Immunology 1994 **81** 79–84

Role of tumour necrosis factor-α in ultraviolet B light-induced dendritic cell migration and suppression of contact hypersensitivity

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SUMMARY

Irradiation with ultraviolet B light (UVB) is known to suppress contact and delayed hypersensitivity responses to a variety of antigens encountered within a short period following exposure. Such irradiation results in loss of Langerhans' cells and in synthesis of tumour necrosis factor-α (TNF-α) in the epidermis. In the present study the effect of broad-band (270–350 nm) and narrow-band (311–312 nm) UVB on the induction of contact hypersensitivity (CH) and on dendritic cell (DC) numbers in draining lymph nodes (DLN) of mice was examined. Broad-band UVB induced the accumulation of DC in DLN and this increase was substantially abrogated by treatment of mice with neutralizing antibody to TNF-α before irradiation. In addition, irradiation before sensitization with oxazolone resulted in a suppressed CH response. The suppression was negated to a considerable extent by TNF-α antibodies, administered before irradiation. Thus, one of the major effects of broad-band UVB is likely to be the synthesis of epidermal TNF-α which, in turn induces the migration of Langerhans' cells to DLN and leads to an impairment of their activity or function. Conversely narrow-band UVB did not result in an accumulation of DC in DLN or in a suppressed CH response. Such irradiation does, however, cause the isomerization from *trans* to *cis*-UCA in the epidermis. *Cis*-UCA has been proposed as a photoreceptor for UV and suppresses immune responses in a variety of experimental systems. Thus *cis*-UCA does not act through TNF-α induction or by influencing DC migration, and other studies indicate that histamine-like receptors in the skin may be inolved.

INTRODUCTION

The induction phase of skin sensitization is associated with the accumulation in lymph nodes draining the exposure site of dendritic cells (DC), many of which bear high levels of antigen.[1-5] There is now clear evidence that these antigen-bearing DC derive from epidermal Langerhans' cells (LC)[6,7] and are able to stimulate efficiently T-lymphocyte activation both *in vivo* and *in vitro*.[2-4,8-10]

Topical sensitization to chemical allergens has been used frequently to investigate the immunosuppression which follows exposure to ultraviolet B light (UVB). Results from a number of experimental systems have demonstrated that such irradiation induces a significant impairment of contact sensitization when mice are subsequently exposed to the chemical allergen at the same or a different site (reviewed in ref. 11). A number of mechanisms has been implicated in the induction of UVB-mediated hyporesponsiveness and immunosuppression, including impaired or altered antigen-presenting cell function,[12-14] the action of suppressor cells[15,16] and/or effects of immunoregulatory molecules such as *cis*-urocanic acid (*cis*-UCA) and prostaglandins.[17,18] A consensus has yet to emerge, however, and in the present study we have sought to examine further the relevance of LC and induced changes in their behaviour for the development of UVB-mediated immunosuppression.

We have reported previously that exposure of mice to a suberythemal dose of broad-band UVB results in the accumulation of DC within lymph nodes draining the treatment site.[19] There is evidence now that tumour necrosis factor-α (TNF-α) provides an important signal, and possibly the sole signal, for the movement of LC from the epidermis.[20] Furthermore, it is apparent that UVB irradiation can stimulate the synthesis and release of this cytokine by keratinocytes.[21] Consequently, we have extended our previous investigations and have examined the influence of TNF-α on UVB-induced changes in the frequency of DC within draining lymph nodes (DLN) and the role of this cytokine in UVB-mediated suppression of contact hypersensitivity (CH). The UVB source used in this and most other studies emits wavelengths from 270 to 350 nm; a preliminary attempt was made to define the action spectrum of UV-induced effects on DC migration by including a narrow-band UVB source (311–312 nm) first developed for the treatment of psoriasis.[22]

Received 20 May 1993; revised 27 July 1993, accepted 18 August 1993.

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MATERIALS AND METHODS

Mice

C3HBu/Kam ($H^{-}2^{k}$) female mice, aged 6–8 weeks, were used throughout. The mice were bred and maintained in the Department of Medical Microbiology Animal House, University of Edinburgh.

UVB irradiation

The dorsal sides of mice were shaved and their ears protected during irradiation with black tape, or they were left unshaved with unprotected ears. The mice were irradiated for 30 min under two Philips TL-20/12 bulbs which gave a dose of 144 mJ/cm^2 in the broad-band range of 270–350 nm, or for 12, 36 or 72 min under a Philips TL-01 lamp which gave a dose of 144, 432 and 864 mJ/cm^2, respectively, in the narrow-band range of 311–312 nm.[22] Irradiance of the two sources at source-skin distance was measured using a filtered photodiode meter[23] which was calibrated for each source against measurements made with a UV-visible spectroradiometer (model 742; Optronics Laboratories Inc., Orlando, FL) across the spectral range 250–400 nm. The relative spectral output of the lamps is shown in Fig. 1. The mice were exposed in separate compartments of a high-sided perspex box to prevent shielding by cage mates.

Induction and expression of CH

Fifty microlitres of 1% 4-ethoxymethylene-2-phenyloxazole-5-one (oxazolone, Ox; Sigma, Poole, U.K.) in acetone:olive oil (4:1) was applied to the shaved dorsal surface of mice. Six days later CH was elicited by challenging the dorsal surface of each ear with 25 μl 0·25% Ox. Ear thickness was measured with a spring loaded caliper 24 hr following challenge and compared with ear thickness prior to challenge.

TNF-α-specific antibody treatment

Rabbit anti-mouse TNF-α, an antiserum from hyperimmune New Zealand rabbits immunized with recombinant murine TNF-α, was purchased from Genzyme Diagnostics (West Malling, U.K.). This has an activity of approximately 1×10^6 neutralizing units per ml and contains no preservative. It was diluted 1:5 with sterile phosphate-buffered saline (PBS) and 100 μl injected i.p. into each mouse, 2 hr prior to each UV irradiation. The same dilution of normal rabbit serum (NRS) was used as a control.

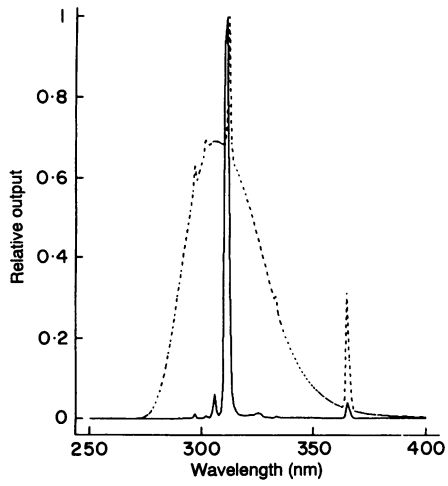

Figure 1. Relative spectral output of Philips TL20/12 (- - -) and TL01 (—) fluorescent sources normalized at 312 nm (Philips, Eindhoven, The Netherlands).

Isolation, identification and enumeration of lymph node DC

The purification and enumeration of DC from pooled, draining auricular lymph nodes has been described previously.[19] In brief, draining auricular lymph nodes were excised, pooled for each experimental group and a single-cell suspension prepared. The cell concentration was adjusted to 5×10^6 per ml in RPMI-1640 growth medium supplemented with 10% heat-inactivated fetal calf serum. DC-enriched populations were prepared by density gradient centrifugation on 14·5% metrizamide. They were washed and resuspended in RPMI-FCS. The number of DC within the low buoyant density fraction was assessed routinely by direct morphological examination using light microscopy. In general, approximately 50% of this population had dendritic morphology. For each experimental group, five counts of DC were made and the mean number of DC present within a single lymph node was calculated.

Statistical evaluation of results

The statistical significance of differences between the means of each experimental group was calculated using Student's t-test. Mean differences were considered to be significant when $P < 0.05$.

RESULTS

Effect of broad- and narrow-band UVB irradiation in the induction of CH

Groups of mice were shaved and irradiated with 144 or 432 mJ/cm^2 narrow-band UVB or with 144 mJ/cm^2 broad-band UVB on 2 consecutive days. Twenty-four hours after the second irradiation, the mice were sensitized on their dorsal skin with 50 μl 1% Ox. Six days later the dorsum of both ears was challenged with 25 μl 0·25% Ox and the increase in ear thickness measured 24 hr later. Groups of non-irradiated mice treated with Ox or the vehicle, in which Ox was dissolved, acted as positive and negative controls respectively. Some negative control mice were challenged with Ox and others with vehicle.

The mean results of two experiments are shown in Fig. 2. It may be seen that while irradiation with the broad-band UV source induced a suppressed CH response, irradiation with the narrow-band UV source did not affect the CH response.

Effect of broad- and narrow-band UVB irradiation on DC accumulation in DLN

Groups of mice were irradiated with a single dose of 144 or 864 mJ/cm^2 narrow-band, 144 mJ/cm^2 broad-band UVB or were left untreated. Forty-eight hours following exposure they were killed, the draining auricular lymph nodes excised and the number of DC per lymph node calculated. It may be seen from Fig. 3 that, while irradiation with broad-band UVB increased the number of DC per lymph node, exposure to neither dose of narrow-band UVB altered the number of DC in DLN significantly.

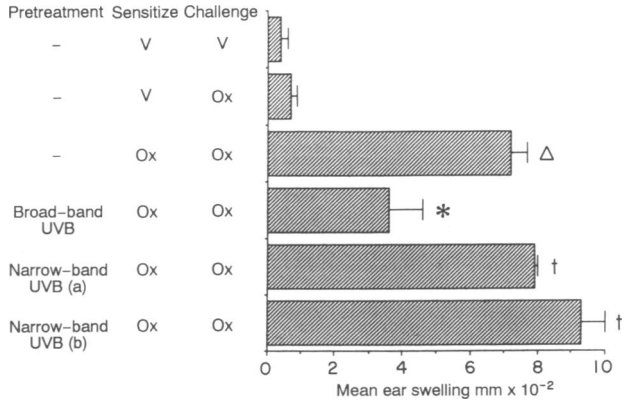

Figure 2. Effect of broad-band (144 mJ/cm^2 × 2) and narrow-band (a, 144 mJ/cm^2 × 2 or b, 432 mJ/cm^2 × 2) UV irradiation on ear swelling response to oxazolone. Bars represent mean ± SEM increase in ear thickness in two independent experiments (10 mice in each group). * Significantly different from group Δ ($P < 0{\cdot}01$); † Not significantly different from group Δ ($P > 0{\cdot}1$). V, vehicle; Ox, oxazolone.

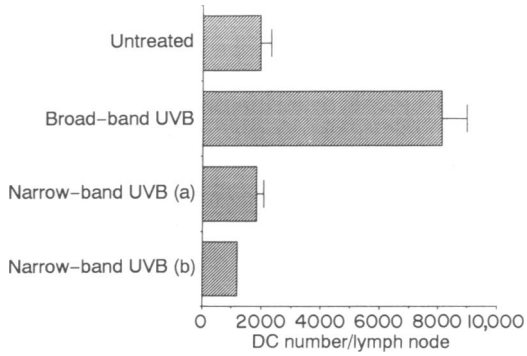

Figure 3. Effect of broad-band (144 mJ/cm^2) and narrow-band (a, 144 mJ/cm^2 or b, 864 mJ/cm^2) UVB irradiation on DC accumulation in DLN 48 hr later. Bars represent the mean ± SEM of three experiments (10 mice in each group) except for the narrow-band UVB group b where a single experiment was performed.

Effect of TNF-α antibodies on UVB-induced DC accumulation in DLN

As TNF-α provides one signal for the migration of DC to DLN, the effect of pretreatment with a rabbit polyclonal antiserum to murine TNF-α on UV-induced accumulation of DC was studied. Two hours prior to irradiating mice with a single dose (144 mJ/cm^2) of broad-band UVB, one group received an i.p. injection of TNF-α antibodies while another received the same volume of normal rabbit serum. Control mice were treated in the same way but were not irradiated. Forty-eight hours later, the mice were killed, the DLN excised and the number of DC counted. Two experiments gave essentially the same results. One is illustrated in Fig. 4 which shows that treatment of the mice with antibodies to TNF-α prior to UVB irradiation reduced considerably the UV-induced migration of DC to lymph nodes.

Effect of TNF-α antibodies on UVB-induced suppression of CH

Having demonstrated above that DC migration to DLN as a result of UVB irradiation was mediated by TNF-α, it was important to confirm that TNF-α played a role in the UV-induced suppression of CH. Accordingly, 2 hr prior to irradiating the backs of shaved mice with a single dose (144 mJ/cm^2) of broad-band UVB on each of 2 consecutive days, the mice were injected with TNF-α antibodies, while another group received normal rabbit serum and a third was left untreated. Two control groups consisted of mice that were similarly injected with TNF-α antibodies or normal rabbit serum but were not irradiated. The mice were sensitized on their dorsal sides with 50 μl 1% Ox 24 hr after the last irradiation. Six days later the dorsum of both ears was challenged with 25 μl 0·25% Ox and the ear swelling response measured 24 hr later.

The results of one experiment presented in Fig. 5 show that administration of TNF-α antibodies prior to UVB irradiation inhibited the UV-induced suppression of CH. Thus, TNF-α production is a critical factor in UVB susceptibility.

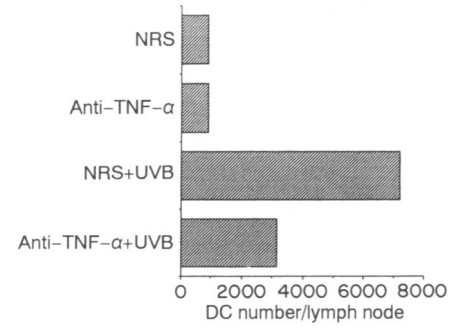

Figure 4. Effect of TNF-α antibodies on DC numbers in DLN 48 hr after UVB irradiation (representative experiment, 10 mice in each group). Mice were injected with TNF antibodies (anti-TNF-α + UVB) or normal rabbit serum (NRS + UVB) 2 hr prior to broad-band UVB irradiation (144 mJ/cm^2). Control mice (NRS or anti-TNF-α) were treated in the same way but were not irradiated.

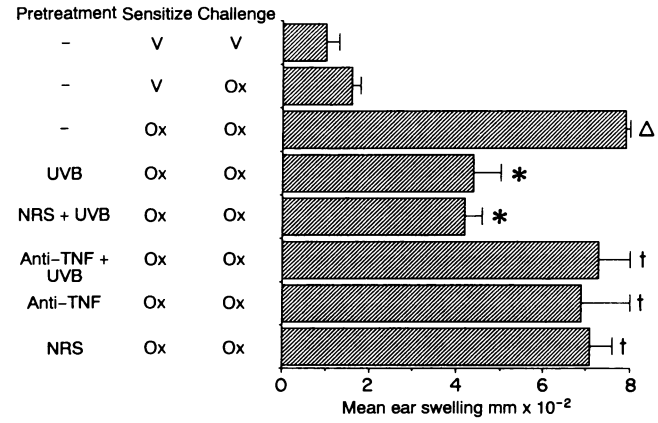

Figure 5. Effect of TNF-α antibodies on UVB-induced suppression of CH to oxazolone (10 mice in each group). Mice were injected with TNF-α antibodies (anti-TNF-α + UVB) or normal rabbit serum (NRS + UVB) 2 hr prior to broad-band UVB irradiation on 2 consecutive days (144 mJ/cm^2). Control mice (anti-TNF-α or NRS) were treated in the same way but not irradiated. Bars represent mean ± SEM ear swelling responses. * Significantly different from group Δ ($P < 0{\cdot}001$). † Not significantly different from group Δ ($P > 0{\cdot}1$). V, vehicle; Ox, oxazolone.

DISCUSSION

The case for UVB-mediated immunosuppression of CH being secondary to effects on epidermal LC has been debated for many years.[11,24] There is no doubt that immunosuppressive doses of UVB have been associated usually with alterations in the density and morphology of LC,[24-27] the implication being that the efficiency of antigen presentation is impaired or the nature of antigen presentation subverted.

It is known that UVB irradiation is able to provoke the synthesis and secretion of TNF-α[21] and it been suggested recently that UV-induced suppression of the induction phase of CH is effected by this cytokine.[28] Our recent studies have shown that intradermal administration of TNF-α stimulates the accumulation of DC in DLN,[20] complementary to a reduction in the density of identifiable LC within the epidermis (I. Kimber and M. Cumberbatch, unpublished observations). In contrast, Streilein *et al.*[29,30] have reported that impairment of skin sensitization to 2,4-dinitrofluorobenzene caused by TNF-α resulted from the immobilization of LC within the epidermis. However, they did find a reduction in the density of epidermal Ia$^+$ LC following intradermal injection of TNF-α.

Consistent with the ability of UVB light to induce the production of TNF-α by epidermal cells, we have demonstrated previously that such treatment results in the accumulation of DC in lymph nodes draining the site of exposure.[19] In the present investigations these observations have been extended to show that the systemic administration of neutralizing antibodies for TNF-α impairs accumulation of DC in DLN following exposure to broad-band UVB. In agreement with studies performed by Yoshikawa and Streilein,[28] we have found that TNF-α antibodies also reverse the UVB-induced suppression of CH. Collectively, these data lead us to propose the following sequence of events. Exposure to UVB results in the local production of TNF-α by keratinocyes and the stimulation of LC migration from the skin. When chemical allergen is applied to the same site subsequently, there are insufficient numbers of responsive or functionally mature LC to handle, transport, process and/or present antigen efficiently to T lymphocytes in the DLN. Compatible with this proposal is the circumstantial evidence that murine LC express the species-restricted receptor for TNF-α[20,31] and the immunocytochemical demonstration that human LC possess the p75 TNF-α receptor protein.[32]

If UVB-mediated suppression of CH is indeed attributable to changes in the behaviour of LC secondary to local production of TNF-α, then it is appropriate to consider the nature of the functional deficit. It is known that the migration of LC from the epidermis to the DLN following skin sensitization is accompanied by changes in the membrane expression of certain molecules necessary for effective antigen presentation. Thus, compared with the epidermal LC from which they derive, the DC which accumulate in the draining nodes after skin sensitization display elevated levels of both MHC class II (Ia) antigens and intercellular adhesion molecule-1 (ICAM-1; CD54).[33,34] Our previous studies lead us to conclude that UVB irradiation fails to prevent the acquisition by migrating LC of enhanced Ia expression. Flow cytometric analyses revealed that the expression of Ia antigen by DC was unchanged following treatment with UVB.[19] Nor is it likely that suppression results simply from a reduced efficiency of antigen transport. Irradiation with UVB did not appear to depress the arrival in DLN of DC bearing FITC.[19] One possibility is that UVB compromises the increased expression by migrating cells of ICAM-1 which is considered essential for the effective interaction of antigen-bearing DC with T lymphocytes.[34,35] In support of this are the results of *in vitro* studies which have shown that UBV inhibits the increased expression of ICAM-1 that is associated normally with the culture of freshly isolated LC.[14,36]

It is possible also that UVB light rather than, or in addition to, impairing LC activity *per se*, may cause changes in antigen processing and/or presentation function which in turn will influence the quality of immune response provoked. In both human and mouse there exists a functional heterogeneity among CD4$^+$ T-helper (Th) cells.[37,38] Two main populations have been identified, designated Th1 and Th2, which differ in terms of the cytokines they secrete following immune activation.[39] Delayed-type hypersensitivity (DTH) reactions are effected by Th1 cells[40,41] It has been shown that exposure to UVB light alters the selectivity of LC for Th cell activation. Irradiated LC were found to lose their ability to stimulate Th1 cells, while fully retaining their capacity to activate Th2 cells.[42] Moreover, they induced long-lived clonal anergy in Th1 populations.[12] It may be, therefore, that UVB causes a changed equilibrium in the immune system and a movement away from the stimulation of the Th1 cell-type responses necessary for CH. Such changes may or may not result from UVB-mediated alterations in the membrane expression by LC of determinants which govern selectivity for CD4$^+$ subpopulations.[13]

In contrast to the broad-band TL-20/12 source, the narrow-band T1-01 source, emitting predominantly at 311–313 nm, did not induce suppression of CH (Fig. 2) or influence the migration of DC to DLN (Fig. 3). In 1983 De Fabo and Noonan suggested that modulations in immune responses following UVB irradiation could be initiated by a specific photoreceptor in the skin, and provided preliminary evidence from the absorption spectrum and wavelength dependence of immunosuppression that UCA was involved.[43] This molecule is found in the stratum corneum as the *trans*-isomer and, on UV irradiation *in vivo* or *in vitro*, converts to the *cis*-isomer in a dose-dependent manner until a photostationary state is reached when *cis*-UCA is about 50% of the total UCA.[44] Recent studies in mice have shown that topical or systemic application of *cis*-UCA suppresses CH[45,46] delayed hypersensitivity,[47] skin allograft rejection and acute graft-versus-host disease.[48] We have compared the potency of the TL-20/12 and TL-01 sources to induce oedema, sunburn cells and isomerization from *trans* to *cis*-UCA in the epidermis of Skh-1 hairless mice.[49] The lamps were equally effective at producing *cis*-UCA, but the TL-01 source was six times less efficient than the TL-20/12 at generating equivalent oedema and sunburn cells. Thus irradiation with the narrow-band source may lead to maximal levels of epidermal *cis*-UCA at doses which lead to minimal skin damage. In the present study it is estimated that a narrow-band UV dose of 144 mJ/cm^2 will induce approximately 35% *cis*-UCA and doses above 400 mJ/cm^2 a photostationary state of approximately 50% *cis*-UCA. Previously, we showed that neither isomer of UCA influenced the migration of DC to DLN.[19] Thus, it is possible that the major effect of the narrow-band UVB irradiation of skin may be UCA isomerization which does not lead to DC migration or suppression of CH responses. This is in contrast to TNF-α induction as a result of broad-band UVB irradiation, and subsequent DC migration. Using a murine model of herpes simplex virus infection, we have shown that *cis*-UCA is highly effective in suppresing delayed hypersensitivity responses to the virus[47] and

have obtained preliminary evidence to indicate that *cis*-UCA may act through histamine-line receptors in the skin.[50] It is possible that immune responses generated in CH and DH may be different, and that *cis*-UCA, whilst modulating DH, may be a poor suppressor of CH compared with UVB irradiation.

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