinoids did not protect against local UV-induced immunosuppression. This may be related to the systemic immunosup-

pression resulting from UV light exposure. It is likely that the prime cause of the local immunosuppression during chemical carcinogenesis is the depletion of local antigen-presenting cells. However, the local immunosuppression following UV light exposure may be due to effects on local antigen-presenting cells as well as other immunosuppressive factors produced following UV irradiation. It is possible that whereas the retinoids protected the local antigen-presenting cells and Thy-1$^+$ DEC, they may not have inhibited the production of immunosuppressive factors during UV irradiation. This would account for the inability of retinoids to protect against UV-induced immunosuppression, whereas we have earlier found them able to protect against TPA-induced immunosuppression.

It is also possible that whereas retinoids maintain the density of these cells during treatment with UV light, they may be non-functional. Existing evidence, however, indicates that retinoids are capable of potentiating the antigen-presenting function of dendritic cells. Splenic dendritic cells have enhanced antigen-presenting function in the presence of low concentration RA.[24]

There have been other reports that retinoids can influence LC. Oral etretinate restores the normal distribution of LC in psoriatic epidermis[25] and RA increases the number of epidermal LC in mouse tail skin.[26] However, to our knowledge there have been no previous studies on the effects of retinoids on Thy-1$^+$ DEC.

The mechanisms by which retinoids preserve the number of LC and Thy-1$^+$ DEC during UV irradiation is unclear. Retinoids may either increase the migration of LC and Thy-1$^+$ DEC into the epidermis, or inhibit UV-induced depletion of dendritic cells from the epidermis or both. In psoriatic skin, oral etretinate has been shown to increase the number of LC in the dermis, which may represent precursors of epidermal LC.[27] LC have been observed to cross the basal lamina of the dermo-epidermal junction during retinoid therapy.[28] Thus retinoids may effect migration of LC into or from the epidermis.

Since retinoids are essential for the maintenance of normal epithelial differentiation,[29] it is possible that they may play a role in maintaining the normal densities of LC and Thy-1$^+$ DEC by affecting cell differentiation. Retinoids may effect the maturation of dendritic cell precursors or induce division of LC and Thy-1$^+$ DEC. Incubation of explanted gingival epithelium with retinol increases the density of LC, probably due to differentiation of connective tissue dendritic cells.[30] Increased numbers of mitotic LC have been observed in patients treated with etretinate.[28] Alternatively it is possible that retinoids may affect production of other mediators such as prostaglandins, which may in turn regulate LC density.

In summary, in this study depletion of local LC and Thy-1$^+$ DEC using daily low doses of UV light filtered to closely resemble the solar spectrum has indicated that these cells are depleted to similar extents. Comparison of two mouse strains which are either amelanotic or pigmented and capable of developing a tan show that melanin is not able to protect these cells from the effects of UV light. However, both topical retinoic acid and temarotene were able to protect LC and Thy-1$^+$ DEC, but not local immunosuppression from the effects of UV light.

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