Effect of Phototherapy and Urocanic Acid Isomers on Natural Killer Cell Function

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Ultraviolet (UV) radiation suppresses a variety of immune responses but it is uncertain whether this action contributes to the effectiveness of phototherapy. Urocanic acid (UCA) has been proposed as a mediator of the immunologic effects of UV. On exposure the naturally occurring *trans*-isomer of UCA in the skin changes into the *cis*-isomer, which has been demonstrated to mimic many of the immunomodulatory effects of UV irradiation. Natural killer (NK) cells play an important role in several immunologic processes and published evidence indicates that their activity is altered by UV irradiation. To ascertain the effect on NK cells of phototherapy used in the treatment of psoriasis, modulation of NK activity in psoriatic patients undergoing broad-band UVB, narrow-band UVB, or psoralen plus (PUVA) regimens was examined. This was compared with NK cell activity in psoriatic patients treated with topical coal tar and in normal subjects receiving broad band UVB.

The NK cell activity of psoriatic and normal subjects was the same over a wide range of effector to target cell ratios. Almost all patients undergoing phototherapy exhibited depressed NK cell activity during or after irradiation, although the timing of the depression varied between the lamps used and may be related to dose. However, patients treated with topical coal tar showed unchanged NK cell activity throughout the therapy. The effect of UCA isomers on NK cell activity *in vitro* was also determined. It was found that *cis*-UCA induced a dose-dependent suppression of NK cell activity in both patients and normal subjects, whereas *trans*-UCA had hardly any effect in either group.

Thus it is possible that there may be a correlation between *cis*-UCA formation in the epidermis and the modulation of NK cell activity that occurs during phototherapy. Key words: UV irradiation/psoriasis/*cis*-urocanic acid. *J Invest Dermatol 101:169–174, 1993*

Natural killer (NK) cells represent a heterogeneous population of large granular lymphocytes that have not been assigned to a single lineage or distinct anatomical location of maturation, and tend to be defined on a functional basis. The majority of NK cells in human peripheral blood are $CD56^+$ $CD16^+$ $CD2^+$ $CD11b^+$ $CD3^-$; other markers, such as CD57, are only expressed on certain NK subsets [1]. They are cytotoxic cells that detect and lyse target cells in a non-major histocompatibility complex (MHC) restricted manner, although MHC class I alleles have been implicated recently in target-cell recognition [2].

There is evidence that NK cells play a substantial role in a number of immunologic processes. These functions include cytotoxic action against tumor cells and virally infected cells [3] and resistance to some bacterial, fungal and parasitic agents [4,5]. In addition they may be involved in rejection of allogeneic grafts [6], and in immune regulation through the secretion of a number of cytokines [7,8]. The receptors involved in target cell recognition by NK cells or the signal-transduction mechanism responsible for effector-cell activation remain largely undefined.

Manuscript received January 5, 1993; accepted for publication April 12, 1993.

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Abbreviations: HSV, herpes simplex virus; MED, minimal erythemal dose; NK, natural killer; PBMC, peripheral blood mononuclear cells; UCA, urocanic acid.

In addition to the well-recognized association between *in vivo* UV exposure and the incidence of squamous cell carcinomas, a number of experimental models show that UV irradiation results in transient suppression of immune responses to antigens that are encountered shortly thereafter. These include systems where NK cell activity is thought to be important in immunity, such as infections with herpes simplex virus (HSV) [9] and murine acquired immunodeficiency virus [10], tumor development [11], and allograft rejection. It is of interest that *in vitro* UV irradiation of human peripheral blood mononuclear cells (PBMC) results in dose-dependent suppression of NK cell activity [12–15]. In addition, there is an indication that exposure to UV and PUVA *in vivo* may result in suppressed NK cell activity [16,17]. Urocanic acid (UCA), found in the stratum corneum, has been proposed as a photoreceptor and mediator of the immunomodulatory effects of UV radiation [18]. On UV exposure, the naturally occurring *trans*-isomer of UCA undergoes a dose-dependent isomerization to *cis*-UCA that has been shown to mimic many of the effects of UV on the immune system (reviewed in [19]).

Psoriasis is a common, genetically determined, hyperproliferative skin disorder in which the immune system is thought to play a critical role [20,21]. UVB phototherapy and PUVA are successfully employed in the management of psoriasis. However, their use is largely empirical and little is known of their mechanisms of action or of their possible effects on the immune system. Conventional broad-band UV sources, such as Sylvania UV6 tubes, emit wavelengths from 270 to 370 nm. Wavelengths less than 300 nm are highly erythemogenic and, in common with those over 315 nm, are relatively ineffective therapeutically [22,23]. Therefore a narrow-

0022-202X/93/$06.00

Table I. Clinical Details of All Subjects Taking Part in the Study

Group	Number of Patients	Mean Age	Age Range	Sex	Skin	Therapy	Mean Number of Treatments at Sample Point (±SEM)	Mean Total UV Dose at Sample Point During Therapy (J_{cm}^{-2} ± SEM)	Mean Total Number of Treatments (±SEM)	Mean Total UV Dose (J_{cm}^{-2} ± SEM)
A	5	41	19–61	1M/4F	Psoriasis	Coal Tar	N/A[a]	N/A	N/A	N/A
B	6	37	31–45	4M/2F	Psoriasis	UVB	11.4 ± 1.8	1.8 ± 0.3	18.9 ± 2.3	5.5 ± 1.3
C	6	48	38–73	2M/4F	Psoriasis	TL01	11.7 ± 0.5	8.0 ± 0.8	21.5 ± 1.5	19.2 ± 2.7
D	6	60	45–72	6M	Psoriasis	PUVA	7.0 ± 0.3	21.6 ± 4.0	15.5 ± 1.5	88.6 ± 18.1
E	7	33	21–52	2M/5F	Normal	Ni1	N/A	N/A	N/A	N/A
F	4	38	32–53	2M/2F	Normal	UVB	13.0 ± 1.1	3.4 ± 0.6	16.5 ± 0.5	6.3 ± 1.3

[a]N/A, not applicable.

band (311–313 nm) source was developed (Philips TL01)§ which has proved effective in the management of psoriasis [24,25].

In the present study the NK cell activities of normal subjects and patients with psoriasis were measured. Secondly the effect of therapeutic regimens employing UV6, TL01, or PUVA therapy on NK cell activity in patients with psoriasis was examined; this was compared with the effect of coal tar therapy in psoriatic patients and with a course of UV6 irradiation in normal subjects. Finally, modulation of NK cell activity in psoriatic patients and normal subjects by UCA isomers *in vitro* was determined.

MATERIALS AND METHODS

Patients Details of patients and normal subjects are summarized in Table I. Groups A, B, E, and F attended the Department of Dermatology, Royal Infirmary of Edinburgh; groups C and D attended the Photobiology Unit, Ninewells Hospital, Dundee. All subjects were in good general health and taking no other photoactive or immunosuppressive medication. Those with psoriasis had chronic plaque psoriasis except for three patients in group C who had guttate psoriasis. Liquid paraffin/white soft paraffin (50/50) or emulsifying ointment were used as emollients between treatments by all psoriatic patients including group A.

Thirty milliliters of venous blood was collected into preservative-free heparin in the early morning. Samples were taken from subjects receiving UV-based therapy (groups B, C, D, and F) immediately before treatment started, after 4 weeks of therapy, and 4 weeks after the last irradiation. Group A patients were treated with increasing concentrations (1–4%) of topical coal tar paste under stockinette dressings. Samples were taken from them before treatment commenced and after 4 weeks of therapy. Group E consisted of normal subjects who received no therapy: samples were taken from four of this group on two occasions, 4 weeks apart.

UV Irradiation All subjects were of skin types II or III. Those in group B received the UVB regimen normally used for treatment of psoriasis in Edinburgh. They were irradiated in a Waldmann 1000 UVB cabinet containing 26 Sylvania UV6 tubes emitting 42% in the UVA waveband, 57.6% in UVB, and approximately 0.3% in UVC. Irradiance of the tubes was measured using a filtered photodiode meter [26], which was calibrated against a spectroradiometer (model 742, Optronic Lab Inc), and the total output (250–400 nm) was 1.4 mW cm^{-2}. Patients were irradiated with incremental doses starting with 37 mJ cm^{-2}, thrice weekly until clear (4–6 weeks). Those in group F were normal volunteers who received the same regimen for 6 weeks except that only their arms were exposed and the remainder of their bodies were draped.

Group C patients had their minimal erythema doses (MED) determined and an initial dose of 70% of MED was given. They were treated 3 times weekly and at each visit the irradiation dose was increased by 40%; the increment was reduced or omitted depending on the occurrence and severity of erythema. Treatment was stopped when psoriatic plaques had resolved to the point of impalpability over all affected sites, excepting minimal residual activity on knees or elbows (15–20 treatments). The irradiation, was given in an upright cubicle incorporating 50 Philips TL01 100W fluorescent lamps. The irradiance was 4.1 mW cm^{-2} and was determined as for the UV6 lamps.

Group D patients received PUVA in a Waldmann 6000 cabinet incorporating 62 Sylvania FR74T12 fluorescent lamps (UVB = 0.2 mW cm^{-2}, UVA = 11.7 mW cm^{-2}). Irradiance was monitored using a Waldmann PUVA meter calibrated at the Regional Medical Physics Department, Durham. All patients had their minimal phototoxic dose determined, and the first irradiation dose was 70% of this. Treatment was then given twice weekly with 40% incremental doses (less in case of erythema) at each visit until minimal residual activity was achieved (15–20 treatments).

Preparation of Peripheral Blood Mononuclear Cells (PBMC) and Phenotyping PBMC were isolated by centrifugation on lymphopaque (Nyegard Ltd, Oslo, Norway). The cells were washed three times and suspended in RPMI 1640 supplemented with antibiotics [27] plus 10% fetal calf serum (tissue culture medium) at 4×10^6 ml^{-1}.

The method for phenotyping of PBMC using a Coulter EPICS "C" flow cytometer has been described previously [27]. The cells were stained with murine monoclonal antibodies to CD1a, CD3, CD4, CD8 (Dakopatts, High Wycombe, UK), CD56, CD57 (Beckton-Dickinson, California), and pan MHC class II (DP, DR, DQ:DA6.231, a gift from Dr K. Guy). An irrelevant antibody was used to determine the background non-specific staining, which was set to approximately 1% of the total histogram. Data were initially collected on a two-parameter histogram measuring forward light scatter. Bit maps were drawn around both the lymphocyte and the monocyte/large granular lymphocyte populations and the percentage of cells exhibiting fluorescent levels higher than background were recorded for these two populations independently.

NK Cell Assay K562 cells were employed as targets in all experiments [28]. One million cells were labeled with 100 μCi sodium chromate (^{51}Cr, Amersham International Ltd, England) for 4 h at 37°C. They were washed three times in 0.01 M phosphate-buffered saline, pH 7.2, suspended in tissue culture medium at 10^5 cells ml^{-1}, and plated at 100 μl/well in round-bottomed microtiter plates (Falcon, Becton Dickinson, Oxford, U.K). Doubling dilutions of PBMC were carried out in tissue-culture medium and 100 μl of each dilution added to quadruplicate wells resulting in effector-to-target-cell ratios from 40:1 to 1.25:1. Spontaneous ^{51}Cr release was determined by adding 100 μl tissue-culture medium to quadruplicate wells and maximum release by adding 100 μl 2% acetic acid to quadruplicate wells. The plates were incubated for 18 h at 37°C in 5% CO_2 in a humidified incubator, then spun at 300 × *g* for 5 min before removing 120 μl supernatant for determination of counts per minute (CPM) using a Packard liquid scintillation counter. The SEM at each ratio was always less than 10% of the mean value. Percent specific ^{51}Cr release was determined by 100 × [(test CPM − spontaneous CPM)/(maximum CPM − spontaneous CPM)].

Urocanic Acid (UCA) *Trans*-UCA was purchased from Sigma (Poole, Dorset). *Cis*-UCA was prepared and purified after UV irradiation of a solution of *trans*-UCA as already described [29]. *Cis*- and *trans*-UCA were dissolved in phosphate-buffered saline at concentrations of 10^{-1} M and 10^{-2} M, respectively, and serial tenfold dilutions to 10^{-8} M carried out in phosphate-buffered saline. Ten microliters of each dilution was added to 0.5 ml PBMC at 10^5 cells ml^{-1}. In addition 100 μl of 10^{-1} M *cis*- or 10^{-2} M *trans*-UCA were added to 0.4 ml PBMC at 1.25×10^5 ml^{-1}. One hundred microliters UCA-treated PBMC were then added to the wells of a microtiter plate and 15 min later 100 μl labeled target cells added at an effector:target cell ratio of 40:1. This resulted in a final concentration of *cis*-UCA of 10^{-2} to 10^{-10} M, and *trans*-UCA of 10^{-3} to 10^{-10} M. Target cells were also incubated alone with the UCA isomers.

Statistical Analysis Differences in NK cell activity of patients were determined by Wilcoxon test or Student *t* test, where appropriate.

RESULTS

NK Cell Activity in Normal Subjects and Patients with Psoriasis Before any treatment commenced, all 23 patients with

§ Van Weelden H, van der Leun JC: Improving the effectiveness of phototherapy for psoriasis (abstr). Br J Dermatol 111:484, 1984.

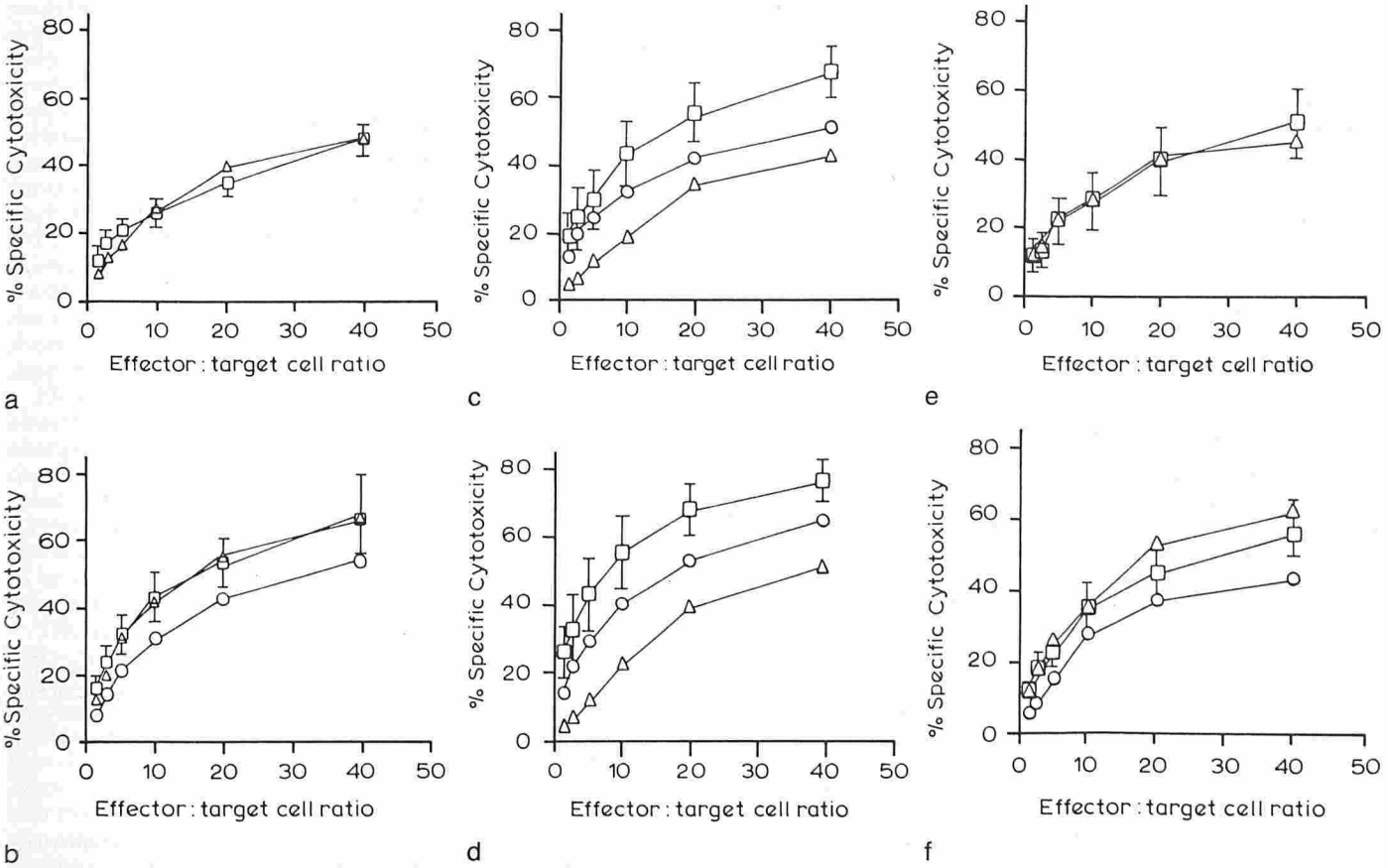

Figure 1. The effect of therapy on NK cell activity in patients with psoriasis and normal subjects. Psoriasis patients receiving coal tar dressing had samples taken on two occasions, before (□) and during (△) treatment *(a)*. Assays were performed before (□), during (△), and after (○) broad-band UV6 *(b)*, narrow band TL01 *(c)*, or PUVA *(d)* therapy of psoriasis patients. Normal subjects, receiving no treatment, had samples taken on two occasions 4 weeks apart (*e*, □ and △). Normal subjects were assayed before (□), during (△), and after (○) UV6 therapy *(f)*. The points represent the mean of five patients in *(a)*, six in *(b–d)*, and four in *(e,f)*. The SEM shown for each group prior to treatment was approximately the same on subsequent occasions *(error bars)*.

psoriasis and 11 normal subjects detailed in Table I were tested for NK cell activity. A comparison of the two groups showed no statistical difference at any effector : target cell ratio. For example, at an effector : target cell ratio of 40 : 1, NK cell activity of normal volunteers was 52.2 ± 5.4 (mean ± SEM) compared with psoriatics 64.1 ± 4.2 ($p = 0.11$, Wilcoxon rank sum test).

Effect of Therapy on NK Cell Activity in Normal Subjects and Patients with Psoriasis All patients showed a marked clinical improvement (to at least minimal residual activity) in the degree of psoriasis irrespective of the treatment used. It should be noted that, although a set of samples was collected after 4 weeks of therapy, in most instances patients required 6 weeks of UV therapy to achieve complete or almost complete clearance of psoriasis.

Figure 1 illustrates the mean NK cell activity before, during, and after therapy in all subjects. Coal tar therapy (group A) had no significant effect on NK cell activity (Fig 1*a*). Patients receiving broad-band UV6 treatment (group B) showed no significant alteration in NK cell activity after 4 weeks of therapy (Fig 1*b*). However, there was some reduction in activity 4 weeks after the last treatment in five of the six patients. This reduction was statistically significant for the group at all effector : target cell ratios ($p = 0.046$, Wilcoxon signed-ranks test). Group C patients, receiving narrow-band TL01 therapy, showed a marked depression in NK cell activity after 4 weeks of therapy (Fig 1*c*); for example, the mean value (± SEM) for the group fell from 66.8 ± 7.1 to 42.0 ± 6.6 at effector : target cell ratio of 40 : 1. This reduction was statistically significant at all effector : target cell ratios ($p = 0.028$, Wilcoxon signed ranks test). Four weeks after the last irradiation the mean NK value for group C had recovered significantly, for example, to 50.9 ± 7.0 at effector : target cell ratio of 40 : 1 ($p = 0.043$, Wilcoxon signed-ranks test). However, it still remained significantly depressed compared with the original value prior to irradiation in five of six patients ($p = 0.043$, Wilcoxon signed-ranks test). Samples were collected from two of the patients a further 4 weeks later, i.e., 8 weeks after the last irradiation. NK cell activity was again increased and had almost returned to the original value. A similar pattern was observed in the six patients receiving PUVA therapy (group D, Fig 1*d*). Following 4 weeks of therapy, all exhibited a marked reduction in NK cell activity; for example, at effector : target cell ratio of 40 : 1 the activity fell from a mean (± SEM) of 76.4 ± 6.1 to 51.6 ± 8.0. The reduction was statistically significant at all effector : target cell ratios but more so if the ratio was less than 40 : 1 ($p = 0.046$ at effector : target cell ratio of 40 : 1, and $p = 0.028$ at effector : target cell ratio less than 40 : 1). Four weeks after the last treatment the activity had recovered but it remained significantly suppressed relative to the original values at effector : target cell ratios less than 40 : 1 ($p = 0.028$, Wilcoxon signed-ranks test). Three patients had their NK cell activity assessed a further 4 weeks later when it had almost returned to normal.

Two groups of normal subjects were also analyzed. The first, part of group E, consisted of four individuals who received no treatment. The second, group F, consisted of four individuals who received a course of UV6 identical to that received by the psoriasis patients in group B, except that only their arms were irradiated. Figure 1*e* shows that there was no fluctuation in NK cell activity in the group E subjects, whereas Fig 1*f* demonstrates that four weeks of UV6 therapy had no significant effect on NK cell activity in group F

subjects. However, 4 weeks after the last irradiation NK cell activity was suppressed in three individuals. This suppression did not reach statistical significance ($p > 0.05$, paired sample t test).

Phenotyping PBMC from all the normal subjects and psoriasis patients were stained with monoclonal antibodies against CD1a, CD3, CD4, CD8, CD57, and MHC class II antigens. There was no difference in any of the markers between normal subjects and patients, and no significant alteration of the markers occurred as a result of therapy (data not shown).

In addition to these antibodies, a further monoclonal against CD56 was employed to determine the number of NK cells present in three patients in group C and four in group D. In the population of cells gated to include large granular lymphocytes and to exclude smaller cells, there was a reduced percentage of $CD56^+$ cells during therapy that rose after therapy had finished [% $CD56^+$ cells before therapy = 77.0 ± 6.5 (mean ± SEM), during therapy = 22.4 ± 3.9, after therapy = 59.7 ± 8.3].

Effect of UCA Isomers on NK Cell Activity *In Vitro* The results above indicate that UV irradiation *in vivo* suppresses NK cell activity and the following experiments were undertaken to determine the effects of UCA on NK cell activity *in vitro*. Because the concentrations of UCA isomers used were relatively high, various tests were carried out to ensure that the isomers were not toxic. In the first place, on four separate occasions, UCA isomers ranging from 10^{-2} to 10^{-10} M were added to wells containing labeled target cells, which were then incubated for 18 h and the supernatant assayed for release of ^{51}Cr. No alteration in background release of ^{51}Cr occurred at any concentration of *cis* or *trans*-UCA. Secondly, effector cells were incubated with UCA isomers for 18 h and the number of viable cells determined by trypan blue exclusion. This was always between 90 and 100%. Thirdly, the effector cells were incubated for 18 h with 10^{-3}, 10^{-6}, or 10^{-9} M *cis*- or *trans*-UCA and then stained overnight with propidium iodide. The number of cells in the S + G_2M stage of cell division were quantitated by flow cytometry using the S-Fit DNA analysis programme (Coulter Electronics Ltd., Luton, U.K.). Approximately 18% of cells were in S + G_2M, and this value was not altered by the presence of UCA isomers.

The percentage suppression of NK cell activity induced by a range of concentrations of *cis*-UCA *in vitro* at an effector : target cell ratio of 20 : 1 was determined for 12 subjects; seven normal volunteers and five untreated patients with psoriasis. *Cis*-UCA had the same effect on PBMC in all of these subjects and Fig 2*a* represents the mean suppression of NK activity in these individuals. The effect of *trans*-UCA was measured in four normal subjects in the same manner (Fig 2*b*). Treatment with *cis*-UCA resulted in a dose-dependent suppression of NK cell activity that was statistically significant at concentrations between 10^{-2} and 10^{-4} M. *Trans*-UCA was slightly suppressive at higher concentrations but this was minimal in comparison with *cis*-UCA and was not statistically significant.

Finally the effect of UCA isomers on the function of lymphocytes other than NK cells was examined. A lymphoproliferation test was carried out in which UCA isomers, in concentrations similar to those used in the NK assay, were added to quintuplicate wells containing PBMC together with sub-optimal concentrations of concanavalin A (1.5 μg/ml) or HSV antigen (0.1 plaque forming unit/cell, UV inactivated), as already outlined [27]. The experiment was done on two separate occasions using PBMC from two healthy donors known to be HSV carriers. In both cases, neither UCA isomer had any consistent or dose-dependent effect on lymphoproliferation (data not shown).

DISCUSSION

Suppressed NK cell activity has been reported in patients with generalized pustular psoriasis and, to a lesser extent, with psoriasis vulgaris [30]. In contrast, Hunyadi *et al* [31] found normal NK cell activity in a group of subjects with chronic plaque psoriasis. Our study included 20 patients with chronic plaque psoriasis and three with guttate psoriasis. No significant difference in NK cell activity was observed in comparison with eleven normal healthy subjects.

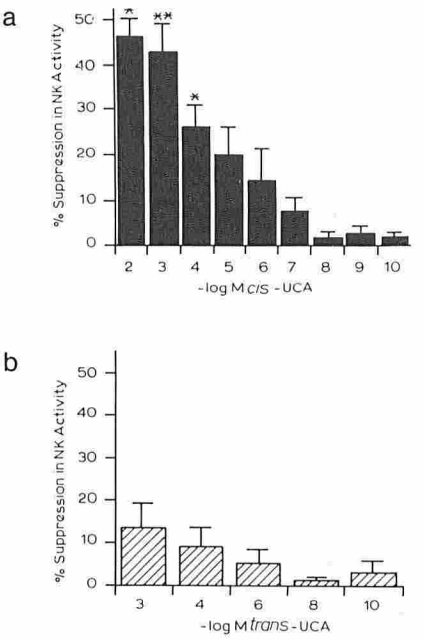

Figure 2. The effect of *cis*-UCA *(a)* and *trans*-UCA *(b)* on NK cell activity *in vitro*. *a* represents the mean ± SEM of 6 – 11 subjects at each UCA concentration and *b* represents the mean ± SEM of four subjects. *$p < 0.05$ and **$p < 0.01$ compared with the mean of the same individuals with no UCA added, paired sample t test.

Coal tar treatment as well as three established UV irradiation regimens used in the management of psoriasis caused a marked improvement in the severity of psoriasis in all cases. However, only patients receiving UV exhibited any suppression in NK cell activity. This suggested that the modulation in NK cell function resulted from UV irradiation and was not secondary to clinical improvement.

Suppressed NK cell activity was found in all patients after 4 weeks of TL01 (group C) or PUVA (group D) therapy. Four weeks after treatment had stopped, NK cell activity had begun to recover although in neither group had it regained its initial level. The kinetics of NK cell modulation were different when the broad-band UVB (UV6) lamp was used (group B). In this case, there was no significant change in NK cell activity following 4 weeks of therapy but suppression had occurred 4 weeks after the completion of the course (Fig 1*b*). It is possible that the suppression of NK cell activity by UV irradiation may be dose dependent. As the TL01 and UVA sources do not emit the highly erythemogenic wavelengths, patients received higher doses of UV in a short period of time compared with group B (Table I). Furthermore, this is suggested by the results from group F (normal subjects undergoing broad-band UVB irradiation of their arms only) as, although a reduction in NK cell activity was recorded in most individuals 4 weeks after the last irradiation, it did not reach statistical significance (Fig 1*f*). A dose-dependent suppression in NK cell activity following solarium exposure has been reported [32]. In that study normal subjects were irradiated with approximately one minimal erythemal dose for 2, 6, or 12 consecutive days. At least six consecutive days of exposure were required to induce suppression of NK cell activity and the suppression was more pronounced following 12 d. Those patients who received 12 whole body exposures still demonstrated suppressed NK cell function 21 d after the last irradiation. Another study examined the effects of PUVA therapy on NK cells in a group of patients with a number of skin disorders, 50% of whom had psoriasis vulgaris [17]. A reduction in NK cell activity was observed during therapy although this tended to return to normal at the higher effector : target cell ratio, if the therapy was continued beyond 25 irradiations.

In addition to the total UV dose, it is possible that the incident

wavelengths of UV are important. In this respect Hersey *et al* [33] reported that 12 1-h exposures to sunlight in a 2-week period had only a minimal effect on NK cell activity. However, further studies revealed that 2 weeks of irradiation with solarium lamps resulted in suppressed activity [34]. The ratio of UVA to UVB emitted by solarium lamps was estimated to be three times higher than that of natural sunlight, and it was shown that the suppression of NK cell function still occurred even if wavelengths in the UVB range were excluded [16,35]. Hence it was concluded that UVA wavelengths (320–400 nm) were solely responsible for the effects on NK cells. However, our present studies do not support this view because the TL01 source that emits narrow-band UVB (311–313 nm) produced suppression of NK cell activity during therapy. *In vitro,* shorter UV wavelengths have been demonstrated to cause more suppression of NK cell activity than longer wavelengths [14].

Hersey *et al* [35] reported minimal changes in the number of circulating $CD16^+$ cells as a result of irradiation and we observed no change in $CD57^+$ cells, suggesting that the total number of circulating NK cells remains unaltered. However preliminary tests indicated that the percentage of large granular lymphocytes expressing CD56 was reduced in patients receiving TL01 or PUVA therapy and this correlated with reduced NK cell activity. CD56 is thought to be an activation marker expressed on NK cells and is found on very few T cells [36].

The mechanism whereby UV irradiation affects NK function is unknown. Possibly there may be a direct effect on NK cells as they circulate through the dermal capillaries that results in permanent functional damage. The recovery of activity may then be dependent on the regeneration of cells from the bone marrow. It is also possible that there may be modulation by another cell type, such as monocytes [37] or NK cells themselves [38]. Alternatively a soluble mediator may be released from epidermal cells on irradiation that enters the circulation and affects NK cell function. Kim *et al* [39] and Brodie and Halliday [40] have reported soluble suppressive factors in murine serum following UV irradiation that are potential candidates. Prostaglandins, induced by UV exposure, are other possibilities as they have been shown to inhibit NK cell activity [41]. *In vitro* epidermal cells release an interleukin-1–like factor that augments NK cell activity. This is called epidermal cell–derived NK cell activity–augmenting factor and may be relevant *in vivo* [42]. Finally, there may be altered regulation of the function of other cells through modified cytokine secretion by NK cells themselves.

One further factor that merits consideration is *cis*-UCA, formed in the epidermis from *trans*-UCA on UV irradiation. We have shown in the present study that *cis*-UCA treatment *in vitro* resulted in a dose-dependent suppression of NK cell activity, whereas *trans*-UCA had little effect. The concentration of *cis*-UCA required to modulate the activity was relatively high (10^{-2} to 10^{-4} M). However, *trans*-UCA is present in considerable amounts in the human epidermis (about 8 μg cm^{-2} [43]) and, on UV exposure, up to approximately 60% converts to the *cis*-isomer in a dose-dependent fashion. We have shown that *cis*-UCA is present in suction blister fluid and it is retained in this compartment for at least 4 weeks after irradiation [44]. Strenuous efforts were made to ensure that the conditions were not toxic for the effector or target cells in the NK assay. Trypan blue exclusion and propidium iodide staining indicated no demonstrable cell death and PBMC were not suppressed in a lymphoproliferation test.

Although the second messenger system involved in NK cell lysis remains to be identified, an increase in intracellular Ca^{++} is associated with NK cell activation and this is thought to be regulated by cyclic adenosine monophosphate [45]. Significantly *cis*-UCA has been shown recently to down-regulate the induction of cyclic adenosine monophosphate by *trans*-UCA or histamine [46] and therefore it is possible that *cis*-UCA may down-regulate the second messenger system of the NK cell.

In this study NK cell activities of normal subjects and psoriatic patients did not differ before treatment, they responded in a similar fashion to UV6 therapy, and they were affected by *cis*-UCA *in vitro* to the same extent. Thus it is reasonable to assume that the modulations noted are relevant to NK cells in general and are not confined to psoriasis.

Finally, because NK cells are thought to play a major role in tumor surveillance, it is of concern that currently employed phototherapies result in significant suppression of NK cell function during treatment with some UV sources and for several weeks following treatment with all UV sources.

JWG was supported by a studentship from the Medical Research Council.

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