Deleterious Effects of Cis-Urocanic Acid and UVB Radiation on Langerhans Cells and on Induction of Contact Hypersensitivity Are Mediated by Tumor Necrosis Factor-Alpha

Iwao Kurimoto and J. Wayne Streilein
Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, Florida, U.S.A.

Ultraviolet B (UVB) light disrupts epidermal Langerhans cells (LC) universally and impairs the induction of contact hypersensitivity (CH) to epicutaneously applied haptens in certain strains of mice. Similar effects are observed when tumor necrosis factor-alpha (TNFα) is injected intradermally (ID) in mice. Trans-urocanic acid (UCA), a photoreceptor for UVB radiation, is known to be immunosuppressive. To determine whether cis-UCA is important in the process by which UVB and/or TNFα act in the skin, cis-UCA was injected ID into C57BL/6, C3H/HeN, BALB/c, and C3H/HeJ mice. Whole mounts of epidermis were removed 5 h later and stained immunochemically with anti-Ia antibodies. Microscopy revealed that Ia-bearing LC had lost their dendrites, had rounded up, and were reduced in number in all strains examined. Moreover, when dinitrofluorobenzene (DNFB) was applied epicutaneously to the injected site, induction of CH was grossly impaired. When neutralizing anti-TNFα antibodies were administered intraperitoneally 2 h prior to ID injection of cis-UCA, the deleterious effects on LC and CH induction were largely reversed. These results indicate that the actions of cis-UCA on LC and on CH induction are very similar to those achieved by ID injections of TNFα and by cutaneous exposure to low-dose UVB. Because the effects of UVB radiation and cis-UCA are reversed by anti-TNFα antibodies, we propose that UVB radiation impairs the induction of CH in mice by converting trans-UCA to cis-UCA within the epidermis; cis-UCA in turn causes the local release of TNFα, which thwarts sensitization by its ability to alter the functional program of epidermal Langerhans cells, thereby preventing the induction of CH. *J Invest Dermatol 99:69S–70S, 1992*

Acute, low-dose irradiation of shaved abdominal skin of mice with ultraviolet B (UVB) light profoundly depletes the epidermis of Langerhans cells (LC) in all strains of mice. However, when dinitrofluorobenzene (DNFB) is applied to the irradiated site, contact hypersensitivity (CH) is induced in some mice [termed UVB-resistant (UVB-R)], but not in others [termed UVB-susceptible (UVB-S)] [1,2]. We have recently demonstrated that intradermal (ID) injection of tumor necrosis factor-alpha (TNFα) achieves the same effect on induction of CH as low-dose UVB radiation [3]. Moreover, the deleterious effects of UVB radiation on induction of CH in UVB-S mice can be abolished by systemic administration of neutralizing anti-TNFα antibodies. These findings have led us to postulate that TNFα is an important and critical molecular mediator of the down-regulatory effects of UVB on CH induction. In order for UVB radiation to achieve this biologic effect, it must interact with a photoreceptor molecule that can transduce the signal into cellular effects. Several investigators have claimed that urocanic acid (UCA), the deaminated form of histidine that is present in very high concentration in the superficial epidermis, is the most likely photoreceptor. Exposure of trans-UCA to UVB radiation in vitro converts the compound into its cis-isoform; moreover, exposure of skin to UVB similarly converts trans-UCA to cis-UCA [4–6]. These investigators have also demonstrated that cis-UCA is immunosuppressive when administered to mice systemically, epicutaneously, or intradermally. We have explored the possibility that cis-UCA is involved in the multi-step pathway by which UVB radiation, via TNFα, impairs CH induction in UVB-S mice.

This work was supported in part by USPHS grant AI-22072 and ACS grant PDT-383.

Reprint requests to: Dr. J. Wayne Streilein, Department of Microbiology and Immunology (R-138), University of Miami School of Medicine, P.O. Box 016960, Miami, Florida 33101.

Abbreviations:
BSA: bovine serum albumin
CH: contact hypersensitivity
DNFB: dinitrofluorobenzene
ID: intradermal
IP: intraperitoneal
LC: Langerhans cells
PBS: phosphate-buffered saline
TNFα: tumor necrosis factor-alpha
UCA: urocanic acid
UVB: ultraviolet B
UVB-R: UVB resistant
UVB-S: UVB susceptible

EXPERIMENTS AND RESULTS

Cis-UCA was prepared according to the method of Ross et al [5]. A thin layer of trans-UCA was UVB irradiated for 8 h. High-performance liquid chromatography demonstrated that approximately 50.5% of the UVB-irradiated UCA (UV-UCA) was converted to the cis isoform.

Panels of four different inbred strains of mice received ID injections (200 μl) of trans-UCA, UV-UCA (1 mg/ml), or phosphate-buffered saline (PBS). Five hours later the injected epidermis was painted with 185 μg DNFB. When the pinnae were challenged with 59 μg DNFB 5 d later, ear-swelling responses of UVB-S mice

0022-202X/92/$05.00

Table I. Effects of ID-Injected Urocanic Acid on Contact Hypersensitivity Induction in UVB-Susceptible and UVB-Resistant Mice[a]

Strain	Mean Ear-Swelling Response (% of positive control)		
	Control	Trans-UCA	UV-UCA
C57BL/6	100	83	32[b]
C3H/HeN	100	66[b]	36[b]
BALB/c	100	76[b]	66[b]
C3H/HeJ	100	80	48[b]

[a] UV-UCA or trans UCA was injected ID (200 μg) into sites painted 5 h later with DNFB (185 μg). Mean car challenge responses 5 d later are presented as percent of positive controls in whom DNFB was initially painted on sites into which PBS had been injected ID.

[b] Values significantly lower than positive control (<0.05).

were 32% (C57BL/6) or 36% (C3H/HeN) of PBS-injected, positive controls, and swelling responses of UVB-R mice were 66% (BALB/c) or 48% (C3H/HeJ) of positive controls (see Table I). CH responses of trans-UCA–treated mice were significantly higher than UV-UCA–treated mice, but usually less than positive controls. We conclude that UCA, especially in the cis-isoform, interfered with the induction of CH when DNFB was painted on injected skin. The impairment was greater among UVB-S mice.

Neutralizing anti-TNFα antibodies, which have been used to alleviate the deleterious effects of UVB radiation on CH induction in UVB-S mice, was examined for its potential to reverse the effects of cis-UCA on CH induction. Panels of C57BL/6 mice received intraperitoneal (IP) injections of 2×10^4 units of anti-TNFα antibodies [controls received irrelevant anti–bovine serum albumin (BSA) antibodies]. Two hours later UV-UCA (200 μg) was injected ID, and 5 h thereafter DNFB (185 μg) was painted directly on the injected sites. When ear challenged with hapten 5 d later, the ears of recipients of anti-TNFα antibodies followed by ID UV-UCA displayed vigorous CH (73% of PBS-injected positive control). By contrast, the ears of recipients of anti-BSA antibodies followed by ID UV-UCA displayed feeble CH (32% of positive control). Thus, antibodies that neutralize TNFα robbed cis-UCA of its capacity to impair CH induction, revealing that cis-UCA acts, at least in part, via TNFα.

The reduction in density and change in morphology of LC that occur within UVB-irradiated skin can be reversed by anti-TNFα antibodies [7]. We wished to determine whether cis-UCA caused similar changes in LC, and if these changes were mediated via TNFα. Two hours after panels of C3H/HeN mice received IP injections of anti-TNFα or anti-BSA antibodies, they received ID injections of 200 μg UV-UCA, trans-UCA, or PBS. Five hours later the skin was excized, stained with anti-Ia^k antibodies, and assessed by fluorescent microscopy. It was found that the number of Ia-bearing epidermal cells was significantly reduced in skin removed from sites of anti-BSA–treated mice that had been injected ID with UV-UCA (75% of PBS ID–injected positive control). By contrast, the number of Ia-bearing epidermal cells was 90% of positive control in skin removed from sites of anti-TNFα–treated mice that had been injected ID with UV-UCA. These results confirm the conclusion of Noonan et al [8] who demonstrated reduction in density of Ia^+ cells in skin treated in vitro with cis-UCA. Our data extend this conclusion by revealing that the cis-UCA effect in vivo is mediated, in part, by TNFα. UV-UCA also caused epidermal LC to lose their dendrites, a change similar to that induced by UVB radiation and ID-injected TNFα. Because dendrites were essentially unchanged (fully formed) in skin of anti-TNFα–treated mice that received ID UV-UCA, we conclude that cis-UCA has two histologically distinct, TNFα-mediated effects on epidermal LC: reduction in absolute number of Ia^+ cells, and loss of dendrites.

CONCLUSIONS

It is relevant and important that UVB radiation, TNFα, and cis-UCA have similar morphologic effects on epidermal LC, and that the effects of all three treatments can be abolished by anti-TNFα antibodies. This constellation of findings suggests that UVB radiation impairs the induction of CH in mice by a multi-step process that is initiated when trans-UCA is converted to cis-UCA in the superficial epidermis. Within the next 2 h epidermal Langerhans cells display morphologic changes similar to those that can be produced promptly (within 5 min) by ID injection of TNFα. These findings are consistent with the hypothesis that cis-UCA, produced by UVB bombardment of the epidermis, binds to a receptor that leads to the intracutaneous release or accumulation of TNFα. One possibility for the source of TNFα is that cis-UCA receptors exist on keratinocytes within the stratum spinosum; when cis-UCA binds, the cells are induced to activate their TNFα genes — which are transcriptionally silent in normal epidermis. The second possibility is that cis-UCA is released from the stratum corneum after UVB radiation and binds to receptors on LC. If that should be the case, then the changes observed in LC must arise from de novo synthesis of TNFα within LC themselves. In either scenario, the greater impairment of CH induction achieved by cis-UCA in UVB-S mice, compared to UVB-R mice, is compatible with the genetic evidence that UVB-R and UVB-S mice contain unique polymorphic alleles at the *Tnfα* and *Lps* loci [3].

REFERENCES

1. Toews GB, Bergstresser PR, Streilein JW: Epidermal Langerhans cell density determines whether contact sensitivity or unresponsiveness follows skin painting with DNFB. J Immunol 124:445–453, 1980
2. Streilein JW, Bergstresser PR: Genetic basis of ultraviolet-B effects on contact hypersensitivity. Immunogenetics 27:252–258, 1988
3. Yoshikawa T, Streilein JW: Genetic basis of the effects of ultraviolet light B on cutaneous immunity. Evidence that polymorphisms at *Tnfα* and *Lps* loci govern susceptibility. Immunogenetics 32:398–405, 1990
4. De Fabo ED, Noonan FP: Mechanism of immune suppression by ultraviolet irradiation in vivo. I. Evidence for the existence of a unique photoreceptor in skin and its role in photoimmunology. J Exp Med 158:84–98, 1983
5. Ross JA, Howie SEM, Norval M, Maingay JP, Simpson J: Ultraviolet irradiated urocanic acid suppressed delayed-type hypersensitivity to herpes simplex virus in mice. J Invest Dermatol 87:630–633, 1986
6. Norval M, Simpson TJ, Ross JA: Urocanic acid and immunosuppression. Photocem Photobiol 50:267–275, 1989
7. Vermeer M, Streilein JW: Ultraviolet-B light induced alterations in epidermal Langerhans cells are mediated by tumor necrosis factor-alpha. Photodermatol Photoimmunol Photomed 7:258–265, 1990
8. Noonan FP, De Fabo EC, Morrison H: Cis-urocanate, a UV irradiation product, decreases the number of Ia-positive Langerhans cells in cultured epidermal sheets (abstr). J Invest Dermatol 84:342, 1985