Vitamin C Abrogates the Deleterious Effects of UVB Radiation on Cutaneous Immunity by a Mechanism That Does *Not* Depend on TNF-α

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Acute low-dose treatment of murine skin with ultra violet B (UVB) light impairs induction of contact hypersensitivity (CH) to dinitrofluorobenzene (DNFB) in certain inbred strains of mice (termed UVB-susceptible), but not in others (termed UVB-resistant), and promotes tolerance. These deleterious effects of ultraviolet radiation (UVR) are mediated in part by TNF-α, which is released from UVR-exposed epidermal and dermal cells. Because UVR damage to skin has also been ascribed in part to the generation of reactive oxygen intermediates (ROIs) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and singlet oxygen ($^1O_2$), we investigated whether vitamin C (ascorbic acid), which can nullify ROIs, prevents the deleterious effects of UVR on the cutaneous immune system. We found that epicutaneous application of vitamin C (10% L-ascorbic acid solution) abrogated the deleterious effects of acute low-dose UVR on induction of CH and prevented the induction of tolerance. Vitamin C, however, did not reverse the effects of TNF-α on CH induction and tolerance. These results indicate that (i) ROIs generated intracutaneously by UVR contribute to the impaired ability of exposed skin to support the induction of CH and to promote the induction of tolerance and (ii) these effects are *not* dependent on TNF-α. *Key words: reactive oxygen intermediates/skin cancer. J Invest Dermatol 109:20–24, 1997*

The vast majority of human skin cancers are promoted by ultraviolet radiation (UVR; Green *et al*, 1976; Vitaliano and Urbach, 1980). Among the components of ultraviolet light, ultraviolet B light (UVB) has been implicated in the process that leads to skin cancers (Vitaliano and Urbach, 1980). In animal models, chronic exposure of mice to high doses of UVR has been found to induce skin cancers (Fox *et al*, 1980; Daynes *et al*, 1981). Moreover, these chronically irradiated mice (i) failed to reject syngeneic UVB-induced tumors (Kripke and Fisher, 1976), (ii) could not be sensitized by reactive haptens (Fox *et al*, 1980), and (iii) displayed systemic tolerance (Fisher and Kripke, 1977).

We have used an acute low-dose UVR protocol (400 J per m^2 per d for 4 consecutive days) to explore the molecular and genetic factors responsible for the deleterious effects of UVR on cutaneous immunity (Toews *et al*, 1980). After four consecutive days of UVR, contact hypersensitivity (CH) to reactive haptens, such as dinitrofluorobenzene (DNFB), is impaired in some strains of mice (UVB-susceptible, UVB-S), but not others (UVB-resistant; Streilein and Bergstresser, 1988). The failed induction of CH in UVB-S mice appears to depend on tumor necrosis factor-α (TNF-α; Yoshikawa and Streilein, 1990), *cis*-urocanic acid (Kurimoto and Streilein, 1992), and α-melanocyte-stimulating hormone (Shimizu and Streilein, 1994a), which are generated in UVB-radiated skin. In addition, acute low-dose UVR of skin also permits hapten to induce specific tolerance by mechanisms independent of TNF-α and α-melanocyte-stimulating hormore (Shimizu and Streilein, 1994a, 1994b). Actually, human beings resemble inbred strains of laboratory mice in that some individuals are UVB-S, whereas others are UVB-resistant (Yoshikawa *et al*, 1990). Thus, UVB susceptibility may be a risk factor for the development of skin cancers (Yoshikawa *et al*, 1990).

Recently, it has been appreciated that UVR-induced damage to skin is mediated in part by reactive oxygen intermediates (ROIs), including superoxide anion, hydrogen peroxide, hydroxyl radical, and singlet oxygen (Black, 1987). Acute UVR to skin leads to intracutaneous production of ROIs (Norins, 1962; Pathak and Stratton, 1968; Nishi *et al*, 1991; Ogura *et al*, 1991; Jurkiewicz and Buettner, 1994) and decreases skin anti-oxidants, including vitamin C (Shindo *et al*, 1993). Vitamin C is a well known ubiquitous anti-oxidant and has a multiplicity of anti-oxidant properties (Halliwell *et al*, 1992; Rice-Evans and Diplock, 1993). The ability of vitamin C to show anti-oxidant properties is related to its rapid reaction with many ROIs and to the fact that the resulting semidehydroascorbate radical is poorly reactive (Halliwell *et al*, 1992). Epicutaneous application of vitamin C to UVR-exposed skin has been shown to overcome the harmful effects of ROIs, including the reduction of UVB-induced erythema and sunburn-cell formation (Darr *et al*, 1992; Werninghaus, 1995) and protection from

Manuscript received April 18, 1996; revised March 19, 1997; accepted for publication March 28, 1997.

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Abbreviations: UVR, ultraviolet B radiation; UVB-S, ultraviolet B light-susceptible; CH, contact hypersensitivity; ROI, reactive oxygen intermediate.

0022-202X/97/$10.50

UVR-induced skin cancer (Dunham *et al*, 1982). In this report, we tested the hypothesis that topical application of vitamin C can prevent the deleterious effects of UVR/TNF-α on the cutaneous immune system.

MATERIALS AND METHODS

Mice Adult C3H/HeN mice (8–12 wk of age) were purchased from Taconic (Germantown, NY). C3H/HeN mice are designated as a UVB-S strain (Streilein and Bergstresser, 1988). Experimental procedures were carried out with the animals under general anesthesia achieved by intraperitoneal injection of pentobarbital. Each control or experimental panel consisted of five mice. All experiments were repeated at least twice.

Reagents L-Ascorbic acid (A.C.S. reagent) was purchased from Aldrich (Milwaukee, WI). Experiments used a 10% (wt/vol) L-ascorbic acid solution in 20% (vol/vol) propylene glycol with 0.5% hydroxypropylcellulose incorporated as thickener. This solution was stable for 1 wk as measured spectrophotometrically (Darr *et al*, 1992). 2,4-Dinitro-1-fluorobenzene (DNFB) was purchased from Sigma (St. Louis, MO). Recombinant mouse TNF-α was purchased from Genzyme (Cambridge, MA).

UVR Dry shaved abdominal skin was exposed to UVB from a bank of four FS-20 fluorescent lamps with a tube-to-target distance of 46 cm, as previously described (Streilein and Bergstresser, 1988). 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 a SEE 240 UVB photodetector, these lamps delivered an average flux of 1.7 J per m^2 per s. Mice were exposed to UVB daily for 4 consecutive days (400 J per m^2 per d). Within 1 h of the final exposure, DNFB was applied to the irradiated site.

Induction and Assay of CH and Tolerance Twenty-five microliters of a 0.5% solution of DNFB (185 μg) in acetone were applied epicutaneously to shaved abdominal skin on day 0. CH was elicited 5 d later by challenging one ear of each mouse with 20 μl of 0.05% DNFB (14.8 μg) in acetone. Ear thickness was measured with an engineer's micrometer 24 and 48 h after challenge and compared with ear thickness prior to challenge. To determine whether tolerance had been induced, mice that initially received DNFB on treated body wall skin received a second sensitizing dose of DNFB (185 μg) on freshly shaved skin surfaces distant from the original site. The ears of the mice were challenged 5 d later and measured 24 and 48 h later, as described above.

Vitamin C and Vehicle Treatment Ten microliters of vitamin C or vehicle were applied epicutaneously to shaved abdominal skin (1 cm^2). The treated area was covered by a Hill Top Chamber (Hilltop Research Inc., Cincinnati, OH) for 3 h, after which it was detached.

TNF-α Treatment Two hundred microliters of phosphate-buffered saline containing 2000 units of TNF-α with 0.1% bovine serum albumin were injected intradermally into shaved abdominal skin immediately before epicutaneous application of DNFB on day 0. This dose of TNF-α was based in part upon the report of Sharpe *et al* (1988) indicating that $<3 \times 10^6$ U of recombinant human TNF-α injected into the footpad of mice induced only mild or no evidence of inflammation.

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 at $p < 0.05$.

RESULTS

Induction of CH Is Not Impaired by Epicutaneous Vitamin C or Its Vehicle We first examined whether vitamin C and/or vehicle when applied epicutaneously impaired the induction of CH by DNFB painted on the same site. Panels of C3H/HeN mice received 10 μl of vitamin C or vehicle on dry shaved abdominal skin. A Hill Top Chamber was used to cover the treated area for 3 h, and then it was removed. These treatments were repeated at the same site for four consecutive days. Twenty-five microliters of 0.5% DNFB (185 μg) were applied epicutaneously to vitamin C (or vehicle)-treated area within 1 h after the final chamber detachment. Positive control mice, treated with neither vitamin C nor vehicle, received the same amount of DNFB on shaved abdominal skin. Five days later, the right ear of each mouse was painted with 20 μl of 0.05% DNFB (14.8 μg), and ear swelling was measured after 24 and 48 h. As shown in **Fig 1**, positive control mice displayed significant ear swelling responses compared with negative control mice. Similarly, both vitamin C-treated and vehicle-treated mice displayed ear swelling responses comparable in magnitude to positive controls ($p > 0.05$). We conclude that neither vitamin C nor vehicle influences the ability of skin to support the induction of CH.

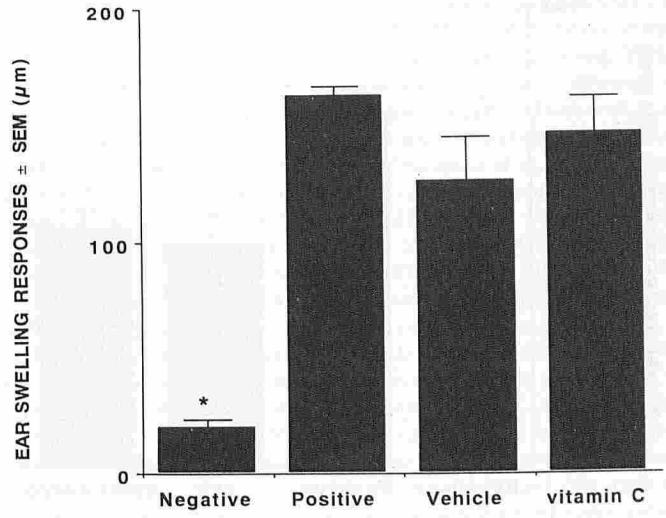

Figure 1. Induction of CH is *not* impaired by epicutaneous vitamin C or its vehicle. Panels of C3H/HeN mice were treated epicutaneously with vitamin C or vehicle for 3 h. Twenty-five microliters of 0.5% DNFB were applied epicutaneously to vitamin C- or vehicle-treated skin within 1 h after the final vitamin C or vehicle treatment. Ears were challenged 5 d later with 20 μl of 0.05% DNFB (14.8 μg) and measured 24 and 48 h later after the challenge. Mean ear swelling responses significantly less than DNFB-sensitized mice (positive control) are indicated with an * (*$p < 0.0001$). *Error bars,* SEM (n = 5).

UVR-impaired Induction of CH Is Not Abrogated by Epicutaneous Vehicle Acute low-dose of UVR impairs the induction of CH in some strains of mice (UVB-S), but not others (UVB-resistant) (Streilein and Bergstresser, 1988). To determine whether the vehicle alone could prevent the deleterious effects of UVR on the induction of CH in UVB-S mice, panels of mice received UVR exposure with or without epicutaneous vehicle treatment for four consecutive days. Twenty-five microliters of 0.5% DNFB (185 μg) were applied epicutaneously to vehicle-treated or nontreated skin within 1 h after the final UVR. Positive control of mice received the same amount of DNFB for sensitization. Ears were challenged 5 d later and measured 24 and 48 h later after the challenge. As revealed in **Fig 2**, mice that received UVR without vehicle treatment displayed reduced ear swelling responses compared with positive control mice. Similarly, mice treated with vehicle prior to each UVR exposure also showed reduced ear swelling responses. We conclude that epicutaneous application of vehicle does not prevent the deleterious effects of UVR on the induction of CH.

Epicutaneous Vitamin C Abrogates UVR-impaired Induction of CH To test the hypothesis that vitamin C can prevent the deleterious effects of UVR on the induction of CH in UVB-S mice, panels of C3H/HeN mice were treated epicutaneously with vitamin C or vehicle 3 h before each UVR exposure on four consecutive days. Twenty-five microliters of 0.5% DNFB (185 μg) were applied epicutaneously to vitamin C- or vehicle-treated skin within 1 h of the final UVR. Ears were challenged 5 d later and measured 24 and 48 h later after the challenge. As revealed in **Fig 3**, mice that received UVR preceded by vehicle treatment displayed reduced ear swelling responses compared with positive control mice that received vehicle treatment without UVR. By contrast, mice treated with vitamin C prior to each UVR exposure developed significant ear swelling responses indistinguishable from positive control. Multiple repetitions of these experiment produced very similar results. Therefore, we conclude that epicutaneous

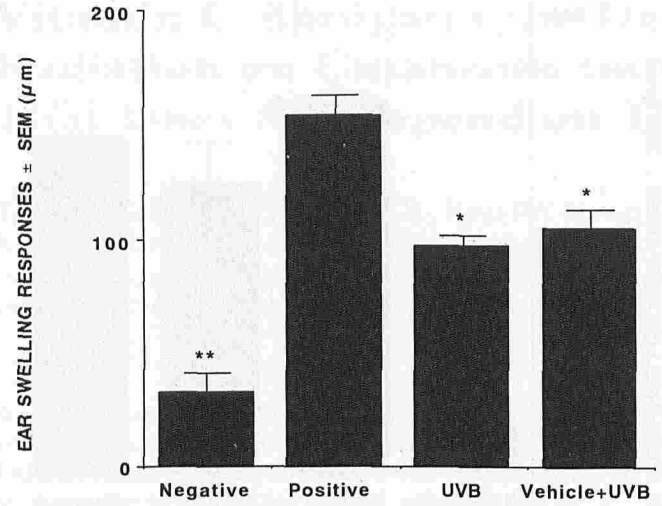

Figure 2. UVR-impaired induction of CH is not abrogated by epicutaneous vehicle. Panels of mice received UVR exposure with or without epicutaneous vehicle treatment for 4 consecutive days. Twenty-five microliters of 0.5% DNFB (185 μg) were applied epicutaneously to vehicle-treated or nontreated skin within 1 h after the final UVR. Positive control mice also received same amount of DNFB for sensitization. Ears were challenged 5 d later with 20 μl of 0.05% DNFB (14.8 μg) and measured 24 and 48 h later after the challenge. Mean ear swelling responses significantly less than DNFB-sensitized vehicle-alone–treated mice (positive control) are indicated with * (*$p < 0.01$; **$p \leq 0.0001$). *Error bars,* SEM (n = 5).

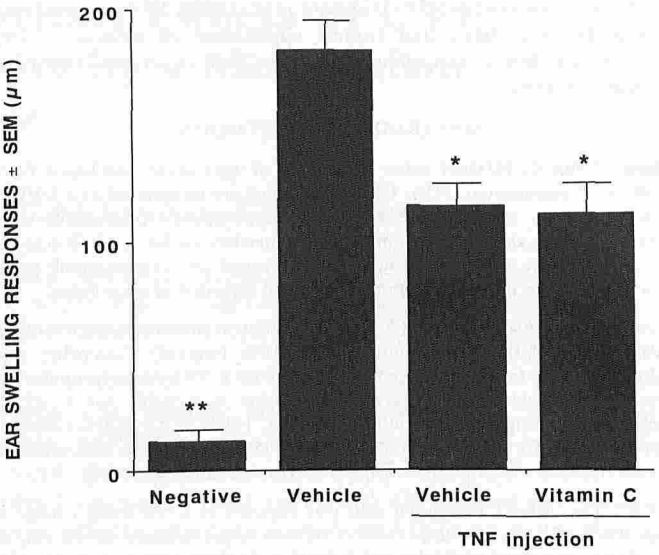

Figure 4. Epicutaneous vitamin C fails to abrogate TNF-α–impaired induction of CH. Panels of mice were treated epicutaneously with vitamin C or vehicle for 3 h for 4 consecutive days. Three hours after the final vitamin C (or vehicle) application, TNF-α (2000 units) was injected intradermally into the treated area followed by sensitization with 25 μl of 0.5% DNFB (185 μg). Ears were challenged 5 d later and measured 24 and 48 h later after the challenge. Mean ear swelling responses significantly less than vehicle-treated mice are indicated with * (*$p < 0.01$; **$p < 0.0001$). *Error bars,* SEM (n = 5).

application of vitamin C can prevent the deleterious effects of UVR on the induction of CH.

Epicutaneous Vitamin C Fails to Abrogate TNF-α-impaired Induction of CH We have previously reported that TNF-α is a major mediator of the deleterious effects of UVR on induction of CH (Yoshikawa and Streilein, 1990). Because ROIs have been shown to promote TNF-α production (Chaudhri and Clark, 1989; Hallahan *et al*, 1989) and TNF-α can generate ROIs (Klebanoff *et al*, 1986; Larrick *et al*, 1987; Nathan, 1987), we hypothesized that ROIs impair CH induction after UVR via a TNF-α-dependent mechanism. To test this hypothesis, panels of mice were treated epicutaneously with vitamin C or vehicle for four consecutive days. Subsequently, TNF-α (2000 units) was injected intradermally into vitamin C- or vehicle-treated skin 3 h after the final vitamin C or vehicle treatment. Immediately after the TNF-α injection, 25 μl of 0.5% DNFB (185 μg) were applied epicutaneously to TNF-α–treated skin. Ears were challenged 5 d later and measured 24 and 48 h after the challenge. The results in **Fig 4** indicate that vehicle-treated mice that then received a TNF-α injection acquired weak CH compared with positive control mice that received vehicle treatment without a TNF-α injection. Similarly, vitamin C-treated mice that received injection of TNF-α also displayed feeble ear swelling responses. We conclude that epicutaneous vitamin C treatment does not abrogate the deleterious effects of TNF-α on induction of CH. This result suggests that UVB-induced ROIs may impair induction of CH directly rather than via the production of TNF-α.

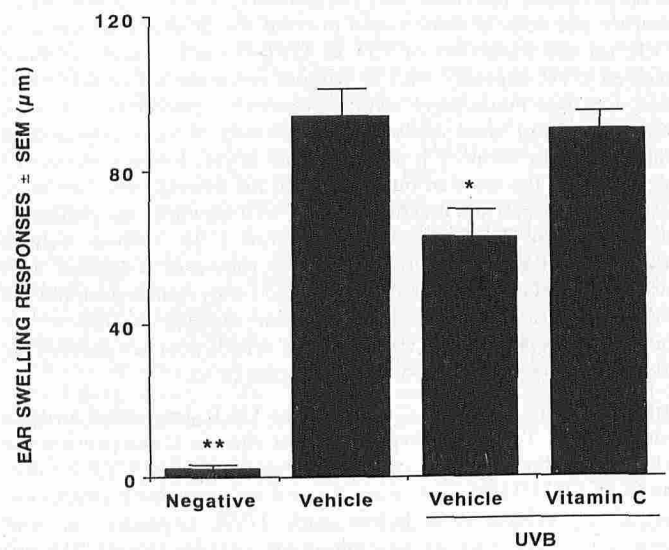

Figure 3. Epicutaneous vitamin C abrogates UVR-impaired induction of CH. Panels of C3H/HeN mice were treated epicutaneously with vitamin C or vehicle 3 h before each UVR (400 J per m^2 per d) for 4 consecutive days. Twenty-five microliters of 0.5% DNFB were applied epicutaneously to vitamin C- or vehicle-treated skin within 1 h after the final UVR exposure. Ears were challenged 5 d later with 20 μl of 0.05% DNFB (14.8 μg) and measured 24 and 48 h later after the challenge. Mean ear swelling responses significantly less than DNFB-sensitized vehicle-alone–treated mice (positive control) are indicated with * (*$0.01 < p < 0.025$; **$p < 0.0001$). *Error bars,* SEM (n = 5).

Epicutaneous Vitamin C Prevents UVR-induced Tolerance Acute low-dose UVR induces hapten-specific tolerance (Elmets *et al*, 1983). To test whether epicutaneous application of vitamin C prevents UVR-induced tolerance, panels of mice were treated epicutaneously with vitamin C or vehicle prior to each UVR exposure for four consecutive days. Twenty-five microliters of 0.5% DNFB (185 μg) were applied epicutaneously to vitamin C- or vehicle-treated skin within 1 h of the final UVR exposure. Positive control mice (designated 0, 14) received epicutaneous applications of vehicle before DNFB treatment. Fourteen days later, 25 μl of 0.5% DNFB (185 μg) were applied epicutaneously to the nonirradiated shaved skin of the back. A second positive control panel (designated 14) received this sensitizing dose of hapten without any prior treatment. Ears of all mice, plus a negative control group, were challenged 5 d later with 20 μl of 0.05% DNFB (14.8 μg) in acetone and measured 24 and 48 h later. The results of a representative experiment are presented in **Fig 5**. Positive control mice

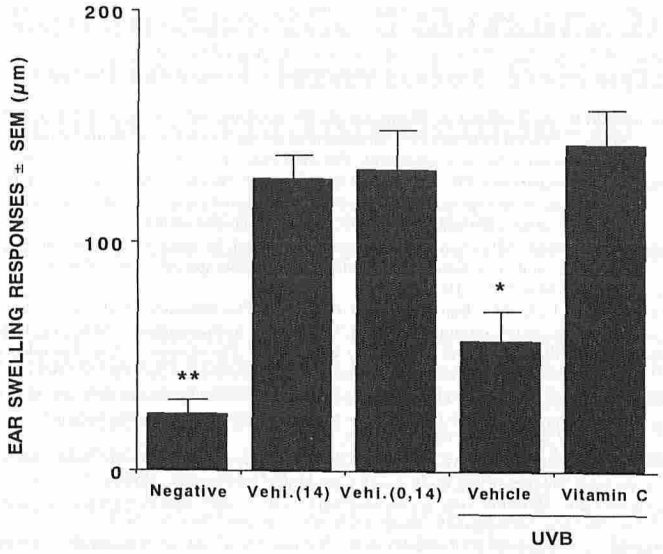

Figure 5. Epicutaneous vitamin C prevents UVR-induced tolerance. Panels of mice were treated epicutaneously with vitamin C or vehicle 3 h before each UVB exposure for 4 consecutive days. Twenty-five microliters of 0.5% DNFB (185 μg) were applied epicutaneously to vitamin C- or vehicle-treated skin within 1 h after the final UVR. Fourteen days later, 25 μl of 0.5% DNFB (185 μg) were applied epicutaneously to nontreated (back) skin. Ears were challenged 5 d later and measured 24 and 48 h later after the challenge. Mean ear swelling responses significantly less than positive controls are indicated with * (*$p < 0.05$; **$p < 0.01$). *Error bars,* SEM (n = 5).

(designated 0, 14), as well as those of second positive control mice (designated 14), had significant ear swelling responses compared with negative control mice ($p < 0.01$). Mice that received UVR preceded by vehicle treatment developed feeble ear swelling responses, i.e., tolerance. Mice that received vitamin C treatment prior to UVR exposure, however, displayed intense ear swelling responses, similar to those of positive control groups. We conclude that (i) ear swelling responses of positive control (designated 0, 14) were similar to those of second positive control (designated 14) and (ii) epicutaneous vitamin C prevented UVR-induced tolerance.

Epicutaneous Vitamin C Fails to Prevent TNF-α-induced Tolerance Although UVR-induced tolerance is not mediated by TNF-α, intradermal injection of TNF-α prior to hapten painting is able to induce hapten-specific tolerance (Shimizu and Streilein, 1994b). To test whether epicutaneous application of vitamin C can prevent TNF-α–induced tolerance, panels of mice were treated epicutaneously with vitamin C or vehicle for four consecutive days. TNF-α (2000 units) was injected intradermally into vitamin C- or vehicle-treated skin 3 h after the final vitamin C or vehicle treatment. Immediately after the TNF-α injection, 25 μl of 0.5% DNFB (185 μg) were applied epicutaneously to TNF-α–treated skin. Positive control mice (designated 0, 14) received epicutaneous applications of vehicle before DNFB treatment. Fourteen days later, 25 μl of 0.5% DNFB (185 μg) were applied epicutaneously to nonirradiated shaved skin of the back. Ears of all mice, plus a negative control group, were challenged 5 d later with 20 μl of 0.05% DNFB (14.8 μg) in acetone and measured 24 and 48 h later. The results of a representative experiment are presented in **Fig 6**. Mice that received a TNF-α injection preceded by vehicle treatment developed feeble ear swelling responses, i.e., tolerance. Mice that received vitamin C treatment prior to TNF-α injection also displayed reduced ear swelling responses, similar to those of vehicle/TNF-α–treated group. Thus, epicutaneous vitamin C did not prevent TNF-α–induced tolerance.

DISCUSSION

UVR to the skin leads to the intracutaneous production of ROIs (Norins, 1962; Pathak and Stratton, 1968; Nishi *et al*, 1991; Ogura *et al*, 1991). UVR also leads to the intracutaneous production of various cytokines (Schwarz *et al*, 1994). ROIs can produce major interrelated derangements of cell metabolism, including DNA strand breakage, increase of intracellular free Ca^{2+}, damage to membrane ion transporters, peroxidation of lipids (Halliwell *et al*, 1992), and the generation of inflammatory cytokines, such as TNF-α (Chaudhri and Clark, 1989; Hallahan *et al*, 1989). Conversely, TNF-α, which is thought to be one of the important mediators of the deleterious effects of UVR on CH induction (Yoshikawa and Streilein, 1990), has been shown to induce formation of ROIs (Klebanoff *et al*, 1986; Larrick *et al*, 1987; Nathan, 1987). Thus, the cutaneous immune system can be disturbed directly by UVR itself, and indirectly by UVR-induced ROIs, and cytokines.

Vitamin C has long been regarded as an anti-oxidant (Halliwell *et al*, 1992) and has been shown to scavenge singlet oxygen and to react rapidly with superoxide, hydroxyl radical, and hydrogen peroxide (Vessey, 1993). Moreover, vitamin C has been shown to reduce the vitamin E free radical back to native vitamin E and, thus, to assist vitamin E in scavenging membrane-bound free radicals (Vessey, 1993). Thus, vitamin C has a multiplicity of anti-oxidant properties (Halliwell *et al*, 1992). We now report that vitamin C can prevent the deleterious effects of UVR on the induction of CH and that vitamin C prevents UVB-induced tolerance. It has been shown that UVR to skin leads to the intracutaneous production of ROIs (Norins, 1962; Pathak and Stratton, 1968; Nishi *et al*, 1991; Ogura *et al*, 1991) and to a reduction of endogenous anti-oxidants (Shindo *et al*, 1993). These factors—increased ROIs and decreased anti-oxidants—are important to the mechanisms by which UVR damages skin, because it has been reported that pretreatment of skin with epicutaneous vitamin C overcomes the deleterious effects of UVR-induced sunburn-cell formation (Darr *et al*, 1992). Moreover, Caceres-Dittmar *et al* (1995) reported that exposure of dendritic cells to hydrogen peroxide mimics the deleterious effects of UVR by inhibiting antigen-presenting function *in vitro*. Our results suggest that disruption of cutaneous immunity must now be included among ROIs-dependent UVR damage.

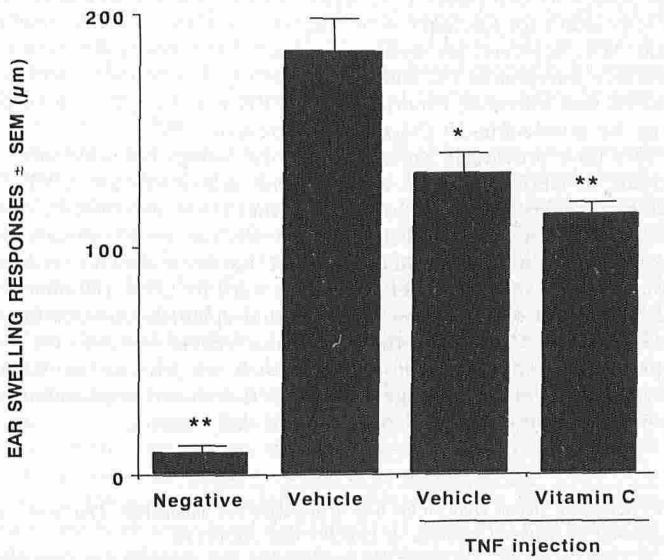

Figure 6. Epicutaneous vitamin C fails to prevent TNF-α–induced tolerance. Panels of mice were treated epicutaneously with vitamin C or vehicle for 3 h for 4 consecutive days. Three hours after the final vitamin C (or vehicle) application, TNF-α (2000 units) was injected intradermally into the treated area followed by sensitization with 25 μl of 0.5% DNFB (185 μg). Fourteen days later, 25 μl of 0.5% DNFB (185 μg) were applied epicutaneously to nontreated (back) skin. Ears were challenged 5 d later and measured 24 and 48 h later after the challenge. Mean ear swelling responses significantly less than vehicle-treated mice are indicated with asterisks (*$p < 0.05$; **$p < 0.01$). *Error bars,* SEM (n = 5).

UVR to skin leads to various skin changes, including DNA damage, induction of cytokine production, inflammatory cell recruitment, and oxidative stress. Much remains to be learned known about the relationship between oxidative stress and other UVB-induced changes. Yoshikawa *et al* (1990) showed that the deleterious effects of UVR on the induction of CH are mediated in part by TNF-α, which is produced in UVB-radiated skin. As discussed above, ROIs have been shown to cause epidermal cells to release TNF-α (Chaudhri and Clark, 1989; Hallahan *et al*, 1989), and TNF-α has been reported to induce the generation of ROIs (Klebanoff *et al*, 1986; Larrick *et al*, 1987; Nathan, 1987). It is interesting to explore whether UVB-induced ROIs mediate their effects on cutaneous immunity through TNF-α. We showed that the deleterious effects of TNF-α on induction of CH are not reversed by epicutaneous vitamin C and, presumably therefore, are not mediated by ROIs. Chaudhri and Clark (1989) reported that hydrogen peroxide and periodate were able to enhance lipopolysaccharide-induced TNF-α production by monocytes, whereas butylated hydroxyanisole, which is an anti-oxidant, suppressed TNF-α synthesis. Moreover, oxidative stress appears to make monocytes produce interleukin-1, which is a proinflammatory cytokine (Kasama *et al*, 1989). Although we have shown that vitamin C prevents the deleterious effects of UVR, but not those of TNF-α, we can not exclude the possibility that UVR-induced ROIs lead to the intracutaneous production of TNF-α, which, in turn, impairs the cutaneous immune system. Because vitamin C works as an anti-oxidant, it may very well prevent ROIs from inducing TNF-α production. Nonetheless, we must also implicate a non-TNF-α–dependent mechanism for some of the deleterious effects of UVR-induced ROIs on cutaneous immunity.

It is of considerable interest that vitamin C prevented UVR-induced tolerance but not TNF-α-induced tolerance. This outcome strongly suggests that the pathway to tolerance after UVR is independent of TNF-α but is related to ROIs generation. The tolerance induced by local treatment with *cis*-urocanic acid is also not reversed by anti-TNF-α antibodies (Shimizu and Streilein, 1994b). Perhaps, UVR-dependent conversion of *trans*-urocanic acid to *cis*-isomer is related to ROIs formation. We are considering the possibility that interaction between *cis*-urocanic acid and ROIs is responsible for tolerance when hapten is applied to UVR-treated skin. We, however, are well aware that UVR induces skin cells to produce interleukin-10, and our laboratory has recently demonstrated that tolerance resulting from UVR is mediated, at least in part, by interleukin-10 (Niizeki and Streilein, 1997).

We have previously shown that human beings resemble inbred strains of laboratory mice in that some individuals are UVB-S, whereas others are UVB-resistant, and that UVB susceptibility may be a risk factor for the development of skin cancers (Yoshikawa *et al*, 1990). Because ingestion of vitamin C has been shown to reduce the incidence of skin cancer induced in mice by UVR (Dunham *et al*, 1982) and our experiments show that epicutaneous treatment with vitamin C prevents the deleterious effects of UVR on the cutaneous immune system of UVB-S mice, we propose that ROIs are involved in the pathogenesis of UVR-induced impairment of cutaneous immunity and UVR-induced skin cancers.

We thank Ms. Ileana Daly for her help in preparing this manuscript. This work was supported by National Institutes of Health Grant AR44130.

REFERENCES

Black HS: Potential involvement of free radical reactions in ultraviolet light-mediated cutaneous damage. *Photochem Photobiol* 46:213–221, 1987

Caceres-Dittmar G, Ariizumi K, Xu S, Tapia FJ, Bergstresser PR, Takashima A: Hydrogen peroxide mediates UV-induced impairment of antigen presentation in a murine epidermal-derived dendritic cell line. *Photochem Photobiol* 62:176–183, 1995

Chaudhri G, Clark IA: Reactive oxygen species facilitate the *in vitro* and *in vivo* lipopolysaccharide-induced release of tumor necrosis factor. *J Immunol* 143:1290–1294, 1989

Darr D, Combs S, Dunston S, Manning T, Pinnell S: Topical vitamin C protects porcine skin from ultraviolet radiation-induced damage. *Br J Dermatol* 127:247–253, 1992

Daynes RA, Bernhard EJ, Gurish MF, Lynch DH: Experimental photoimmunology: immunologic ramifications of UV-induced carcinogenesis. *J Invest Dermatol* 77:77–85, 1981

Dunham WB, Zuckerkandl E, Reynolds R, Willoughby R, Marcuson R, Barth R, Pauling L: Effects of intake of L-ascorbic acid on the incidence of dermal neoplasms induced in mice by ultraviolet light. *Proc Natl Acad Sci USA* 79:7532–7536, 1982

Elmets CA, Bergstresser PR, Tigelaar RE, Wood PJ, Streilein JW: Analysis of the mechanism of unresponsiveness produced by haptens painted on skin exposed to low dose ultraviolet radiation. *J Exp Med* 158:781–794, 1983

Fisher MS, Kripke ML: Systemic alternation induced in mice by ultraviolet light irradiation and its relationship to ultraviolet carcinogenesis. *Proc Natl Acad Sci USA* 74:1688–1692, 1977

Fox IJ, Perry LL, Sy MS, Benacerraf B, Greene MI: The influence of ultraviolet light irradiation on the immune system. *Clin Immunol Immunopathol* 17:141–155, 1980

Green AES, Findley GB, Klenk KF, Wilson WM, Mo T: The ultraviolet dose dependence of non-melanoma skin cancer incidence. *Photochem Photobiol* 24:353–362, 1976

Hallahan DE, Spriggs DR, Beckett MA, Kufe DW, Weichselbaum RR. Increased tumor necrosis factor α mRNA after cellular exposure to ionizing radiation. *Proc Natl Acad Sci USA* 86:10104–10107, 1989

Halliwell B, Gutteridge JMC, Cross CE: Free radicals, antioxidants, and human disease: where are we now? *J Lab Clin Med* 119:598–620, 1992

Jurkiewicz BA, Buettner GR: Ultraviolet light-induced free radical formation in skin: an electron paramagnetic study. *Photochem Photobiol* 59:1–4, 1994

Kasama T, Kobayashi K, Fukushima T, Tabata M, Ohno I, Negishi M, Ide H, Takahashi T, Niwa Y: Production of interleukin 1-like factor from human peripheral blood monocytes and polymorphonuclear leukocytes by superoxide anion: the role of interleukin 1 and reactive oxygen species in inflamed sites. *Clin Immunol Immunopathol* 53:439–448, 1989

Klebanoff S, Vadas MA, Harlan JM, Sparks LH, Gamble JR, Agosti JM, Waltersdorph AM: Stimulation of neutrophils by tumor necrosis factor. *J Immunol* 136:4220–4225, 1986

Kurimoto I, Streilein JW: *cis*-Urocanic acid suppression of contact hypersensitivity induction is mediated via tumor necrosis factor-α. *J Immunol* 148:3072–3078, 1992

Kripke ML, Fisher MS: Immunologic parameters of ultraviolet carcinogenesis. *J Natl Cancer Inst* 57:211–215, 1976

Larrick JW, Graham D, Toy K, Lin LS, Senyk G, Fendly BM: Recombinant tumor necrosis factor causes activation of human granulocytes. *Blood* 69:640–644, 1987

Nathan CF: Neutrophil activation on biological surfaces: massive secretion of hydrogen peroxide in response to products of macrophages and lymphocytes. *J Clin Invest* 80:1550–1560, 1987

Niizeki H, Streilein JW: Hapten-specific tolerance induced by acute, low-dose ultraviolet B radiation of skin is mediated via Interleukin-10. *J Invest Dermatol* 109:25–30, 1997

Nishi J, Ogura R, Sugiyama M, Hidaka T, Kohno M: Involvement of active oxygen in lipid peroxide radical reaction of epidermal homogenate following ultraviolet light exposure. *J Invest Dermatol* 97:115–119, 1991

Norins AL: Free radical formation in the skin following exposure to ultraviolet light. *J Invest Dermatol* 39:445–448, 1962

Ogura R, Sugiyama M, Nishi J, Haramaki N: Mechanism of lipid radical formation following exposure of epidermal homogenate to ultraviolet light. *J Invest Dermatol* 97:1044–1047, 1991

Pathak MA, Stratton K: Free radicals in human skin before and after exposure to light. *Arch Biochem Biophys* 123:468–476, 1968

Rice-Evans CA, Diplock AT: Current status of antioxidant therapy. *Free Radical Biol Med* 15:77–96, 1993

Schwarz T, Urbanski A, Luger TA: Ultraviolet light and epidermal cell-derived cytokines. In: Luger TA, Schwarz T (eds.). *Epidermal Growth Factors and Cytokines*. Marcel Dekker, New York, 1994, pp 303–324

Sharpe RJ, Margolis RJ, Askari M, Amento EP, Granstein, RD: Induction of dermal and subcutaneous inflammation by recombinant cachectin/tumor necrosis factor (TNFα) in the mouse. *J Invest Dermatol* 91:353–357, 1988

Shimizu T, Streilein JW: Influence of alpha-melanocyte stimulating hormone on induction of contact hypersensitivity and tolerance. *J Dermatol Sci* 8:187–193, 1994a

Shimizu T, Streilein JW: Evidence that ultraviolet B radiation induces tolerance and impairs induction of contact hypersensitivity by different mechanisms. *Immunology* 82:140–148, 1994b

Shindo Y, Witt E, Packer L: Antioxidant defense mechanisms in murine epidermis and dermis and their responses to ultraviolet light. *J Invest Dermatol 100*:260–265, 1993

Streilein JW, Bergstresser PR: Genetic basis of ultraviolet-B effects on contact hypersensitivity. *Immunogenetics* 27:252–258, 1988

Toews GB, Bergstresser PR, Streilein JW: Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. *J Immunol* 124:445–453, 1980

Vessey DA: The cutaneous antioxidant system. In: Fuchs J, Packer L (eds.). *Oxidative Stress in Dermatology*. Marcel Dekker, New York, 1993, pp 81–103

Vitaliano PP, Urbach F: The relative importance of risk factors in nonmelanoma carcinoma. *Arch Dermatol* 116:454–456, 1980

Werninghaus K: Role of antioxidants in reducing photodamage. In: Gilchrest B (ed.) *Photodamage*. Blackwell Science, 1995, pp 249–258

Yoshikawa T, Rae V, Bruins-Slot W, Van den Berg JW, Taylor JR, Streilein JW: Susceptibility to effects of UVB radiation on induction of contact hypersensitivity as a risk factor for skin cancer in humans. *J Invest Dermatol* 95:530–536, 1990

Yoshikawa T, Streilein JW: Genetic basis of the effects of ultraviolet light B on cutaneous immunity: Evidence that polymorphism at the *Tnfa* and *Lps* loci governs susceptibility. *Immunogenetics* 32:398–405, 1990