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Suppression of contact hypersensitivity in mice by ultraviolet irradiation is associated with defective antigen presentation

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Summary. A single dose of radiation from FS40 sunlamps results in systemic depression of delayed-type hypersenstivity (DTH) to 2-chloro-1,3,5-trinitrobenzene (TNCB) and 1-fluoro-2,4-dinitrobenzene (DNFB). Immunosuppression is proportional to the $\log_{10}$ dose of radiation and exhibits a delayed time course. Animals sensitized one day after ultraviolet (u.v.) treatment respond normally, but sensitization 3–15 days after treatment results in about 70% suppression of the DTH response. The dose response of DTH in normal and u.v.-treated animals to 1,3,5-trinitrophenyl (TNP) conjugated adherent splenocytes from normal or u.v.-treated donors was investigated. When normal mice were immunized with TNP-conjugated adherent splenocytes from normal or u.v.-treated donors, a DTH response could be elicited in these animals by injection of TNP-conjugated splenocytes into the ear. However, u.v.-irradiated recipients could not be sensitized by TNP-conjugated adherent cells from u.v.-treated mice but were sensitized by such cells from normal mice. Lysed, TNP-conjugated, normal adherent splenocytes did not immunize u.v.-irradiated recipients, but did immunize normal recipients. These results confirmed that antigen presentation is deficient in u.v.-treated mice. The time of appearance of the antigen-presenting defect in the spleen cells of u.v.-treated mice was the same as for the depression of contact sensitivity, strengthening the evidence for a causal relationship between defective antigen presentation and depression of contact sensitivity.

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INTRODUCTION

Recently, it was demonstrated that spleen cells from ultraviolet (u.v.)-treated animals are deficient in antigen-presenting ability (Greene, Sy, Kripke & Benacerraf, 1979). 1,3,5 Trinitrophenyl (TNP)-conjugated adherent splenocytes from normal animals induced a delayed-type hypersensitivity (DTH) response to TNP in both normal and u.v.-treated mice; however, similarly conjugated adherent spleen cells from u.v.-treated mice could not immunize u.v.-treated recipients, as determined by lack of DTH response, although they immunized normal recipients to a certain extent. Injection of u.v.-treated mice with TNP-conjugated cells from u.v.-treated, but not from normal animals was associated with the production of antigen-specific suppressor T lymphocytes. It was argued that these suppressor cells arose as a result of the ineffective or altered antigen-presenting ability of the adherent splenocytes from u.v.-treated animals.

In order to investigate further the alteration in antigen-presenting cell (APC) function induced by u.v. irradiation of mice, we wished to determine whether

the u.v.-induced depression of DTH to contact sensitizers actually was mediated by this alteration in APC function. We established the dose response and time course of the depression of contact hypersensitivity (CHS) induced by u.v. radiation, and we correlated the latter with the time of appearance of the antigen-presenting defect. Using the method of Smith & Miller (1979), we immunized mice with lysed hapten-conjugated cells to establish the role of host reprocessing of antigen in our system and to further validate the finding that antigen presentation is defective.

MATERIALS AND METHODS

Mice

Specific pathogen-free animals of the BALB/cAnN strain were obtained from the Frederick Cancer Research Center's Animal Production Area. The animals were between 6 weeks and 3 months of age at the start of experiments. Within each experiment, all animals were age- and sex-matched.

Ultraviolet irradiation

Animals with shaved backs were irradiated with a bank of six FS40 sunlamps that delivered a non-cosine-corrected dose rate of 2 J/m^2/sec as described previously (De Fabo & Kripke, 1979). The mice were placed in separate compartments during irradiation to prevent shielding by cage mates. Their ears were covered during irradiation with black insulating tape. The ears of control mice also were taped. All u.v. treatments were given as a single unfractionated dose.

Preparation and TNP conjugation of spleen-cell populations

Adherent cells were prepared and conjugated with TNP as described previously (Greene, Sugimoto & Benacerraf, 1978). The viability of these cell preparations was $>85\%$.

Preparation of lysed TNP-conjugated cells

Adherent spleen cells prepared and conjugated with TNP as described above were lysed by resuspending a cell pellet of known cell number in a small amount of medium and adding distilled water. The resulting suspension was frozen and thawed three times in alcohol/dry ice, thereby eliminating all viable cells. The suspension containing a known number of cell equivalents was injected subcutaneously (s.c.) in the same manner as the conjugated cells.

Immunizations

Two methods of immunization were used. In the first procedure, mice were painted on the shaved abdomen with 100 μl of a 5% solution of 2-chloro-1,3,5-trinitrobenzene (TNCB) in acetone or 30 μl of a 0·5% solution of 1-fluoro-2,4 dinitrobenzene (DNFB) in acetone. In the second procedure, a 0·1 ml volume of adherent spleen cells, conjugated with TNP, was injected s.c. into each flank of the recipient.

Challenge

Animals sensitized on the abdomen with TNCB or DNFB solutions and unsensitized age-matched controls were challenged 5–7 days later on the ear with antigen in acetone solution (Asherson & Zembala, 1974). Five microlitres of 1% TNCB or 0·2% DNFB were applied to each surface of both ears; ear thickness was measured with an engineer's micrometer (Model No. 7309, Mitutoyo, Japan) before and 24 hr after application of the challenge dose. The ear swelling obtained was compared with the ear swelling of control mice that had been challenged, but not sensitized. There were five mice in each experimental group.

Animals that had been sensitized with TNP-conjugated adherent cells and unsensitized age-matched controls were challenged with TNP-conjugated unfractionated spleen cells 5 days after sensitization as described previously (Greene *et al.*, 1979), except that the cells for challenge were injected into the ear instead of the footpad. TNP-conjugated spleen cells (1×10^7) suspended in 100 μl HBSS were injected into the base of the pinna of the right ear. A dose of 100 μl of unconjugated spleen cells was injected into the contralateral ear. Ear thickness was measured at a position above the site of injection both before and 24 hr after injection. Net ear swelling was calculated by subtracting swelling of the right ear (injected with TNP-conjugated cells) from swelling of the left ear (injected with unconjugated cells), as is done in the footpad assay (Greene *et al.*, 1979).

To assess the depression of contact hypersensitivity by u.v. treatment, we determined the net ear swelling (ear thickness of sensitized mice minus ear thickness of unsensitized mice) of control and u.v.-treated groups of animals. The percentage of control response = $(A-B)/(C-D) \times 100$ where treatment of mice is as follows: A: u.v., TNCB; B: u.v., no TNCB; C: no u.v., TNCB; D: no u.v., no TNCB.

RESULTS

Dose–response and time course of ultraviolet-induced depression of contact hypersensitivity (CHS)

Chronic u.v. treatment with FS40 sunlamps (three 1-hr treatments per week for 4 weeks) has been shown to depress the ability of BALB/c mice (Jessup, Hanna, Palaszynski & Kripke, 1978) and C3H$^-$ mice (Kripke, Lofgreen, Beard, Jessup & Fisher, 1977) to be immunized by an s.c. injection of dinitrochlorobenzene and to induce an alteration in APC function (Greene *et al.*, 1979). As shown in Table 1, dose fractionation of u.v. irradiation is not necessary for suppression of contact hypersensitivity to occur. Treatment of BALB/c mice with a single 3-hr irradiation, followed by sensitization 5 days later, depressed the CHS response to both TNCB and DNFB. The dose of u.v. required for 50% suppression of the response to TNCB obtained from Fig. 1 is 1900 J/m^2. Similar results were obtained using C3H$^-$ mice (data not shown). This single u.v. treatment routinely depressed the response to TNCB of mice of both strains by about 70%, relative to unirradiated animals. It should be emphasized that this suppression is systemic and not due to local effects of u.v. on the sensitizing or challenge sites, since neither of these sites was exposed directly to u.v. irradiation.

The immune suppression is related linearly to the $\log_{10}$ of the u.v. dose (Fig. 1). The slopes of the lines for TNCB and DNFB are similar, implying that the mechanism of suppression is the same for both antigens.

After u.v. treatment, more than 24 hr must elapse before application of the contact sensitizer in order for suppression to occur. Figure 2 illustrates that animals sensitized 1 day after u.v. exposure have a normal CHS response. However, mice sensitized 3 or more days after u.v. treatment respond poorly to challenge, and this unresponsive state persists for at least 2 weeks after u.v. irradiation.

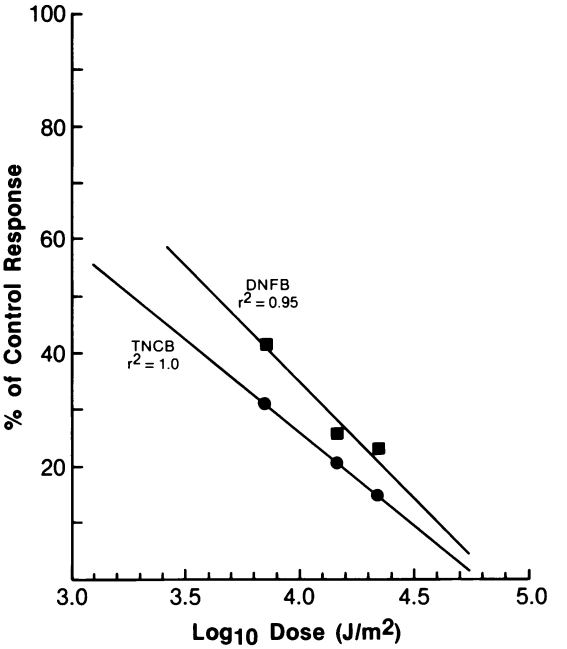

Figure 1. Dose response of suppression by u.v. irradiation. Animals were sensitized 5 days after u.v.. r^2 is the correlation coefficient.

Table 1. Depression of contact hypersensitivity to TNCB and DNFB in BALB/c mice given a single dose of u.v. irradiation with FS40 sunlamps

u.v. Exposure	Sensitization†	Ear swelling ± SE (cm × 10^{-3}) Unsensitized	Sensitized	Net ear swelling (cm × 10^{-3})	% of control response
Nil	TNCB	5·8 ± 0·7	17·7 ± 1·3	11·9	100
3 hr*	TNCB	6·3 ± 0·4	9·5 ± 0·8	3·2	26
Nil	DNFB	5·2 ± 1·1	28·7 ± 1·2	23·5	100
3 hr*	DNFB	3·6 ± 0·4	9·0 ± 0·9	5·4	23

* Total dose: 2·16 × 10^4 J/m^2.
† Mice were painted on the shaved abdomen 5 days after u.v. treatment.

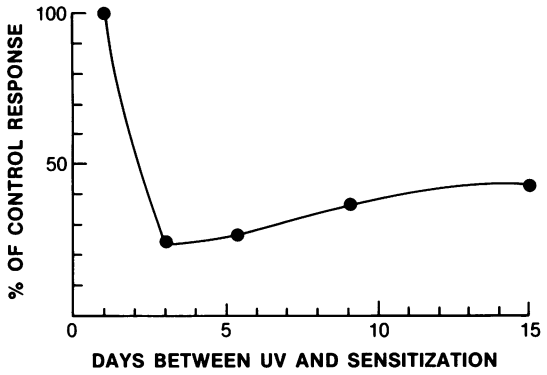

Figure 2. Time course of depression of CHS after u.v. irradiation. A 3-hr treatment of u.v. was given.

Altered antigen presentation in ultraviolet-treated mice

Having established these parameters for the suppression of CHS by u.v. treatment, we wished to see whether the alteration in APC from u.v.-treated mice exhibited the same characteristics. As shown in Fig. 3, $5–20 \times 10^6$ TNP-conjugated adherent splenocytes from normal mice (TNP-normal cells) immunized the normal recipients. The same number of TNP-conjugated adherent spleen cells from animals given a 3-hr u.v. exposure 5 days earlier (TNP-u.v. cells) also immunized normal recipients; this response to TNP-u.v. cells appeared somewhat less than the response of normal mice to TNP-normal cells, but these differences were within experimental error. A different picture emerged when mice given a 3-hr u.v. treatment 5 days earlier were immunized in this manner. TNP-normal cells could immunize u.v.-irradiated recipients to the same extent as normal recipients, but a greater number of cells (1×10^7) was necessary to achieve similar levels of sensitization. TNP-u.v. cells could not immunize the u.v.-treated recipients at any of the cell doses used ($1–20 \times 10^6$ cells). These results confirm and extend those previously reported for studies using chronically u.v.-irradiated animals (Greene *et al.*, 1979) and demonstrate that a single 3-hr exposure of mice to FS40 sunlamps also induces an alteration in APC function.

Next, we investigated the time of appearance of the alteration in APC activity. Adherent spleen cells were collected from mice 1 day or 3 days after u.v. irradiation. These cells were conjugated with TNP and used to immunize normal or u.v.-irradiated recipients (treated with u.v. 5 days earlier). As is illustrated in Fig. 4, adherent cells collected and conjugated with

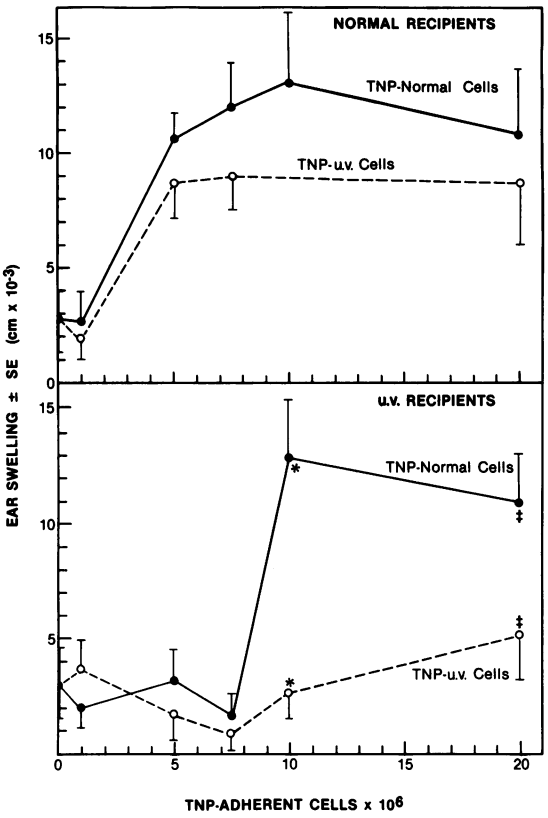

Figure 3. Immunization of normal and u.v.-treated recipients with TNP-conjugated splenic adherent cells from normal or u.v.-irradiated donors. Both u.v.-treated donors and recipients had 3 hr u.v. 5 days previously. (*‡) Values significantly different between immunizations with normal and u.v. cells ($P < 0.05$, Student's t test).

TNP 1 day after u.v. treatment of the donors immunized normal and u.v.-treated recipients to the same extent. In contrast, APC from mice irradiated 3 days earlier were unable to sensitize u.v.-irradiated recipients, although they immunized normal recipients. This delayed onset of altered APC function correlates precisely with the time course of the suppression of CHS by u.v. irradiation (Fig. 2).

One of the problems in interpreting these and the previous studies as showing defective antigen presentation in u.v.-treated mice is the ability of these cells to sensitize normal recipients. If these cells are deficient or altered in their antigen-presenting capacity, why is this alteration exhibited primarily in u.v.-treated reci-

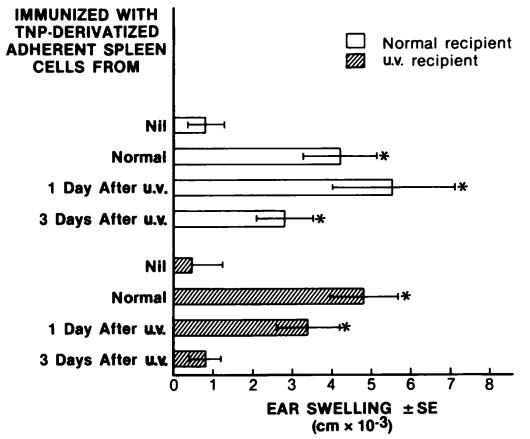

Figure 4. Time course of antigen-presenting defect in splenic adherent cells after u.v. treatment. Ultraviolet-irradiated spleen cell donors and recipients had 3 hr u.v.; the recipients were irradiated 5 days before immunization with 30×10^6 conjugated cells. * Significantly different from non-immunized control ($P < 0{\cdot}05$).

pients and not in normal recipients? Greene *et al.* (1979) suggested that this discrepancy was due to reprocessing of the antigen by cells of the normal recipient. To test this hypothesis, we used lysed, TNP-conjugated adherent cells to immunize normal and u.v.-irradiated recipients, reasoning that reprocessing of the TNP in the recipient would be required for immunization to occur. The results of this experiment are depicted in Fig. 5. Normal recipients gave DTH responses after immunization with either lysed or intact TNP-conjugated cells from normal or u.v.-treated animals. Ultraviolet-treated recipients, however, could not be immunized by lysed TNP-conjugated cells from either normal or u.v.-treated donors. These results strongly support the hypothesis that TNP-conjugated cells from u.v.-treated donors sensitize normal recipients because the antigen is re-utilized by endogenous, normal APC; this reaction does not occur in u.v.-treated recipients as these mice have no normal APC to carry out this function.

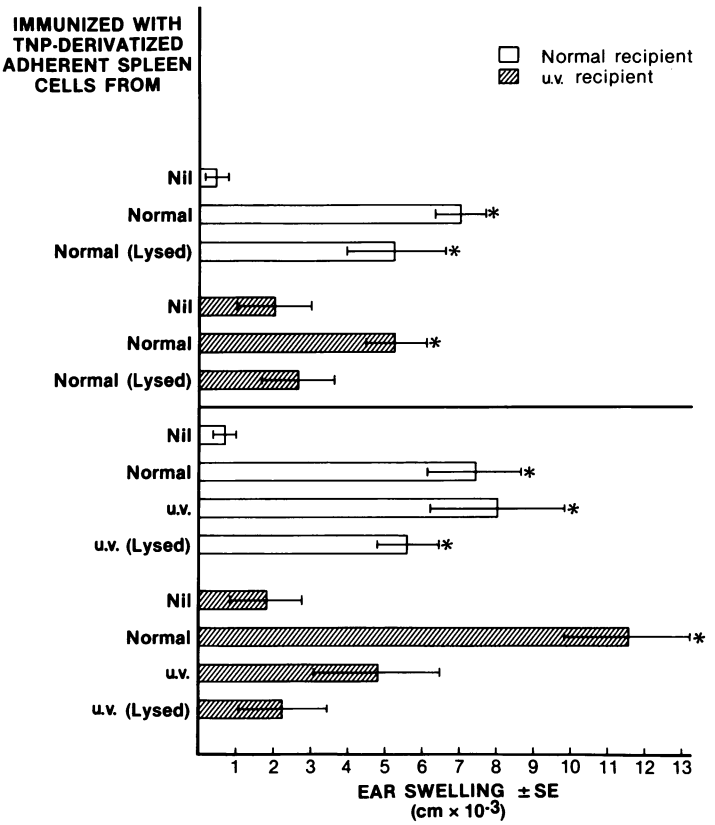

Figure 5. Immunization of normal and u.v.-treated recipients with intact or lysed splenic adherent cells. Ultraviolet irradiation as for Fig. 3; immunization with 20×10^6 TNP-conjugated cells (upper panel) or 25×10^6 cells (lower panel). * Significantly different from non-immunized control ($P < 0{\cdot}05$).

DISCUSSION

These studies demonstrate that classical CHS induced by painting mouse skin with TNCB or DNFB is suppressed by prior exposure of the animal to u.v. radiation. This finding is consistent with earlier experiments in which dinitrochlorobenzene was injected s.c. into mice given multiple exposures to u.v. radiation (Jessup *et al.*, 1978). These studies show further that fractionated doses of u.v. are not required for the suppression to occur and that the suppression is linearly related to the $\log_{10}$ dose of radiation. These findings are similar to those of De Fabo & Kripke (1979) who investigated the dose–response relationship between u.v. exposure and the induction of susceptibility to challenge with u.v.-induced tumours. Here, also, the proportion of tumour-susceptible animals increased linearly with the $\log_{10}$ dose of u.v. radiation, and the dose–response relationship was independent of the way in which the dose was delivered (multiple *v.* single treatments). These two similarities in the dose–response characteristics between suppression of CHS and suppression of tumour rejection provide some support for the possibility that these two responses to u.v. radiation may share a common mechanism.

The doses of u.v. (2–20 kJ/m^2) we have used here to suppress CHS are considerably less than the dose used in previous experiments to suppress tumour rejection (80–90 kJ/m^2; De Fabo & Kripke, 1979) and are much less than the 400–500 kJ/m^2 required to produce primary skin cancers in mice (Kripke, 1977). Recent results (De Fabo & Noonan, 1980) have shown that wavelengths of u.v. which do not cause erythema or gross skin damage (265–275 nm) are the most effective in suppressing CHS, suggesting that immunosuppression following irradiation with sunlamps is not a consequence of these damaging effects of the radiation.

The suppression of CHS that we observe following u.v. treatment is a systemic phenomenon. First, neither the site of sensitization nor the challenge site of the test animals was exposed to u.v. radiation. Second, altered antigen-presenting cells were recovered from the spleen following irradiation of the shaved dorsal epidermis. Third, sensitization within the 24 hr after u.v. exposure results in normal levels of CHS, and suppression requires a 1- to 3-day expression period between u.v. exposure and sensitization before it is detectable. This differs from the local suppression described by Toews, Bergstresser & Streilein (1980) in which mice were sensitized by painting skin that had been exposed directly to u.v. radiation. This procedure prevented contact sensitization, but showed no time delay in manifestation after u.v. treatment. Also, the inhibition of CHS was localized to the u.v.-treated area, since sensitization of an unexposed site led to the development of CHS. The absence of a systemic effect of u.v. exposure in these experiments of Toews *et al.* (1980) is almost certainly due to the very low dose of u.v. used, approximately five-fold lower than the dose with which we obtained 50% suppression.

The prediction that the systemic depression of CHS by u.v. exposure is mediated by an alteration in APC function (Greene *et al.*, 1979) is supported by three of our results. First, both effects could be induced following a single 3-hr exposure to u.v. radiation. Second, the delayed onset of suppression of CHS following u.v. treatment was mimicked closely by the time course of the appearance of altered APC in the spleens of irradiated mice. Third, and most compelling, is the ability to reverse the depression of CHS by presenting antigen on intact, but not lysed, splenic adherent cells from normal donors. This result strongly suggests that the APC alteration is responsible for the inability of u.v.-treated mice to be contact sensitized. This conclusion is also consistent with the earlier cell transfer studies of Jessup *et al.* (1978), which indicated an afferent rather than an efferent block to sensitization in u.v.-treated mice.

An important question that remains to be addressed is how irradiation of mouse skin can lead to the appearance of altered APC in the spleen. It is possible that direct irradiation of Langerhans cells with subsequent migration of these cells into the circulation could be involved, or a soluble mediator produced in the skin by u.v. radiation could account for the systemic nature of the alteration. Alternatively, the direct irradiation of blood monocytes in superficial capillaries could be responsible. The delayed time course of the manifestation of the systemic effect after u.v. irradiation is consistent with the first two of these explanations, but makes the third possibility less likely.

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REFERENCES

ASHERSON G.L. & ZEMBALA M. (1974) Suppression of contact sensitivity by T cells in the mouse. I. Demonstration that suppressor cells act on the effector stage of contact sensitivity; and their induction following *in vitro* exposure to antigen. *Proc. R. Soc. London. [Biol.]*, **187,** 329.

DE FABO E.C. & KRIPKE M.L. (1979) Dose–response characteristics of immunologic unresponsiveness to UV-induced tumors produced by UV irradiation of mice. *Photochem. Photobiol.* **30,** 385.

DE FABO E.C. & NOONAN F.P. (1980) Suppression of cell-mediated immunity in BALB/c mice by monochromatic UV. *Proc. VIIIth Annual Meeting of the American Society for Photobiology*, p. 132.

GREENE M.I., SUGIMOTO M. & BENACERRAF B. (1978) Mechanisms of regulation of cell-mediated immune responses. I. Effect of the route of immunization with TNP-coupled syngeneic cells on the induction and suppression of contact sensitivity to picryl chloride. *J. Immunol.* **120,** 1604.

GREENE M.I., SY M.S., KRIPKE M. & BENACERRAF B. (1979) Impairment of antigen-presenting cell function by ultraviolet radiation. *Proc. natn. Acad. Sci. U.S.A.* **76,** 6591.

JESSUP J.M., HANNA N., PALASZYNSKI E. & KRIPKE M.L. (1978) Mechanisms of depressed reactivity to dinitrochlorobenzene and ultraviolet-induced tumors during ultraviolet radiation. *Proc. natn. Acad. Sci. U.S.A.* **76,** 6591. 105.

KRIPKE M.L. (1977) Latency, histology and antigenicity of tumors induced by ultraviolet light in three inbred mouse strains. *Cancer Res.* **37,** 1395.

KRIPKE M.L., LOFGREEN J.S., BEARD J., JESSUP J. & FISHER M.S. (1977) *In vivo* immune responses of mice during carcinogenesis by ultraviolet radiation. *J. natn. Cancer Inst.* **59,** 1227.

SMITH F.I. & MILLER J.F.A.P. (1979) Delayed-type hypersensitivity to allogeneic cells in mice. III. Sensitivity to cell-surface antigens coded by the major histocompatibility complex and by other genes. *J. exp. Med.* **150,** 965.

TOEWS G.B., BERGSTRESSER P.R. & STREILEIN J.W. (1980) Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. *J. Immunol.* **124,** 445.