

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*

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The role of ultraviolet (UV)¹ radiation in carcinogenesis in mice appears to be twofold: (a) a neoplastic transforming event producing fibrosarcomas and squamous cell carcinomas, and (b) a selective, systemic, immunosuppressive effect. While the former has been known and studied for some time (1, 2), the latter has only recently begun to be described in detail (3–13). For example, it was observed several years ago that UV-induced tumors in mice could not be transferred to syngeneic recipients unless the latter were also given UV irradiation (3, 4). It is now known that the ability of such tumor cells to grow in a UV-irradiated syngeneic host is associated with the presence in spleen and lymph nodes of suppressor T lymphocytes specific for UV-induced tumors as a group. Not only do these suppressor cells prevent immunologic rejection of UV tumors on transplantation into syngeneic recipients (3–6), but they also significantly reduce the latent period of tumor appearance during UV carcinogenesis (12). A second UV-induced immunosuppressive system has also recently been described in which UV irradiation of mice leads to the systemic suppression of contact hypersensitivity (CHS) (7). This suppression is also associated with the generation of antigen-specific suppressor cells, but in this case specific for the contact sensitizer. The mechanism appears to involve a UV-induced defect in antigen uptake, processing and/or presentation such that, in response to antigen, suppressor T cells are generated in preference to immune cells (8, 9).

Photobiologic studies of these two UV-induced immunosuppressive effects show three similarities: (a) the same effective waveband, i.e., 250–320 nm (7, 11); (b) the same independence of dose rate and dose fractionation (7, 10, 11); and (c) similar shape of dose-response curves (7, 10). In addition, a time delay of

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¹ Abbreviations used in this paper: CHS, contact hypersensitivity; DNFB, 1-fluoro-2,4-dinitrobenzene; IR, infrared; TNCB, 2-chloro-1,3,5-trinitrobenzene; UCA, urocanic acid; UV, ultraviolet.

24–72 h after irradiation is required for expression of the immunosuppressive effect (9, 11). The actual dose of UV from FS40 sunlamps needed to induce 50% suppression of CHS is ~ 13 times less than that needed to induce tumor susceptibility in 50% of the UV-irradiated animals (13). The photobiologic and immunologic similarities of these two UV-induced immunosuppressive effects suggest they may share a common step(s).

A major question to be considered is how irradiation of mice with UV wavelengths that will not penetrate beyond the dermis leads to the formation of antigen-specific suppressor cells in the spleen and lymph nodes. Clearly, the first step in this process, the transduction of UV radiation into a biochemical signal leading to immunosuppression, is a key one. Furthermore, the photobiologic similarities between the two immunosuppressive systems cited above implies that at least part of the shared step(s) may involve the initial light-absorbing event.

The light source used to induce these immunosuppressive events is the FS40 sunlamp, emitting radiation primarily (65%) in the 250–320 nm range (13). One of the major difficulties of using these lamps is that, at the doses necessary to suppress either CHS or tumor rejection, very considerable erythema and gross skin damage occur (10). It was possible that the immunosuppression observed was a byproduct of a UV-induced inflammatory response. To rule out this possibility, and to establish whether there is a primary photoreceptor and, if so, its chemical nature, we determined an *in vivo* action spectrum for UV-induced immunosuppression of CHS in BALB/c mice. In theory, the shape of the action spectrum should be exactly congruent with the *in vivo* absorption spectrum of the putative photoreceptor (14), although in heterogeneous molecules, e.g., DNA or proteins, the action spectrum of the particular function may not match the absorption spectrum of the whole molecule, but rather that of the moiety which is the actual chromophore (15). The energy requirement, much smaller for the suppression of CHS than for the suppression of tumor rejection, suggested that such an action spectrum could be determined.

Another important reason for determining such an action spectrum is suggested by the increased concern over potential destruction of stratospheric ozone (16) by human-directed activities. Decreases in stratospheric ozone are expected to result in enhanced levels of UVB (290–320 nm) reaching the earth's surface, and concomitant increases in skin cancer are predicted (17). Thus, given the immunologic changes induced by UV radiation described above, and in the event that humans might also be subject to these changes, knowledge of the wavelength spectrum for UV-induced immunosuppression could prove valuable in studies of human cancer.

Materials and Methods

Mice. Specific pathogen-free female mice of the BALB/c AnN strain were supplied by the Animal Production Area of the NCI Frederick Cancer Research Facility. All animals were 8 wk old at the start of experiments.

Optical Dispersion System. For the production of narrow-band UV radiation, a unique system previously developed and described (18) was used. The system, located in an air-conditioned (20°C) dark room, consisted of narrow bandpass UV interference filters of half bandwidth 2–3 nm (except 250 nm, half bandwidth 10 nm) (Corion Corp., Waltham, MA) coupled to a 2.5 kW xenon arc. The filters transmit 10–20% of the incident energy

and block transmission of wavelengths outside the main bandpass to $<10^{-3}$ from X ray to the far infrared (IR). All filters had a center wavelength tolerance of ± 0.5 nm (Fig. 1). A relatively large area of narrow-band irradiation ($50\text{--}60\text{ cm}^2$) was produced with irradiances of 0.025 to 0.5 W/m^2 depending on the filter used, permitting total dorsal irradiation of three mice simultaneously. Wavelengths used for this study ranged from 250 to 320 nm in $5\text{--}15$ nm steps. All filters were routinely checked for changes in transmission spectra using a Cary Spectrophotometer (Model 17) and replaced if such occurred.

Irradiance measurements for each of the wavelengths were made using an International Light Radiometer (Model 700) coupled to a cosine-corrected UVB detector (No. PT171C) containing a WB320 interference filter or cosine-corrected "solar blind" UVC detector (No. PT171D). Nine measurements were taken across the field before and after each exposure and the average used to calculate the dose. Additional measurements were made for the 305-nm and 320-nm filters using an Optronics Spectroradiometer (No. 742; Optronics Laboratories, Orlando, FL). Levels of irradiance were $<10^{-3}$ of the peak value for wavelengths shorter than 300 nm using the 305-nm filter or for wavelengths shorter than 315 nm using the 320-nm filter. Measurements with the two radiometers agreed to within 20% .

Induction, Measurement, and Effect of UV on CHS. This was performed as previously described (7). Briefly, $100\text{ }\mu\text{l}$ of a 5% solution in acetone of 2-chloro-1,3,5-trinitrobenzene (TNCB) or $20\text{ }\mu\text{l}$ of an 0.5% solution of 1-fluoro-2,4-dinitrobenzene (DNFB) in acetone was applied to the shaved abdomen. 5 d later, a challenge dose of $5\text{ }\mu\text{l}$ of a 1% TNCB or of a 0.2% DNFB solution in acetone was applied to each surface of each ear. Ear thickness was measured with an engineer's micrometer (No. 7309; Mitutoyo, Japan) before and 24 h after application of the challenge dose.

12 mice were used for each experiment; two groups of 3 mice each were irradiated, and there were two corresponding control groups, also of 3 mice each. Animals were sensitized 5 d after UV, and challenged by ear painting a further 5 d later. Percent of control response was determined as $[(A - B)/(C - D)] \times 100$, where treatments were (a) UV, TNCB; (b) UV only; (c) TNCB; or (d) nil.

Detection of CHS Suppressor Cells after Narrow-band UV Irradiation. The presence of suppressor cells in the spleens of UV-irradiated TNCB-treated mice was determined by the ability of these cells on transfer into a naive mouse to decrease the induction of contact hypersensitivity (7).

Dose-Response Determination for the Suppression of CHS by UV Radiation. Mice were shaved on the back ($\sim 10\text{ cm}^2$) with electric clippers immediately before irradiation. The ears were covered with electrical tape and the mice placed in a quartz-covered lucite container (11.5 cm diameter $\times 5\text{ cm}$ depth) that had air holes in the sides and three individual compartments. The mice were unable to turn over, thus the abdomen was protected from UV. Control mice were placed in an identical container and kept in the dark for the same period of time as those for the UV irradiation. Both control and UV containers were set atop turntables rotating at 3 rpm . For each wavelength tested, exposure times ranged

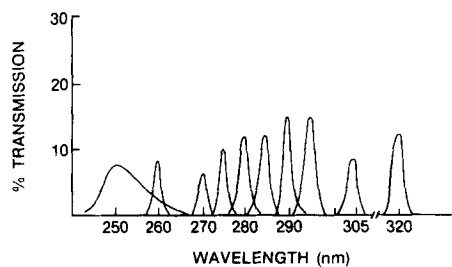


FIGURE 1. Transmission characteristics determined using a Cary Spectrophotometer Model No. 17 of the narrow-band interference filters used to determine the action spectrum. Transmission of actinic radiation outside the main bandpass was $<10^{-3}$ from the X ray to the far IR.

from 1 to 12 h. Exposures of >6 h were fractionated into two equal treatments on successive days. Immediately following treatment, mice were returned to their conventional cages.

Sensitivity Changes. To minimize possible variations in sensitivity due to endogenous rhythm changes or other light/dark perturbations all mice were routinely kept on a 12 h light/12 h dark cycle (light cycle 6 AM–6 PM). All mice used in an experiment were at least 3 h into their light cycle before treatment, which was always completed at least 3 h before their light cycle would end.

Statistical Treatment of Results. For each wavelength, percent of control response for each individual mouse was plotted vs. the logarithm of the dose and linear regressions were calculated to assess the relationship of the responses to the radiation received. The dose required to produce a 50% response and the standard error of this dose were estimated (19). These doses and their standard error limits were then quantum corrected. The values for each wavelength were normalized to the value at 270 nm, and the action spectrum plotted.

Tape Stripping Experiments to Remove Stratum Corneum. Hair was plucked from dorsal skin under metaphane anesthesia; the majority of the stratum corneum was then removed from the plucked area by tape stripping four times (3M Magic Transparent Tape) (20). Histologic examination confirmed that most of the stratum corneum was removed, but that nucleated epidermal cells remained. The UV source was a bank of six germicidal lamps, emitting ~90% of their energy at 254 nm. The mice were placed in dividers in conventional cages as previously described for FS40 irradiation (10). The average irradiance at mouse level was 5 W/m². This irradiance takes into account screening by wire cage tops, sides, and dividers.

Results

Systemic Suppression of CHS to TNCB by Narrow-band UV Radiation and Detection of CHS Suppressor Cells after Irradiation. Previously, the active waveband of FS40 sunlamps that suppresses CHS associated with specific suppressor T cell formation was shown to be 250–320 nm (7). The first question was whether narrow-band UV in this region showed a similar suppressive effect. Table I (*top*) shows that prior irradiation with UV of 270 ± 1.5 nm caused a systemic suppression of CHS as shown for irradiation by FS40 sunlamps. It must be emphasized that, since the site of sensitization (abdomen), and the site of challenge (ears) are protected from direct UV radiation, we are investigating here the systemic effects of UV radiation on the immune system, mediated chiefly by radiation incident on the shaved dorsal surface of the mouse.

That this suppression was associated with the generation of antigen-specific suppressor cells is shown in the lower half of Table I. Transfer of spleen cells from mice treated with narrow-band UV and TNCB into naive recipients immediately before sensitization decreased the CHS response to TNCB (lower left half; line 4 significantly lower than line 2). These suppressor cells are antigen specific. Animals that were given cells from mice that had been irradiated with narrow-band UV and then given TNCB, gave a normal response to DNFB (lower right half; line 4 and line 2 not significantly different).

The doses of 270-nm irradiation used caused no visible gross skin damage or erythema such as seen with FS40 irradiation. During this entire study, the only wavelength to produce visible skin damage and erythema was 295 nm.

Dose Response Curves. Fig. 2 shows the dose response curves from 250 to 320 nm determined as described in Materials and Methods. The results indicate that, at all wavelengths, suppression is proportional to log₁₀ dose and that a clear

TABLE I
*Suppression of CHS to TNCB by Narrow-band UV Radiation (270 ± 1.5 nm) (Upper);
 Association with Antigen-specific Suppressor Cells (Lower)*

UV treatment		Ear swelling $\pm$ SEM (cm $\times$ 10 ⁻³)		Percent of control	
Time of irradiation	Dose (J/m ²)	No TNCB	TNCB		
Nil	Nil	4.7 $\pm$ 0.6	20.2 $\pm$ 1.7	100	
4 h	370	5.2 $\pm$ 0.3	12.0 $\pm$ 0.8	44	
Spleen cells given*		Sensitization of recipients	Ear swelling $\pm$ SEM (cm $\times$ 10 ⁻³) TNCB challenge	Sensitization of recipients	Ear swelling $\pm$ SEM (cm $\times$ 10 ⁻³) DNFB challenge
1. Nil		Nil	4.1 $\pm$ 0.2	1. Nil	1.1 $\pm$ 0.3
2. Nil		TNCB	28.6 $\pm$ 0.6	2. DNFB	8.1 $\pm$ 1.2
3. 8 $\times$ 10 ⁷ (TNCB)		TNCB	24.3 $\pm$ 1.1 ‡(P > 0.2)	3. DNFB	10.5 $\pm$ 1.6 ‡(P > 0.2)
4. 8 $\times$ 10 ⁷ (UV, TNCB)		TNCB	18.0 $\pm$ 1.8 ‡(P < 0.001)	4. DNFB	8.8 $\pm$ 1.2 ‡(P > 0.2)

* Single-cell suspensions of spleen cells from either sensitized (TNCB) or UV-treated, then sensitized (UV, TNCB) mice were prepared 4 d after TNCB treatment and given intravenously to naive recipients which were then sensitized with either TNCB or DNFB as indicated. Dose of UV given to donors was $\sim 485 \text{ J}/\text{m}^2$ of 270 ± 1.5 nm radiation, followed 3 d later by TNCB sensitization as described in Materials and Methods.

$\dagger$ P values compared to value for mice treated with sensitizer alone (line 2).

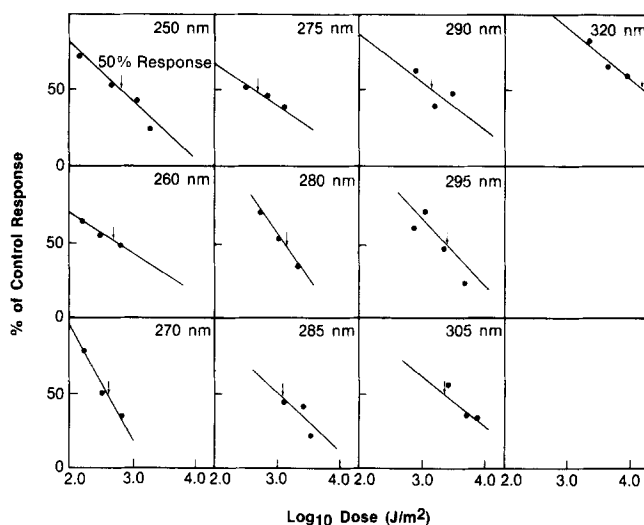


FIGURE 2. Dose-response curves for suppression of CHS by narrow-bands of UV from 250 to 320 nm. The individual points represent the mean of 2–4 separate experiments; each experiment consisted of 12 mice including controls (see Materials and Methods). Straight lines were plotted from equations determined by first-order regression analysis of the data (see Materials and Methods). All lines had a slope significantly different from zero at $P < 0.05$. The arrow represents the dose needed to produce 50% suppression.

difference in wavelength effectiveness exists.

Action Spectrum. The action spectrum (Fig. 3) derived from the dose-response curves as described in Materials and Methods has (a) a broad peak between 260 and 270 nm, (b) a shoulder at 280–290 nm, and (c) a steady decline to ~3% of maximum at 320 nm. Since the primary purpose of a biologic action spectrum study is to describe the absorption characteristics of an unknown photoreceptor, the action spectrum should be compared with the absorption spectra (or, in some cases the action spectra—see Discussion) or compounds known to be in the tissue under study. As Fig. 3 shows, there is either good or partial agreement between this action spectrum and the absorption spectrum (or action spectrum) of at least two UV-absorbing compounds known to be in mammalian skin. We cannot completely rule out however, the possibility that other compounds with similar absorption spectra might also be present in mammalian skin.

Tape-Stripping Experiments. To identify the location of the photoreceptor, skin stripping experiments were performed. Fig. 4 shows that tape stripping the dorsal surface four times immediately before irradiation prevents suppression. Previously (20) stripping was shown to cause histologic changes in the epidermis

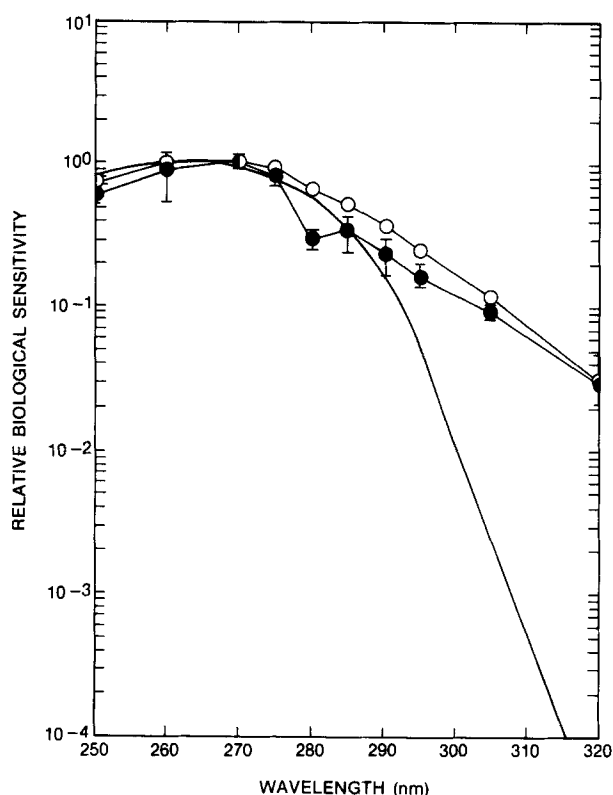


FIGURE 3. In vivo action spectrum for the induction of systemic suppression of CHS (●). Points on the action spectrum represent the reciprocal of the number of photons (m^2/photon) required to produce 50% suppression, normalized to 270 nm. Bars represent ± 1 SEM. For purposes of comparison the DNA action spectrum (—) (34) and the absorption spectrum of urocanic acid (UCA) (○) (see Discussion) (48), are also shown.

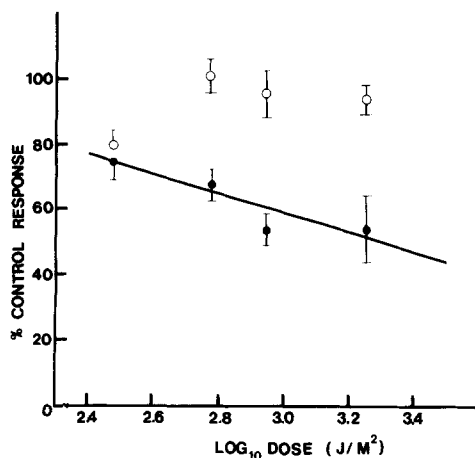


FIGURE 4. Pooled results of three individual experiments (consisting of at least two experiments at each dose) to determine the effect of prior tape stripping (four times) on UV-induced immunosuppression by 254-nm radiation; (●) no tape stripping, (○) tape stripped. The individual values for each mouse in each experiment were expressed as percent control and the mean $\pm$ SEM determined at each dose. The line shown is the calculated regression line. Tape stripping alone had no significant effect on systemic CHS, or on the background ear swelling (no sensitization) of control or UV-treated mice.

that begin within 1 h and reach a maximum by 24 h. To minimize the possibility of these effects altering the optical properties of the skin, stripping was carried out immediately before irradiation, and a UV source of high irradiance was used such that irradiation times were 6 min or less. Since we confirmed histologically that tape stripping four times removed the majority of the stratum corneum while not grossly altering the other epidermal layers, we interpret the results in Fig. 4 as suggesting that the photoreceptor is superficially located in the upper epidermis and most likely lies in the stratum corneum.

Discussion

This study demonstrates that narrow-band UV radiation between 250 and 320 nm can, like broad-band UV radiation, cause a systemic suppression of contact hypersensitivity to TNCB. Furthermore this suppression is independent of gross skin damage and erythema, which clearly shows that UV-induced suppression of CHS is not a by-product of a UV-induced inflammatory response. It also indicates that we are not dealing simply with direct damage by UV radiation.

The essential findings are that UV radiation between 250 and 320 nm can lead to the formation of suppressor cells specific for some antigens and that within this activating waveband a clear-cut differential in wavelength effectiveness occurs. This second finding suggests that a specific photoreceptor mediates this effect. Apparently this putative photoreceptor is different from the photoreceptor usually associated with UV effects, such as cell killing or viral inactivation (see below). The results of the tape-stripping experiment (Fig. 4), in which removing the stratum corneum prevented immune suppression, support the idea of a unique photoreceptor and indicate that this compound is located superficially

in the epidermis, probably in the stratum corneum itself. The next question is how UV irradiation of mouse skin by wavelengths that do not penetrate the dermis lead to the formation of antigen-specific T suppressor cells in the spleen and lymph nodes.

To answer this question we discuss some mechanisms that might induce suppression by UV radiation, based on the findings cited above and some of our previous work (13). An antigen-presenting cell defect in the spleens of mice irradiated with broad-band UV radiation has been demonstrated, (21), and it is presumably by way of this defect that introduction of an antigen leads to the generation of specific suppressor cells (22–24). Although in this study we have not formally demonstrated an antigen-presenting cell defect in mice irradiated with narrow-band UV radiation, the finding of suppressor cells suggests a common mechanism with suppression by broad-band UV radiation. Consequently in one model direct UV radiation of antigen-presenting cells as they circulate through the capillaries of the epidermal-dermal junction could generate an antigen-presenting cell defect (21). In support of this model, UV irradiation of these cells *in vitro* depresses their ability to present antigen (13, 21, 24). *In vitro* irradiation of these cells, however, may not necessarily mimic *in vivo* effects (13, 24). Furthermore two pieces of evidence in this paper argue against this model. First, the most effective wavelengths, 260–270 nm, are those most absorbed by the epidermis (25). Thus, an effect in the skin capillaries, which are screened by the epidermis and by the capillary wall, is unlikely to show an action spectrum such as we have described. Second, removing the stratum corneum reverses suppression. This is also inconsistent with a photoreceptor located at the level of the capillaries, in which case removing this superficial layer would increase rather than decrease the effectiveness of UV radiation.

A second model proposes that UV radiation damages epidermal Langerhans cells, thereby mediating immunosuppression directly or by a homeostatic mechanism and creating a relative deficiency in antigen-presenting cells. As the major immunologic cells in the epidermis, Langerhans cells must be considered as the possible initiator of these UV-induced immunosuppressive processes. We have addressed this question and present, in a future paper, evidence against the involvement of Langerhans cells in the primary UV-absorbing event leading to immunosuppression.²

An alternative model, based on the evidence presented here which suggests the existence of a unique photoreceptor located superficially in the skin, proposes that altered antigen presentation and consequently T suppressor cell formation is due to the formation of a specific photoproduct after UV absorption by the photoreceptor. Because the dose-response curves for immunosuppression (Fig. 2) apparently display simple one-hit kinetics, they should be considered differently from the typical dose-response curves seen in cell-killing or -survival studies (26, 27). Instead of behaving as a biological "target" exhibiting, e.g., multihit-multitarget inactivation kinetics (27, 28) this target acts more like a biological signal transducer. Photosensory transducers such as rhodopsin, bacteriorhodop-

² Noonan, F. P., C. Bucana, D. N. Sauder, and E. C. De Fabo. 1983. The UV effects on number and morphology of epidermal Langerhans cells and the UV-induced suppression of contact hypersensitivity have different wavelength dependencies. Manuscript submitted for publication.

sin, phytochrome, and the blue-light receptor are well known and their biological as well as evolutionary significance amply described (14, 29–32). We believe the photoreceptor proposed here acts in a similar way. After absorbing UV radiation it generates a specific biochemical signal that leads ultimately to some observable response. In our system this signal could be considered a unique photoproduct capable of entering the immune system and eventually leading to T suppressor cell formation. This signal presumably is associated with the induction of an antigen-processing defect because induction of such a defect is associated with specific T suppressor cell formation (8, 22, 23). The finding of a clearly defined action spectrum for suppression of CHS suggests that such a scheme is plausible.

The tape-stripping experiments in which removal of the stratum corneum before UV irradiation prevented suppression (Fig. 4) as well as our earlier observation that removing the epidermis immediately after UV treatment also prevented immunosuppression (13) give some support for such a model.

It is formally possible that tape-stripping before UV exposure removes not the photoreceptor per se, but a factor necessary for subsequent steps in generating immunosuppression. However, a mechanism by which this could occur is difficult to imagine particularly because this factor would lie above the level of the photoreceptor and most likely interfere with UV absorption (see discussion of scattering below).

The shape of the action spectrum should correspond to the *in vivo* absorption spectrum of the unknown photoreceptor (14, 31). Thus different skin compounds can be assessed as candidates for the photoreceptor based on the congruence between their absorption spectrum and the action spectrum, their location in the skin, and their photochemical properties. However, because of difficulties associated with interpreting biological action spectra (14, 31), the following should be noted. (a) Transmission and forward-scattering studies on mouse epidermis show that radiation of 320 nm penetrates 10 times better than radiation of 270 nm (25). Thus potential screening by compounds that absorb in this waveband could present a special problem. It means that the action spectrum could be lower by as much as a factor of 10 at 270 nm, which would alter its interpretation. However, because removing the stratum corneum prevented rather than induced suppression, as might be expected were the photoreceptor screened by the stratum corneum, it is highly unlikely that significant screening occurred. (b) Although this study suggests that a photoreceptor mediating immunosuppression of CHS by UV radiation exists in mouse skin and that it is most likely located in the uppermost layers of the epidermis, its exact identity and location are not definitively established; biochemical and genetic approaches are necessary to do this. Notwithstanding these two points, some inferences can be made about the photoreceptors' chemical nature.

Nucleic acids in general and DNA in particular are unlikely candidates for being the photoreceptor. A close fit of the action spectrum presented here to the DNA action spectrum (33) is seen up to 285 nm (Fig. 3). For longer wavelengths, however, these action spectra begin to deviate significantly from each other. For example, Fig. 3 shows that at 320 nm the action spectra differ by more than 300-fold. Recent studies show that not all the bases in DNA absorb radiation equally. For wavelengths longer than 300 nm the relative absorption

of DNA increases as a function of guanine-cytosine content, thereby raising the absorption of DNA in this wavelength region (15). Although this may account for the differences between DNA absorption and some action spectra that are similar to DNA (15), it is unlikely to account for the large differences seen in Fig. 3. Apparently free nucleic acid bases are ruled out also because they do not absorb appreciably at wavelengths longer than ~ 300 nm (15, 26). Also analysis of stratum corneum shows that DNA and RNA for the most part do not exist in this tissue (34).

Although DNA does not appear to be the direct absorber of UV photons inducing antigen-specific immunosuppression of CHS, UV effects of DNA seem closely associated with photocarcinogenesis (reference 16, chapt. 3 and appendix G). Therefore it is conceivable that the UV-activated photoreceptor proposed here mediates immunosuppression, whereas UV-irradiated DNA mediates transformation. An interaction between these two events could conceivably be responsible for tumor outgrowth in the skin.

Recently (35) an absorption spectrum for isolated stratum corneum from hairless mouse epidermis was published, which corresponds closely to the action spectrum of this study and is consistent with our hypothesis that the photoreceptor is probably located in the stratum corneum. The stratum corneum itself is made up largely of terminally differentiated cells from the epidermis that contain mainly an insoluble disulfide-linked proteinaceous complex, keratin, consisting of two components, keratin filaments and filaggrin, a histidine-rich protein (36). In proteins the major amino acids that absorb UV radiation in our region of interest (250–320 nm) are tyrosine and tryptophan (peaks at 275, 280 nm, respectively), phenylalanine (peak at 257 nm), and cystine (shoulder at 240–260 nm) (37). All of these amino acids in neutral or aqueous solution show little or no extinction at wavelengths longer than ~ 314 nm (37). Consistent with this, the absorption spectrum of mouse keratin shows a peak at 278 nm and no extinction at 320 nm (P. W. Steinert, personal communication). None of these compounds, which are known to be present in the stratum corneum, give absorption spectra that match the action spectrum of this study. We interpret this lack of correspondence to indicate that neither keratin nor any of its UV-absorbing amino acids are likely to be the photoreceptor for UV-induced immunosuppression.

Although keratin makes up the bulk of the stratum corneum, UV-absorbing lipids are also present. However, we are aware of none with a UV-absorption spectrum that matches the action spectrum for suppression. One important UV-absorbing epidermal lipid, 7-dehydrocholesterol, shows peaks at 295, 282, and 271 nm, (38) and little or no absorption at wavelengths longer than 300 nm. Prostaglandins, another group of epidermal lipids, are stimulated to increase in concentration by wavelengths of 360 nm as well as by 254 nm (39). Thus this association with UV does not correspond with our observation that immunosuppression is induced only by wavelengths shorter than 320 nm (11, 13).

A major component of the epidermis, that shows absorption similar to the action spectrum for immunosuppression (Fig. 3) and is its principal acid-soluble UV-absorbing compound, is urocanic acid (UCA) (40, 41). It is formed in a single-step deamination of histidine catalyzed by the enzyme histidine-ammonia

lyase (Histidase, E.C. 4.3.1.3) (40, 42, 43). Histidase is detectable in relatively high amounts only in two types of mammalian tissue, liver and epidermis (40, 43). In the liver histidine can be deaminated by histidase to UCA and then further catabolized by urocanase; an alternative pathway converts histidine to imidazolepyruvic acid and other imidazole metabolites. In the epidermis however, urocanase is missing and further catabolism of histidine beyond its deamination to UCA does not occur (43, 45). Consequently, a relatively large amount of UCA accumulates (44, 45). Perhaps significantly, in the epidermis histidase activity and hence UCA formation are almost completely restricted to the stratum corneum, with the final amount of UCA formed depending on the interaction of several factors (40). Obviously then, UCA represents a major component of the stratum corneum. Although no clear-cut function for UCA has been established, it has been proposed that this compound acts as a natural sunscreen (44, 46). Serious doubt, however, has recently been raised about this hypothesis because of the much greater efficiency (by a factor of ~ 3) in the production of UCA in fur-bearing animals than in humans (40). Photochemically, UCA has the following properties: (a) a UV-dependent $\text{trans} \rightleftharpoons \text{cis}$ isomerization (47), (b) a capability of forming photodimers (48), and (c) a capability to bind to DNA in a UV-dependent manner (49). Isomerization to the cis isomer produces a more water-soluble form.

Accordingly, based on (a) the closer fit of the action spectrum to UCA than to DNA or epidermal protein or lipid, (b) its location in the stratum corneum, the removal of which prevents immunosuppression, and (c) its photochemical properties, we propose that UCA may be the photoreceptor for the UV induction of immunologic unresponsiveness to contact sensitivity. In support of our hypothesis we have recently obtained new data using mice whose stratum corneum were left intact but with urocanic acid content greatly reduced ($<10\%$ of normal mice). These mice, but not their counterparts containing normal levels of urocanic acid, were unable to be immunosuppressed, by varying amounts of UV radiation, to a TNCB-induced CHS response (50). The kinetics of this response were very similar to those seen for the tape-stripping experiment (Fig. 4). Finally, because of the immunologic and photobiologic similarities between the UV-induced suppression of CHS and the UV-induced suppression of tumor rejection (see introduction), this postulated photoreceptor may also play a role in photocarcinogenesis via the production of tumor-specific suppressor cells.

How might such a photoreceptor initiate a systemic immunologic suppression? As postulated above, a UV-induced photoproduct, or possibly a secondary product formed from an interaction between the activated photoreceptor and the epidermis, enters the systemic circulation and alters either the antigen-presenting ability of cells in situ or their distribution, creating a deficiency in available antigen-presenting cells. After this change, introduction of an antigen (e.g., TNCB) directs the production of T suppressor cells specific for that antigen. Although further studies are needed to implicate UCA as the initiator of this sequence of events, we have obtained preliminary results by inducing immunosuppression of CHS after applying UCA exogenously to mouse skin under appropriate conditions (unpublished results).

Whatever the chemical nature of the photoreceptor or its mode of action, the

data presented in this paper suggest that it exists and that it probably is located in the stratum corneum. Furthermore this photoreceptor links relatively low levels of UV radiation, including wavelengths in the solar UVB range to important systemic immunologic alterations. The evolutionary significance of such a mechanism could be to regulate against uncontrolled autoimmune attack on sun-damaged skin by triggering the production of suppressor cells. The concept that mammalian epidermis contains a photoreceptor capable of mediating interaction between sunlight and the immune system is new and perhaps important. Accordingly, we suggest that such a photoreceptor may play a fundamental role in photocarcinogenesis and in immune diseases associated with UV radiation.

Summary

UV irradiation of mice causes a systemic immune alteration that can be detected either by suppression of the immunologic rejection of UV-induced tumors, or by suppression of contact hypersensitivity (CHS). Suppression of these two immunologic responses has similar photobiologic characteristics and in both cases is associated with the generation of antigen-specific suppressor T cells. To identify whether a specific photoreceptor for this effect exists, the relative wavelength effectiveness (action spectrum) was determined for the UV-induced suppression of CHS.

Narrow bands of UV (half bandwidth 3 nm) were used at 10 wavelengths from 250 to 320 nm to obtain dose-response curves. Irradiation with each of these bands of UV caused dose-dependent immunosuppression of CHS, but with differing effectiveness. Immunosuppression was clearly separable from the generation of gross skin damage and inflammation. Further, immunosuppression by the most effective wavelength (270 nm) was associated with the generation of antigen-specific suppressor cells.

The action spectrum derived from the dose-response curves has a maximum between 260 and 270 nm, a shoulder at 280–290 nm, and declines steadily to ~3% of maximum at 320 nm. The finding of such a clearly defined wavelength dependence implies the presence of a specific photoreceptor for this effect. Removing the stratum corneum by tape stripping before UV irradiation prevented the suppression of CHS using 254-nm radiation, suggesting the photoreceptor is superficially located in the skin.

A number of epidermal compounds with absorption spectra similar to the action spectrum are discussed and evaluated with respect to their potential for being the photoreceptor. Based on (a) the close fit of its absorption spectrum to the action spectrum, (b) its superficial location in the stratum corneum, and (c) its photochemical properties, the hypothesis is advanced that the photoreceptor for systemic UV-induced immunosuppression of contact hypersensitivity may be urocanic acid. As such, it may also play a role in UV-induced carcinogenesis via the production of tumor-specific suppressor cells.

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