

## Chapter 10

Summary and General discussion

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It has been well recognized that exposure to ultraviolet B (UVB), as in sunlight, can modulate the immune system in animals and humans. Animal infection models have demonstrated that exposure to UVB suppresses the resistance to several infectious agents, *i.e.* bacteria, parasites, viruses and fungi, and increases the morbidity and mortality. Importantly, the effects of UV exposure are not restricted to skin-associated infections, but also concern non-skin-associated, *i.e.* systemic infections<sup>1-14</sup>.

Risk analysis, by extrapolating animal data obtained from infection models to the human situation, indicated that exposure to UV at doses relevant for outdoor exposure impairs the human immune system sufficiently to induce reduced resistance to infections<sup>15-17</sup>. Since extrapolation relies on many assumptions, experimental studies with human volunteers needed to be performed. For ethical reasons it is obvious that infection studies cannot be performed in humans. Epidemiological studies have provided some information on effects of sun exposure on infections, but are far from conclusive<sup>18-20</sup>.

Vaccination, which relies on the principle of development of an immune response to agents derived from infectious organisms, can be performed and effects of UV can thus be studied. Induction of antibody responses requires the involvement of major components of the immune system, and alteration of antibody levels by exogenous factors such as UV radiation may thereby reflect an altered immune system<sup>21,22</sup>.

The aim of this study was to investigate the effects of UVB exposure on the development of immune responses after vaccination against hepatitis B to improve the risk estimation of the immunosuppressive effects of UVB exposure and as a consequence altered resistance to infections in humans.

### Hepatitis B vaccination models

First, effects of UVB exposure on hepatitis B vaccination responses were established in a mouse model (chapter 2). Therefore two different mouse strains, BALB/c and C57Bl/6, were exposed to different doses of UVB, after which they were vaccinated intramuscularly with a commercial available hepatitis B vaccine, Engerix-B<sup>®</sup>, that contains a recombinant surface antigen. This vaccine contains aluminium hydroxide as an adjuvant, which can shift T cell responses in a Th2 direction<sup>23</sup>. Hence, the mice developed Th1 responses (DTH and IgG2a) as well as Th2 responses (IgG1), providing a good system for studying effects of UVB exposure on both arms of the immune system.

UVB preexposure suppressed mainly the cellular immune response although the IgG2a antibody titers were impaired also. The extent of this

UVB-induced immunosuppression depends on the immune response induced after this vaccination, which depends *a.o.* on the H2-histocompatibility complex<sup>24</sup>. In addition, the susceptibility to UVB-induced immunosuppression differs between different mouse strains<sup>25</sup>. These findings implicated a possible effect of UVB exposure on hepatitis B vaccination responses in humans.

However, the human volunteer study, described in chapter 3, revealed that exposure to UVB for 5 consecutive days with one personally determined minimal erythema dose per day prior to hepatitis B vaccination, did not significantly alter the cellular and humoral immune responses against hepatitis B surface antigen. Remarkably, natural killer cell activity (non-specific immunity) and contact hypersensitivity responses against diphenylcyclopropenone (specific immunity) were suppressed in these same volunteers indicating that the UVB exposure protocol was sufficient to induce immunosuppression.

### **Cytokine polymorphisms and their role in immune responses and UVB-induced immunomodulation in the hepatitis B vaccination model in man**

Effects of UV exposure on immune responses in different strains of mice revealed that mice differ in their immune response evoked upon the vaccination, and differ in their susceptibility to UVB-induced immunosuppression (chapter 2). Differences in immune responses have in part been ascribed to differences in cytokine levels due to inheritable single nucleotide polymorphisms (SNPs) present within regulatory elements of cytokine genes<sup>26-30</sup>. It was hypothesized that cytokine polymorphisms could reveal interindividual differences in hepatitis B vaccination responses as well as differences in susceptibility to UVB-induced immunomodulation of these vaccination responses in humans. In chapter 4 it was demonstrated that both the humoral and cellular immune response to hepatitis B surface antigen (HBsAg) were significantly increased in individuals possessing the minor allelic variant of IL-1 $\beta$  (+3953). As described in chapter 5, possession of this minor allelic variant of IL-1 $\beta$  resulted in significantly suppressed antibody responses to hepatitis B after exposure to UVB. Increased minimal erythema dose values, which resulted in higher absolute UVB exposures, were observed in these same individuals. In other words, the cytokine polymorphism IL-1 $\beta$  plays a significant role in the susceptibility to UVB-induced acute skin effects as well as in immunomodulation in humans. It is known that individuals homozygous for the IL-1 $\beta$  (+3953) allele produce approximately four-fold and heterozygous cells produce approximately two-

fold more IL-1 $\beta$  compared to individuals homozygous for the wildtype allele<sup>26</sup>. The increase in antibody titers and lymphocyte proliferation against hepatitis B in individuals with the IL-1 $\beta$  polymorphism can be explained by the immunostimulatory properties of IL-1 $\beta$ . IL-1 $\beta$  is able to stimulate B cell growth and differentiation<sup>31</sup> and to increase primary antibody responses<sup>31-33</sup>. In addition, IL-1 $\beta$  upregulates Th1 cytokines, including IL-2<sup>34</sup>, resulting in enhanced T cell proliferation<sup>35</sup>. If IL-1 $\beta$  is immunostimulatory, how can the suppressed antibody titers of individuals with the IL- $\beta$  polymorphism after exposure to UVB then be explained? Probably, another mechanism will be initiated by exposure to UVB, and ultimately result in immunosuppression. UVB radiation is known to increase IL-1 by keratinocytes<sup>36</sup>, and increased IL-1 levels have been found in serum of human volunteers after total body UVB irradiation<sup>37</sup>. PGE<sub>2</sub> that promotes a Th2 immune response, by suppression of IL-12 production and stimulating the production of IL-10<sup>38</sup>, is induced after UVB radiation by keratinocyte-derived IL-1<sup>39</sup>. It has been suggested that UVB radiation activates a cytokine cascade, involving PGE<sub>2</sub>, IL-4 and IL-10<sup>40</sup>, which will ultimately result in immunosuppression<sup>41</sup>. It might be hypothesized that individuals that produce more IL- $\beta$ , ultimately show more immunosuppression after exposure to UVB. The reason why upregulated IL-1 $\beta$  after UVB radiation initiates immunosuppression remains to be investigated.

It might be postulated that SNPs reveal interindividual differences in UV-induced modulation of immune responses and that these need to be taken into account. For use of vaccination responses as indicators of immunomodulating effects by exogenous factors, such genetic variations in cytokine genes are important for a proper evaluation.

### UVB-induced mediators

Effects of UVB exposure on the immune system are initiated by the absorption of UV photons by certain photoreceptors in the skin, such as urocanic acid (UCA)<sup>42</sup> and DNA<sup>43</sup>. As a consequence, different cytokines, chemokines, prostaglandins and neuropeptides are produced that are involved in UV-induced immunomodulation<sup>44-48</sup>. UV converts UCA, present in the skin as the *trans*-isomer, to the *cis*-isomer<sup>49</sup>. In animal models, *cis*-UCA has been demonstrated to be an important mediator of UVB-induced immunosuppression<sup>50</sup>. Chapter 6 describes that *cis*-UCA levels were significantly increased in human Finn chamber skin samples, sampled in the context of the earlier described human volunteer study, after exposure to UVB. The *cis*-UCA increment depended on the dose of UVB received. Correlations with the

hepatitis B vaccination responses showed that individuals with high *cis*-UCA levels revealed low lymphocyte proliferation responses against hepatitis B. This indicated that *cis*-UCA might be an important mediator of UVB-induced immunosuppression in humans also. In addition, significant effects of UVB exposure on hepatitis B vaccination responses in the human volunteer study were not observed when the total studied population was taken into account. However, when individuals with high *cis*-UCA levels were taken into account than deleterious effects of UVB on vaccination responses could be observed. This indicates that individuals differ in their susceptibility to UVB-induced immunomodulation. Thus, differences in susceptibility to UVB might be determined by differences in immune responses due to SNPs (chapter 5), and also due to differences in UVB-induced UCA isomerisation and its immunological consequences (chapter 6).

Another mediator in the mechanism of UVB-induced immunomodulation, which has recently gained a lot of attention, is calcitonin gene-related peptide. In animal models it has been demonstrated that CGRP plays a role in UVB-induced immunosuppression<sup>48,51,52</sup>. In chapter 7, it was demonstrated that CGRP levels can be measured in human Finn chamber skin samples, and are dose-dependently increased after exposure to UVB. Differences in CGRP levels could also reveal interindividual differences regarding susceptibility to UVB-induced immunomodulation and need therefore to be further investigated.

#### **Other factors involved in modulation of vaccination responses and the possible consequences for extrapolation from mice to humans**

The vaccine used in the murine and the human hepatitis B vaccination model, Engerix-B<sup>®</sup>, contains aluminium hydroxide, which is known to skew the type of immune response to Th2<sup>23</sup>. UVB exposure, primarily affects Th1 immune responses<sup>53-55</sup>, although effects of UVB on Th2 immune responses have been described also<sup>1,6,56,57</sup>. In the murine model significant decreased vaccination responses were found, although only the Th1-associated immunity was affected. The human volunteer study did not reveal any significant effect, although susceptible individuals (*i.e.* with the IL-1 $\beta$  polymorphism or with high *cis*-UCA levels) showed lower vaccination responses. In chapter 8, it was investigated using the mouse model whether different adjuvants, which induce different type of immune responses, are differentially affected by UVB exposure. It was concluded that different adjuvants indeed induce different immune responses; yet UVB exposure affects the development of immunity against hepatitis B regardless the type of adjuvant used.

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The differences between the murine and the human hepatitis B vaccination models can be ascribed to i) interspecies differences ii) difference in sensitivity to UVB exposure and possibly to iii) difference in history of sunlight exposure. Mice have never seen sunlight during their lifetime, and humans have been exposed to sunlight from an early age onwards. In addition, mice are nocturnal animals. Whether “adaptation” to UVB offers an explanation of the difference between the mouse and the human hepatitis B vaccination model, was investigated by chronic UVB exposure of BALB/c mice, after which they received an immunosuppressive protocol with UVB (chapter 9). Effects of this chronic exposure protocol were established on hepatitis B vaccination responses, and on contact hypersensitivity (CHS) responses. It was demonstrated that the “adaptation protocol” could prevent the UVB-induced immunosuppression of hepatitis B vaccination responses, but not the UVB-induced suppression of CHS responses. This might explain why no significant effects on the hepatitis B vaccination responses in the human volunteer study were found, but significant suppression of the contact type hypersensitivity responses was demonstrated. However, individuals with the IL-1 $\beta$  polymorphism or high *cis*-UCA levels showed suppressed responses. Are these individuals then not or less adapted to the deleterious effects of UVB? This needs to be further investigated before we can ascribe the differences between the two hepatitis B vaccination models to “immunological adaptation” to UVB.

### Concluding remarks and future research

The objective of this study was to further improve the risk assessment of the immunosuppressive effects of UVB exposure and as a consequence altered resistance to infections in humans. In this study the response to a hepatitis B vaccine was used as a model for responses to infectious agents. It was demonstrated that the human immune system can be affected by UVB exposure, which suggests that resistance to infections might be altered by UVB exposure also.

It can be concluded, that vaccination responses can be a readout parameter for immunomodulating effects of UV exposure. Hence, when vaccination studies are used as a model to determine the effects of exogenous factors on the human immune system, the dose of the vaccine and the adjuvant should be considered. It is known that not all individuals react equally to the hepatitis B vaccine. The response to the hepatitis B vaccine is correlated to the type of HLA-DR<sup>58,59</sup>. The dose of the vaccine is chosen in such a way, that all individuals develop a sufficient immune

response. The level of UVB-induced immunosuppression depends on the dose of the antigen<sup>60</sup>, so it might be that vaccination with lower doses of vaccine might reveal significant effects of UV exposure in the overall population. This should be further investigated since vaccination with lower doses of vaccine is suggested to reduce the cost of vaccination against hepatitis B<sup>61,62</sup>. This vaccination with a lower dose of vaccine also occurs via the skin, which increases the risk that vaccination responses are suppressed after UV exposure. As described in chapter 8, vaccination against hepatitis B in the mouse model was significantly suppressed after exposure to UVB regardless the type of adjuvant used. Yet, whether different adjuvants influence the susceptibility to UVB-induced immunosuppression in humans remains to be established. In addition, regarding the lower vaccination responses in the human subpopulations, the effect of UVB exposure on the level of protection after vaccination remains to be established. It is not likely that protection is severely, if at all, affected. Yet, this needs to be further elucidated. An infection model, in which UVB-irradiated vaccinated mice are infected with the infectious agent, would be a good model to investigate the level of protection. However, mice cannot be infected by the hepatitis B virus since they are not the natural host<sup>63</sup>, so another vaccine and infectious agent has to be used.

To improve the risk assessment of the effects of UV on the resistance to infectious diseases in humans by extrapolating data obtained in the murine hepatitis B vaccination model to the human situation is difficult to establish. Interspecies (between mouse and humans) and interindividual differences (in the human population) in types of immune response and in susceptibility to UVB-induced immunomodulation are factors that make the risk assessment more difficult. Interindividual differences in humans might be revealed by cytokine polymorphism determination or determination of UV-induced "immunosuppressive" mediators. However, recently many cytokine polymorphisms have been discovered, and it should be further investigated which polymorphisms designate the susceptibility to UVB-induced immunomodulation. The same is true for UV-induced "immunosuppressive" mediators, which might serve as a biomarker. The question remains, at what level does an "immunosuppressive" mediator indicate that UVB-induced immunosuppression is severe enough to have health consequences regarding decreased resistance to infections?

In addition, adaptation to UV should be considered also. Further investigation should be performed to determine the effects of chronic irradiation and adaptation to UV on the immune system. Adaptation of the skin has been described extensively<sup>64,65</sup>, but whether the effects described in

chapter 9 are due to adaptation of the immune system to the deleterious effects of UV, remains to be further investigated.

## REFERENCES

1. Brown EL, Rivas JM, Ullrich SE, Young CR, Norris SJ, Kripke ML: Modulation of immunity to *Borrelia burgdorferi* by ultraviolet irradiation: differential effect on Th1 and Th2 immune responses. *Eur J Immunol* **25**: 3017-3022, 1995
2. Denkins Y, Fidler IJ, Kripke ML: Exposure of mice to UVB radiation suppresses delayed hypersensitivity to *Candida albicans*. *Photochem Photobiol* **49**: 615-619, 1989
3. Garssen J, Van Der Vliet H, De Klerk A, Goettsch W, Dormans JAMA, Bruggeman CA, Osterhaus ADME, Van Loveren H: A rat cytomegalovirus infection model as a tool for immunotoxicity testing. *Eur J Pharmacol Envir Toxic* **292**: 223-231, 1995
4. Garssen J, Van Der Molen R, De Klerk A, Norval M, Van Loveren H: Effects of UV irradiation on skin and nonskin-associated herpes simplex virus infections in rats. *Photochem Photobiol* **72**: 645-651, 2000
5. Giannini MSH: Suppression of pathogenesis in cutaneous Leishmaniasis by UV-irradiation. *Infect Immunol* **51**: 838-843, 1986
6. Goettsch W, Garssen J, Deijns A, De Gruijl FR and Van Loveren H: UVB exposure impairs resistance to infections with *Trichinella spiralis*. *Env Health Perspectives* **102**: 298-301, 1994
7. Goettsch W, Garssen J, De Klerk A, Herremans TMPT, Dortant P, De Gruijl FR, Van Loveren H: Effects of Ultraviolet-B exposure on the resistance to *Listeria monocytogenes* in the rat. *Photochem Photobiol* **63**: 672-679, 1996
8. Jeevan A, Evans R, Brown EL, Kripke ML: Effect of local ultraviolet irradiation on infections of mice with *Candida albicans*, *Mycobacterium bovis BCG*, and *Schistosoma mansoni*. *J Invest Dermatol* **99**: 59-64, 1992
9. Jeevan A, Gilliam K, Heard H, Kripke ML: Effects of ultraviolet radiation on the pathogenesis of *Mycobacterium lepreum* infection in mice. *Exp Dermatol* **1**: 152-160, 1992
10. Letvin NL, Kauffman RS, Finberg R: T lymphocyte immunity to reovirus: cellular requirements for generation and role in clearance of primary infections. *J Immunol* **127**: 2334-2339, 1981
11. Norval M, El-Ghorr AA: UV and mouse models of herpes simplex virus infection. *Photochem Photobiol* **64**: 242-245, 1996
12. Ryan LK, Neldon DL, Bishop LR, Gilmour MI, Daniels MJ, Sailstad DM, Selgrade MJ: Exposure to ultraviolet radiation enhances mortality and pathology associated with influenza virus infection in mice. *Photochem Photobiol* **72**: 497-507, 2000
13. Ward MW, Sailstad DM, Andrews DA, Boykin EH, Selgrade MJ: Effects of ultraviolet radiation (UVR) on the allergic respiratory responses of BALB/c mice to a fungal allergen. *Toxicol Sci* **60S**: 207, 2001
14. Yamamoto K, Ito R, Koura M, Kamiyama T: UV-B irradiation increases susceptibility of mice to malarial infection. *Infect Immun* **68**: 2353-2355, 2000

15. Goettsch W, Garssen J, Slob W, de Gruijl FR, Van Loveren H: Risk assessment for the harmful effects of UVB radiation on the immunological resistance to infectious diseases. *Environ Health Perspect* **106**: 71-77, 1998
16. Goettsch W, Hurks HM, Garssen J, Mommaas AM, Slob W, Hoekman J, Pierik F, Roholl PJ, Van Loveren H: Comparative immunotoxicology of ultraviolet B exposure I. Effects of in vitro and in situ ultraviolet B exposure on the functional activity and morphology of Langerhans cells in the skin of different species. *Br J Dermatol* **139**: 230-238, 1998
17. Garssen J, Norval M, Van Loveren H: UV-B induced immunomodulation: a health risk. *Polar Research* **18**: 339-343, 2000
18. Bouwes Bavinck JN, De Boer A, Vermeer BJ, Harteveldt MM, Van Der Woude FJ, Claas FH, Wolterbeek R, Vandenbroucke JP: Sunlight, keratotic skin lesions and skin cancer in renal transplant recipients. *Br J Dermatol* **129**: 242-249, 1993
19. Termorshuizen F, Boland GJ, De Gruijl FR, Garssen J, Van Loveren H, Van Hattum J: Influence of season on antibody response to high dose rDNA hepatitis B vaccine: effect of exposure to solar UVR? *Eur J Gastroenterol Hepatol* **11**: A94-A95, 1999
20. Termorshuizen F, Geskus RB, Roos MT, Coutinho RA, Van Loveren H: Seasonal influences on immunological parameters in HIV-infected homosexual men: searching for the immunomodulating effects of sunlight. *Int J Hyg Environ Health* **205**: 379-384, 2002
21. Van Loveren H, Germolec D, Koren HS, Luster MI, Nolan C, Repetto R, Smith E, Vos JG, Vogt RF: Report of the Bilthoven Symposium: Advancement of epidemiological studies in assessing the human health effects of immunotoxic agents in the environment and the workplace. *Biomarkers* **4**: 135-157, 1999
22. Van Loveren H, Van Amsterdam JGC, Vandebriel RJ, Kimmman TG, Rümke HC, Steerenberg PS, Vos JG: Vaccine-induced antibody responses as parameters of the influence of endogenous and environmental factors. *Environ Health Persp* **109**: 757-764, 2001
23. Gupta RK: Aluminium compounds as vaccine adjuvants. *Adv Drug Deliv Rev* **32**: 155-172, 1998
24. Neurath AR, Stark D, Strick N, Sproul P: H-2 linked genetic control of immune responsiveness to hepatitis B surface antigen (HBsAg) in mice. *J Med Virol* **12**: 227-236, 1983
25. Noonan FP, Hoffman HA: Susceptibility to immunosuppression by ultraviolet B radiation in the mouse. *Immunogenetics* **39**: 29-39, 1994
26. Pociot F, Molvig L, Wogensen L, Warsaae H, Nerup J: A TagI polymorphism in the human interleukin beta (IL-1beta) gene correlates with IL-1beta secretion in vitro. *Eur J Clin Invest* **22**: 396-402, 1992
27. Mandrup-Poulsen T, Pociot F, Molvig J, Shapiro L, Nilsson P, Emdal T, Roder M, Kjems LL, Dinarello CA, Nerup J: Monokine antagonism is reduced in patients with IDDM. *Diabetes* **43**: 1242-1247, 1994
28. Danis VA, Millington M, Hyland VJ, Grennan D: Cytokine production by normal human monocytes: inter-subject variation and relationship to an IL-1 receptor antagonist (IL-1RA) gene polymorphism. *Clin Exp Immunol* **99**: 303-310, 1995
29. Perrey C, Pravica V, Sinnot PJ, Hutchinson IV: Genotyping for polymorphisms in interferon-gamma, interleukin-10, transforming growth factor-beta-1 and

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- tumour necrosis factor-alpha genes: a technical report. *Transpl Immunol* **6**: 193-197, 1998
30. Engebretson SP, Lamster IB, Herrera-Abreu M, Celenti RS, Timms JM, Chaudhary AG, Di Giovine FS, Kornman KS: The influence of interleukin gene polymorphism on expression of interleukin-1 beta and tumor necrosis factor-alpha in periodontal tissue and gingival crevicular fluid. *J Periodontol* **70**: 567-573, 1999
  31. Reed SG, Pihl DL, Conlon PJ, Grabstein KH: IL-1 as adjuvant. Role of the T cells in the augmentation of specific antibody production by recombinant human IL-1 $\beta$ . *J Immunol* **142**: 3129-3134, 1989
  32. Hoffman MK, Gilbert KM, Hirst JA, Scheid M: An essential role for interleukin-1 and a dual function for interleukin 2 in the immune response of murine B lymphocytes to sheep erythrocytes. *J Mol Cell Immunol* **3**: 29-37, 1987
  33. Frasca D, Boraschi S, Baschieri S, Bossu P, Tagliabue A, Adorini L, Doria G: In vivo restoration of T cell functions by human IL-1 $\beta$  or its 167-171 nonapeptide in immunodepressed mice. *J Immunol* **141**: 2651-2660, 1998
  34. Cork MJ, Crane AM, Duff GW: Genetic control of cytokines: Cytokine gene polymorphisms in Alopecia Areata. *Dermatol Clin* **14**: 671-678, 1996
  35. Knoller I, Hoffmann UJ, Werchau HH, Konig W: Effects of mitogens and lymphokines on the regulation of the immune response to HBs antigen in vitro. *Immunology* **71**: 352-357, 1990
  36. Ansel JC, Luger TA, Green I: The effect of in vitro and in vivo UV irradiation on the production of ETAF activity by human and murine keratinocytes. *J Invest Dermatol* **81**: 519-523, 1983
  37. Granstein RD, Sauder DN: Whole-body exposure to ultraviolet radiation results in increased serum interleukin-1 activity in humans. *Lymphokine Res* **6**: 187-193, 1987
  38. Kalinski P, Hilkens CM, Snijders A, Snijdewint FG, Kapsenberg ML: IL-12-deficient dendritic cells, generated in the presence of prostaglandin E2, promote type 2 cytokine production in maturing human naive T helper cells. *J Immunol* **159**: 28-35, 1997.
  39. Pentland AP, Mahoney MG: Keratinocyte prostaglandin synthesis is enhanced by IL-1. *J Invest Dermatol* **94**: 43-46, 1990
  40. Shreedhar V, Giese T, Sung VW, Ullrich SE: A cytokine cascade including prostaglandin E2, IL-4 and IL-10 is responsible for UV-induced systemic immune suppression. *J Immunol* **160**: 3783-3789, 1998
  41. Rivas JM, Ullrich SE: Systemic suppression of DTH by supernatants from UV-irradiated keratinocytes: an essential role for interleukin-10. *J Immunol* **149**: 3865-3871, 1992
  42. Noonan FP, De Fabo EC: Immunosuppression by UVB radiation: initiation by urocanic acid. *Immunol Today* **13**: 250-254, 1992
  43. Kripke ML, Cox PA, Alas LG, Yarosh DB: Pyrimidine dimers in DNA initiate systemic immuno-suppression in UV-irradiated mice. *Proc Natl Acad USA* **89**: 7516-7520, 1992
  44. Luger TA, Schwarz T: Effects of UV-light on cytokines and neuroendocrine hormones. In: Krutmann J, Elmetts C (eds.). *Photoimmunology*. Oxford: Blackwell science, pp. 55-76, 1995

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45. Takashima A, Bergstresser PR: Impact of UVB radiation on the epidermal cytokine network. *Photochem Photobiol* **63**: 397-400, 1996
46. Boonstra A, Savelkoul HF: The role of cytokines in ultraviolet-B induced immunosuppression. *Eur Cytokine Netw* **8**: 117-123, 1997
47. Scholzen TE, Brzoska T, Kalden DH, O'Reilly F, Armstrong CA, Luger TA, Ansel JC: Effect of ultraviolet light on the release of neuropeptides and neuroendocrine hormones in the skin: mediators of photodermatitis and cutaneous inflammation. *J Invest Dermatol Symp Proc* **4**: 55-60, 1999
48. Garssen J, Buckley TL, Van Loveren H: A role for neuropeptides in UVB-induced systemic immunosuppression. *Photochem Photobiol* **68**: 205-210, 1998
49. Morrison H: Photochemistry and photobiology of urocanic acid. *Photodermatol* **2**: 158-165, 1985
50. Norval M, Gibbs NK, Gilmour J: The role of urocanic acid in UV-induced immunosuppression: recent advances (1992-1994). *Photochem Photobiol* **62**: 209-217, 1995
51. Gillardon F, Moll I, Michel S, Benrath J, Weihe E, Zimmermann M: Calcitonin gene-related peptide and nitric oxide are involved in ultraviolet radiation-induced immunosuppression. *Eur J Pharmacol* **293**: 395-400, 1995
52. Niizeki HP, Allard P, Streilein JW: Calcitonin gene-related peptide is necessary for ultraviolet-B-impaired induction of contact hypersensitivity. *J Immunol* **159**: 5183-5186, 1997
53. Araneo B, Dowell T, Moon HB, Daynes RA: Regulation of murine lymphocyte production in vivo. UV radiation exposure depresses IL-2 and enhances IL-4 production by T cells through an IL-1 dependent mechanism. *J Immunol* **143**: 1737-1744, 1989
54. Simon JC, Mosmann T, Edelbaum D, Schopf E, Bergstresser PR, Cruz PD: In vivo evidence that ultraviolet-B-induced suppression of allergic contact sensitivity is associated with functional inactivation of Th1 cells. *Photodermatol Photoimmunol Photomed* **10**: 206-211, 1994
55. Ullrich SE: Does exposure to UV radiation induce a shift to a Th-2-like immune reaction? *Photochem Photobiol* **64**: 254-258, 1996
56. Garssen J, Vandebriel RJ, De Gruijl FR, Wolvers D, Van Dijk M, Fluitman A, Van Loveren H: UVB exposure-induced systemic modulation of Th1 and Th2 mediated immune responses. *Immunology* **97**: 506-514, 1999
57. Van Loveren H, Boonstra A, Van Dijk M, Fluitman A, Savelkoul HF, Garssen J: UV exposure alters respiratory allergic responses in mice. *Photochem Photobiol* **72**: 253-259, 2000
58. Caillat-Zucman S, Gimenez JJ, Wambergue F, Albouze G, Lebki B, Naret C, Moynot A, Jungers P, Bach JF: Distinct HLA class II alleles determine antibody response to vaccination with hepatitis B surface antigen. *Kidney Int* **53**: 1626-1630, 1998
59. Desombere I, Willems A, Leroux-Roels G: Response to hepatitis B vaccine: multiple HLA genes are involved. *Tissue Antigens* **51**: 593-604, 1998
60. Miyauchi H, Horio T: Ultraviolet B-induced local suppression of contact hypersensitivity is modulated by ultraviolet irradiation and hapten application. *J Invest Dermatol* **104**: 364-369, 1995

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61. Nagafuchi S, Kashiwagi S, Imayama S, Hayashi J, Niho Y: Intradermal administration of viral vaccines. *Rev Med Virol* **8**: 97-111, 1998
62. Rivey MP, Peterson J: Intradermal hepatitis B vaccination. *DICP* **25**: 628-634, 1991
63. Pugh JC, Bassendine MF: Molecular biology of hepadnavirus replication. *Br Med Bull* **46**: 329-353, 1990
64. Kaidbey KH, Kligman AM: Sunburn protection by longwave ultraviolet radiation-induced pigmentation. *Arch Dermatol* **114**: 46-48, 1978
65. Bruls WA, Van Weelden H, Van Der Leun JC: Transmission of UV-radiation through human epidermal layers as a factor influencing the minimal erythema dose. *Photochem Photobiol* **39**: 63-67, 1984