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Tumour necrosis factor-alpha mediates ultraviolet light B-enhanced expression of contact hypersensitivity

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SUMMARY

Acute, low-dose ultraviolet B radiation (UVB) impairs the induction of contact hypersensitivity (CH) to dinitrochlorobenzene (DNCB) in certain inbred strains of mice (termed UVB-susceptible), but not in others (termed UVB-resistant). By contrast, exposure of mouse ear skin to an identical regimen of UVB has been reported to exaggerate the expression of CH. Recently, tumour necrosis factor-alpha (TNF-α) has been demonstrated to mediate the deleterious effects of UVB on CH induction, presumably through local release of TNF-α within UVB exposed skin. The present studies were conducted to determine whether TNF-α also mediates the exaggerated expression of CH induced by UVB radiation. It was found that TNF-α, injected intradermally at the ear challenge site, enhanced the expression of CH to DNFB in conventionally sensitized mice. Interestingly, TNF-α was able to amplify the expression of CH in the ears of both UVB-susceptible strains of mice, and UVB-resistant strains. However, anti-TNF-α antibodies neutralized UVB-enhanced CH in UVB-susceptible mice, but not in UVB-resistant mice. These findings support the proposition that TNF-α, released from UVB-exposed epidermal cells, is a critical mediator of the effects of UVB radiation on induction and expression of contact hypersensitivity. The effects of UVB radiation, intradermal (ID) TNF-α, and/or epicutaneously applied DNFB on epidermal Langerhans' cells were also evaluated and compared. Whereas epicutaneously applied DNFB alone profoundly depleted the epidermis of Langerhans' cells, DNFB painted on UVB-exposed or TNF-α-treated skin was much less effective at eliminating normal appearing Langerhans' cells. These results suggest that one direct effect of TNF-α on Langerhans' cells may be to immobilize these antigen-presenting cells transiently within the epidermis. It is proposed that this immobilization has the paradoxical effects (a) of interfering with sensitization, by preventing hapten-bearing Langerhans' cells from migrating to the draining lymph node, while at the same time (b) of amplifying CH expression by lengthening the interval of hapten retention and presentation with the epidermis.

INTRODUCTION

Acute, low-dose ultraviolet B (UVB) irradiation of mouse skin impairs the induction of contact hypersensitivity (CH) if hapten is painted on the site immediately after the last exposure.[1,2] This phenomenon appears to be genetically determined since impaired CH following UVB exposure occurs in some strains of mice (termed UVB-susceptible), but not in others (UVB-resistant).[3] At least two separate genetic loci govern this polygenic trait—*Tnfα* and *Lps*. Appropriate alleles at these loci ($Tnf\alpha^b$ and Lps^n) are required to produce the UVB-susceptibility phenotype.[4] Since vigorous CH develops in UVB-susceptible mice which are exposed to UVB and given anti-tumour necrosis factor-alpha (anti-TNF-α) antibodies prior to hapten application, it has been proposed that UVB susceptibility (impairment of CH induction) is mediated by excessive intracutaneous production or release of TNF-α. In support of this view, it has been reported that intradermal injection of TNF-α prior to painting the injected site with hapten also yields grossly impaired CH.[4] It has been suspected that a UVB-induced abnormality in regulation of TNF-α transcription and/or translation gives rise to excessive local TNF-α secretion by epidermal cells: keratinocytes or Langerhans' cells. The exact mechanisms by which excess intraepidermal TNF-α impairs CH induction remains speculative, although an effect on epidermal Langerhans' cells has been postulated.

Whereas low-dose UVB impairs the *induction* of CH, this irradiance exaggerates the *expression* of CH in mice.[5] This paradoxical and unexpected observation has defied a suitable

Abbreviations: CH, contact hypersensitivity; DNFB, dinitrofluorobenzene; ID, intradermal; i.p., intraperitoneal; PBS, phosphate-buffered saline; TNF-α, tumour necrosis factor-alpha.

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explanation. Since the effects of UVB radiation on CH induction appear to be mediated by TNF-α, we examined the possibility that this cytokine might also mediate the amplifying effects of UVB on CH expression. Our results indicate that this is the case, and they suggest that an effect of TNF-α on the motility of Langerhans' cells may be the basis for the opposite consequences if UVB on expression and induction of CH.

MATERIALS AND METHODS

Mice

Female mice, 8–12 weeks of age, of the following strains were used: C57BL/6, a UVB-susceptible strain, and BALB/c, a UVB-resistant strain.[3,4] These mice were produced and maintained in our domestic colony. Each control or experimental panel consisted of five mice.

Induction and expression of CH

Twenty-five microlitres 0·5% dinitrofluorobenzene (DNFB) (125 μg in a 4:1 acetone; olive oil mixture) was applied to the shaved abdominal cutaneous surface of mice on Day 0 as previously described.[1] CH was elicited on Day 5 by challenging one ear of each mouse with 20 μl 0·2% (40 μg) DNFB. The extent of ear swelling was used as a measure of CH. Ear thickness was measured with an engineer's micrometer 24 hr following challenge and compared with ear thickness prior to challenge.

UVB radiation

Mouse ears were exposed to UVB from a bank of four FS-20 fluorescent lamps with a tube to target distance of 46 cm as previously described.[1] These bulbs have a broad emission spectrum (250–400 nm), and high output was primarily in the UVB range (290–320 nm). As measured by an IL 700 radiometer with an SEE 240 UVB photodetector, these lamps delivered an average flux of 1·4 J/m^3/second. Mouse ears were exposed to UVB daily for 4 consecutive days (400 J/m^2/day). Within 1 hr of the final exposure, DNFB was applied to the irradiated site.

TNF-α

Mouse recombinant TNF-α was purchased from Genzyme (Boston, MA). The specific activity of the preparation was 4×10^7 U/mg (which was assayed on L-929 cells in the presence of actinomycin-D). The dose of TNF-α which we chose for intradermal (ID) injection was based in part upon the recent report by Sharpe and co-workers[6] who determined that < 60 ng human recombinant TNF-α injected into the footpad of mice induced only a mild acute inflammation. In our experiments, mice received intrapinnae injections of 0·05 ml phosphate-buffered saline (PBS) containing 50 ng TNF-α immediately before epicutaneous application of DNFB. Hapten was painted directly over and exclusively upon the ID injection sites. No evidence of inflammation was observed within 24–48 hr at ID injection sites of TNF-α unless hapten was applied.

TNF-α-specific antibodies

Rabbit anti-mouse TNF-α antiserum from hyperimmune New Zealand rabbits immunized with recombinant murine TNF-α was purchased from Genzyme. This antiserum, which has neutralizing activity of approximately 1×10^6 neutralizing U/ml, has been sterile filtered using 0·22 micron filter, and contains no preservatives. Rabbit anti-bovine serum albumin (BSA) was purchased from ICN Immunobiologicals (Lisle, IL).

Determination of epidermal Langerhans' cells

Epidermis was separated from dermis by incubation in ethylenediaminetetraacetate as described previously.[1,3] The epidermis was stained with monoclonal anti-I-A^d antibody (Becton Dickinson, Mountain View, CA) and evaluated under epifluorescent microscopy. With the aid of an eyepiece with a 1mm^2 grid, a minimum of 10 fields was counted for each sample to enumerate the number of positively stained cells present. The data are presented as mean ± standard error of the mean.

Statistical evaluation of results

The statistical significance of differences in the means of each experimental group was calculated with Student's *t*-test. Mean differences were considered to be significant when $P < 0{\cdot}05$.

RESULTS

Effects of UVB radiation on CH expression

We first wished to confirm that acute, low-dose UVB radiation of pinnae of mice causes enhanced expression of CH. In addition, we wanted to know whether the effect could be observed in both UVB-resistant and UVB-susceptible strains of mice. Panels of C57BL/6 (UVB-susceptible) and BALB/c (UVB-resistant) mice received sensitizing doses of DNFB (125μg) to shaved abdominal skin. Four days later, the animals were anaesthetized and their ears (dorsal surface) were irradiated with UVB (40 mJ/cm^2). This procedure was repeated on each of the successive days. One hour after the last dose of UVB, DNFB (40 μg) was applied to the dorsal surface of the ears. Positive control mice were sensitized and ear challenged without exposure to UVB, and negative control panels of naive mice received 4 daily exposures of UVB radiation on their ears prior to challenge with DNFB. As displayed in Fig. 1, UVB irradiation of ears of BALB/c produced enhanced (+57%) expression of CH, compared to unirradiated controls. Similarly, UVB irradiation enhanced the expression of CH in ears of C57BL/6 mice (+80%). We conclude that UVB irradiation, in an acute low-dose regimen, amplifies the expression of CH in both UVB-resistant and UVB-susceptible mice.

Effects of intradermal TNF-α on CH expression

If the effects of UVB radiation on CH are mediated by TNF-α, then it would be anticipated that intradermally injected TNF-α would also exaggerate the expression of CH, as did UVB. Accordingly, panels of BALB/c and C57BL/6 mice were sensitized on abdominal skin with DNFB. Seven days later groups of sensitized mice received 50 ng of TNF-α beneath the epidermis of the dorsal surface of the pinnae. Immediately afterwards, DNFB (40 μg) was painted on the injected site. Control mice received ID injections of PBS rather than TNF-α. When the ear swellings were measured 24 hs later, it was found that ears of UVB-resistant BALB/c mice that received TNF-α ID expressed CH of significantly greater intensity (+57%) than ears treated with PBS (Fig. 2). In identical fashion, TNF-α injected into the pinnae amplified CH expression in UVB-susceptible C57BL/6 mice.

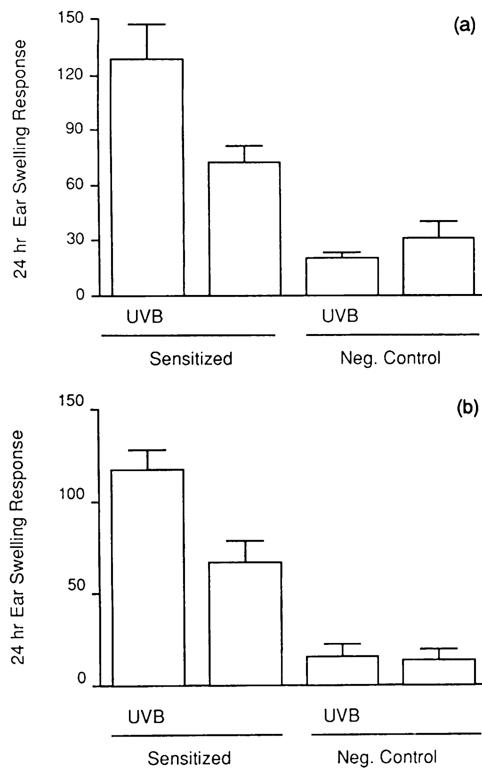

Figure 1. Effect of UVB radiation on expression of CH in BALB/c (a) and C57BL/6 (b) mice. Panels of five mice each were sensitized on Day 0 with 125 μg DNFB on shaved abdominal skin. Starting on Day 4, ear pinna received four daily exposures of UVB (400 J/m^2). Immediately afterwards, DNFB (40 μg) was painted on the dorsal surfaces of the irradiated sites. Bars represent mean ($\pm$SE of the mean) ear swelling responses 24 hr after hapten application expressed as $\times 10^{-3}$ mm. Negative controls were only ear challenged. Responses of UVB-treated ears are significantly greater than non-UVB-treated ears in sensitized mice ($P<0{\cdot}05$), and both responses are significantly greater than negative controls ($P<0{\cdot}02$).

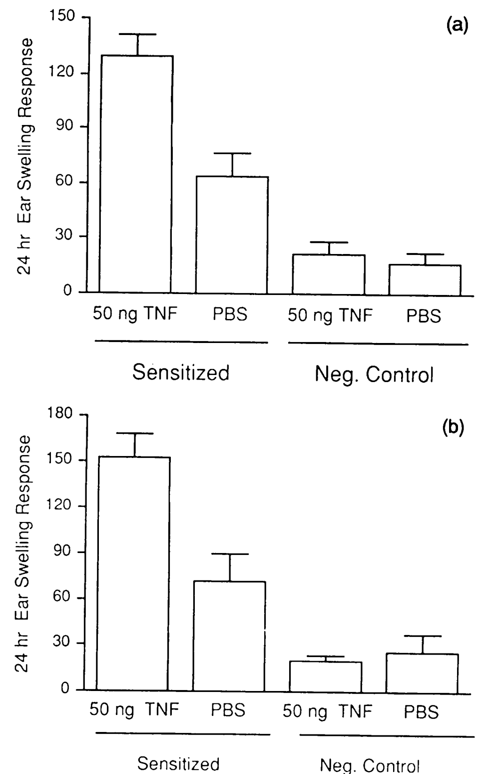

Figure 2. Effect of intrapinna injection of TNF-α (50 ng) on expression of CH in BALB/c (a) and C57BL/6 (b) mice. Panels of five mice each were sensitized as described in Fig. 1. On Day 7, TNF-α (50 ng or PBS — control) was injected beneath the epidermis of the dorsal surface of the ear. Immediately afterwards DNFB was painted on the surface above the injected site. Ear swelling responses were assayed as described in Fig. 1. Responses of pinnae injected with TNF-α are significantly greater than PBS-injected ears of sensitized mice ($P<0{\cdot}05$), and both are significantly greater than negative controls ($P<0{\cdot}02$).

In separate experiments, TNF-α injected ID at a distant site was tested for its capacity to enhance the expression of CH. TNF-α (50 ng) was injected into the left ear pinnae of one group of BALB/c mice that had been immunized epicutaneously with DNFB (125 μg) 5 days previously. In a second group of DNFB-sensitized mice, TNF-α (50 ng) was injected into the right ear pinnae. Immediately thereafter, DNFB (40 μg) was painted on the right ears of both groups of mice. The results are presented in Fig 3, and indicate that only challenged ears that had received ID TNF-α displayed enhanced CH. We conclude that local TNF-α at the site of hapten challenge amplifies the expression of CH in immune mice, but similar amounts of TNF-α injected at distant sites have no comparable effect.

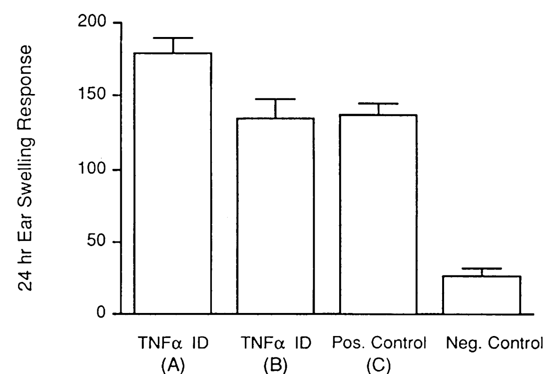

Figure 3. Effect of local and distant injections of TNF-α (50 ng) on expression of CH in BALB/c mice. Panels of five mice each were sensitized as described in Fig. 1. On Day 5, TNF-α (50 ng or PBS — control) was injected into the left ear (A) or right ear (B) pinnae. Immediately afterwards, DNFB was painted on the right ear pinnae of all mice. Ear swelling responses were assayed as described in Fig. 1. Responses of pinnae injected with TNF-α and painted with DNFB are significantly greater than PBS-injected ears of sensitized mice (B, $P<0{\cdot}05$) or DNFB painted ears of mice receiving TNF-α ID in their left (unpainted) ears (C, $P<0{\cdot}05$).

Effect of anti-TNF-α antibodies on CH expression exaggerated by UVB radiation

Neutralizing anti-TNF-α antibodies have been shown to reverse the deleterious effects of UVB on the induction of CH in UVB-susceptible mice.[4] We next investigated whether the ability of UVB radiation to exaggerate CH expression could be nullified with anti-TNF-α antibodies. BALB/c and C57BL/6 mice were sensitized to DNFB; 4 days later their ears were exposed to UVB

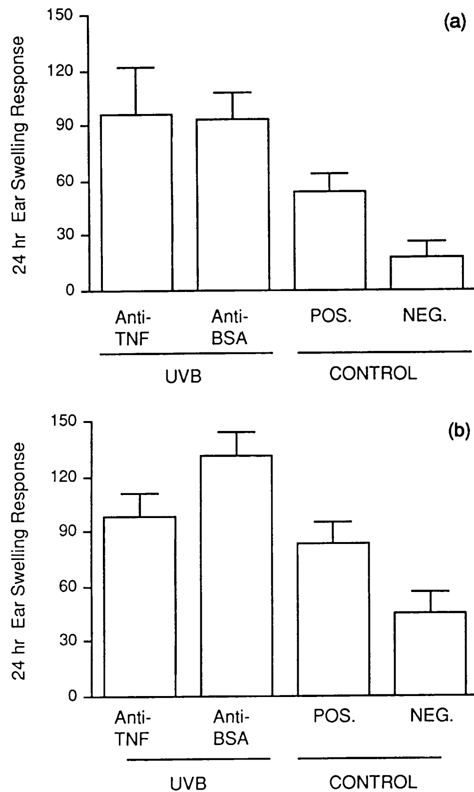

Figure 4. Effect of neutralizing anti-TNF-α antibodies on expression of CH following UVB radiation of ears of BALB/c (a) and C57BL/6 (b) mice. Panels of mice were sensitized and their pinnae irradiated with UVB as described in Fig. 1. Six hours prior to last dose of UVB radiation, panels of mice received anti-TNF-α (10^{-4} U) or anti-BSA antibodies intraperitoneally. The ears were challenged with DNFB and ear swelling responses were measured as described in Fig. 1. Responses of sensitized BALB/c mice treated with anti-TNF-α and anti-BSA are virtually identical, and significantly greater than responses of positive control ($P<0{\cdot}05$) and negative control mice ($P<0{\cdot}02$), whereas responses of sensitized C57BL/6 mice treated with anti-TNF-α are similar to the positive control mice, but significantly less than anti-BSA treated mice ($P<0{\cdot}05$).

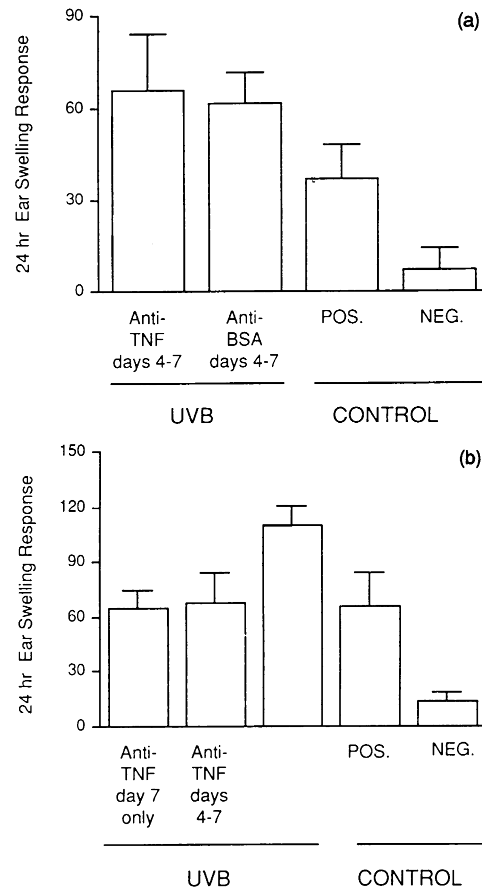

Figure 5. Effect of repeated injections of neutralizing anti-TNF-α antibodies on expression of CH following UVB radiation of ears of BALB/c (a) and C57BL/6 (b) mice. Experimental protocol was similar to that described in Fig. 3 except that anti-TNF-α or anti-BSA antibodies were injected intraperitoneally on Days 4–7 post-sensitization. Responses of anti-TNF-α and anti-BSA-treated sensitized BALB/c mice are significantly greater than positive control mice ($P<0{\cdot}05$), whereas responses of C57BL/6 mice are significantly reduced compared to the anti-BSA-treated mice ($P<0{\cdot}05$), but similar to responses of positive control mice.

radiation for 4 successive days. Six hours prior to the last UVB exposure, these mice received 10^4 neutralizing units of anti-TNF-α antibodies intraperitoneally (i.p.). Control mice received anti-BSA antibodies i.p. The ears of all mice were challenged with DNFB 7 hr after the last dose of UVB. As the results displayed in Fig. 4 reveal, anti-TNF-α prevented the UVB-induced enhancement of CH in C57BL/6 mice, but not in BALB/c. The inability of anti-TNF-α to reverse the effects of UVB on CH expression was unexpected, since 50 ng TNF-α injected ID in this strain mimicked the effects of UVB. In a more intensive treatment regime, BALB/c and C57BL/6 mice that were sensitized to DNFB through abdominal skin received four daily doses of anti-TNF-α antibodies i.p., each dose being administered 6hr prior to the daily exposure of their ears to UVB radiation. The results are presented in Fig. 5. Challenge of UVB-exposed ears of BALB/c mice with DNFB revealed exaggerated CH responses, whether they were treated i.p. with anti-BSA antibodies or anti-TNF-α. By contrast, either one or four injections of anti-TNF-α antibodies reversed the enhancing properties of UVB radiation on CH expression in C57BL/6 mice. Thus, although treatment with anti-TNF-α antibodies confirms that TNF-α alone is sufficient to account for enhanced CH expression secondary to UVB radiation in C57BL/6 mice, a similar conclusion cannot be reached for BALB/c mice.

TNF-α is a pleiotropic cytokine with many effects that are regarded as proinflammatory. The capacity of anti-TNF-α antibodies to reverse the enhancement of CH expression by UVB radiation in C57BL/6 mice raised the possibility that anti-TNF-α antibodies might be directly immunosuppressive in their own right. To examine this issue, panels of C57BL/6 mice were sensitized on abdominal skin with DNFB (125 μg) and then ear challenged with DNFB (40 μg) 5 days later. One panel of mice received anti-TNF-α antibodies intraperitoneally 6 hr prior to the ear challenge. When the ears of these mice were measured 24 hr later (see Fig. 6), the intensity of CH expression in the ears of mice that received anti-TNF-α antibodies was virtually identical to that of positive control mice. This indicates that anti-TNF-α antibodies are not inherently immunosuppressive, and is consistent with the view that the ability of anti-TNF-α antibodies to

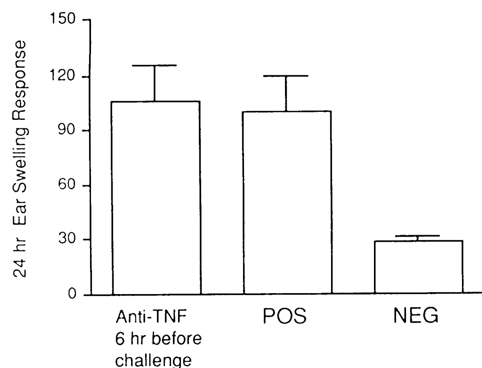

Figure 6. Effect of anti-TNF-α on expression of contact hypersensitivity in C57BL/6 mice. Panels of mice were sensitized on abdominal skin with DNFB as described in Fig. 1. One panel of mice received 10^{4} U of anti-TNF-α antibodies intraperitoneally 6 hr prior to ear challenge with DNFB. Responses of anti-TNF-α-treated, sensitized mice are indistinguishable from responses of untreated sensitized mice, and both are significantly greater than the negative control mice ($P<0{\cdot}02$).

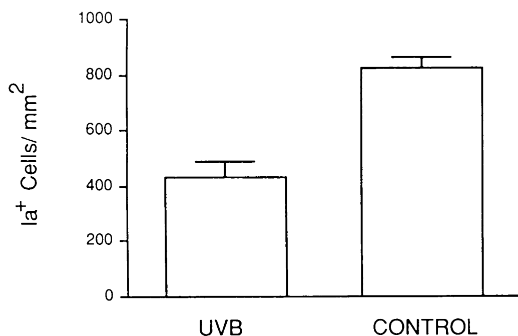

Figure 7. Effect of UVB radiation on density of Ia^+ cells in epidermis of pinnae. Epidermal sheets removed from ears of four BALB/c mice immediately after the fourth daily dose of UVB were assayed for density of Ia^{d+}, expressed as a number of positive cells/mm^2; control ears were unirradiated. Density of cells in UVB-treated skin significantly less than in unirradiated skin ($P<0{\cdot}01$).

prevent UVB-induced enhancement of CH expression is a direct consequence of their ability to neutralize excess TNF-α released by UVB radiation of skin.

Effects of UVB on epidermal Langerhans' cells in pinnae

Exposure of murine abdominal skin to acute, low-dose UVB radiation depletes the epidermis of virtually all normal-appearing Langerhans' cells in both UVB-susceptible and UVB-resistant mice.[1,3] Since UVB irradiation also enhances the expression of CH in ears, we examined the morphological consequences of UVB irradiation on epidermal Langerhans' cells of mouse ears. Epidermal sheets were removed from ears of BALB/c mice immediately after the last of four daily exposures to UVB. The epidermis was stained with fluorescein-conjugated anti-Ia^d antibodies and examined by fluorescent microscopy. As the results expressed in Fig. 7 indicate, UVB depleted the ear epidermis of Ia^+ cells, but the extent of depletion was much less than that achieved by UVB irradiation of abdominal skin.[1]

Table 1. Effect of TNF-α on density of Langerhans' cells in epidermis painted with DNFB

Intradermal injection	Dose of DNFB*		
	None	40 μg	125 μg
TNF-α (50 ng)	812±46†	706±40	650±25
PBS	804±41	364±32‡	350±35‡

* DNFB applied epicutaneously 24 hr prior to immunohistochemical evaluation of epidermis. TNF-α or PBS injected intradermally 5 min prior to DNFB application.

† Mean ± standard error of mean of I-A^{d+} epidermal cells/mm^2.

‡ Significantly less than TNF-α treated sites ($P<0{\cdot}01$).

Most of the Ia^+ cells that persisted in UVB-exposed ear epidermis were altered in shape, with short stubby dendrites and a rounded cell body. Similar changes have been described after a single UVB exposure or TNF-α injection of abdominal skin. The contour of the mouse ear, and the fact that we did not attempt to shave the ears prior to UVB exposure probably conspired to reduce the effective dose of UVB delivered to the epidermis, compared to abdominal skin. Nonetheless, UVB radiation altered ear epidermal Langerhans' cells in a fashion qualitatively similar to its effects on Langerhans' cells of abdominal skin.

Studies bearing on the mechanism of action of TNF-α

We have recently reported that ID injection of TNF-α (50 ng) can cause a modest reduction in the density of Ia-bearing cells (approximately 30% reduction) of the epidermis within 5 min, but that the number and morphology of epidermal Ia^+ cells was restored to normal levels within 24 hr.[7] These results imply (but do not prove) that effacement of surface markers, rather than loss of cells, is responsible for the acute changes observed in Ia^+ epidermal cells after TNF-α injection. Bergstresser *et al.*[8] and Hunziker and Winkelmann[9] have reported independently that epicutaneous applications of DNFB can produce a much more significant and sustained reduction in the local density of Ia^+ cells. Since haptens are known to be carried by Langerhans' cells to the draining lymph node, the reduction in Ia^+ cells after hapten is applied may be due, at lease in part, to an absolute loss of Langerhans' cells due to emigration from the epidermis. We wondered whether TNF-α (and UVB radiation) might have the capacity to alter surface marker expression on Langerhans' cells, but without causing the cells to migrate away from the skin. To test this possibility, TNF-α (50 ng) or PBS was injected intradermally into body wall skin of BALB/c mice. Five minutes later the epidermal surface overlying the injection sites was painted with DNFB (125 or 40 μg). Skin was removed 24 hr later and the epidermis was assayed for density of Ia^+ cells. The results are presented in Table 1. DNFB (40 and 125 μg) elicited a significant and persistant reduction (>55%) of Ia^+ cells that was still evident 24 hr later (PBS control). Pretreatment of skin with TNF-α prior to DNFB application mitigated the hapten-induced effect, since many more Langerhans' cells (>80%) were

evident in TNF-α-treated skin 24 hr after hapten application. These results provide circumstantial evidence that local TNF-α may prevent Langerhans' cells from migrating from the epidermis in response to a hapten stimulus. We believe that this effect of TNF-α on Langerhans' cells may explain the paradoxical effects of this cytokine (and presumably the effects of acute low-dose UVB radiation) on the induction and expression of contact hypersensitivity.

DISCUSSION

These studies demonstrate that enhanced expression of contact hypersensitivity on the ears of specifically sensitized mice can be a consequence of direct intrapinnal injection of TNF-α as well as of exposure of ears to acute low-dose UVB radiation. Circumstantial evidence that TNF-α mediates the UVB-induced effect has been provided by the finding that systemic administration of neutralizing anti-TNF-α prevented UVB treatment from enhancing CH expression in UVB-susceptible C57BL/6 mice. We are aware that our results differ from those reported recently by Piguet *et al.*[10] These investigators claimed that anti-TNF antibodies prevented the expression of CH elicited in sensitized mice by trinitrochlorobenzene. In comparing that study with ours, we note differences in the strains of mice studied (they used CBA, we used BALB/c, C3H, C57BL/6), the hapten (they used TNCB, we used DNFB), and the anti-TNF-α antibodies. Moreover, the Piguet *et al.* paper does not indicate the route of an anti-TNF antibody administration. One or more of these factors could account for the disparity between our results and those of Piguet *et al.* Since our studies have been conducted in numerous genetically different strains of mice, we feel confident in concluding that locally injected TNF-α amplifies the intensity of CH elicited by DNFB in specifically sensitized mice.

It is paradoxical that UVB radiation can cause exaggerated expression of CH since low-dose UVB radiation has been found to impair the induction of contact hypersensitivity to epicutaneously applied hapten.[1,2] Since our findings reveal that UVB radiation of mouse ears has considerably less effect on local Langerhans' cells (approximately 50% decrease of Ia^+ cells) than radiation of body wall skin (usually >90% decrease), and since intradermal injection of TNF-α achieves only a minor reduction (30%) in density of local epidermal Ia^+ cells[4], we have investigated whether the capacity of UVB and TNF-α to enhance CH expression is dependent upon a common mode of action on Langerhans' cells.

At a theoretical level, enhanced expression of CH could result from (a) an increase in amount of antigen in the challenge application and/or an increase in the local capacity of epidermal cells to present antigen to sensitized T cells; (b) an increase in the number and/or reactivity of effector T cells that reach or are recruited to the challenge site; (c) an increase in local non-specific dermal inflammation. Concerning the last possibility, the acute low-dose UVB regimen used in our experiments causes a mild inflammation in the dermis and induces hyperkeratotic changes in the epidermis (data not shown). Thus, UVB radiation could enhance CH expression merely by elicitation of inflammation at the site. However, this cannot explain the enhancing properties of ID-injected TNF-α since the dose of TNF-α employed (50 ng) in our experiments caused neither clinical nor microscopic evidence of inflammation (data not shown). Moreover, this dose is less than the minimum dose of TNF-α found by Sharpe *et al*[6] to induce microscopically detectable inflammation in mouse skin. We do not therefore, favour the view that enhanced CH expression following exposure to UVB and TNF-α results primarily from the creation of a local inflammatory response prior to hapten application.

As mentioned previously, TNF-α is an enormously pleitropic cytokine. It has the capacity to alter the endothelial surfaces of vessels by up-regulating expression of cell adhesion molecules that serve as ligands for circulating lymphocytes, monocytes and granulocytes.[11] In addition, Norris *et al.* have recently reported that UVB radiation, TNF-α and interferon-gamma (IFN-γ) induce the expression of ICAM-1 on keratinocytes.[12] Interestingly, in the Norris study the onset of UVB-induced ICAM-1 expression was delayed by 24 hr after exposure. These properties of TNF-α and UVB radiation lead to the possibility that the enhanced expression of CH induced by these agents may relate, on the one hand, to increased recruitment of effector T cells and inflammatory cells through the TNF-α-modified dermal microvasculature, and, on the other hand, to induced expression of cell adhesion molecules on keratinocytes which could have the effect of promoting leucocyte migration into the epidermis.

TNF-α is one of two cytokines that participate in the phenomenon of macrophage migration inhibition.[13] Macrophage migration inhibitory factor (MIF) is thought to represent the combined actions of TNF-α and IFN-γ. This property of TNF-α may be particularly pertinent to the ability of the cytokine to enhance CH expression. Our experiments have demonstrated that if TNF-α was injected locally immediately prior to hapten application significantly more Ia^+ cells were present in the epidermis 24 hr later. Since there is good circumstantial evidence to suggest that the dramatic reduction in Ia^+ cells in epidermis that has been painted with hapten may be due to emigration of Langerhans' cells from the site,[14-16] we believe that TNF-α acts to prevent that emigration. If this is the relevant effect of TNF-α on the enhancement of CH expression by UVB and by TNF-α itself, then we would speculate that TNF-α enhances CH expression by retaining hapten-bearing Langerhans' cells at the challenge site, thereby increasing the local density of antigen-presenting cells (and presumably antigen) for activation of effector T cells. The finding of Norris *et al.*[12] that expression of ICAM-1 on keratinocytes is eventually up-regulated after exposure to UVB is consistent with this view.

Since UVB radiation is chiefly, but not exclusively, absorbed within the epidermis, the tendency is to consider only the effects of UVB radiation (and TNF-α) on epidermal cells as the reason for enhanced expression of CH. However, CH reactions take place simultaneously in *both* the epidermal and dermal compartments, and a small portion of UVB radiation does reach the upper dermis.[17] In fact, in our experiments TNF-α was injected *intradermally* to achieve the CH-enhancing effect. Therefore, we can hardly exclude a dermal locus of action for TNF-α (and UVB) in considering the exaggerated CH response. It is probable that TNF-α-induced up-regulation of cell adhesion molecules on dermal endothelial cells promotes emigration of blood-borne cells that participate in contact hypersensitivity responses, and it is possible that TNF-α-induced activation of dermal macrophages, perhaps with the subsequent release of other proinflammatory cytokines [interleukin (IL)-6, IL-1, etc.], is also relevant.

Having rationalized a role for TNF-α (and UVB via local release of TNF-α) in enhanced expression of CH, the paradoxical ability of TNF-α (and UVB radiation in susceptible strains of mice) to impair the induction of CH must be addressed. Most of the proinflammatory actions of TNF-α already mentioned help to explain enhanced induction of CH, but they do not so easily explain why TNF-α impairs the induction of CH. To help explain this paradox, we draw upon the finding that the density of Ia^+ epidermal cells 24 hr after ID injection of TNF-α and application of DNFB is significantly greater than that in skin injected with PBS prior to hapten application. We interpret this result to mean that TNF-α can immobilize Langerhans' cells (at least transiently) within the epidermis, much as MIF can immobilize macrophages from migrating. If that is the case, then it is this effect of TNF-α on epidermal Langerhans' cells that could account for the ability of this cytokine (a) to impair CH induction—since hapten-bearing Langerhans' cells are prevented from migrating to the regional lymph node where initial activation of unprimed T cells must occur, and (b) to enhance CH expression—since retention of hapten-bearing Langerhans' cells exaggerates temporally the antigen-presenting capabilities of the epidermis to circulating CH effector cells. This contention, that the paradoxical effects of TNF-α on CH induction and expression are dictated by its effects on Langerhans' cells, is supported by our recent report that intradermally injected TNF-α prevents CH induction if DNFB is painted epicutaneously, but not if the hapten is injected directly into the dermis.[18]

If this interpretation is correct—that TNF-α and UVB radiation (indirectly) immobilize hapten-bearing Langerhans' cells in the epidermis during most of the 24 hr after skin painting with hapten, the failure of CH to be induced implies that the hapten-bearing cells, that are responsible for initiating T-cell activation in the draining lymph node, do not reach that node in sufficient numbers during this critical time. In fact, evidence suggests that if this early opportunity to induce T-cell activation is missed, sensitization completely fails, and tolerance ensues instead. The classic experiments of Macher and Chase[19] yielded similar results and conclusions, since in their hands excision of the hapten application site within 24 hr prevented CH induction, but not tolerance. We have reported comparable results recently from experiments in mice.[20] It is relevant and interesting that excision of the site of hapten application within 1 hr (as well as prior exposure of skin to acute low-dose UVB radiation), not only fails to permit sensitization, but leads to the induction of hapten-specific unresponsiveness. If enhanced expression of CH following UVB radiation or ID TNF-α is also due primarily to changes wrought among epidermal Langerhans' cells, then retention of significant numbers of these cells at the challenge site during the first 24 hr post-hapten application suggests that sustained hapten presentation can serve to attract and activate more hapten-specific T cells within the skin than occurs in non-UVB-irradiated (or TNF-α-treated) skin (from which hapten-induced Langerhans' cell emigration is rapid and extensive). This result further suggests that the magnitude of CH responses can be influenced not only by the dose of hapten in the challenge, but by the length of time hapten is retained *in immunogenic fashion* (i.e. on Langerhans' cells) within the epidermis.

While ID TNF-α enhances expression of CH in both UVB-susceptible and UVB-resistant strains of mice, anti-TNF-α antibodies reversed the enhancing effects of UVB radiation *only* in the former. Although our data are insufficient to permit us to explain this difference, a recent report from Fong and Mosmann may be relevant.[21] These investigators have generated Th1 clones from BALB/c and C57BL/6 mice, and examined their relative capacities to mediate delayed hypersensitivity responses. It was found that BALB/c-derived clones relied heavilty upon their own secreted IFN-γ to mediate the DH response, whereas C57BL/6-derived clones were able to mediate DH even when the IFN-γ they secreted was completely neutralized. This finding implies that cell-mediated immune responses in C57BL/6 mice may be less reliant upon IFN-γ and more dependent upon lymphotoxin/TNF-α, than in BALB/c mice. It is possible therefore that the reason anti-TNF-α antibodies are so successful at neutralizing UVB-induced CH enhancement in C57BL/6 mice (but not in BALB/c mice) is that in the former the CH response can be mediated primarily by lymphotoxin and/or TNF-α.

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