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Mechanisms underlying UV-induced immune suppression: implications for sunscreen design

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Abstract: The ultraviolet (UV) radiation present in sunlight is immune-suppressive. Recently we showed that solar-simulated UV radiation (UVA + UVB; 295–400 nm), applied after immunization, suppressed immunological memory and the elicitation of delayed-type hypersensitivity to the common opportunistic pathogen, *Candida albicans*. Further, we found that wavelengths in the UVA region of the solar spectrum (320–400 nm), devoid of UVB, were equally effective in activating immune suppression as UVA + UVB radiation. Here we report on the mechanisms involved. No immune suppression was found in UV-irradiated mice injected with monoclonal anti-interleukin (IL)-10 antibody, or mice exposed to solar-simulated UV radiation and injected with recombinant IL-12. Antigen-specific suppressor T cells were found in the spleens of mice exposed to UVA + UVB radiation. Applying liposomes containing bacteriophage T4N5 to the skin of mice exposed to solar-simulated UVA + UVB radiation or mice exposed to UVA radiation blocked immune suppression, demonstrating an essential role for UV-induced DNA damage in the suppression of established immune reactions. These findings indicate that UV radiation activates similar immunological pathways to suppress the induction of, or the elicitation of, the immune response.

Stephen E. Ullrich, Margaret L. Kripke and Honnavara N. Ananthaswamy

Department of Immunology, The University of Texas, M. D. Anderson Cancer Center, Houston, TX, USA

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Stephen E. Ullrich
Department of Immunology-178
The University of Texas
M. D. Anderson Cancer Center
1515 Holcombe Blvd
Houston
TX 77030
USA
Tel.: +1 713 792 8593
Fax: +1 713 7451633
E-mail: sullrich@mdanderson.org

Introduction

Protection against the harmful effects of solar UV radiation is important for preserving human health and well-being. Excessive exposure to UV radiation causes sunburn, skin cancer, immune suppression and skin aging. In recent decades, lifestyle changes have resulted in increasing exposure of people in industrialized nations to ambient UV radiation, and this trend is expected to increase in future decades as a result of stratospheric ozone depletion.

The development and widespread use of chemical sunscreens has helped to reduce at least some of the deleterious effects of UV radiation on human skin. Sunscreens are highly effective in protecting against sunburn, and they are thought to protect against the induction of skin cancer, mainly by reducing DNA damage caused by UV radiation. The relative effectiveness of sunscreens is generally evaluated on the basis of their ability to prevent erythema in human skin, the so-called sunburn protection factor (SPF). Erythema provides a simple, rapid endpoint for measuring the attenuation of exposure to solar radiation by sunscreens. However, its ability to predict the protective effect of sunscreens on other endpoints of UV exposure, such as skin cancer and immune suppression, depends on the similarities of the action spectra and threshold doses for these effects. Because the action spectra for skin cancer induction and immune suppression in humans are not known, it is possible that a sunscreen could protect against erythema and be relatively ineffective in protecting against another effect of UV radiation. Therefore, it is desirable to develop other endpoints for measuring attenuation of UV radiation effects by sunscreens. The long-term goal of our studies was to determine whether endpoints other than SPF would be more accurate predictors of the effectiveness of sunscreens efficacy. We chose to examine the mechanisms involved in photoimmu-

nosuppression and measure the effectiveness of sunscreens in blocking immune suppression.

Experimental model system

In these experiments we measured the effects of UV exposure on established immune reactions, such as the elicitation phase of delayed-type hypersensitivity and immunological memory (1,2). In the majority of experiments performed in the past to measure an effect of UV radiation on the immune response, the radiation was given to naive animals. Of equal, if not greater concern, however, is the ability of UV exposure to suppress established immune responses. Moyal *et al.* (3) and Halliday and coworkers (4) found that solar-simulated UV radiation suppressed delayed-type hypersensitivity to recall antigens. In addition, UV radiation suppressed contact allergy in individuals presensitized to nickel (5). These experiments, carried out with human volunteers, not only confirmed the initial animal data showing that UV radiation suppresses established immune reactions (6,7), but made an important contribution to photoimmunology by indicating that solar UVA radiation played a role in activating immune suppression. The focus of our experiments was to use our mouse model to understand the mechanisms underlying UV-induced suppression of established immune responses, paying particular attention to the wavelengths involved, the immunological mechanisms involved and the ability of sunscreens to prevent photoimmune suppression.

Results

We were particularly interested in determining which wavelengths within the UV region of the solar spectrum suppressed established immune reactions. Mice (C3H/HeN) were first immunized with *Candida albicans* and then exposed to UV radiation 7–9 days post-immunization. Three different Schott filters were placed on the Xenon UV solar simulator to provide three different types of radiation. Mice were irradiated with solar-simulated UVA + UVB radiation (WG 320/1 mm), UVA only (WG 335/3 mm) or UVA I only (WG 360/2 mm). We found that the dose–response curves for immune suppression observed in mice exposed to UVA + UVB, or UVA only, were identical. On the other hand, no immune suppression was noted when the mice were irradiated with UVA I only. These data indicate that the UVA II present in the solar-simulated radiation was responsible for suppressing established immune reactions. Fifty per cent immune suppression was noted when the mice were exposed to 80 kJ/m^2 UVA radiation,

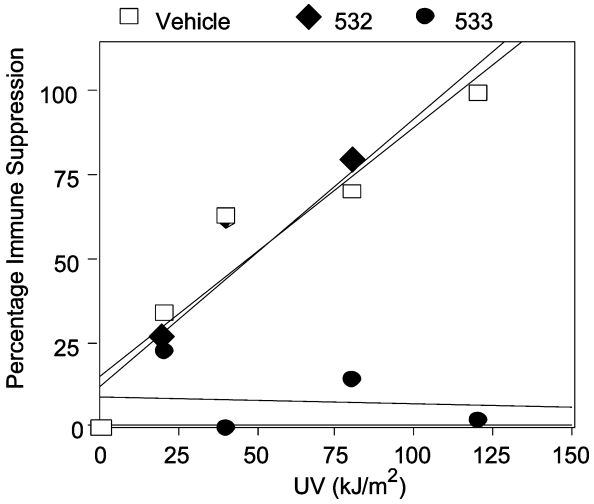

Figure 1. A UVA-absorbing sunscreen affords immune protection. Two different sunscreen preparations were applied to mice 30 min prior to UV exposure. The percentage immune suppression generated in the presence of the vehicle (□); sunscreen P533 (◆); or sunscreen P532(●) was plotted vs the dose of UV (WG-320) applied. $^*P < 0.01$; Student's *t*-test vs the PC. Reproduced from Nghiem *et al.* (2001) UVA radiation suppresses an established immune reaction: implications for sunscreen design. J Invest Dermatol **117**: 1193 with permission.

a dose achieved easily in 60–90 min outdoors on a bright sunny day during the summer in Houston. These findings were confirmed by a sunscreen protection experiment (Fig. 1). Two sunscreens were used: P532, that only absorbs in the UVB region of the spectrum, and P533, a sunscreen that absorbs both UVA and UVB. Both sunscreens had a similar SPF (Table 1). No immune protection was observed when P532 was applied to the mice prior to UV exposure. The degree of immune suppression found was identical to that seen in mice treated with the vehicle and exposed to solar-simulated UV radiation. An entirely different outcome was observed when the UVA + UVB absorbing sunscreen (P533) was applied. In this case, no immune suppression was observed when P533 was applied to the skin of the mice 30 min prior to UV exposure. These data indicate that UVA radiation is the critical wavelength for suppressing established immune reactions and confirm that a sunscreen that absorbs UVA will provide complete immune protection (1).

Our next series of experiments was designed to determine whether different immunological mechanisms are involved in suppressing the induction and the elicitation of delayed-type hypersensitivity. We started out by asking a series of questions using our previous work as a guide. We know that the initiating event in UV-induced immune suppression is UV-induced pyrimidine dimer formation. This begins a cascade of events, including cytokine synthesis and the migration of

Table 1. Sunscreens used in this study

Sunscreen	SPF	Composition (%)				
		Uvinul[a] N539 (UVB)	Parsol[b] MCX (UVB)	Eusolex[c] 232 (UVB)	Mexoryl[d] SX (UVA)	Parsol[e] 1789 (UVA)
Vehicle[f]	0	–	–	–	–	–
P532	15	0	8	2	0	0
P533	15	9	0	0.3	0.7	3

[a]Uninul N-539, octocrylene.
[b]Eusolex 232, phenylbenzimidazole sulfonic acid.
[c]Parsol MXC, octyl methoxycinnamate.
[d]Parsol 1789, butyl methoxydibenzoylmethane
[e]Mexoryl SX, terephthalylidene dicamphor sulfonic acid.
[f]Oil-in-water emulsion(10% silicone, 2% organic esters, 15% polyol; mineral oil free).

UV-damaged Langerhans cells to draining lymph nodes, which results in the immunosuppressive signal being transmitted from the skin to the immune system. The ultimate result of the signal cascade is impaired systemic antigen presentation, due probably to the failure of antigen-presenting cells to secrete biologically active IL-12 (reviewed in (8)). Are the same mechanisms involved in UV-induced suppression of established immune reactions? The answer is ''yes''. Pyrimidine dimers are found in the skin of mice exposed to UVA radiation and applying liposomes containing the DNA excision repair enzyme T4N5 to the skin of mice exposed to UVA radiation blocks the suppression of elicitation, indicating that UV-induced DNA damage is the initiating event. Cytokines are involved in transmitting the immunosuppressive signal from the skin to the immune system. No immune suppression was found in UV-irradiated mice injected with neutralizing anti-IL-10 antibody, or mice exposed to solar-simulated UV and injected with recombinant IL-12. Antigen-specific, $CD4^+$ suppressor T cells were found in the lymphoid organs of mice exposed to UVA radiation post-immunization (2).

Summary and conclusions

Using optical filters to achieve a three- to fourfold reduction in the amount of UVB present, we found that UVA radiation alone suppresses established immune reactions, thereby confirming earlier work using broad-spectrum sunscreens (5,9,10). Our findings also address the issue of whether SPF provides an adequate estimation of a sunscreen's immune protective ability. Although we used two sunscreens with equal SPFs, only the UVA-absorbing sunscreen afforded any degree of immune protection. This was predicted from our wavelength dependency study that indicated that UVA and not UVB suppresses established immune reactions (1). In view of the fact that protection against erythema and sunburn is provided primarily by UVB filters it is not surprising that SPF does not equal immune protection when the immunological endpoint used (i.e. the elicitation of DTH) is suppressed by a different wavelength of light. The implication on sunscreen design is obvious. Based on previous data, showing that UVB suppresses the induction of immunity, and in concert with the findings summarized here, complete immune protection requires a sunscreen that absorbs both UVB and UVA radiation.

Whereas different photobiological mechanisms are involved in suppressing the induction of immunity (UVB radiation) vs the elicitation of immunity (UVA II), the immunological mechanisms involved are similar. Perhaps the most critical finding from the point of developing new endpoints for measuring photoimmunosuppression was the observation that repairing pyrimidine dimer formation *in vivo* blocks the activation of immune suppression. This is true regardless of whether the immunological endpoint employed is the induction of immunity as shown previously (11) or suppressing established immune reactions (2). In view of the fact that sunscreens have been shown to block UV-induced pyrimidine dimer formation (12), our findings suggest that blocking UV-induced DNA damage can serve as an early endpoint for measuring immune protection by sunscreens.

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