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Ultraviolet A Induces Immunosuppression, Protection or Memory Enhancement Depending on Dose, while Ultraviolet B is Immunosuppressive and Tolerogenic over a Large Dose Range

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UVR-induced immunosuppression contributes to skin cancer. The aim was to construct accurate dose response curves for primary and secondary contact sensitivity for solar-simulated UVR (ssUVR; 290–400nm), UVA and UVB as the role of UVA in immunosuppression is controversial. We used a xenon arc source. The mice were immobilised, enabling accurate dosing. C57BL/6 mice were immunosuppressed at half the dose of ssUVR required to cause sunburn but not by higher doses (up to the sunburn dose). Thus, ssUVR causes systemic immunosuppression only over a narrow, low dose range. UVA caused suppression at low but not high doses whereas UVB induced immunosuppression at all doses tested. 8 weeks later the mice were resensitised to assess tolerance. Mice exposed to the minimum immunosuppressive dose of ssUVR prior to primary sensitisation were tolerant to re-sensitisation. However, at higher doses of ssUVR, these mice were protected from tolerance. Interestingly, while low doses of UVA caused immunosuppression, even lower doses enhanced the response to the second sensitisation. Higher doses of UVA had no affect. UVB induced tolerance in a dose related manner. Thus, ssUVR only induces immunosuppression and tolerance over a narrow dose range. Both UVA and UVB are immunosuppressive at this dose, while higher doses of UVA protect from the suppressive effects of UVB. Surprisingly very low doses of UVA enhanced memory development. Thus UVR has complex effects on the immune system depending on dose and spectrum.

Key Words: Immunosuppression, contact sensitivity, Ultraviolet A, Ultraviolet B, tolerance, immunological memory

INTRODUCTION

UV* radiation is divided into 3 wavebands, UVC (<290 nm), UVB (290-320 nm) and UVA (320-400 nm). UVC has the highest energy, and hence is the most biologically damaging, but is blocked by the stratospheric ozone layer and does not reach the Earth's surface. Sunlight is therefore a mixture of UVB and UVA. One way UV radiation causes skin cancer is by suppressing the natural immune system, so that we are not able to immunologically destroy cancer cells [1].

Local immunosuppression results from the irradiation of an area of skin with UV (eg dorsal trunk) and is characterised by the inability to mount an immune response to an antigen applied to the same skin site (dorsal trunk). Systemic immunosuppression on the other hand is when one site receives the irradiation (eg dorsal trunk) but the antigen is applied to a site distal to that which received UV (eg abdomen). This project investigates systemic immunosuppression. In mice and humans, local immunosuppression appears to be caused by disruption to the function of Langerhans cells, which are epidermal dendritic antigen presenting cells at the irradiated site [2]. Langerhans cells take up antigen and migrate to local lymph nodes where they induce an immune response. Disruption to these cells is an important suppressive event as it inhibits the activation of an effective immune response [3]. Systemic immunosuppression is caused by absorption of UV by one or more photoreceptors at the irradiated site culminating in activation of regulatory T cells with suppressor activity [4].

Whereas there is agreement in the literature that UVB induces immunosuppression, the effect of UVA on the immune system remains controversial. We have previously found that chronic exposure to low dose UVA causes suppression of contact hypersensitivity, and this was accompanied by a depletion in epidermal Langerhans cells (Figure 1) in mice [5]. These effects of UVA could be

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prevented by topical application of Vitamin E [6]. We have also shown that UV-induced nitric oxide production is an important mediator of Langerhans cell loss and immunosuppression [7] and that low-dose UVA causes immunosuppression in humans [8].

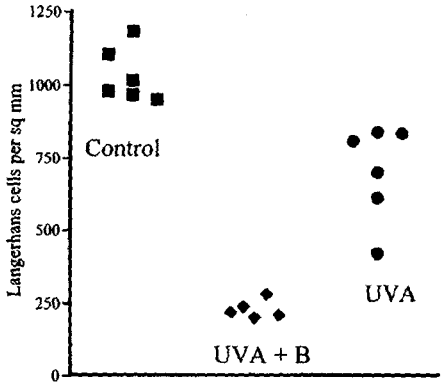

Figure 1. Both UVA and UV containing a mixture of UVA and UVB reduce the number of Langerhans cells.

However the effects of UVA on the immune system are unclear. Reeve and colleagues have found that high doses of UVA protect the immune system from UVB-induced immunosuppression [9]. UVA upregulation of interferon [10] and heme oxygenase [11] were identified to be important for this immunoprotective effect of UVA. To address this controversy we constructed dose response curves for immunomodulation caused by UVB, UVA and solar-simulated UV. We studied effects on the induction of primary and secondary contact hypersensitivity.

MATERIALS AND METHODS

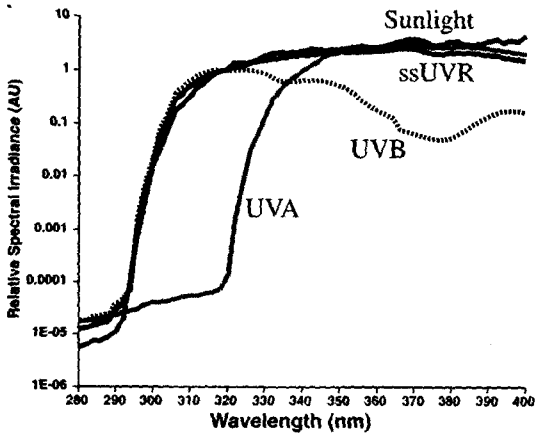

Figure 2. Spectra of the solar-simulated UV (ssUVR), UVA and UVB emitted from the Oriel solar simulator are compared with sunlight. Spectra used in these studies.

C57BL/6 mice were irradiated on their dorsal trunk for 3 consecutive days using an Oriel solar simulator. The spectrum emitted by this source is shown in Figure 2. The doses of UV are shown in the results figures.

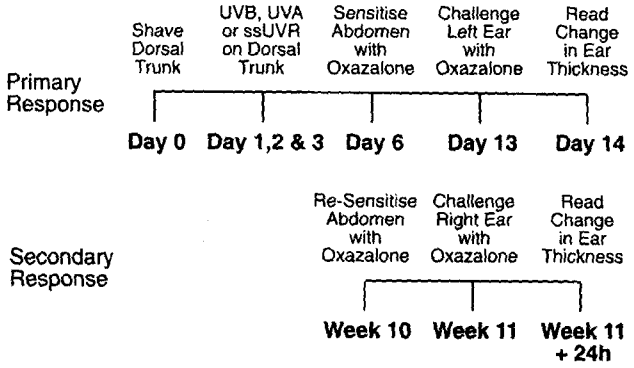

Figure 3. Experimental design.

To study the suppression of primary immunity the mice were contact sensitised with oxazolone applied to the abdomen 3 days after the final irradiation. Primary contact sensitivity was then assessed. As shown in Figure 3, the mice were then rested until week 10 when they were resensitized to assess the effects of the UV exposure on secondary immunity.

RESULTS AND DISCUSSION

As shown in Figure 2, sunlight does not contain any UVC (wavelengths below 290 nm), and the UVB component (up to 320 nm) is at a much lower intensity than UVA (up to 400 nm). On a photon-to-photon basis UVB and C have the most energy and mediate their immunsuppressive effects through direct absorption by a chromophore. UVA on the other hand, can penetrate deeper into the layers of skin. The Oriel xenon arc solar simulator used in these studies delivers a spectrum essentially identical to that of sunlight (ssUVR). Also, the shape of the UVB spectrum, and the shape of the UVA spectrum closely match those parts of the solar spectrum. As this source has an intense output with automatically timed shutters, irradiation times are accurate and short, enabling precise doses to be delivered to temporarily immobilised mice. Because we have now been able to construct, for the first time, accurate dose response curves with defined spectra, we have observed electromagnetic UV spectra to have a previously unknown intricate effect on the immune system.

The data shown in Figure 4 is a pool of 3 experiments with C57BL/6 mice. The top graph shows the

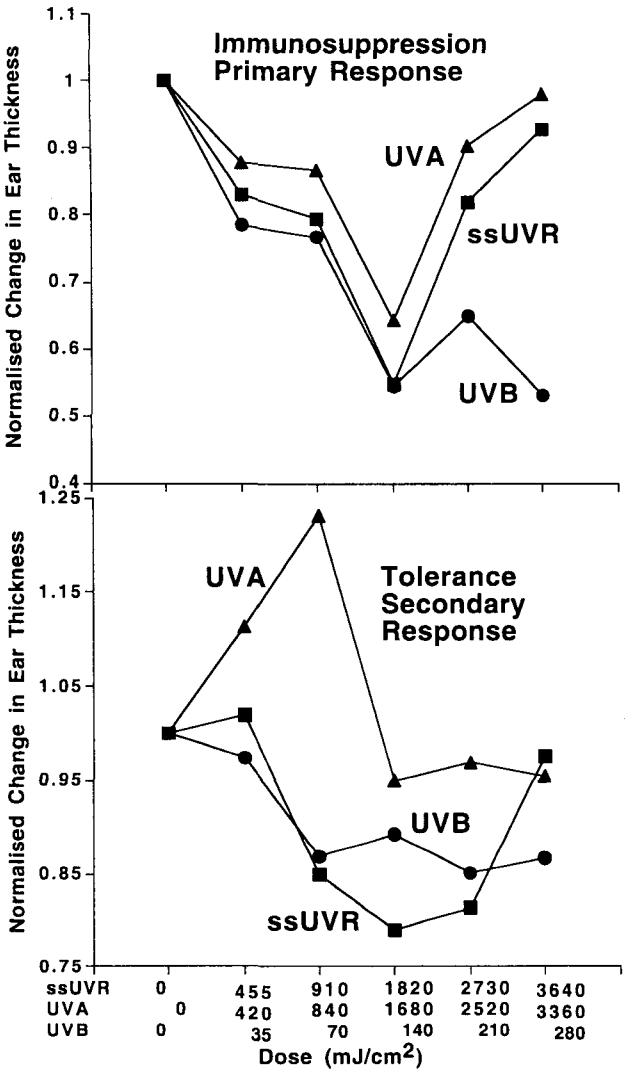

Figure 4. Contact hypersensitivity responses in groups of C57BL/6 mice irradiated with the doses of UVB, UVA, or solar-simulated (ssUVR). Top figure: UV modulation of the primary response. Bottom figure: UV modulation of secondary immune responses.

primary immune response where mice were irradiated with doses of either ssUV, UVB or UVA for 3 consecutive days on their dorsal trunk followed by sensitisation on their abdomen 3 days later as a test for systemic immunosuppression. Mice were then ear challenged 7 days later for assessment of this primary response. In the bottom graph the same mice were re-sensitised 8 weeks later and re-challenged to assess the secondary immune response (the effect of the primary response on the ability to respond to the same antigen a second time). The UV doses are in mJ/cm^2, ssUVR is the summation of the UVB and UVA components, which were also given separately. The highest dose of ssUVR delivered to the mice is only 1 minimum erythemal dose (MED; the minimum amount of ssUVR which causes barely detectable sunburn); therefore humans could easily be exposed to these doses during normal daily activities and the doses studied are all relatively low. Consistent with a considerable amount of literature, UVB caused a dose-related suppression of both the primary and secondary immune responses (both immunosuppression and tolerance). Only 0.25 MED UVB was needed to cause both immunosupression and tolerance.

UV suppression of secondary responses determines whether primary exposure to antigen following UV irradiation failed to activate effector cells or instead activated regulatory T cells which suppress a second response to the antigen (Figure 5). As UVB suppressed secondary immunity it must have resulted in the activation of regulatory T cells as reported previously [12].

We found that doses of UVA up to 840 did not modulate primary immunity, while 1680 but not higher doses was immunosuppressive. This bell-shaped dose response curve is consistent with previous data that we have obtained in humans, showing that low, but not high cumulative doses of UVA are immunosuppressive [8]. This is also consistent with data showing that high dose UVA is immunoprotective [9]. It is also consistent with previous data by our group that low doses of UVA are immunosuppressive [5].

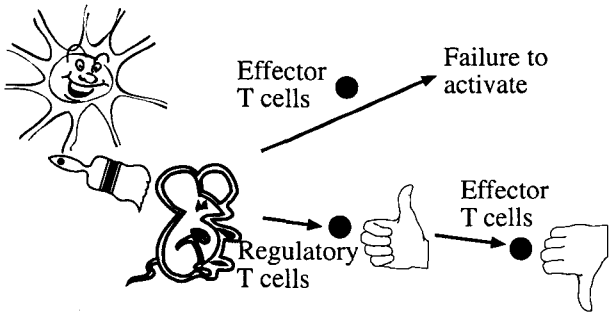

Figure 5. Schematic diagram of UV suppression of primary and secondary immunity. UV can result in a failure of effector T cells to become activated, or can activate regulatory T cells which inhibit effector T cells.

A completely novel finding is that UVA doses lower than those which caused immunosuppression enhanced secondary immunity. Low dose UVA augmented memory development. This is important when considering the effects of sunlight on immunomodulation. UVA did not cause tolerance, and higher doses of UVA did not modulate secondary immunity.

ssUVR induced immunosuppression at 1820, consistent with the combined immunosuppressive effects

of UVA and UVB, and at higher doses ssUVR was not immunosuppressive, showing that UVA at this dose prevented the immunosuppressive effects of UVB. Tolerance was only induced by a narrow dose range of ssUVR, the protective effect of higher dose UVA, and memory enhancement by low dose UVA restricting the ability of UVB in the ssUVR to induce tolerance as summarised in Figure 6.

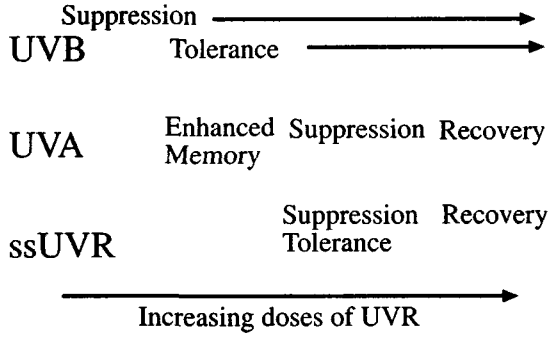

Figure 6. Summary of results showing relative doses at which UVB and UVA modulate the immune system.

CONCLUSION

Clearly different UV spectra switch on different signals at different doses, and sunlight can no longer be regarded as immunosuppressive at all doses. It is our hypothesis that these dose responses result from differing effects on effector or regulatory T cell activation. Whereas UVB was found to activate suppressive regulatory T cells, low dose UVA enhanced secondary immunity, probably by enhancing the survival of antigen activated long-lived memory T cells (Figure 7). This would result in a larger response upon subsequent contact with antigen. However at higher doses UVA switched on different responses resulting in immunosuppression, and at even higher doses initiated different responses resulting in immunoprotection. Sunlight appears to have an effect which is additive of the combined effects of UVA and UVB (Figure 6).

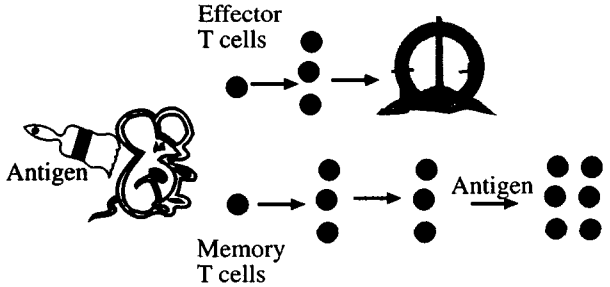

Figure 7. Antigen exposure results in the activation of short-lived effector T cells and long-lived memory T cells.

REFERENCES

1. Kripke, M. L. (1994) Ultraviolet radiation and immunology: Something new under the sun - Presidential address. *Cancer Res.* 54, 6102-6105.

2. Streilein, J. W., J. R. Taylor, V. Vincek, et al. (1994) Immune Surveillance and Sunlight-Induced Skin Cancer. *Immunol Today.* 15, 174-179.

3. Halliday, G. M., K. A. Odling, J. C. Ruby, H. K. Muller (1988) Suppressor cell activation and enhanced skin allograft survival after tumor promotor but not initiator induced depletion of cutaneous Langerhans cells. *J Invest Dermatol.* 90, 293-297.

4. Shreedhar, V. K., M. W. Pride, Y. Sun, M. L. Kripke, F. M. Strickland (1998) Origin and Characteristics Of Ultraviolet-B Radiation-Induced Suppressor T Lymphocytes. *J Immunol.* 161, 1327-1335.

5. Bestak, R., G. M. Halliday (1996) Chronic low-dose UVA irradiation induces local suppression of contact hypersensitivity, Langerhans cell depletion and suppressor cell activation in C3H/HeJ mice. *Photochem Photobiol.* 64, 969-974.

6. Yuen, K. S., G. M. Halliday (1997) Alpha-Tocopherol, an Inhibitor Of Epidermal Lipid Peroxidation, Prevents Ultraviolet Radiation From Suppressing the Skin Immune System. *Photochem Photobiol.* 65, 587-592.

7. Yuen, K. S., M. R. Nearn, G. M. Halliday (2002) Nitric oxide-mediated depletion of Langerhans cells from the epidermis may be involved in UVA radiation-induced immunosuppression. *Nitric Oxide.* 6, 313-318.

8. Damian, D. L., R. S. Barnetson, G. M. Halliday (1999) Low-dose UVA and UVB have different time courses for suppression of contact hypersensitivity to a recall antigen in humans. *J Invest Dermatol..* 112:939-944.

9. Reeve, V. E., M. Bosnic, C. Boehmwilcox, N. Nishimura, R. D. Ley (1998) Ultraviolet A Radiation (320-400 Nm) Protects Hairless Mice From Immunosuppression Induced By Ultraviolet B Radiation (280-320 Nm) or Cis-Urocanic Acid. *Int Arch Allergy Immunol.* 115, 316-322.

10. Reeve, V. E., M. Bosnic, N. Nishimura (1999) Interferon-gamma is involved in photoimmunoprotection by UVA (320-400 nm) radiation in mice. *J Invest Dermatol.* 112, 945-50.

11. Reeve, V. E., R. M. Tyrrell (1999) Heme oxygenase induction mediates the photoimmunoprotective activity of UVA radiation in the mouse. *PNAS.* 96, 9317-9321.

12. Glass, M. J., J. W. Streilein (1990) UVB radiation and DNFB skin painting induce suppressor cells universally in mice. *J Invest Dermatol.* 94, 273-278.