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The role of interleukin-4 in ultraviolet B light-induced immunosuppression

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SUMMARY

Prolonged exposure to ultraviolet light (UV) is known to lead to premature skin ageing, increased incidence of cataract and a high risk of developing skin cancers. UV-B irradiation, even if given as a single suberythemal dose, suppresses some immune responses, possibly reducing the production of T helper (Th) 1 cytokines [interleukin (IL)-2 and interferon-γ] and augmenting Th2 cytokines (IL-4, IL-5 and IL-10) in mice. We investigated the role of IL-4 in UV-B induced immunomodulation using IL-4 knockout (IL-$4^{-/-}$) mice and the parent strain Bb129. Suberythemal UV-B irradiation (1440 J/m^2) led to a reduction in the density and antigen presenting ability of Langerhans' cells in the epidermis of both normal and IL-$4^{-/-}$ mice. Exposure also induced an accumulation of $CD4^+$ and $CD8^+$ lymphocytes as well as dendritic cells in the lymph nodes draining the irradiated site in both strains. The proliferation of lymph node cells in response to the mitogen concanavalin A was enhanced in the IL-$4^{-/-}$ mice compared with the parent strain. Following UV-B exposure, this proliferation was increased in lymph node cells of parent mice but was significantly suppressed in the IL-$4^{-/-}$ mice. The contact hypersensitivity (CH) response to oxazolone was suppressed to the same extent by UV-B irradiation in both strains. In the parent mice, infected with herpes simplex virus (HSV) following UV-B exposure and challenged subsequently with inactivated virus, the delayed hypersensitivity (DH) response was suppressed by about 50% compared with unirradiated mice; no such suppression in DH occurred in irradiated IL-$4^{-/-}$ mice infected with HSV. Thus, IL-4 may be an important mediator of the UV-B-induced suppression in DH but not in CH, where other cytokines may be involved or may compensate for the lack of IL–4.

INTRODUCTION

Ultraviolet light (UV) exposure, even if given as a single suberythemal dose, can suppress cell-mediated immunity.[1] With the discovery of seasonal holes in the ozone layer,[2] concern has arisen over the increased levels of UV-B radiation reaching the Earth's surface which may lead not only to an increased incidence of skin tumours but also to a higher susceptibility to infectious diseases. UV-B induced immunosuppression can be measured in human subjects and experimental animals by utilizing antigen presentation assays as well as contact hypersensitivity (CH) and delayed hypersensitivity (DH) tests. Exposure reduces the density and antigen presenting ability of epidermal Langerhans' cells (LC) at the site of irradiation.[3] CH responses[4] and DH responses[5] to various antigens are both suppressed by UV-B radiation. The role of individual cytokines in mediating the immunosuppressive effects of UV radiation is poorly understood. There is evidence from *in vitro* and *in vivo* murine systems that UV-B irradiation may abrogate a T helper (Th) 1 response[6] while promoting a Th2 response, thus leading to suppression of cell-mediated immunity.[7] For example, Simon *et al.*[8] observed that while normal LC could present antigen to both Th1 and Th2 clones, UV-irradiated LC were unable to present to Th1 clones, but retained their ability to present to Th2 cells. There was a reduction in the production of the Th1 cytokines (interleukin (IL)-2 and interferon (IFN)-γ) and an augmentation of Th2 cytokines (IL-4, IL-5 and IL-10) in irradiated mice.[7] Keratinocytes are now known to be the source of many of the epidermal cytokines, including IL-1α, IL-3, IL-6, IL-8, IL-10, IL-15 and tumour necrosis factor (TNF)-α, which are all reported to be up-regulated following UV-B irradiation (reviewed by Takashima and Bergstresser[9]). Some of these cytokines, such as IL-1α,[10] IL-10[11] and TNF-α,[12] suppress the antigen presentation function of LC, thus suppressing the local epidermal immune response. Many

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Abbreviations: Con A, concanavalin A; CH, contact hypersensitivity; DC, dendritic cells; DH, delayed hypersensitivity; EC, epidermal cells; FBS, fetal bovine serum; HSV, herpes simplex virus; IFN, interferon; IL, interleukin; LC, Langerhans' cells; MED, minimum erythema dose; MSLR, mixed skin lymphocyte reaction; PFU, plaque-forming unit; Th, T helper; TNCB, trinitrochlorobenzene; TNF, tumour necrosis factor; UV, ultraviolet light.

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cytokines and other mediators are probably involved in UV-B-induced immunosuppression and IL-4 may be one of the candidates, as antibody to IL-4 has been shown to block the UV-induced suppression of the DH response.[13]

IL-4 is a B- and T-cell growth and differentiation factor which is involved in a wide variety of events (reviewed by O'Garra and Spits[14]). IL-4 is produced mainly by Th2 cells and, to a lesser extent, by $CD8^+$ T cells, mast cells, basophils and $CD4^-CD8^-\alpha\beta$ T-cell receptor positive (TCR^+) T cells. IL-4 exerts its effects on a large number of cell types; for example causing B cells to switch to immunoglobulin G1 (IgG1) and IgE production, inducing Th2 cells to produce Th2 cytokines, inhibiting IFN-γ production by Th1 cells, down-regulating Ia expression on monocytes, up-regulating Ia expression on B cells, up-regulating IL-4 receptor expression on lymphocytes, up-regulating vascular cell adhesion molecule-1 (VCAM-1) expression on endothelial cells, and inducing naive T cells to differentiate into Th2 cells.[14] IL-4, therefore, plays a key role in the development of the allergic reaction, suppresses the action of Th1 cells and inhibits monocyte functions that are related to cellular immune responses.[14] Keratinocytes express IL-4 receptors but it is not known whether they produce IL-4 themselves. Mast cells are probably the main source of IL-4 in the skin.[14] Controversy surrounds the role of IL-4 in the CH response. Gautam *et al.*[15] reported that an injection of IL-4 on the day of challenge suppressed the CH response to trinitrochlorobenzene (TNCB) and that alternatively, an injection of antibody to IL-4 on that day enhanced the response. Thus it was concluded that IL-4 plays a role in down-regulating the CH response. On the other hand, Salerno *et al.*[16] have found that an injection of antibody to IL-4 suppressed the CH response to TNCB, evidence that IL-4 may be an essential cytokine for the generation of the effector phase of a CH response. In addition, Muller *et al.*[17] concluded that IL-4 may mediate local tissue inflammation because antibody to IL-4 blocked the swelling response obtained following injection of Th2 cells *in vivo*. Other reports, however, have indicated no role for IL-4 in this response.[18,19] A clarification of the importance of IL-4 in the CH response and, more generally, in its participation in UV-induced immunosuppression is required.

Genetic manipulation has led recently to the production of IL-4 gene knockout mice ($IL\text{-}4^{-/-}$).[20] $CD4^+$ T cells from these mice failed to produce Th2-derived cytokines after *in vitro* stimulation. Significantly reduced levels of Th2 cytokines (IL-5, IL-9 and IL-10) were produced by $CD4^+$ T cells derived from nematode infected $IL\text{-}4^{-/-}$ mice. Twenty-fold reduced IgG1 levels and no detectable IgE were also reported.[20] We investigated the role of IL-4 in UV-B-induced immunosuppression using these $IL\text{-}4^{-/-}$ mice and the parent strain Bb129 ($IL\text{-}4^{+/+}$) in a variety of immunological tests in an attempt to assess the role of IL-4 under these circumstances.

MATERIALS AND METHODS

Animals

The parent strain Bb129 and homozygous $IL\text{-}4^{-/-}$ mice were kindly supplied by Dr Bluethmann, Hoffmann-La Roche, Basel, Switzerland. Female mice, aged 8–15 weeks, were used in all experiments. They were bred and maintained in the departmental animal facility where they were kept in a 12 hr light–dark cycle (fluorescent light tubes with covers) in standard mouse boxes, and had unlimited access to food and water. Cervical dislocation was performed to kill the mice. Female Balb/c mice, aged 8–10 weeks, were used to provide responder cells for the mixed skin lymphocyte reaction (MSLR), outlined below.

UV irradiation

The mice were irradiated on their dorsal surfaces immediately following shaving with electric clippers. They were immobilized using Hypnorm anaesthesia (Janssen, High Wycombe, UK). The ears of the mice were protected from UV radiation with paper card only for the hypersensitivity experiments. Broadband UV-B was provided by a bank of two TL20 W/12 lamps (Philips, Eindhoven, the Netherlands) with an output range of 270–350 nm (peak 308 nm).[5] The irradiance was 8 $\mu W/cm^2$ and a 30 min exposure corresponded to 1440 J/m^2 or 0·9 minimum erythema dose (MED).

ATPase staining of epidermal sheets

Parent strain and $IL\text{-}4^{-/-}$ mice (four mice per group) were irradiated with 0·9 MED UV-B. Twenty-four hours after irradiation, one ear from each mouse was removed, split and epidermal sheets from the dorsal surface only were stained for ATPase as previously described, using ADP as substrate.[21] The number of $ATPase^+$ cells were counted in at least 20 fields per group (1 field = 0·1 mm^2).

Mixed skin lymphocyte reaction (MSLR)

The other ear from each of the above mentioned mice was removed for preparation of epidermal cells (EC) using a 1% trypsin solution. Spleen cells (4×10^5 cells/well from Balb/c mice) and EC (1×10^5 cells/well from the $IL\text{-}4^{-/-}$ or $IL\text{-}4^{+/+}$ mice), were incubated together in complete RPMI medium containing 10% fetal bovine serum (FBS) (Gibco, Paisley, UK) at 37° in 96-well plates as previously described.[22] [^{3}H]thymidine incorporation (0·7 μCi per well; specific activity 84 Ci/mmol, Amersham International, Amersham, UK) was measured after 6 days in culture. The net proliferative response was calculated as the mean of 10 replicate wells after subtracting the mean of the negative control proliferation consisting of spleen cells alone cultured in complete RPMI medium.

Accumulation of dendritic cells (DC) in draining lymph nodes

Four mice per group were UV-B irradiated with a dose of 1440 J/m^2. Twenty-four hours after irradiation, the mice were killed and their auricular, axillary and inguinal lymph nodes, draining the UV-irradiated skin, were removed and pooled. A single cell suspension was made and the lymph node cells counted microscopically. Some of the cells were used for phenotyping or lymphoproliferation, as outlined below. The DC were purified on a 14·5% metrizamide cushion as previously described,[23] except that no fetal bovine serum (FBS) was used in the RPMI medium, and that the DC were counted microscopically. The number of DC per lymph node was deduced after a mean of four cell counts was calculated for each group.[23]

Phenotyping of lymphocytes in lymph nodes

The phenotypes of lymph node cells were determined as previously described.[24] Rat monoclonal antibodies to mouse

CD4 (1/10) and CD8 (1/40) markers (Serotec, Oxford, UK) were used, as well as an isotype matched rat anti-human IFN-γ as a negative control. The cells were analysed by an EPICS XL flow cytometer (Coulter, Luton, UK). The lymphocyte population (around 80% of lymph node cells) were gated on the basis of size and granularity, and were examined separately from the other cell populations.

Lymphoproliferation assay

Twenty-four hours after UV exposure, lymph node cells were plated out at 2×10^5 lymphocytes per well, in 200 µl complete RPMI medium containing 10% FBS, in 10 replicate wells of round bottom 96-well plates and incubated in the presence of 1 µg per well concanavalin A (Con A, Sigma, Poole, UK) for 2 days at 37°. [^{3}H]thymidine (0·7 µCi per well) was added and the incubation continued for a further 24 hr before harvesting the cells and counting. Control background proliferation without Con A was subtracted from the proliferation with Con A prior to presentation of the data.

Contact hypersensitivity (CH) assay

The CH response to oxazolone was measured as previously outlined by us.[25] Briefly, seven mice per group were UV-B irradiated with 1440 J/m^2 on their shaved backs, while their ears were protected from the radiation. Twenty-four hours later, the UV irradiation was repeated. One day later all mice received a sensitizing dose of 100 µl 1% oxazolone on their shaved backs, except for the negative control group which received vehicle alone (olive oil : acetone, 1 : 4). Eight days after the sensitization step, ear thicknesses were measured and all the mice were challenged with 25 µl per ear of 0·5% oxazolone on the dorsal surface. One day after the challenge, the ear thicknesses were measured again. All the groups were coded and the mean increase in ear thickness for each individual mouse was calculated before calculating the mean ear swelling for each group of mice.

Delayed type hypersensitivity (DH) assay

The DH response to HSV was measured as described elsewhere.[26] Briefly, eight mice per group were UV-B irradiated with 1440 J/m^2 on their shaved backs, while their ears were protected from the radiation. Three days later, all mice except a control group were infected with $2{\cdot}5 \times 10^6$ plaque-forming units (PFU) of HSV type 1 subcutaneously in the back of the neck. Ten days after the infection, ear thicknesses were measured and all the mice were challenged intrapinnally with 6×10^5 erstwhile PFU per ear of UV-inactivated HSV in a 10 µl volume. One day after the challenge, the ear thicknesses were measured again. All the groups were coded and the mean increase in ear thickness for each individual mouse was calculated before calculating the mean ear swelling for each group of mice.

RESULTS

The density of ATPase positive LC and their antigen-presentation function

The density of LC in the epidermis of the IL-4$^{+/+}$ and the IL-4$^{-/-}$ mice was found to be similar at around 500 LC per mm^2 of epidermis. This number was halved in both strains of mice following UV-B irradiation (Fig. 1a) indicating that

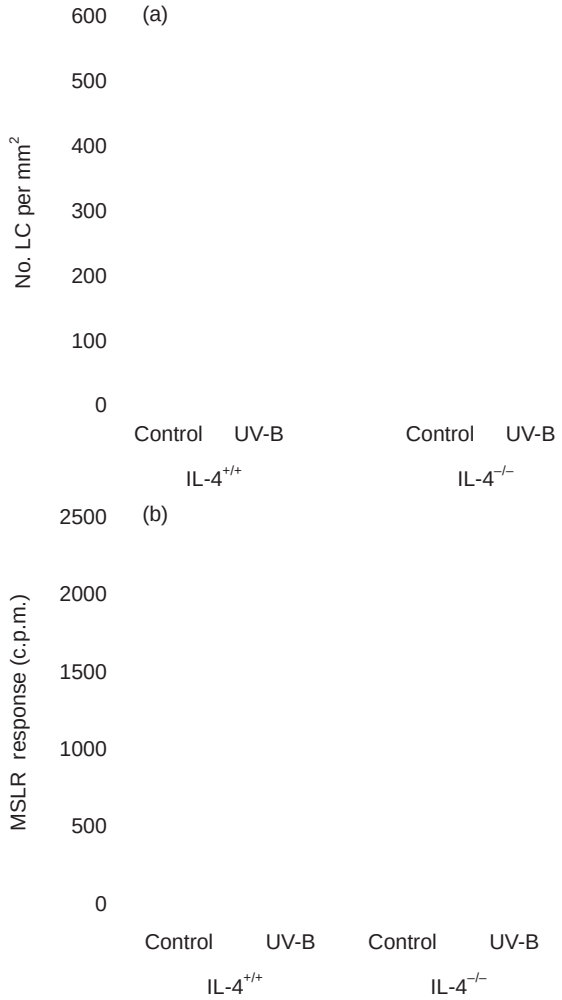

Figure 1. (a) UV-B induced a reduction in Langerhans' cell (LC) numbers in the parent Bb129 strain (IL-4$^{+/+}$) and the IL-4 knockout mice (IL-4$^{-/-}$). Control mice were not irradiated but were otherwise treated the same way as the irradiated mice which received 1440 J/m^2 UV-B. The number of ATPase$^+$ cells per mm^2 was counted 24 hr after irradiation. The error bars depict the standard error of the means. * = $P < 0{\cdot}001$ compared with the relevant control groups using the Student's *t*-test. (b) UV-B irradiation induced a reduction in epidermal antigen presenting cell function as measured by the mixed skin lymphocyte reaction (MSLR). The specific proliferative response after subtracting the background proliferation is shown. The bars indicate the standard error of the means. * represents the statistical significance ($P < 0{\cdot}001$) of the data from the irradiated groups when compared with the control groups. The background proliferation of spleen cells alone was 78 c.p.m. Background proliferation of epidermal cells alone was 135 c.p.m. for the IL-4$^{+/+}$ mice and 218 c.p.m. for the IL-4$^{-/-}$ mice.

IL-4 was unlikely to play a role in regulating the number of epidermal LC or in the UV-B induced depletion of these cells. The same result was obtained when this experiment was repeated.

UV-B irradiation suppressed the antigen presentation function of EC, as assessed by the MSLR by 57% for the IL-4$^{+/+}$ and by 74% for the IL-4$^{-/-}$ mice (Fig. 1b). Among the unirradiated controls, a higher proliferation was evident in the IL-4$^{-/-}$ compared with the IL-4$^{+/+}$ mice. This enhanced

response was not observed when the experiment was repeated, while the UV-B-induced suppression of the MSLR was still detected at 36% for the IL-$4^{+/+}$ and at 34% for the IL-$4^{-/-}$ mice ($P<0{\cdot}05$).

Lymph node cells

The lymph nodes of IL-$4^{+/+}$ or IL-$4^{-/-}$ mice contained very similar numbers of cells and UV-B irradiation led to a doubling of this number in both strains (Fig. 2a). Similarly, the number of DC per lymph node was also the same in IL-$4^{+/+}$ and IL-$4^{-/-}$ mice, and this number was significantly increased 24 hr after UV-B irradiation in both cases (Fig. 2b).

A slightly higher percentage of $CD4^+$ and $CD8^+$ lymphocytes was observed in the lymph nodes of IL-$4^{-/-}$ compared with those of the IL-$4^{+/+}$ mice (Fig. 3). These increases were not statistically significant ($P>0{\cdot}05$) although the same pattern was detected on three separate occasions. The percentage of $CD4^+$ and $CD8^+$ cells in lymph nodes was not significantly altered by UV-B irradiation in either strain (Fig. 3).

However, the proliferation of lymph node cells in response to Con A did differ between the two strains of mice. A significantly higher proliferation occurred in the IL-$4^{-/-}$ compared with the IL-$4^{+/+}$ mice, and UV-B irradiation led to an enhanced proliferative response in the IL-$4^{+/+}$ mice but to down-regulation of this response in the IL-$4^{-/-}$ mice (Fig. 4). This experiment was repeated three times with the same result. IL-4 may, therefore, play a role in the control of the proliferation of lymph node cells and its alteration by UV-B irradiation.

CH assay

The CH response to oxazolone was the same in IL-$4^{+/+}$ and IL-$4^{-/-}$ mice (Fig. 5). However, a higher concentration of oxazolone was needed in order to elicit a CH response in both strains of mice than is required by other strains such as C3H/HeN or Balb/c. Our standard technique,[24] using a sensitizing dose of 50 µl of 1% oxazolone followed by a challenge with 25 µl of 0·25% oxazolone normally leads to an ear swelling response of around 0·2 mm. In order to induce a similar ear swelling response in the IL-$4^{+/+}$ and IL-$4^{-/-}$ mice, a sensitizing dose of 100 µl of 1% oxazolone followed by a challenge of 25 µl of 0·5% was required. This probably reflected a strain variation and was not due to the IL-4 gene. The effect of UV exposure on the CH response was the same in the IL-$4^{+/+}$ and IL-$4^{-/-}$ mice. UV-B irradiation suppressed the CH

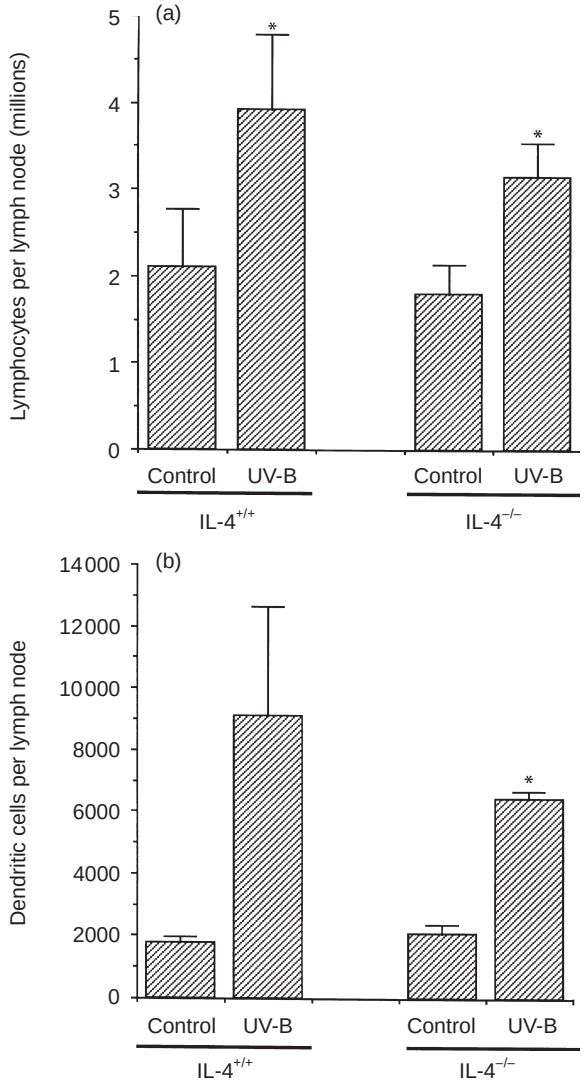

Figure 2. (a) UV-B-induced an increase in the number of cells in lymph nodes draining the irradiated site in the IL-$4^{+/+}$ and IL-$4^{-/-}$ mice. (b) An accumulation of dendritic cells in draining lymph nodes was also evident 24 hr after 1440 J/m^2 UV-B exposure in these two strains. The data presented in (a) and (b) is the mean of three experiments and the error bars indicate the standard error of the means. * indicates a significant difference between the control and irradiated groups ($P<0{\cdot}05$).

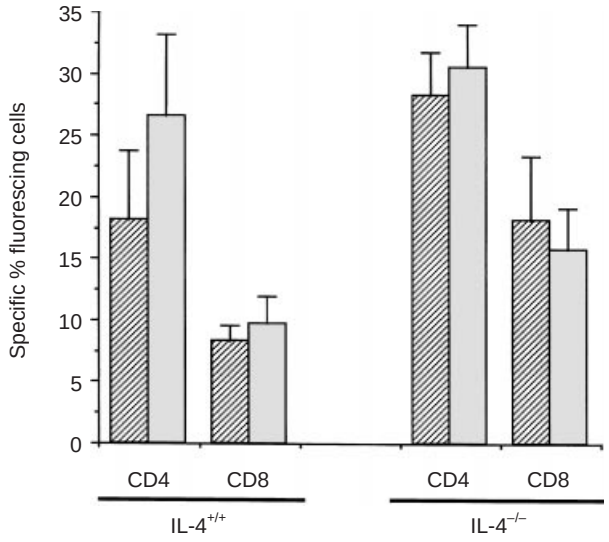

Figure 3. The phenotype of lymphocytes in lymph nodes draining the irradiated site in the IL-$4^{+/+}$ and IL-$4^{-/-}$ mice was not significantly altered by 1440 J/m^2 of UV-B exposure. The specific fluorescence after subtracting the background fluorescence obtained with an isotype matched control is shown. The data are derived from three experiments and the error bars indicate the standard error of the means. Hatched histograms represent unirradiated controls and dotted histograms represent UV-B-irradiated mice.

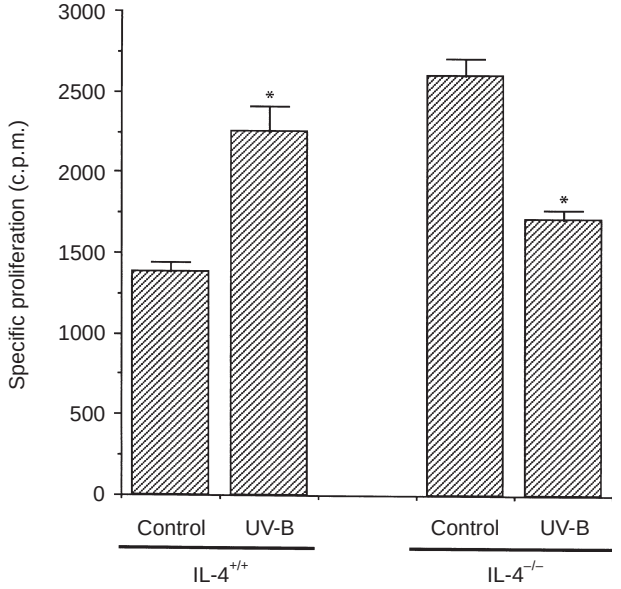

Figure 4. The specific proliferation of lymph node cells in response to Con A was enhanced in the IL-$4^{-/-}$ mice compared with IL-$4^{+/+}$ mice ($P<0{\cdot}05$). Following 1440 J/m^2 of UV-B exposure the proliferation in IL-$4^{+/+}$ mice was increased, while that in the IL-$4^{-/-}$ mice was significantly suppressed. The background proliferation of lymph node cells alone ranged between 294 and 464 c.p.m. for the IL-$4^{+/+}$ mice and between 726 and 914 c.p.m. for the IL-$4^{-/-}$ mice. The data represent the mean of 10 replicate wells from one experiment and the error bars indicate the standard error of the means. * indicates a significant difference between the control and irradiated groups ($P<0{\cdot}05$).

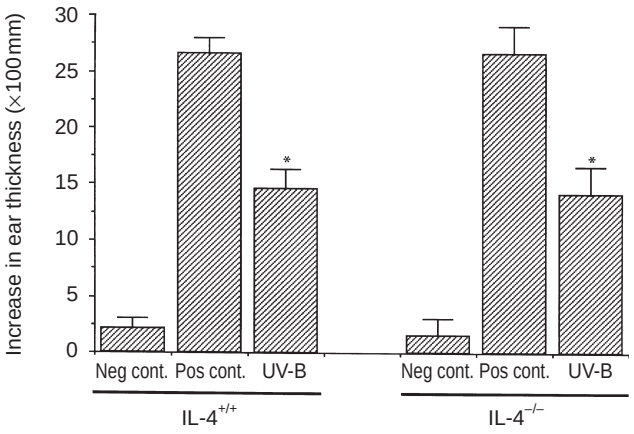

Figure 5. IL-4 played no role in generating the CH response nor in the UV-B induced suppression of the CH response. The IL-$4^{+/+}$ and IL-$4^{-/-}$ mice developed a similar ear swelling response and in both cases this response was suppressed by UV-B irradiation. The mean increase in ear thickness of seven mice per group and the standard errors of the mean are shown. The positive control group was sensitized and challenged with oxazolone while the negative control group was sensitized with vehicle alone, followed by challenge with oxazolone. The error bars depict the standard error of the means. *$P<0{\cdot}005$ compared with the positive control groups.

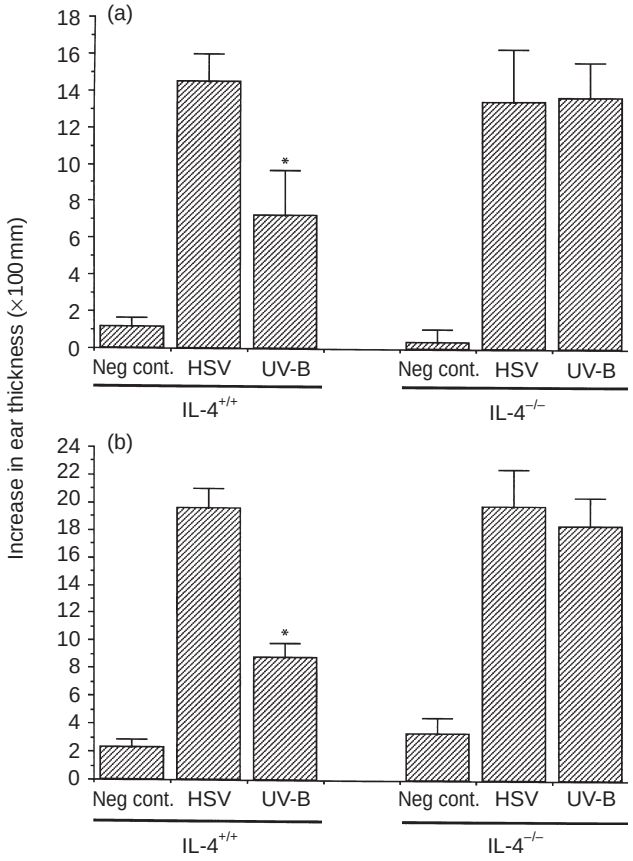

Figure 6. The DH response to live (a) and inactivated (b) HSV. The IL-$4^{+/+}$ and IL-$4^{-/-}$ mice were both capable of raising a strong DH response to HSV. This response was suppressed by UV-B in the IL-$4^{+/+}$ mice but not in the IL-$4^{-/-}$ mice. Ear swelling DH response and the standard error of the mean are shown. Neg cont, negative control group which was injected with PBS instead of HSV; HSV, positive control group which was infected with $2{\cdot}5\times10^6$ PFU HSV; UV-B, this group was UV-B irradiated (1440 J/m^2) once, 3 days prior to infection with HSV. All mice were challenged with inactivated HSV. *$P<0{\cdot}05$ compared with the 'HSV' group.

response to a similar extent in both strains (Fig. 5). A similar result was obtained when this experiment was repeated indicating that IL-4 may play no role in the CH response or its suppression by UV-B.

DH assay

The DH response to HSV was the same in IL-$4^{+/+}$ and IL-$4^{-/-}$ mice. UV-B irradiation suppressed this response by 55% in the IL-$4^{+/+}$ mice but could not suppress it in the IL-$4^{-/-}$ mice (Fig. 6a). This was indicative of a role for IL-4 in the UV-B-induced suppression of the DH response. This result was confirmed when the experiment was repeated. In order to rule out the possibility that this result was due to the IL-$4^{-/-}$ mice responding differently to the viral infection rather than due to the immune system of these mice, the DH experiment was repeated on one more occasion but utilizing the same amount of inactivated virus as a sensitizing antigen instead of infectious virus. The same result was again obtained (Fig. 6b).

DISCUSSION

UV-B irradiation results in a suppression of various immune functions. Rivas and Ullrich[13] reported that antibody to IL-4 blocked the UV-B-induced suppression of the DH response indicating that IL-4 may be a key cytokine in the UV suppression of the immune system. Mice lacking the IL-4 gene were used to explore this hypothesis. The IL-$4^{-/-}$ mice were healthy and phenotypically indistinguishable from the IL-$4^{+/+}$ strain.

The density of LC found in the epidermis of the IL-$4^{+/+}$ and IL-$4^{-/-}$ mice was identical. The maturation and kinetics of these epidermal antigen-presenting cells therefore, were unlikely to be controlled by IL-4. This cytokine did not seem to play a major role in the development of most of the immune functions tested, as the IL-$4^{-/-}$ mice were capable of developing a strong CH and DH response and demonstrated efficient epidermal antigen presentation, indicating that the skin immune system of these mice was not compromised by the lack of IL-4. This finding is in agreement with a published report which concluded that IL-4 was either not essential, or its functions could be superseded by other cytokines, in the development of immune responses.[27] In three experiments, the proliferation of lymph node cells in response to the mitogen Con A was consistently higher in the mice lacking the IL-4 gene. IL-4 may, therefore, play some role in regulating this response and it would be interesting to analyse cytokine production in the supernatants of such cultures compared with the parent strain.

Suberythemal UV-B irradiation led to a reduction in the density and antigen presentation function of epidermal LC in both the parent strain and IL-$4^{-/-}$ mice. Similarly, UV exposure induced an accumulation of $CD4^+$ and $CD8^+$ lymphocytes as well as dendritic cells in the lymph nodes draining the irradiated site in both strains. UV-B irradiation also resulted in a suppression of the CH response of the IL-4 knockout and parent strain. Therefore, IL-4 is unlikely to be important in these particular UV-induced alterations of the immune system. It is possible, however, that other cytokines have overlapping roles with IL-4 so that these will compensate in the absence of IL-4 and a normal response will be observed. Our CH findings agree with those of Berg *et al.*[19] suggesting that IL-4 plays no direct role in generating or suppressing the CH response to oxazolone.

Following exposure to UV-B, an enhancement was observed in the Con A proliferation of lymph node cells of IL-$4^{+/+}$ mice but a suppression in the lymph node cells of IL-$4^{-/-}$ mice. This probably indicates a complex interaction between this cytokine and the UV modulation of lymphoproliferation. Such modulation has been previously observed with keratinocyte-derived cytokines, which can have both positive and negative effects on LC function.[28] A cytokine analysis of the supernatant medium from these proliferating cells may aid in elucidating the function of IL-4 in regulating the proliferation of lymph node cells.

UV-B irradiation was unable to suppress the DH response to HSV in the IL$4^{-/-}$ mice. IL-4 may therefore play a direct or indirect role in the UV-induced suppression of the DH response. This is in contrast to the data obtained for the CH response to oxazolone. This result could not be attributed to the IL$4^{-/-}$ mice being infected to a different extent by the HSV than the IL-$4^{+/+}$ mice, since it did not make any difference whether live or inactivated virus was used to sensitize the mice. The different results obtained with the CH and DH responses may have been due to the differing protocols utilized in the experiments (CH: mice irradiated on days 1 and 2 and sensitized on day 3; DH: mice irradiated once and infected 3 days later). A further DH experiment, performed using the same irradiation doses and infecting at the same time as for the CH, showed that this was not the case. In agreement with the earlier data, this experiment confirmed that UV irradiation suppressed the DH response in the parent strain mice but not in the IL$4^{-/-}$ mice (data not shown). In a separate experiment, a 'systemic' CH assay was also performed, where the sensitizer was applied to the unirradiated abdomen instead of the irradiated back. This protocol mimics more closely the DH assay, where the virus is injected subcutaneously, then becomes systemic and thus is not only present at the irradiated site. This experiment again confirmed earlier data that the CH response was suppressed in both strains of mice (data not shown).

These findings provide evidence adding to the published results[13,25] that the CH response differs from the DH response in the pathways that generate them and the mechanisms which suppress them. A previous study has also shown that IL-4 (and IL-10) are involved in the UV-B induced suppression of DH but not CH. As mentioned above, Rivas and Ullrich[13] utilized antibodies to IL-4 or IL-10 to block the ability of UV-induced suppressor T cells to suppress the DH response on transfer to recipient mice. Neither antibody, however, was able to block the UV suppression of the CH response, further consolidating our findings.

The final immunological outcome is likely to be determined by a complex balance between several cytokines and other factors. Many cytokines have reciprocal activities and some duplicate each other's effector functions. UV-B irradiation may lead to an altered balance of cytokines which, depending on dose or timing, can affect several immune parameters. Whether the same changes occur in human subjects as in inbred mouse strains is not known. It is interesting to note that dermal mast cells, by releasing histamine and cytokines, play a vital role in the induction of systemic immunosuppression by UV-B radiation (Dr Prue Hart, personal communication). IL-4 may be one of the cytokines released by mast cells following UV-B exposure and may function in conjunction with histamine. We have shown in this study that IL-4 is required for the successful suppression of the DH response but not for the other skin immune responses tested. The mechanism by which IL-4 mediates or facilitates the suppression of DH is being investigated currently.

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