The effect of ultraviolet B irradiation and urocanic acid isomers on dendritic cell migration

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SUMMARY

Irradiation with ultraviolet-B light (UV-B) suppresses some cell-mediated immune responses to a variety of antigens, including contact sensitizers. Following UV irradiation there is modulation of Langerhans’ cells’ markers and keratinocytes are induced to synthesize and secrete tumour necrosis factor-α (TNF-α). Cis-urocanic acid (cis-UCA) has been suggested as a photoreceptor for UV and has been demonstrated to suppress immune responses in several experimental systems. UCA is found naturally in the stratum corneum as the trans-isomer and converts to the cis-isomer on irradiation. In the present study the migration of dendritic cells (DC) to lymph nodes following UV-B irradiation or epicutaneous application of UCA isomers was examined in unsensitized mice and mice sensitized with fluorescein isothiocyanate (FITC). It was found that UV-B irradiation alone induced DC migration to draining lymph nodes (DLN) and that UV-B irradiation prior to skin sensitization at the same site enhanced DC migration. A maximum number of DC was present in DLN 48 hr following irradiation. In sensitized mice, the percentage of DC bearing FITC and the quantity of FITC per DC was unaltered by prior UV exposure. In contrast, neither isomer of UCA had any significant effect on DC numbers in sensitized or unsensitized mice. It was concluded that UV-B irradiation induced the migration of DC from the epidermis to draining lymph nodes, an effect possibly mediated by TNF-α release, while UCA may act by a different mechanism, perhaps via histamine-like receptors in the epidermis.

INTRODUCTION

Following epicutaneous exposure of mice to skin-sensitizing chemicals there is a rapid accumulation of dendritic cells (DC) in the draining lymph nodes (DLN). A significant proportion of the DC found within DLN bear antigen suggesting that they may originate from the epidermis and may have an antigen-presenting function. Topical exposure of mice to 2,4-dinitrofluorobenzene (DNFB) causes a temporary but marked depletion of Langerhans’ cells (LC) from the epidermis. In addition at least some DC which accumulate in DLN following contact sensitization contain Birbeck granules, characteristic of LC. These and other studies indicate that the antigen-bearing DC in DLN are derived from epidermal LC which travel to the lymph nodes as afferent lymph. Recently Kripke et al. have corroborated this hypothesis by showing that cells from DLN of athymic nude mice, grafted with allogeneic skin and contact sensitized through the graft, contain antigen-bearing DC of graft donor origin. However, it is possible that, while LC initiate contact sensitization under normal circumstances, skin cells other than LC may be capable of inducing the sensitization particularly when the potential of LC to present antigen has been abrogated.

Antigen-bearing DC in DLN are potent stimulators of both primary and secondary T-lymphocyte proliferative responses in vitro and efficiently induce contact sensitization in naive animals. A correlation is found between the number of DC which are present in DLN 24 hr after skin sensitization and the extent of the primary lymphocyte proliferative response. Irradiation with ultraviolet-B light (UV-B) is known to suppress some cell-mediated immune responses to a variety of antigens including contact sensitizers (reviewed in ref. 13). It has been speculated that a UV-induced loss of LC is responsible for suppression as epidermal LC numbers in UV-irradiated skin decrease in parallel with a decrease in contact hypersensitivity (CH) responses. However, a number of other studies have not shown such a correlation although there is evidence for the modulation of LC markers after UV irradiation.
It has been reported that UV-B irradiation impairs the induction of CH in some (UV susceptible), but not other (UV resistant), genetically defined strains of mice.\(^{18}\) Susceptibility to UV-B is dictated by alleles at the Lps and Tnfα loci which influence the amount of tumour necrosis factor-α (TNF-α) produced in response to UV-B.\(^ {19} \) Recently, TNF-α has been shown to act as an important mediator of the suppressive effects of UV-B on the induction of CH.\(^ {19} \) Interestingly, there is now evidence that keratinocytes synthesize and release TNF-α after UV-B irradiation.\(^ {20} \)

It is likely that there is a photoreceptor in skin which mediates the effects of UV irradiation on the immune system. One candidate, first proposed by De Fabo and Noonan,\(^ {21} \) is urocanic acid (UCA), found naturally in the stratum corneum as the trans-isomer, which converts to the cis-isomer on irradiation. There is considerable evidence from several experimental systems that cis-UCA plays an important role in UV-induced immunosuppression. For example, cis-UCA has been shown to modify antigen-presenting cell function in vivo,\(^ {22} \) to suppress delayed hypersensitivity (DH) responses in a murine model of herpes simplex virus infection,\(^ {23, 24} \) to delay rejection of transplantation allografts\(^ {25, 26} \) and to enhance UV-induced tumour yield and malignancy in the hairless mouse.\(^ {27} \)

In the present study the effects of UV irradiation and UCA isomers on the migration of DC to DLN have been examined in unsensitized and sensitized mice. In addition the carriage of antigen and expression of Ia antigens by the DC have been measured.

**MATERIALS AND METHODS**

**Mice**

C3H Bu/Kam (H-2\(^ {b}\)) female mice, aged 6–8 weeks were used throughout. The mice were bred and maintained in the Dept. of Medical Microbiology Animal House, University of Edinburgh, U.K.

**UV-B irradiation**

Mice were irradiated for 30 min under two Philips TL20/12 bulbs which gave a dose of 144 ml/cm\(^ {2}\) in the range of 270–350 nm. They were irradiated in separate compartments of a high-sided perspex box to prevent shielding by cage mates. They were unshaved and their ears unprotected.

**UCA treatment**

Trans-UCA (Sigma Chemical Co., Poole, U.K.) or cis-UCA (obtained by preparative thin layer chromatography of UV-B-irradiated trans-UCA)\(^ {28} \) was dissolved at a concentration of 40 mg/ml in dimethylsulphoxide (DMSO) at 37° for 5 min. The solution was then diluted 10-fold in acetone and 25 μl applied topically, to the dorsal surface of both ears. An equal volume of the appropriate vehicle was applied in the same way to control mice.

**Contact sensitization**

Fluorescein isothiocyanate (FITC; Sigma) was used as a 1% solution in 1:1 acetone: dibutylphthalate. Mice received 25 μl of FITC or an equal volume of the appropriate vehicle on the dorsum of both ears 18 hr before killing.

**Isolation, identification and enumeration of lymph nodes**

Draining auricular lymph nodes were excised, pooled for each experimental group and a single-cell suspension of lymph node cells was prepared by mechanical disaggregation through a 200-mesh stainless steel gauze. Viable cell counts were performed using 0.5% trypan blue and the cell concentration adjusted to 5 x 10\(^ {6}\) cells/ml in RPMI-1640 growth medium (Flow Laboratories, Irvine, U.K.) supplemented with 5 x 10\(^ {-5}\) m 2-mercaptoethanol, 100 IU/ml penicillin, 2 mM l-glutamine, 100 μg/ml gentamicin, 20 μg/ml fungizone and 10% heat-inactivated foetal calf serum (RPMI-FCS). DC-enriched populations were prepared by density gradient centrifugation as described previously.\(^ {2} \) Briefly, 8 ml of the cell suspension was added to 10 ml conical-bottom test tubes and was gently underlayed with 2 ml of 14.5% metrizamide (Nygaard, Oslo, Norway) in RPMI-FCS. The tubes were then centrifuged for 15 min (600 g) at room temperature. The DC-enriched population accumulating at the interface was collected, washed once 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. For each experimental group, five counts were made and the mean number of DC present within a single lymph node was calculated.

**Analysis of FITC-bearing DC**

DC-enriched preparations derived from draining auricular lymph nodes of mice whose ears had been exposed to UV-B irradiation or painted with UCA isomers prior to sensitization with FITC, were analysed on an EPICS C flow cytometer (Coulter Electronics, Luton, U.K.) equipped with a 100 mW argon laser tuned to 488 nm wavelength. A total of 5000 cells from each sample was analysed at a flow rate of 200 cells/second.

DC were identified on a two-parameter histogram measuring size and side-scatter and then green fluorescence analysed from a bit map onto a 252 channel histogram using log amplification. The percentage of FITC+ cells within this population was measured and also antigen density/cell (fluorescence intensity) by mean channel analysis.

**Ia antigen staining of DC and FITC-bearing DC**

DC-enriched preparations derived from draining auricular lymph nodes, were washed and 5 x 10\(^ {6}\) cells stained with anti-mouse Ia monoclonal antibody (clone 11.5.2.1.9; ECACC, Porton Down, Salisbury, U.K.) at a dilution of 1:100 for 1 hr on ice. The cells were then washed and stained with either a sheep anti-mouse IgG [F(ab')\(^ {2}\)fragment] labelled with FITC (Sigma) at a dilution of 1:40 or a sheep anti-mouse IgG labelled with phycoerythrin (Sigma) at a dilution of 1:40 for 40 min on ice. Simultaneously, 5 x 10\(^ {6}\) cells from DC-enriched preparations were incubated with an irrelevant antibody followed by FITC or phycoerythrin-labelled secondary antibody to act as background controls in the EPICS analysis. The cells were analysed in an EPICS C flow cytometer (Coulter Electronics) equipped with 5 W argon laser operating at 488 nm wavelength. To measure the percentage of DC expressing Ia, the cells were labelled with anti-Ia and FITC secondary antibody before analysis as outlined in the section above. To measure Ia expression on DC after sensitization with FITC, the cells were labelled with anti-Ia and phycoerythrin secondary antibody and a double-colour analysis carried out in the following manner. Dichroic mirrors were used to separate light into the 90° light
scatter detector (500 long pass) and into both red (560 short pass followed by a 575/25-band pass filter) and green (530/30 band pass) fluorescence detectors. Any residual spectral overlap of green fluorescence into the red detector was removed by electronic compensation.

**RESULTS**

**Effect of UV-B irradiation and cis or trans-UCA on DC accumulation in DLN of unsensitized mice**

After irradiating mice or painting their ears with cis or trans-UCA, the mice were then killed, their auricular lymph nodes excised and the number of DC present in each lymph node was estimated. Table 1, which shows the results of four experiments, demonstrates that neither isomer of UCA has a consistent effect on inducing DC migration to DLN, whereas UV-B irradiation increases DC numbers within the DLN.

**Time-course of the effect of UV-B irradiation and cis-UCA on DC migration and Ia expression**

The number of DC present within the DLN of mice at various times following a single dose of UV-B irradiation of 144 mJ/cm² was calculated. Figure 1 illustrates that DC migration to DLN starts to take place between 12 and 24 hr, and reaches a maximum at 48 hr before decreasing. On the other hand, at various times after the cutaneous application of cis-UCA, the numbers of DC were unaltered (Fig. 1).

The number of DC expressing Ia antigens in the DLN at various times after UV-B irradiation was examined; the results are shown in Table 2. The same mean intensity of staining per cell was found throughout the experiment (data not shown).

**Influence of UV-B irradiation and cis or trans-UCA on DC migration induced by FITC**

Irradiated mice or mice painted with UCA isomers were sensitized on their ears with FITC 18 hr before killing. Their auricular lymph nodes were removed and the number of DC present in each lymph node was measured. It can be seen from Table 3, which shows the results of four independent experiments, that whilst the isomers have no effect on DC migration induced by FITC, UV-B irradiation increases DC migration to the DLN considerably.

**Influence of UV-B irradiation on the percentage of DC carrying FITC and Ia expression in the DLN**

The draining auricular lymph nodes of mice, whose ears had been exposed to UV-B radiation prior to sensitization with FITC, were examined for the percentage of DC bearing FITC (Table 4). UV-B irradiation which increased DC migration induced by FITC did not influence the percentage of DC carrying FITC. The mean intensity of staining/cell of FITC-bearing DC was found to be unaltered by UV-B irradiation. It can therefore be concluded that UV-B irradiation prior to sensitization increases the number of FITC-bearing DC accumulating in the DLN.

It can be seen from Table 4 that UV-B irradiation has no effect on the expression of Ia molecules by DC accumulating in

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**Table 1. Effect of UV-B irradiation and cis or trans-UCA on DC migration to DLN**

<table>
<thead>
<tr>
<th>Treatment at 66 and 42 hr prior to analysis</th>
<th>DC count/lymph node experiment</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1*</td>
<td>2*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>2850</td>
<td>2860</td>
</tr>
<tr>
<td>cis-UCA</td>
<td>5162</td>
<td>3050</td>
</tr>
<tr>
<td>trans-UCA</td>
<td>7040</td>
<td>4446</td>
</tr>
<tr>
<td>UV-B (144 mJ/cm²)</td>
<td>10,005</td>
<td>9344</td>
</tr>
</tbody>
</table>

* 200 μg of UCA was painted onto each ear.
† 100 μg of UCA was painted onto each ear.
‡ Significantly different from control group (P < 0.001 by Student’s t-test).

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**Table 2. The effect of UV-B irradiation on Ia expression in DC from DLN**

<table>
<thead>
<tr>
<th>Time after UV-B irradiation (hr)</th>
<th>% of cells within the gated population expressing Ia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated DC</td>
<td>93·8</td>
</tr>
<tr>
<td>12</td>
<td>83·6</td>
</tr>
<tr>
<td>24</td>
<td>92·0</td>
</tr>
<tr>
<td>36</td>
<td>94·7</td>
</tr>
<tr>
<td>48</td>
<td>91·8</td>
</tr>
<tr>
<td>60</td>
<td>86·2</td>
</tr>
<tr>
<td>72</td>
<td>91·5</td>
</tr>
</tbody>
</table>

At various times after UV irradiation (single dose of 144 mJ/cm²) the mice were killed, their auricular lymph nodes removed and the enriched DC were then stained for Ia.
Table 3. Influence of UV-B irradiation and cis or trans-UCA on DC migration to DLN induced by FITC

<table>
<thead>
<tr>
<th>Treatment at 66 and 42 hr</th>
<th>FITC at 18 hr</th>
<th>DC count/lymph node experiment</th>
<th>Mean ± SD</th>
<th>Statistical significance (by Student’s t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Vehicle</td>
<td>–</td>
<td>6750</td>
<td>8775</td>
<td>4788</td>
</tr>
<tr>
<td>Vehicle*</td>
<td>+</td>
<td>12,222</td>
<td>13,754</td>
<td>10,196</td>
</tr>
<tr>
<td>100 µg cis-UCA</td>
<td>+</td>
<td>15,238</td>
<td>16,406</td>
<td>9111</td>
</tr>
<tr>
<td>100µg trans-UCA</td>
<td>+</td>
<td>14,175</td>
<td>19,511</td>
<td>11,500</td>
</tr>
<tr>
<td>UV-B (144 mJ/cm²)</td>
<td>+</td>
<td>23,490</td>
<td>20,174</td>
<td>16,422</td>
</tr>
</tbody>
</table>

† Significance of difference from vehicle group.*
NS, not significantly different from vehicle group.

Table 4. Influence of UV-B irradiation on the percentage of DC carrying FITC and expressing Ia

<table>
<thead>
<tr>
<th>Treatment at 66 and 42 hr</th>
<th>FITC at 18 hr</th>
<th>DC count/lymph node % of cells within gated population bearing FITC</th>
<th>% of cells within* gated population bearing FITC</th>
<th>% of cells within† gated population expressing Ia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>–</td>
<td>8125</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Vehicle*</td>
<td>+</td>
<td>13,294</td>
<td>40</td>
<td>38 ± 8</td>
</tr>
<tr>
<td>UV-B (144 mJ/cm²)</td>
<td>+</td>
<td>19,600</td>
<td>34</td>
<td>38 ± 5</td>
</tr>
</tbody>
</table>

* Expressed as arithmetic mean of four experiments ± 1 SD.
† Expressed as arithmetic mean of two experiments ± 1 SD.

the DLN as a result of FITC induction. In addition, by using double-colour fluorescence, UV-B irradiation did not alter Ia expression on FITC-bearing DC (data not shown).

DISCUSSION

The results reported here demonstrate that UV-B irradiation alone, or prior to skin sensitization at the same site, induces or enhances DC migration to DLN. In addition, our data show that cis-UCA is unable to induce similar changes, indicating that the mechanism of its action may be different from irradiation.

Since LC are the major antigen-presenting cells of the skin and play a crucial role in the induction of CH responses, they are excellent candidates to mediate the immunosuppressive effects of UV-B irradiation. It has been suggested that the immobilization of LC at UV-B-irradiated sites may contribute to the failure of mice to develop CH when hapten is painted subsequently on these sites. Bigby et al. reported that there were no hapten-bearing antigen-presenting cells in DLN, if the skin on which the hapten was painted had been exposed previously to UV-B. It is known that UV-B irradiation stimulates the synthesis and secretion of TNF-α by keratinocytes. Vermeer and Streilein found that TNF-α inhibited the induction of CH in DNFB. Based on this observation and the demonstration that UV-B or intradermal TNF-α alters the morphology of epidermal LC, Vermeer and Streilein concluded that TNF-α prevents effective sensitization following UV-B irradiation by immobilizing LC within the epidermis. In contrast to these findings, the data presented here indicate that UV-B irradiation increases the number of migrating DC induced by FITC, while the percentage of DC bearing antigen in DLN is unaltered. Also, we have found that treatment of mice with dexamethasone, a transcriptional inhibitor of TNF-α, prior to UV irradiation inhibits DC migration to DLN (A. M. Moodycliffe, I. Kimber and M. Norval, unpublished data). The recent demonstration by Cumberbatch and Kimber that TNF-α induces DC migration to DLN, supports the concept that one of the effects of UV-B exposure is to induce DC accumulation in DLN by stimulating TNF-α release. Further support is provided by Vermeer and Streilein who have shown that both intradermal TNF-α and UV-B irradiation reduce the density of Ia+ cells in the epidermis, suggesting that UV-B exposure may induce LC migration through the local release of TNF-α.

It is not possible to conclude from our results whether or not the induction of DC migration by UV-B radiation to DLN, either by itself or in conjunction with some other important components(s), is responsible for UV-B induced suppression of CH responses. However, we have shown that, following UV-B irradiation, there was a greater number of antigen-bearing DC entering the DLN than would occur with skin sensitization alone. Ia expression was unaffected suggesting that antigen-presenting cell function may not be lost, although it could be altered. There is evidence, that during migration from the skin to lymphoid tissue, LC are subject to phenotypic and functional maturation. The functional maturation of LC in vitro is stimulated by granulocyte–macrophage colony-stimulating factor, a product of keratinocytes. It is possible that, following UV irradiation, the migrating DC do not have time to mature into fully functional antigen-presenting cells. T-cell interactions with DC may then be affected, for example, by altered ability to...
synthesize and secrete accessory molecules, such as interleukin-1 (IL-1) and IL-6, or by altered expression of appropriate adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and LFA-1. Indeed it has been shown recently that membrane ICAM-1 is expressed on lymph node DC, while it is present in only very low amounts on epidermal LC; increased ICAM-1 expression may be necessary for the development of LC into effective antigen-presenting cells.35

Unlike UV-B irradiation, there was little, if any, effect on DC migration induced by the UCA isomers even at larger than physiological doses (100–200 μg/mouse). C3H mice contain approximately 20 μg/cm² UCA in the epidermis, most as the trans-isomer, and about 50% converts into the cis-isomer after UV-B irradiation of 144 mJ/cm².36 The result indicates that cis-UCA may mediate its effect on the immune system in a rather different way than via TNF-α production and DC migration. Indeed cis-UCA is a poor suppressor of CH compared with UV-B irradiation, while it is highly effective at suppressing DH responses. In the model of HSV infection cis-UCA suppresses DH even at doses of as little as 1 μg/mouse.37 By using structural analogues of UCA and histamine receptor antagonists and antagonists, we have shown that cis-UCA is likely to act via histamine-like receptors in the epidermis.38,39 In addition there is some evidence that immune responses generated in DH and CH may be different. For example irradiation of keratinocytes with UV of different wavebands induced the release of mediators which were selective in their ability to induce suppression of DH and CH.38

REFERENCES


31. **Cumiskey M. & Kimber I.** (1992) Dermal tumour necrosis factor-α induces dendritic cell migration to draining lymph nodes, and possibly provides one stimulus for Langerhans' cell migration. *Immunology, 75*, 257.


