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Invited Review

Estimation of the effect of increasing UVB exposure on the human immune system and related resistance to infectious diseases and tumours

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Abstract

Exposure to UV light has, besides some beneficial effects (vitamin D production), many harmful effects on human health. UVB irradiation has been shown to suppress both systemic and local immune responses to a variety of antigens, including some microorganisms. However, it is still not known whether such immunomodulating effects may lead to an increase in the number and severity of certain tumours and/or infections in humans. We report herein the data provided by a project that was funded by the European Union (Programme Environment), and that was aimed at the estimation of the risk associated with increased UVB exposure due to ozone depletion regarding the deleterious effects on the immune system and related resistance to tumours and infections in humans. The data, obtained by the different research groups involved, were assembled and used to calculate for the first time a risk assessment for increased environmental exposure to UVB in human subjects.

Keywords: Ultraviolet light; Immunosuppression; Photocarcinogenesis; Infections; Urocanic acid; DNA damage

1. Introduction and aim of the study

Much attention has focused recently on the decrease in the upper atmosphere of the ozone layer which serves as a protective shield against ultraviolet (UV) light reaching the earth's surface. Exposure to UV light, especially UVB (280–315 nm), has many harmful effects on animal and human health. Two decades ago it was demonstrated that the induction of skin tumours by chronic UVB exposure was due to a combination of the genotoxic (mutagenic) effects together with a decrease in immune surveillance against the tumour cells. Since then, UVB irradiation has been shown to suppress both systemic and local immune responses to a variety of antigens, including several microorganisms. It is not known at the present time whether such effects may lead to an increase in the number of cases of tumours and/or the severity of infectious diseases in humans and such information is difficult to obtain except by large-scale retrospective epidemiological studies, made even more complex by the inability to quantitate past exposure to sunlight. However, our approach has been to consider a variety of systems in rodent models and then, from the data generated, to develop a mathematical model allowing an estimate to be made of the effect of increased environmental UVB exposure on immunity to one systemic bacterial infection in human subjects.

In the present review, these systems, which formed the basis of a recent EC-funded collaboration, are outlined. In the first place, the cutaneous chromophores for UV radiation, urocanic acid (UCA) and DNA, suggested as initiators of the changes leading to immunomodulation, were investigated. Secondly, the effects of UVB exposure on the skin and basal immune parameters in mice, rats and human subjects were evaluated, including studies of non-melanoma skin can-

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cers in mice and humans. Thirdly, several rodent models of infectious diseases, some using microorganisms that were skin associated and others that were systemic, were developed, and the effect of UV exposure on specific immune responses determined in each case. The data obtained from these approaches were then assembled and used to calculate for the first time a risk assessment for increased environmental exposure to UVB in human subjects.

Throughout the studies the output from the sources of broadband UVB radiation was measured with UVX radiometers fitted with a UVX31 detector (UVL Products Inc., UK). These had been calibrated centrally against spectroradiometers, thus allowing valid comparisons regarding UVB dosimetry to be made between the results obtained by one group and another.

2. Chromophores for UV-induced immunosuppression

2.1. Urocanic acid (UCA)

UCA (4-imidazoleacrylic acid) is formed in the upper layers of mammalian epidermis as the *trans*-isomer. On UV irradiation it converts to the *cis*-isomer in a dose-dependent manner until an equilibrium is reached when *cis*-UCA comprises about 30–70% of the total UCA. In 1983 De Fabo and Noonan [1] proposed that UCA could act as a major chromophore for UV radiation in the skin and so initiate a chain of events leading to immunosuppression. They constructed an action spectrum (wavelength dependence) for the inhibition of contact hypersensitivity (CH) in mice. This peaked at 270 nm with a 10-fold decreased effect at 315 nm, and closely matched the in vitro absorption spectrum of UCA. If the stratum corneum was stripped from the animals before UV exposure, no suppression in CH resulted. Since then many investigations have sought to substantiate the role of UCA in UV-induced immunosuppression and these were reviewed most recently by Norval et al. [2].

At a particular wavelength the degree of *trans*-to-*cis*-UCA photoisomerization is dependent on two factors: (a) the absorption of *trans*-UCA and (b) the quantum yield ($\phi_{t\rightarrow c}$ = probability of *trans*-to-*cis* isomerization occurring after the absorption of one photon of radiation). For many photochemical reactions, values are constant across different wavelengths. If this is the case, the action spectrum for the photochemical reaction is solely dependent on the absorption spectrum of the initial molecule. However, work by Morrison et al. [3] showed that the in vitro $\phi_{t\rightarrow c}$ for UCA was wavelength dependent with $\phi_{t\rightarrow c}$ at 270 nm being almost 10 times lower than at 313 nm. Thus, although the *trans*-UCA absorption spectrum peaks at 270 nm and tails off into the UVA II (320–340 nm), the quantum yields are increasing over the same spectral range. If $\phi_{t\rightarrow c}$ values are wavelength dependent, it is probable that the action spectrum for the reaction would not match the absorption spectrum of *trans*-UCA in vitro. To test this hypothesis, aqueous solutions (6 mM and 15 μM) of *trans*-UCA were irradiated with narrow half-bandwidth (3 nm) radiation from a monochromator, and *cis*-UCA production analysed using high-performance liquid chromatography (HPLC) [4,5]. The slopes of the UV dose–response curves at each waveband were used to construct an action spectrum for *cis*-UCA production. The resulting curves showed that this action spectrum was highly dependent on the initial *trans*-UCA concentration. The action spectrum at 6 mM peaked at 310–315 nm but was artificially biased towards higher wavelengths because of the opacity of the UCA solutions at 270–300 nm [4]. A more accurate estimation of the wavelength dependence for *cis*-UCA production was made at 15 μM and was found to peak at 280 nm, as shown in Fig. 1 [5].

To demonstrate the action spectrum for *trans*-to-*cis* photoisomerization in vivo, outbred Skh-1 hairless albino mice were anaesthetized and were irradiated with narrow half-bandwidth radiation. UCA was extracted from skin samples and isomers monitored by HPLC. Data analysis and construction of the action spectrum were as described for the in vitro studies above. The resulting action spectrum peaked at 310–315 nm (Fig. 1) [4].

Thus the action spectra for *cis*-UCA production in vitro (15 μM) and in mouse skin in vivo peak at 280 and 310–315 nm, respectively. Both of these spectra are red-shifted compared with the absorption peak of UCA at 268 nm. The original proposal that UCA might be a unique chromophore for UV suppression of CH was largely based on the similarity between the action spectrum for this effect and the absorption spectrum of *trans*-UCA and assumed that the absorption of *trans*-UCA also reflected the action spectrum for its photoisomerization [1]. Our data do not support this assumption (Fig. 1). The marked deviation of the in vivo action spectra

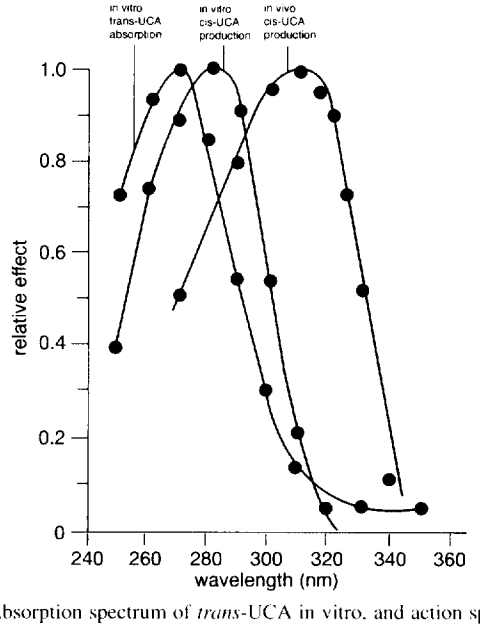

Fig. 1. Absorption spectrum of *trans*-UCA in vitro, and action spectra for production of *cis*-UCA (15 μg ml^{-1}) in vitro and in mouse skin in vivo. All spectra normalized at their own peaks.

for production of *cis*-UCA and the reported action spectra for inhibition of CH suggests that the proposal that UCA is a unique chromophore and mediator of UV suppression of CH may be an oversimplification. There is much evidence that *cis*-UCA has immunosuppressive properties that mimic the effects of UV radiation [2] and therefore UCA should probably still be considered as one of several chromophores that may contribute to the complex processes of UV immunosuppression.

In human subjects, our studies showed that UCA isomerization occurs in the epidermis after irradiation with either broadband (TL12) or narrowband (TL01) UVB phototherapy sources [6]. Exposure to the combination of psoralens and UVA radiation during PUVA photochemotherapy for psoriasis also resulted in UCA photoisomerization [7]. However, in vitro studies suggest that it is probably the very small amount of UVB radiation emitted by the so-called UVA fluorescent sources used in PUVA that is active in UCA isomerization rather than any 8-MOP photosensititization. As 8-MOP photosensitization is crucial to both the therapeutic and immunosuppressive effects of PUVA, it is highly doubtful that UCA has any role in these PUVA effects [8]. The action spectrum for UCA photoisomerization in human skin is yet to be reported. We have found that the peak of the absorption spectrum of UCA when in contact with isolated human stratum corneum shifts from 270 to about 290 nm [5] and there is an early indication from our in vivo studies that, like the mouse action spectrum described above, the human action spectrum may also be red-shifted compared to the *trans*-UCA absorption spectrum in vitro.

In Italian volunteers of skin type III to V, the total content of UCA and percentage of *cis*-UCA have been investigated in two different sites, one exposed (forearm) and one unexposed (buttocks) to natural sunlight throughout the year (C. De Simone and D. Cerimele, unpublished). The total UCA content decreased in June and then increased in September, to reach the maximum value in March, in both the photoexposed and non-photoexposed areas (Table 1). The percentage of *cis*-UCA was higher in the exposed body site than in the unexposed one at all the times investigated. In the exposed areas the content of *cis*-UCA had a minimum value in March, which increased in June, then decreased in September. The inter-subject variation in UCA content was high and this was confirmed in another study using a cohort of subjects in Scotland, in which the UCA isomer concentration at 10 body sites was analysed [9].

Up till now, most experimental systems to test the immunosuppressive effects of *cis*-UCA have been developed in the mouse. Comparative studies were therefore undertaken to examine its properties in rats, first by UCA analysis of skin samples, and secondly by measuring its effects in vivo (J. Garssen and H. Van Loveren, unpublished results). It was demonstrated that *trans*-UCA could be isomerized by a single exposure of rats to UVB radiation. Isomerization was greater in the ears than in the dorsal skin. The highest percentage of *cis*-UCA was found in the ears and was approximately 30–40% of the total amount of UCA, which is comparable to other species. The total UCA concentration was not affected by UVB exposure. Analysis of the dorsal skin from experiments in which rats were exposed daily to UVB radiation of between 0.125 and 0.5 minimum erythema dose (MED) for periods ranging from three to 42 days demonstrated a low percentage (i.e., $< 10\%$) of *cis*-UCA. Thus there is the possibility that some kind of adaptation may occur with respect to UCA isomerization as a result of frequent UV irradiation, although this suggestion is not substantiated by the analysis noted above of UCA isomers in human subjects exposed to natural sunlight during the summer months [9].

Rats were injected subcutaneously with *trans*- or *cis*-UCA (50–200 μg) three times a week for a period of four weeks. Subsequently they were examined for changes in various immune parameters. Their body weight and the weight of lymphoid organs were unchanged. In the thymus an increase in cortical apoptosis was demonstrated in the *cis*-UCA-injected groups compared with the control groups. The in vitro proliferation of splenocytes induced by the mitogens concanavalin A (Con A), phytohaemagglutinin (PHA) and pokeweed mitogen was not affected by the *cis*- or *trans*-UCA treatment. In addition, the mixed lymphocyte reaction (MLR) was not altered. Thus no evidence was obtained to indicate that *cis*-UCA administered subcutaneously modulates these particular immune responses in the rat.

However, in contrast to these results, *cis*-UCA had significant activity in a model of *Trichinella spiralis* infection in the rat (J. Garssen and H. Van Loveren, unpublished). As described in Section 6.2 below, UV exposure lowers resistance to oral infection with this worm. Similarly, if rats were injected subcutaneously with *cis*-UCA three times a week for four weeks starting one week before oral *T. spiralis* infection, the delayed-type hypersensitivity (DTH) to *Trichinella* antigen was significantly impaired (Fig. 2). *Trans*-UCA did not have this effect. In addition, the number of *T. spiralis* worms

Table 1
Urocanic acid (UCA) content and percentage as *cis*-UCA in the skin of healthy volunteers ($n = 15$) throughout the year

	Photoexposed site		Non-photoexposed site	
	UCA (nM/cm^2) (±S.D.)	% *cis*-UCA (±S.D.)	UCA (nM/cm^2) (±S.D.)	% *cis*-UCA (±S.D.)
March	7.11 ± 2.74	13.51 ± 25.15	9.68 ± 6.60	0.40 ± 0.57
June	4.81 ± 1.88	41.05 ± 22.17	4.84 ± 2.72	15.90 ± 13.40
September	6.30 ± 2.15	22.10 ± 10.97	6.12 ± 3.50	11.57 ± 9.35

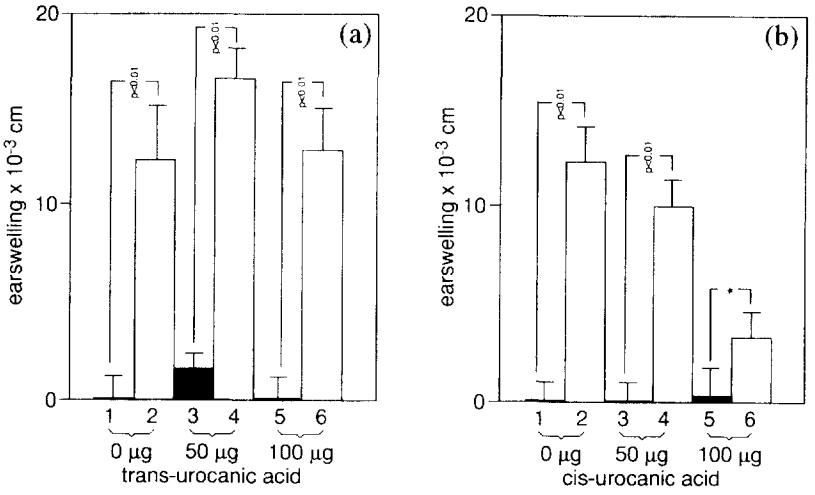

Fig. 2. Effect of treatment of rats with UCA isomers (three times a week for four weeks starting one week prior to infection) on delayed-type hypersensitivity response to *T. spiralis* antigen 17 days after infection (black bars, uninfected animals (1,3,5); open bars, infected animals (2,4,6); * not significant).

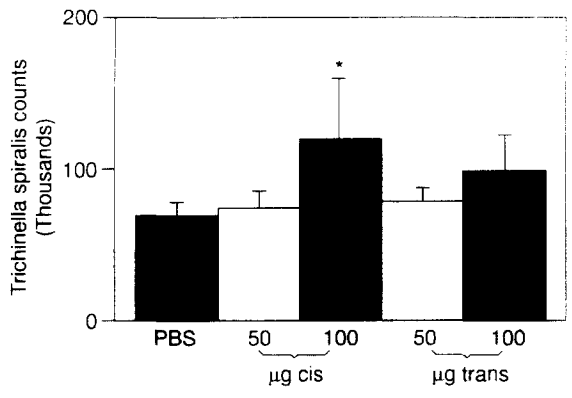

Fig. 3. Effect of treatment of rats with UCA isomers (three times a week for four weeks starting one week prior to infection) on *Trichinella spiralis* counts in cascasses 45 days after oral infection (* $p<0.05$ compared to control group).

isolated from the carcasses 45 days after infection was significantly increased in the *cis*-UCA-treated animals only (Fig. 3). This is a particularly interesting finding, as *T. spiralis* induces a systemic infection, the larvae spreading from the gut to muscle tissues, and is not skin associated. The cellular immune response is strictly T-cell dependent. Thus *cis*-UCA, formed by photoisomerization in the epidermis, appears to be capable of initiating a cascade of responses which then lead to the suppression of specific systemic immunity.

2.2. DNA

UV radiation is absorbed in the skin, especially in the epidermis. After exposure, the number of major histocompatibility complex (MHC) class II$^+$, ATPase$^+$ Langerhans cells and Thy-1$^+$ dendritic epidermal cells (DETC) in the epidermis of rodents decreases. Whether this decrease is due to migration of these cells or to loss of membrane markers is not clear. To address this question, the monoclonal antibody H3 [10] directed against cyclobutyl thymine dimers — a form of DNA damage that is specifically induced by UV radiation — was used to investigate whether H3$^+$ cells are present in the draining lymph nodes of the skin following UV exposure of hairless, inbred mice (HRA/Skh) [11]. After a single dose of UV irradiation of mice, H3$^+$ cells were detected in the paracortex of the draining lymph nodes. No positive cells were found in the blood of UV-exposed mice. These results suggest that H3$^+$ cells in the lymph nodes originate from the skin. The number of H3$^+$ cells in the draining lymph nodes increased in the first 24 h after irradiation and then stabilized. Immunohistochemical double staining revealed that all H3$^+$ cells (i.e., cells with thymine dimers) were MHC class II$^+$, and that only a fraction of the cells were NLDC-145 positive, i.e., cells with a dendritic morphology. No Vγ3 T-cell-receptor-bearing cells, i.e., cells that might be similar to dendritic epidermal T cells, could be found in the lymph nodes after UV irradiation of the skin.

From the above and other published work (for example, Refs. [12–14]) it is clear that both DNA damage and UCA isomerization are involved in UVB-induced immunosuppression. It is possible that UCA isomers may interact with DNA or bind to DNA or that both photoreceptors may act independently. We have not found any indication of binding of *cis*-UCA to DNA. The relative importance of both photoreceptors may depend on the wavelength and dose of UV radiation, the antigen involved and its route of administration, in addition to the species studied.

3. Primary skin effects induced by UV exposure

3.1. Effects in murine skin

Although broadband UVB irradiation has been shown to induce selective immunosuppression in a variety of experi-

mental systems, the wavelength dependence of the immunomodulation and the primary events in the skin remain unclear. In one study [15] three UV lamps were used at suberythemal doses to determine their relative local effect in the skin of C3H/HeN mice. The lamps were a conventional broadband UVB source (270–350 nm, Philips TL12), a narrowband UVB source (311–312 nm, Philips TL01) and a UVA source (320–400 nm). Their effect on the photoisomerization of *trans*-to-*cis*-UCA, on the density of Langerhans cells and on the ability of epidermal cells to stimulate autologous lymphocytes in the mixed skin lymphocyte reaction (MSLR) was ascertained. Broadband UVB irradiation was more efficient than narrowband UVB at reducing the density and function of Langerhans cells, while UVA irradiation was least effective. These changes were most pronounced immediately following irradiation, were dose dependent and were only detected in UV-exposed areas of the skin. There was a close correlation between the UV-induced reduction in Langerhans cell density and the formation of *cis*-UCA in the epidermis. Such a correlation was not detected between the reduction in the MSLR response following UV irradiation in vivo and *cis*-UCA formation. Thus *cis*-UCA plays a role as a photoreceptor and mediator of some, but not all, of the local effects of UV on the cutaneous immune system.

In a second study, the effects of chronic and acute irradiation with the narrowband UVB (TL01) lamp and broadband UVB (TL12) lamp on various parameters in the skin of mice were compared [16]. C3H mice were irradiated three times a week for up to six weeks with either 500 or 1000 J m^{-2} broadband UVB, or 3000 J m^{-2} narrowband UVB. Each dose was suberythemal. The number of Langerhans cells in the epidermis was reduced by over 50% after two weeks of irradiation with the TL12 source and by 20% following TL01 irradiation. Continued irradiation for up to six weeks resulted in no further decrease in Langerhans cell numbers in the case of the TL12 source but a steady decline to 40% in the case of the TL01 source. Sunburn cells were detected following irradiation with both sources, but the numbers were very low in comparison with acute exposure. TL12 exposure resulted in a doubling of the thickness of the epidermis throughout the six weeks of irradiation, while TL01 exposure did not alter epidermal thickness. The epidermal concentration of *cis*-UCA started to decline to unirradiated values after four weeks of TL01 exposure, despite continued irradiation. As observed following a single exposure, the CH response was significantly reduced after six weeks of UVB irradiation but was not affected by TL01 exposure, indicating no correlation between *cis*-UCA levels and CH responses. Total serum immunoglobulin levels remained unchanged throughout the six weeks of TL12 or TL01 irradiation, but IgE titres significantly increased in all cases in the first two weeks of irradiation, indicating a possible shift to a T helper 2 (Th2) cytokine profile. IgE levels started to return to normal at later times. Thus chronic broadband UVB exposure induces a number of cutaneous and systemic responses which are likely to be dose dependent, while chronic TL01 exposure induces only some of these responses.

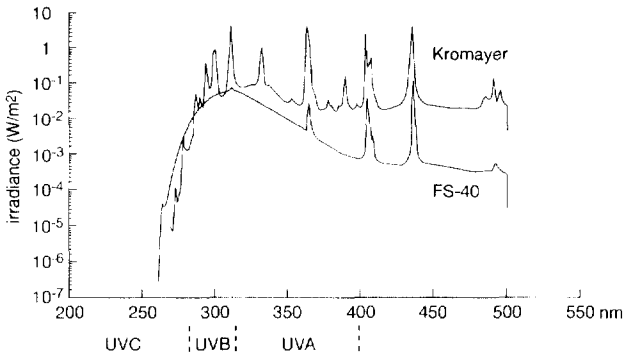

Fig. 4. Emission spectra for the FS40 and the Kromayer lamps.

3.2. Comparison of effects in the skin of different species

Rats and mice were exposed to UVB radiation daily for five consecutive days using two different broadband UVB sources, the Kromayer (on four small areas of the shaved flank) and the FS40 (on the shaved back) lamps [17]. The emission spectra of these lamps are given in Fig. 4.

Human volunteers were similarly exposed, but to the Kromayer lamp only, on discrete areas of their backs. The advantage of using the Kromayer (handheld) lamp is that it is very easy to expose volunteers locally (e.g., 2 cm^2 exposure spots). After irradiation, acanthosis, hyperkeratosis, inflammation and sunburn cells were observed in a dose-dependent fashion in the three species tested. The number of MHC class II^+ cells was lower in the epidermis due to a depletion of Langerhans cells. The inflammatory response in all three species was characterized by infiltration of MHC class II^+ monocytes/macrophages and T cells. The UVB-induced influx of MHC class II^+ macrophages in the dermis, and sometimes the epidermis, was positively correlated with the increased expression of intercellular adhesion molecule-1 (ICAM-1) and lymphocyte-function-associated antigen-1 (LFA-1) in these layers. The interspecies variation for the in vivo UVB-induced effects in the skin was modest. There was a relatively large variation between different human volunteers (intraspecies) for the UVB-induced effects.

4. UVB and immune functions

The effects of UVB on several different specific and non-specific immune parameters were investigated in mice, rats and human subjects. Comparisons could then be made between the responses in the different species to determine whether the data obtained in rodent models were applicable to humans.

4.1. Effects on basal immune functions in mice

Both CH and DTH responses are suppressed by UV irradiation prior to exposure to the antigen. Two types of antigen-

specific T cells are needed for the elicitation of CH [18]. They act in an obligate sequence to mediate the early initiating and late effector phases of CH, which are accompanied by skin-swelling responses at 2 and 24 h after challenge, respectively. The magnitude of the late ear swelling depends on that of the early swelling.

The influence of UV radiation on both phases of CH to picryl chloride was studied [19]. Mice were exposed to suberythemal doses on the shaved backs for four consecutive days and were sensitized on non-irradiated skin four days later. Four days after that, they were challenged on the ears, and swelling was measured 2, 4 and 24 h after challenge. Both the early and late phases of CH were significantly suppressed in UV-irradiated, sensitized mice (Fig. 5(a)). Transfer of immune lymphoid cells from donor mice that were sensitized four days previously induced the early and late components of CH in naive recipients after challenge. Transfer of immune lymphoid cells from donors that were sensitized one day previously induced the early component of CH only. UV irradiation of donor mice significantly reduced the capacity of the immune lymphoid cells to induce both phases of CH.

DTH responses in mice can also be suppressed by UVB exposure. This was shown in a murine model of HSV infection [20], and, as for CH, the suppression could be transferred with splenic T cells to recipient mice already infected with HSV. The early swelling response on elicitation of DTH to HSV is at 1 h after challenge. UV irradiation prior to primary infection with the virus suppressed both the early and the late response at 24 h (Fig. 5(b)). Additionally, it was found that epicutaneous treatment of mice with *cis*-UCA before HSV infection suppressed both phases of the DTH response [21].

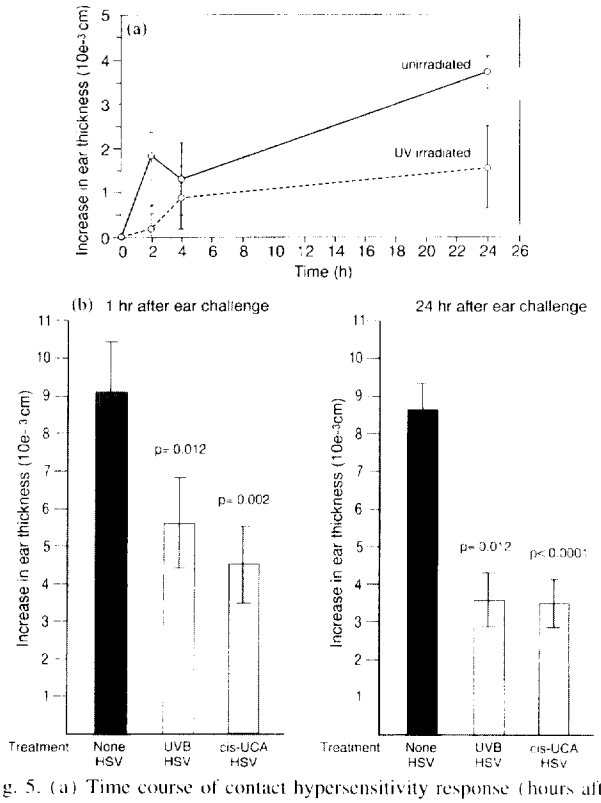

Fig. 5. (a) Time course of contact hypersensitivity response (hours after ear challenge) to picryl chloride in UV-exposed animals (UV) and non-exposed animals (* $p<0.05$) (from Ref. [19] with permission from *J. Invest. Derm.*). (b) Delayed-type hypersensitivity response to herpes simplex virus in UV-irradiated, *cis*-UCA-treated and control mice.

4.2. Effects on basal immune functions in rats

After exposure of rats to suberythemal doses of UVB radiation (FS40) daily for seven days, ranging from 0.125 to 0.5 MED, there was dose-dependent impairment of basal immune functions, such as the MLR and natural killer (NK) cell activity of splenocytes [22]. In contrast, lymphoproliferative responses to the mitogens Con A, PHA and pokeweed mitogen were enhanced. Immunoglobulin titres were not altered by the irradiation, except for a significant decrease in IgE serum titre after three days of UVB exposure. Analysis of subsets of lymphocytes in the spleen, using flow cytometry, indicated that the suppression of NK and MLR activity was not due to a change in the number of T lymphocytes or to a change in $CD4^+/CD8^+$ ratio in the spleen. After more prolonged exposure to UVB radiation, the activity of lymphocytes, as tested in NK and MLR assays, returned to control levels, indicating some form of protective adaptation, such as epidermal thickening or pigmentation.

4.3. Effects on basal immune functions in man

Several investigations of immune function in patients with psoriasis undergoing phototherapy or chemophototherapy, and in normal subjects undergoing phototherapy were undertaken. Initially, psoriatics were shown to have percentages of subsets of circulating peripheral blood mononuclear cells (PBMC), complement components and immunoglobulin isotypes within normal ranges and their NK cell activity was also normal. The epidermis contained approximately three times the quantity of total UCA per unit area of normal subjects, perhaps reflecting the abnormal thickening that occurs in psoriasis, even in uninvolved skin.

In the first study [7], samples were taken from psoriatic patients before starting treatment (broadband UVB, PUVA or coal tar), after four weeks of therapy and four weeks after completing it, and from normal subjects following broadband UVB. In vitro lymphoproliferative responses to the mitogen Con A and to HSV were unchanged by any of the treatments, as were the percentage subsets of PBMC. The functional ability of epidermal cells to present HSV was suppressed during UVB irradiation, while that of the adherent cells from the peripheral blood remained unaltered by the therapy. During the UVB irradiation, the percentage of *cis*-UCA rose in both the epidermis and the suction blister fluid of all subjects and it remained elevated in the suction blister fluid for several weeks after therapy had terminated. Whether any of these changes in immune parameters contribute to the success of the phototherapy remains uncertain.

In the second study [23], modulation in NK cell activity in psoriasis patients undergoing broadband UVB, narrowband UVB (311–313 nm, TL01 source) and PUVA regimens was examined. A comparison was made with psoriatics treated with coal tar and normal subjects receiving broadband UVB. The group treated with coal tar showed unchanged NK cell activity throughout therapy. In contrast, most subjects undergoing phototherapy exhibited depressed NK cell activity during or after irradiation, with the timing of the suppression varying between the lamps used, a factor which may be related to dose. The mechanism of UV-induced suppression of NK activity is uncertain but evidence was obtained to indicate that *cis*-UCA induced a dose-dependent suppression of NK cell activity when added to cultures of PBMC in vitro. *Trans*-UCA had hardly any effect. Thus it was concluded that *cis*-UCA formed in the epidermis as a result of UVR may modulate NK cell activity to account for the suppression that occurs during phototherapy [23].

The third and most recent approach has been to concentrate on changes in several immune parameters at the initiation of phototherapy [6]. The main reason for undertaking this study was to compare the results in human subjects with those obtained in rats exposed daily to UVB where seven days of irradiation inhibited NK and MLR responses and enhanced lymphoproliferative stimulation by mitogens, but these activities returned to control values after further exposures (see Section 4.2 above). The responses of patients with chronic plaque psoriasis were therefore compared immediately before starting therapy and after one week of broadband UVB, narrowband UVB or PUVA therapy. Broadband UVB and PUVA had no effect on NK cell activity, but a reduction was found in the group receiving TL01. In vitro lymphoproliferative responses to mitogens and HSV did not alter with therapy, except there was a significant increase in some mitogen responses in the TL01 group. Generally no change in overall percentage of subsets of circulating mononuclear cells was found in any group. The higher dose and particular wavebands emitted by the TL01 source may be inducing significant modulation in several immune parameters very early in phototherapy, which may contribute to the success of treatment with this lamp.

An extension of these studies aimed to establish whether, as a result of UVB therapy, Th1 responses were suppressed with enhancement of Th2 responses, as has been reported in several in vitro systems [24,25] and in mice [26]. Patients with psoriasis undergoing broadband UVB or TL01 treatment were monitored before and after one to four weeks of therapy [27]. First, plasma IgE and IgG subclasses were assayed and no significant change occurred. Secondly, the PBMC of the patients was stimulated in vitro for 48 hours with PHA; proliferation was measured by the incorporation of tritiated thymidine and the supernatants assayed for the presence of various cytokines characteristic of Th1 (IFN-γ, IL-2) and Th2 (IL-10) activities. Lymphoproliferation was not consistently affected by four weeks of broadband UVB therapy and there was no change in the production of IL-2, IL-10 or IFN-γ. In contrast, four weeks of TL01 therapy significantly suppressed lymphoproliferative responses. In addition, the production of IL-2, IL-10 and IFN-γ was lowered after one week of TL01 therapy and this was even more apparent after the treatment had extended to four weeks. Thus, although no evidence was obtained to indicate that broadband UVB therapy affected Th subsets in psoriasis, TL01 therapy inhibited the activity of both Th1 and Th2 subsets, while not altering plasma antibody concentrations.

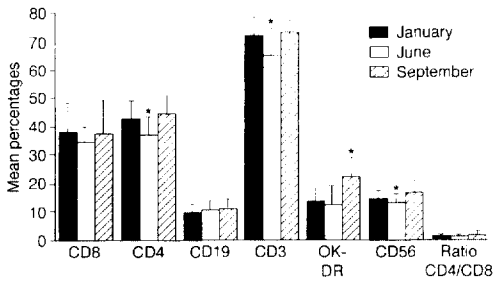

Fig. 6. Lymphocyte subsets in 15 healthy volunteers at different times of the year (* $p < 0.05$ compared to January).

Modifications in PBMC have been reported after acute exposure of human subjects to UV [28,29]. Further investigations were carried out to monitor circulating PBMC subsets in healthy Italian volunteers, skin type III to V, at three different times in the year: January (winter), June (beginning of summer) and September (end of summer) and the results are shown in Fig. 6 (C. De Simone and D. Cerimele, unpublished results). In June a significant decrease in the percentages of $CD3^+$ and $CD4^+$ was observed; these values returned to normal in September. In September $HLA\text{-}DR^+$ and NK ($CD56^+$) cells increased significantly to return to normal values in January. It is not known if UCA isomerization may play a role in inducing these changes (see Section 2.1 above). In addition, the effect of acute exposure to the sun was evaluated in 13 healthy Italian volunteers, skin type III to V, by comparing percentages of CD3 (T cells), CD4 (T helper cells), CD8 (T cytotoxic/suppressor cells), CD19 (B cells), CD25 (IL-2 receptor), CD56 (NK cells) and DR-positive cells (human MHC class II positive cells) before, 12 h after, and seven days after a one hour (1–2 p.m.) sun exposure in Rome in June. A transient increase in DR^+ cells was found 12 h after exposure ($p < 0.01$). $CD56^+$ cells decreased immediately after sun exposure and were still low after seven days ($p < 0.05$). Total UCA did not change in either exposed or non-exposed body sites; however, the percentage of *cis*-UCA increased at 12 h in both exposed and non-exposed sites, with decreasing values at seven days although still higher than the pre-exposure amounts.

Flow cytometric assays were also carried out on PBMC in patients with multiple basal cell carcinomas (MBCC), together with an analysis of their epidermal UCA content, and these results are described in Section 5.2 below.

4.4. Comparison of susceptibility to UVB between rodents and man

In the first place, lymphocytes from blood and/or spleen of rats, mice and human subjects were exposed to different doses of UV in vitro. The functional activity of these lymphocytes was determined using assays for mitogen proliferation and the MLR. These experiments demonstrated that in vitro irradiation caused a dose-dependent decrease of the MLR activity [30]. The viability of lymphocytes and proliferative responses to mitogens were also decreased by exposure, but were less severely affected than the MLR. The lymphocytes of rats were more sensitive to the effects of UV than lymphocytes of mice and humans. However, the interspecies variation was limited.

Secondly, the UVB-induced morphological and functional changes in the skin of mice, rats and humans were investigated [17]. The morphological studies concentrated on Langerhans cells using confocal laser scanning microscopy. Changes were found in mouse and rat skin after in situ exposure of pieces of skin to high doses of broadband UVB radiation (3–9 kJ m^{-2}). Similar UVB doses failed to induce alterations in the morphological structure of human Langerhans cells.

Thirdly, alterations in the function of epidermal cells as antigen-presenting cells were monitored, using the MSLR (Fig. 7). In vitro UVB exposure of epidermal cells, derived from the skin of different species, revealed that low doses impaired the alloreactivity of these cells dose dependently; mouse epidermal cells were most UVB susceptible, while human cells were least UVB susceptible [31]. For suppression of the alloreactive capacity of epidermal cells after in situ UVB exposure of pieces of skin tissue, higher doses of UVB radiation were needed in all species tested; again mouse epidermal cells were most UVB susceptible, and human epidermal cells were least UVB susceptible. Differences in susceptibility for UVB-induced changes in the alloreactive capacity of epidermal cells were similar after in situ and after in vitro exposure experiments. It was concluded that epidermal cells, especially Langerhans cells, from the skin of rodents are more susceptible to UVB-induced deleterious effects than epidermal cells derived from human skin.

5. UVB-induced skin tumours

5.1. In mice

A failure in cellular immunity is known to play an important role in the formation of skin carcinomas induced by chronic UVB irradiation. An investigation was therefore carried out into the changes in subpopulations of lymphoid cells in the epidermis during the course of exposure. MHC class II cells became bigger by rounding up, and their number

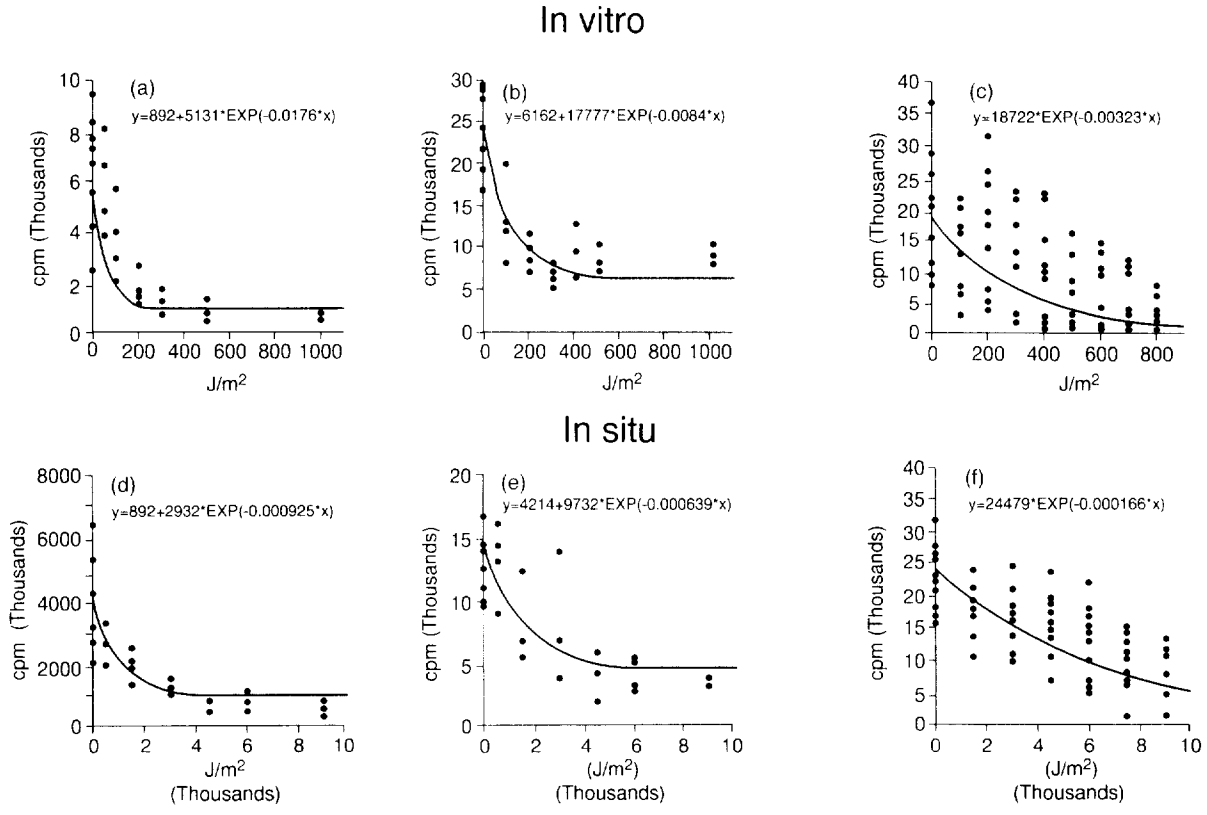

Fig. 7. MSLR responses after in vitro (a, mice; b, rats; c, humans) and in situ (d, mice; e, rats; f, humans) exposure to UVB. Non-linear regression curves are fitted in all graphs, demonstrating the mathematical relationship between exposure and response (from Ref. [31] with permission from *Photochemistry and Photobiology*).

increased. Thy-1$^+$ cells also increased in size in the first week, and then returned to normal. The Thy-1$^+$ cells were initially dominated by Vγ3$^-$ CD4$^-$ CD8$^-$ cells, which disappeared after the first week and reappeared at week seven just before the tumour became apparent. An influx of CD4$^-$ and CD8$^+$ Thy-1$^+$ cells started in the first week, and the number of CD8$^+$ cells peaked around seven weeks. Thus the UV irradiation caused considerable changes in lymphoid cell subpopulations in the epidermis; most of these alterations preceded the appearance of tumours and some, like the influx of Thy-1$^+$ Vγ3$^-$ CD4$^-$ CD8$^-$ cells, coincided with the onset of tumour growth.

To gain more insight into the changes in the immune system which may be responsible for the failure to reject the tumour cells, alterations in lymphoid cell subpopulations in the spleen and skin draining lymph nodes were compared with those occurring in the epidermis during chronic UVB irradiation [32]. In both spleen and lymph nodes, the total number of cells increased and all subpopulations investigated increased. The increase in the lymph nodes occurred early, at the onset of the exposures, while the increase in the spleen occurred much later, i.e., at the time the skin tumours (diameter > 1 mm), induced by the radiation, were observed. In lymph nodes there was a percentage increase in MHC class II$^+$ cells and a decrease in CD3$^+$ cells. The latter was caused by a percentage decrease of CD8$^+$ cells, while the percentage of CD4$^+$ cells remained constant. In the spleen there was a slight percentage decrease of MHC class II$^+$ cells and almost no change in CD3$^-$ cells. Within the CD3$^+$ cell population there was again a percentage decrease of CD8$^+$ cells, while the percentage of CD4$^+$ cells remained constant. Therefore, the ratio MHC class II:CD3 increased in the lymph nodes and decreased very slightly in the spleen, while the CD4$^+$:CD8$^+$ ratios increased in the lymph nodes as well as in the spleen. All the observed shifts in subpopulations, in the lymph nodes as well as in the spleen, were already present before the tumours started to appear. The decrease in CD8$^+$ numbers in the spleen and lymph nodes, with consequent increase in the epidermis, may indicate a migration of this cell subset to the skin as a result of chronic UV exposure.

To understand further the role of the immune system in the UVB-induced systemic suppression of host resistance to skin cancer, the total period of daily exposure to UVB radiation that was necessary to render hairless (HRA/Skh) mice incapable of rejecting highly antigenic implants of an UVB-induced syngeneic squamous cell carcinoma cell line was investigated [33]. Two groups of mice were used, each chronically exposed to different daily UV doses. The median tumour induction time for 1 mm tumours, which had already been established in a closely related outbred strain (Skh-HR1), was verified for the mice used in the study. At different time points in the course of daily exposure, the mice were injected intradermally at unirradiated sites with tumour cells, and were not irradiated further. In both groups tested, tumour acceptance occurred considerably before the induction of primary tumours. There was no relationship between the previously established time course of changes in lymphoid cell subpopulations in the spleen and the skin draining lymph nodes, outlined above, and the time the tumour transplants were accepted. However, the disappearance of Vγ3 DETC from the irradiated epidermis after two weeks of exposure appeared to correlate with the time of transplant acceptance.

5.2. *In human subjects*

Prolonged exposure to solar radiation in sunny areas of southern Europe is a significant risk factor for the development of skin cancer. Individuals with multiple skin carcinomas may be considered a susceptible population for the development of further skin cancers. For this reason a group of 46 patients affected by MBCC (two to eight per subject) living in central Italy has been investigated to test the possible relationship between genetic and immunological factors in the pathogenesis of UVB-induced skin carcinomas (D. Cerimele, unpublished results). UCA analysis was performed using samples collected from a sun-exposed and an unexposed skin site. In addition, PBMC subsets were examined by flow cytometry. Two sets of controls were used: the first consisted of 50 healthy blood donors living in the same geographical area (central and southern Italy), and the second was healthy blood donors living in Spain, a geographically equivalent area.

It was found that the total amount of epidermal UCA and the percentage of *cis*-UCA were significantly higher in the patients compared with the healthy controls, in both exposed and unexposed sites. In addition, the percentage of CD8$^+$ cells in the peripheral blood was significantly higher than in the controls, while the percentages of CD3, CD4 and CD19 did not differ between the patients and the controls. The increased numbers of CD8$^+$ cells in the blood may represent the migration of this cell population from the lymph nodes and spleen to the skin, a suggestion made in Section 5.1 above for events occuring during UV-induced carcinogenesis in the mouse.

30 patients, living in central Italy and affected by MBCC, and 220 healthy age-matched controls were typed for 72 HLA-A, -B, -C, and MHC Class II antigens (16 HLA-A, 34 HLA-B, 7 HLA-Cw, 12 HLA-DR and 3 HLA-DQ) (D. Cerimele, unpublished results). HLA typing was performed by a standard two-stage microtoxicity test using enriched B-cell suspensions. The results obtained with the class I antigens are shown in Table 2. No significant modifications were found in the HLA-A antigens. With regard to B antigens, B17, which is present in 23.6% of normal controls, decreased to 6.6% in the patients with MBCC. Amongst the subsets of B17, the frequency of B58 decreased from 21 to 6.6% for the MBCC, while the values of B57 remained unaffected. The frequency of Cw3, which was 7.3% in the controls, was increased to 23.3% for the patients. For Class II antigens, the frequency of DR-1, which was 18.1% in the controls, was significantly increased to 40% for MBCC.

Table 2
HLA antigen frequencies (%) in 30 patients with multiple basal cell carcinomas (MBCC) and 220 controls

	MBCC	Controls	p value	Relative risk
B17	6.6	23.6	n.s.	0.23
B57	0.0	1.8	n.s.	
B58	6.6	21	0.024	
Cw3	23.3	7.3	0.004	3.88
DR1	40.0	18.1	0.005	3.00

These results show a negative association of B17 (mostly B58) and a positive association of Cw3 and DR-1 with MBCC. It is interesting to note that B58 shows higher frequencies in negroid populations which are relatively resistant to skin cancer. In addition, the frequency of B58 is lower in northern European populations, which are more susceptible to sunlight-induced cutaneous carcinomas. B17 is an antigen commonly associated with psoriasis, a disease which has a negative correlation with skin cancer. An increased frequency of DR-1 antigen has been observed in xeroderma pigmentosum, where a deficiency in DNA repair mechanisms is highly associated with sunlight-induced skin cancers. Thus no direct correlation between immunological and genetic findings was established in this study. It is likely that more than one system of defence against the development of skin tumours could be operating in people exposed to solar irradiation: (1) genetic factors linked to HLA-B17 antigen, and (2) immunological factors linked to an increase of $CD8^+$ cells.

6. UVB and infectious diseases

6.1. Skin-associated infections (herpes simplex virus in mice and rats)

UVB irradiation is known to have diverse effects on microbial infections and those occurring in the case of viruses are reviewed in Ref. [34]. One of the few instances where UV is recognized to affect clinical symptoms in human subjects is orolabial herpes simplex virus (HSV) infections, when sudden exposure to sunlight can lead to reactivation of latent virus and recrudescence of cold sores.

Previous studies have indicated that suberythemal UVB irradiation of C3H mice before primary infection with HSV type 1 does not result in increased morbidity or mortality, but a suppressed DTH to the virus can be demonstrated (reviewed in Ref. [35]). Any effect of UV radiation on pathogenesis during secondary epidermal HSV infection has not been previously examined. Mice were immunized by subcutaneous injection of inactivated HSV and, five days later, one group was UVB irradiated. The next day all mice were challenged epidermally with HSV. Most of the mice (92%) in the irradiated group developed severe lesions, whilst 59% of the non-irradiated group had mild lesions and 30% no lesions [36]. After challenge in either group, infectious virus was not isolated from the adrenal glands, a preferential site for viral replication. In addition, the DTH to the virus was not affected by the UV exposure. The numbers of lymphocytes and dendritic cells in the lymph nodes draining the site of epidermal infection were increased in the UV group compared with the non-irradiated group. Following challenge, the percentage of $CD4^+$ and $CD8^+$ lymphocytes in lymph nodes was unaltered but the MHC Class II expression on dendritic cells in these lymph nodes was reduced by UV exposure. The in vitro lymphoproliferative response of lymph node cells revealed a suppressed response to HSV and to the mitogen, Con A, in the irradiated group. Thus UV irradiation prior to epidermal secondary infection with HSV led to more severe infections due, perhaps, to a modulation in local antigen presentation.

In the case of rats, UVB exposure before epicutaneous infection with HSV type 1 led to decreased cellular immune responses to the virus as measured by lymphocyte stimulation and DTH tests [17]. Irradiation after infection did not affect cellular immunity to HSV-1, but increased the number of HSV-1 specific skin lesions. Thus the timing of irradiation with respect to infection is important for the induction of immunosuppression and skin lesions.

6.2. Non-skin-associated infections (cytomegalovirus, Listeria monocytogenes and Trichinella spiralis in rats)

Rats exposed to suberythemal doses of UVB radiation using the Kromayer lamp before or after intraperitoneal infection with rat cytomegalovirus (RCMV) had increased virus titres in the salivary glands, a preferential site for viral replication. This was particularly marked 26 days after infection [37].

In the case of *Listeria monocytogenes*, non-specific phagocytotic and killing responses, as well as specific T-cell-mediated responses, are important in resistance. A rat infection model with this pathogen was used to analyse the immunosuppressive activity of UVB exposure [38]. Rats were irradiated with suberythemal doses of UVB for five or seven consecutive days, using the Kromayer or FS40 lamps, respectively. Subsequently, the rats were infected subcutaneously or intravenously with viable *Listeria*. UVB exposure resulted in an increased number of bacteria in the spleen four days after infection. Specific lymphocyte proliferation assays as well as DTH responses demonstrated that T-cell-mediated immunity to *Listeria* was impaired by UVB when measured four and eight days after infection. Additionally, UVB exposure decreased the phagocytotic activity of macrophages. This study demonstrates that suberythemal doses of UVB radiation cause a delay in the clearance of *Listeria* from the spleen of rats, and that this is probably due to impaired non-specific phagocytosis of the bacteria by macrophages and impaired activity of *Listeria*-specific T cells.

Other rats were orally infected with *T. spiralis* larvae and were exposed to suberythemal doses of UVB radiation daily for five days (Kromayer lamp) or seven days (FS40 lamps)

Table 3
Predicted effects of ozone reduction on the biologically effective irradiance for immune suppression (at noon under clear skies) (RAF = radiation amplification factor). Parts of this Table are from De Fabo et al. [40] and Garssen et al. [31] with permission from *Photochemistry and Photobiology*

Latitude	Ozone (dobson units)	% Decrease in ozone	Biologically effective irradiance (BEI) (W m^{-2})	% Increase in BEI	RAF	Calculated time (min.) for 50% immunosuppression
60°N January	368.5	0	0.0087	0.0		3349
	350.1	5	0.0091	4.2	0.84	3201
	331.7	10	0.0095	8.7	0.87	3066
	294.8	20	0.0103	18.8	0.94	2828
60°S January	333.8	0	0.174	0.0		167
	317.1	5	0.179	2.7	0.54	163
	300.4	10	0.184	5.6	0.56	158
	267.0	20	0.196	12.1	0.61	149
40°N January	335.6	0	0.073	0.0		399
	318.8	5	0.075	3.0	0.60	389
	302.0	10	0.078	6.3	0.63	373
	268.5	20	0.083	13.5	0.68	351
40°N July	307.9	0	0.278	0.0		104
	292.5	5	0.285	2.5	0.50	102
	277.1	10	0.292	5.3	0.53	100
	246.3	20	0.310	11.5	0.58	94
20°S January	262.6	0	0.332	0.0		88
	249.5	5	0.341	2.5	0.50	85
	236.3	10	0.350	5.3	0.53	84
	210.1	20	0.370	11.4	0.57	78
20°S July	262.1	0	0.196	0.0		149
	249.0	5	0.201	2.6	0.52	145
	235.9	10	0.206	5.4	0.54	142
	209.7	20	0.218	11.7	0.59	134

for different periods before or after infection [39]. A significant increase in the number of *Trichinella* larvae was found in the carcasses of rats that were UVB irradiated in the second week after infection. This is the time at which larvae are spreading from the gut to the muscle tissue. An increase in the number of larvae was also detected histologically in the tongue in the same groups of rats. In addition, the lymphoproliferative response to *T. spiralis* was reduced. Thus exposure to UVB radiation has suppressed the resistance to this parasitic infection. In contrast, there was no correlation between UVB-induced suppression of the resistance to these worms and altered levels of *Trichinella*-specific IgM, IgG and IgE antibodies [38].

Therefore in each of the three above examples ranging from worms to viruses, all of which cause systemic infections, UVB irradiation lowers specific cell-mediated immune responses to the pathogen, resulting in increased numbers of microorganisms.

7. An example of risk evaluation with respect to increased exposure to UVB

Risk assessment comprises four steps: hazard identification, dose–response assessment, exposure assessment and risk characterization. In this study, the effects of UVB radiation on basal immune functions and the immunological resistance to infectious diseases and tumours in rodents were analysed as part of this strategy [31]. In a parallelogram approach, non-threshold mathematical methods were used in order to estimate the risk for the human population after increased exposure to UVB radiation as, for ethical reasons, it is not possible to investigate directly the effects of UV light on the resistance to infections and tumours in man. Thus data obtained from animal studies need to be extrapolated to the human situation. It was decided to take uncertainty (safety) factors into account for inter- and intraspecies variation. In general, these factors are arbitrarily chosen to be 10, but in the present project were calculated using experiments in which human volunteers and rodents or cells from these species were exposed to UV light, using exactly the same UV source. Dose–response studies indicated that, for the interspecies variation, a factor of three to four was sufficient instead of a factor of 10. Variation between individuals within one species (humans) was calculated as a factor of 0.5 [31].

Using a worst-case strategy, it was estimated, using biologically effective doses of sunlight as calculated by De Fabo et al. [40] in 1990, that exposure for approximately 100 min (local noon) at 40°N in July could lead to 50% suppression of specific T-cell-mediated responses to *L. monocytogenes* in human subjects ([31], Table 3). Whether this will also lead to a decreased resistance is uncertain, although in rats 50% suppression of cellular immunity results in a significant impairment of the resistance. A 5% decrease in the thickness

of the ozone layer would shorten this exposure time by approximately 2.5%. These data demonstrate that UVB radiation, in doses relevant to natural exposure, may affect the immunological resistance to infectious diseases in humans. Further experimental studies, in which the relationship between UVB exposure and infectious load is investigated, are essential. In addition, large-scale epidemiological studies may answer the important question of whether UVB exposure impairs the incidence and/or severity of infectious disease in the human population.

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