UV-induced Immune Suppression in Humans: Wavelength Dependencies, Possible Mechanisms and Relation to Non-melanoma Skin Cancer

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LIST OF ABBREVIATIONS:

BCC	basal cell carcinoma
CD	cluster of differentiation
CTLA-4	cytotoxic T lymphocytes antigen
DNCB	dinitrochlorobenzene
DPCP	diphenylcyclopropanone
IFN	interferon
IL	interleukin
MED	minimal erythema doses
LPS	lipopolysaccharide
SCC	squamous cell carcinoma
Th	T helper
TNF-α	tumour necrosis factor alpha
UCA	urocanic acid
UV	ultraviolet
UVA	ultraviolet A (320–400 nm)
UVA-I	long-wave ultraviolet A (340–400 nm)
UVA-II	short-wave ultraviolet A (320–340 nm)
UVB	ultraviolet B (280–320 nm)
UVB-R	ultraviolet B resistant
UVB-S	ultraviolet B susceptible

TABLE OF CONTENTS

This thesis is based on the following papers, which will be referred to by their Roman numbers:

I. L Skov, H Hansen, JNWN Barker, J Simon, O Baadsgaard: Contrasting effects of UVA and UVB exposure on induction of contact sensitivity in human skin. Clin Exp Immunol, 1997; 107: 585–588.

II. L Skov, H Hansen, M Allen, L Villadsen, M Norval, JNWN Barker, J Simon, O Baadsgaard: Contrasting effects of ultraviolet A1 and ultraviolet B exposure on the induction of tumour necrosis factor-α in human skin. Br J Dermatol, 1998; 138: 216–220.

III. L Skov, L Villadsen, BK Ersbøll, JC Simon, JNWN Barker, O Baadsgaard: Long-wave UVA offers partial protection against UVB-induced immune suppression in human skin. APMIS 2000; 108: 825–30.

IV. L Skov, H Hansen, HC Dittmar, JNWN Barker, JC Simon, O Baadsgaard: Susceptibility to effects of UVB irradiation on the induction of contact sensitivity, relevance of number and function of Langerhans cells and epidermal macrophages. Photochem Photobiol, 1998; 67: 714–719.

V. M Allen, L Skov, R Barber, R Trembath, J Simon, O Baadsgaard, JNWN Barker: Ultraviolet B induced suppression of induction of contact sensitivity in human skin is not associated with tumour necrosis factor-alpha-308 or interleukin-10 genetic polymorphisms. Br J Dermatol, 1998; 139: 225–229.

VI. L Skov, MH Allen, B Bang, D Francis, JNWN Barker, O Baadsgaard: Basal cell carcinoma is associated with high TNF-α release but not with TNF-α polymorphism at position -308. Exp Dermatol, 2003; 12: 772–776.

PREFACE

The present thesis is based on investigations conducted during my employment at the Department of Dermatology, Gentofte Hospital, Denmark.

I am gratefull to Dr. Ole Baadsgaard for introducing me to research in immunodermatology and for continuous inspiration and encouragement. My special thanks to Professor Jonathan Barker, London and Professor Jan Simon, Leipzig for many stimulating discussions and for collaboration for many years.

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I want to thank Mette Ramm for technical assistance and staff members at the Department of Dermatology at Gentofte Hospital for interest in my work and help.

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INTRODUCTION

Non-melanoma skin cancer (basal cell carcinoma (BCC) and squamous cell carcinoma (SCC)) is the most frequent malignant condition in the white population and epidemiologic studies indicate that chronical exposure to ultraviolet (UV) radiation from sunlight is the principal cause of skin cancer (1). The solar UV wavelength consists of UVC (200–280 nm), UVB (280–320 nm) and UVA (320–400 nm). Only UVA and UVB radiation is present in terrestrial sunlight and of biological relevance as the shorter solar wavelengths are blocked by stratospheric ozone. The exposure to UV radiation is increasing due to altered recreational behaviour and as a result of stratospheric ozone depletion (2). The main carcinogenic effect of the solar UV radiation is caused by direct DNA damage or indirectly by generation of reactive oxygen species, which can also cause DNA mutations, reviewed by De Gruijl (3). Only UVB radiation and not UVA radiation induces direct DNA damage and UVB radiation is generally believed to be the main cause of skin cancer (4). However, besides DNA damage, UV radiation of the skin induces immunosuppression, sunburn and skin aging. The UV-induced immunosuppression plays an important role in the development of non-melanoma skin cancer in mice (5, 6) and a similar role is suspected in humans supported by the increasing number of non-melanoma skin cancers seen among patients undergoing organ transplantation (7). UVB-induced immunosuppression not only plays a role in relation to skin cancer, UVB exposure has been demonstrated to impair immune dependent resistance to bacterial, viral, parasitic and fungal infection (8–10) and consequently has important implications for susceptibility to infectious diseases and vaccine responses. Sunlight is also the principal cause of the most lethal of the skin cancers, the cutaneous malignant melanoma which differs in many ways from non-melanoma skin cancer. The direct target of the sunlight and the wavelength responsible for induction of malignant melanoma is still not identified, mainly due to lack of good animal models. Malignant melanoma is associated with intense sporadic exposure to sunlight during childhood and the incidence is not increasing following organ transplantation (11). Only UV-induced immunosuppression in humans and the relation to non-melanoma skin cancer will therefore be discussed in this thesis.

Most studies on UV-induced immunosuppression are performed in mice. The aims of this thesis were to study in humans wavelength dependencies of UV-induced immunosuppression in a contact hypersensitivity model, possible mechanisms for UVB-induced immunosuppression, and a possible relation between UV-induced immunosuppression and non-melanoma skin cancer.

BACKGROUND

The main interest in photoimmunology started about 25 years ago with the observation that UV irradiation inhibits the immunological rejection of transplanted skin tumours. In mice, UVB-induced tumours are highly immunogenic and fail to grow when transplanted to syngenic mice (5). In contrast, when mice are exposed to subcarcinogenic doses of UV radiation before transplantation, tumours grow progressively (6) due to induction of antigen-specific lymphocytes which down-regulate the tumour immune responses (12, 13) (Fig. 1). How these antigen specific T cells are activated is unknown. One theory is that tumour antigens induced by UV irradiation are inappropriately presented through UV exposed skin resulting in down-regulatory mechanisms that inhibit immune responses against the incipient tumour and allow it to grow. Similarly UV exposure of rodents have been shown to interfere with another immunological *in vivo* model, namely the induction of contact hypersensitivity to epicutaneous antigens (14) and delayed type hypersensitivity to intracutaneous antigens (15). For practical reasons this model has been used both in man and mice as a model for induction of tumour antigens and have been used to study the UV-induced effect on the immune responses.

LOCAL AND SYSTEMIC IMMUNOSUPPRESSION

In mice two models of UV-induced immunosuppression have been studied namely local and systemic immunosuppression (14, 16). In local immunosuppression low doses of UV exposure induce unresponsiveness when haptens are painted on the site of UV exposure. In systemic immunosuppression high doses of UV exposure also affect immune responses to haptens applied at a distant non-UV-exposed site. Sensitization through UV-exposed or non-exposed skin in either local or systemic immunosuppression does not only induce unresponsiveness to the haptens, but leads to tolerance, since it was not possible to sensitize the mice with the same antigen at a later time point. Neither was the UV-induced unresponsiveness due to general immunosuppression since the mice could be sensitized to a non related antigen (14). The tolerance could be transferred with T cells from lymph nodes and spleen. Supernatant and destroyed cells were not able to transfer tolerance and the mice reacted only to the specific antigen. Therefore the tolerance seems to be due to induction of antigen specific T cells. Transfer of tolerance was observed in both the local (17) and the systemic model (18). Due to problems with purification and cloning of the cells, the nature of the regulator T cells has not been known until recently. Now several types of regulatory T cells have been described mainly belonging to the CD4 type T cells, expressing CD25, CTLA-4 and secreting interleukin-10 (IL-10) upon stimulation (19–21). Despite the fact that both UV-induced local and systemic immunosuppression leads to induction of antigen specific T cells, the UV-induced tolerance in the two models seems to involve different mechanisms. TNF-α and the antigen presenting capacity in the skin seem to be critically involved in local immunosuppression whereas IL-10 seems to be involved in systemic immunosuppression (see below).

In humans UV-induced local immunosuppression and the contact hypersensitivity model have been used in most studies. Studies in humans have clearly shown that UVB irradiation interferes with the induction of contact hypersensitivity to antigens applied on UVB-exposed skin (22–25) and a subset of the humans who fail to develop contact hypersensitivity after UVB irradiation also develop tolerance (23).

POSSIBLE CHROMOPHORES FOR UV-INDUCED IMMUNOSUPPRESSION

Several UV-absorbing chromophores in the skin have been identified but the most important UVB absorbing chromophore in the skin is nuclear DNA. Following UV irradiation the most frequent photoproducts in the skin are pyrimidine dimers and (6,4)-photoproducts (19, 26). Reducing the py-

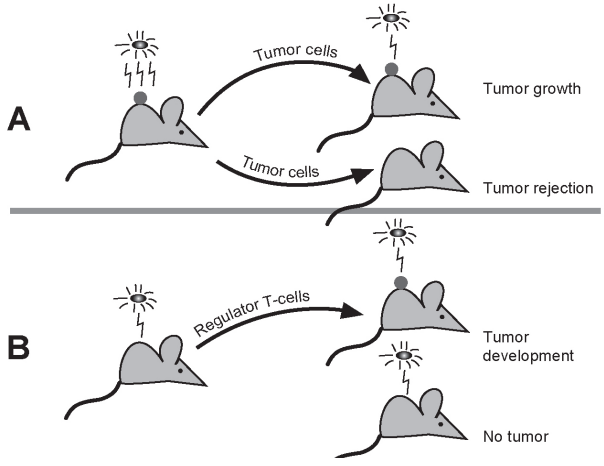

Fig. 1. In mice tumors are induced by chronic UV-irradiation. The tumors are highly immunogenic and are rejected when transplanted to normal syngeneic mice. In contrast, when such tumors are transplanted to syngeneic mice irradiated with subcarcinogenic doses of UV irradiation the tumors grow progressively (1A). The depressed immune response against UV-induced tumors can be transferred to naïve mice with T regulator cells from UV irradiated mice leading to rapid tumor development (1B). Reproduced with permission from Ugeskrift for Læger (Bang et Skov 2000; 162/50: 6862).

rimidine dimer formation significantly antagonized the immunosuppressive effect of UVB irradiation by blocking the inhibition of the induction of both contact hypersensitivity and delayed hypersensitivity (27, 28). DNA damage has been shown to trigger release of the two cytokines important for UV-induced immunosuppression namely IL-10 and TNF-α since topical treatment with DNA repair enzymes reduced the secretion of IL-10 and TNF-α from keratinocytes *in vitro* and in human skin (29–31).

Another UV-absorbing chromophore in the skin is urocanic acid (UCA). UCA is generated from histidine and accumulated in the skin due to lack of enzymes which catabolize UCA. UCA exists in two isoforms trans-UCA and cis-UCA. Trans-UCA is found in non-exposed epidermis but is converted to cis-UCA following irradiation. Injection of cis-UCA has been shown to suppress induction of delayed type hypersensitivity (32) and contact hypersensitivity (33) responses and removal of stratum corneum by tape stripping was associated with loss of UV-induced immunosuppression (34). The role of cis-UCA is complex since, in a more recent study where antibodies against cis-UCA were used, the authors found no effect of the anti-cis-UCA on UV-induced suppression of contact hypersensitivity. However, anti-cis-UCA completely restored the UV-induced suppression of delayed hypersensitivity and both in the case of contact and delayed hypersensitivity induction of regulator cells was inhibited (35).

Lastly, UV radiation has also been shown directly to affect cell membrane and to trigger surface receptors, reviewed by (36).

POSSIBLE MECHANISM FOR UV-INDUCED IMMUNOSUPPRESSION

In both local and systemic immunosuppression regulator T cells are activated. The exact mechanism for this activation is not known but seems to involve several factors which differ in local and systemic immunosuppression.

In local immunosuppression the antigen presenting cells in the skin and the cytokine TNF-α are critically involved. From studies in mice the local immunosuppression seems to be partly genetically determined as suppression of contact hypersensitivity was observed in some, called susceptible, but not all strains (37). UVB susceptibility is a genetically determined trait under the control of up to three independent loci (38) and in mice two of these have been identified as the TNF-α and lipopolysaccharide (LPS) loci (39). TNF-α is upregulated in the skin following UVB irradiation (40). TNF-α injected into the dermis of mice mimics the effect of UVB irradiation on induction of contact hypersensitivity and neutralizing TNF-α antibody abrogates the UVB-induced suppression of contact hypersensitivity (39). However, TNF-α alone is not responsible for UVB-induced local immunosuppression, since injection of neutralizing TNF-α antibodies could not prevent UVB-induced tolerance (41) and UVB-induced suppression of contact hypersensitivity responses was achieved in a TNF-receptor (p55)-deficient mice (42).

In normal human epidermis the Langerhans cell is the principal antigen presenting cell. UVB irradiation of the skin leads to reduced number and altered morphology and function of the Langerhans cells (14). Whether the reduced number is mainly due to migration to the regional lymph nodes or to apoptosis is still not known. UVB irradiation of Langerhans cells suppresses the expression of the surface molecules MHC II, ICAM-1 and B7 (43–46). The changes in surface molecules in combination with changes in the cytokine pattern following UVB irradiation changes the Langerhans cells from being capable of stimulating both T helper (Th) 1 and Th2 type T cells to mainly stimulate Th2 type T cells (47, 48). In addition, UVB irradiation of the skin induces epidermal macrophages (CD1a-,DR+,CD11b+,CD36+) (49, 50), which are the major source of IL-10 in human skin (51) and neutrophils (IL-4+,CD11b+,CD15+,CD36-) capable of releasing IL-4, further supporting the stimulation of Th2 type T cells (52).

UV-induced systemic immunosuppression, where a high dose of UVB irradiation affects the immune function at a distant non-irradiated site, seems mainly to involve IL-10. First it was found that supernatant from UVB irradiated keratinocytes injected into mice inhibited induction of contact hypersensitivity and delayed hypersensitivity responses (53, 54). Several cytokines may be responsible for inhibiting delayed type hypersensitivity responses, but IL-10 seems to be important since antibodies against IL-10 block the inhibitory effect of the supernatant (54). In mice, keratinocytes are the main source of IL-10, but in human skin the UVB-induced epidermal macrophage is also a high releaser of IL-10 (51). Other cytokines including IL-4 (52) and IL-12 (55) may be involved leading to a change in T cell activation from Th1 to Th2 type T cells.

That activation of Th2 type T cells is involved in both local and systemic immunosuppression is supported by the finding that injection of IL-12, which favours development of Th1 type T cells, prior to UVB exposure suppresses the immunosuppressive effect of UVB irradiation in both models (56, 57). Another mechanism by which IL-12 may prevent UVB-induced immunosuppression has recently been described. The authors found that prevention of UVB-induced immunosuppression by IL-12 was dependent on DNA repair in the contact hypersensitivity model (58). Previously IL-12 injection was shown to exhibit the capacity to remove UV-induced DNA-damage (59).

UVA EXPOSURE AND IMMUNOSUPPRESSION

UVB exposure of the skin causes DNA damage and is the most important factor for development of skin cancer.

Therefore, in the main part of the studies investigating UV-induced immunosuppression UVB radiation has been used. As a consequence of altered social behaviour, the western population is exposed to increasing doses of UV radiation including UVA radiation. In addition to solar UVA the increased exposure to UVA is amplified by the use of UVA tanning salons, UVA phototherapy (especially long-wave UVA) and the used of UVB sun screens. The UVA spectrum consists of short wave UVA (UVA-II, 320–340 nm) and long-wave UVA (UVA-I, 340–400 nm). Only a few studies have looked at UVA irradiation and immunosuppression, but these studies indicate a differential effect of UVB and UVA irradiation on immunosuppression. UVA radiation has less carcinogenic potential and a differential effect on DNA. Both UVB and UVA irradiation of the skin depletes Langerhans cells, but following UVA-I there is a rapid recovery of Langerhans cell function (60). UVB irradiation of the skin leads to epidermal infiltration of neutrophils and immunosuppressive macrophages (49, 50). UVA-II irradiation of the skin also leads to infiltration of epidermal macrophages, like UVB irradiation, but in contrast to UVA-I irradiation (61). Only one group has determined the effect of UVA-II irradiation on induction of contact hypersensitivity and has found an effect comparable to that of UVB irradiation (61). The modulatory effect of UVA-I irradiation on the induction of contact hypersensitivity has previously not been studied, but since UVA-I exposure in contrast to UVB and UVA-II exposure does not induce epidermal macrophages, one might expect a differential effect of UVA-II and UVA-I irradiation.

UV-INDUCED IMMUNOSUPPRESSION IN RELATION TO NON-MELANOMA SKIN CANCER

The importance of UV-induced immunosuppression for induction of non-melanoma skin cancer has not been finally proven. However, three major lines of evidence support this hypothesis. First, epidemiological studies report that there is a substantially increased incidence in skin cancer of the sun-exposed body parts of immune-suppressed transplant patients as compared to those of the general population (62). In one study, the overall incidence of squamous cell carcinoma was 250 times higher and that of basal cell carcinoma 10 times higher when compared with the general population (7). This indicates a link between immunosuppression and skin cancer induction. Secondly, exposing mice to subcarcinogenic doses of UV radiation suppresses their immune response and permits the outgrowth of highly antigenic UV-induced skin tumours (6). Finally, studies with non-melanoma skin cancer patients have indicated that UV-induced immunosuppression is a major risk factor for skin cancer induction (24). The study of Yoshikawa (24) is the only experimental study which has looked at UV-induced suppression of contact hypersensitivity in skin cancer patients. Using local UVB irradiation before sensitization, 40% of healthy age-matched volunteers were not sensitized on UVB-exposed skin but among patients with previous non-melanoma skin cancer the number was as high as 93% (24). In mice the susceptibility to UV-induced immunosuppression is genetically determined and this may also be the case in humans. Only few studies have looked for possible mechanisms involved in UVB-induced immunosuppression in non-melanoma skin cancer patients.

AIMS

UVB irradiation of the skin causes immunomodulation in both mouse and man but has mainly been investigated in mice whereas investigations in the immunomodulatory effect of UVA irradiation on the skin have been sparse. The aim of this thesis was to study in humans the wavelength dependencies of UV-induced immunosuppression, possible mechanisms for UVB-induced immunosuppression, and a possible relation between UV-induced immunosuppression and non-melanoma skin cancer.

More specifically the aims were:

Effects on the immune system of UVA-I versus UVB irradiation in humans

- Does UVA-I irradiation of human skin interfere with the induction of contact sensitivity?
- Does UVB and UVA-I irradiation of human skin induce release of TNF-α, IL-10 and photoisomerization of UCA?
- Does UVA-I protect against UVB-induced immunosuppression?

UVB-induced suppression of the induction of contact hypersensitivity in humans

- To determine possible mechanisms for the differential sensitivity to UVB-induced immunosuppression, especially
  - o the antigen presenting capacity in the skin
  - o TNF-α and IL-10 polymorphism

TNF-α and skin cancer

- To determine whether there is an association between non-melanoma skin cancer and TNF-α release and TNF promoter polymorphism

EFFECTS ON THE IMMUNE SYSTEM OF UVA-I VERSUS UVB IRRADIATION IN HUMANS

WAVELENGTH-SPECIFIC EFFECT ON INDUCTION OF CONTACT HYPERSENSITIVITY IN HUMAN SKIN (STUDY I)

Several humans studies have confirmed the findings in mice that low dose UVB irradiation induces local immunosuppression and reduces the immunization rates to epicutaneous antigens (22–25, 63). Only few studies in mice have looked at UVA radiation and contact hypersensitivity and with conflicting results (64, 65). We wanted therefore to examine the effect of long-wave UVA on induction of contact hypersensitivity in humans. We have chosen to compare the effect of UVA-I with UVB irradiation since a previous study in humans with UVA-I and UVB irradiation has shown differential effect on the antigen presenting cells in the skin (66). We used a highly sensitive method for assessing contact hypersensitivity in human skin as described by Friedmann et al (67). Healthy human volunteers were randomly assigned to receive sensitization with diphenylcyclopropanone (DPCP) on non-UV-exposed normal skin or sensitization with DPCP on skin exposed to three minimal erythema doses (MED) of either UVA-I or UVB. Three weeks after sensitization all volunteers were challenged with DPCP. The challenge reactions were scored clinically and the increase in skin thickness measured. Sensitization on UVB-exposed skin reduced the immunization rate compared with sensitization on non-irradiated skin as previously described. In contrast, sensitization on skin exposed to UVA-I radiation did not result in a decreased immunization rate as compared with non-irradiated skin, (Fig. 2). One may argue that we have used a low UVA-I dose. However, in this study we used photo-biologically equivalent doses of UVB and UVA radiation judged by erythema. In agreement with .revious findings (66), the doses of UVA radiation used that were necessary for inducing erythema were about 1000 times higher than the doses of UVB radiation.

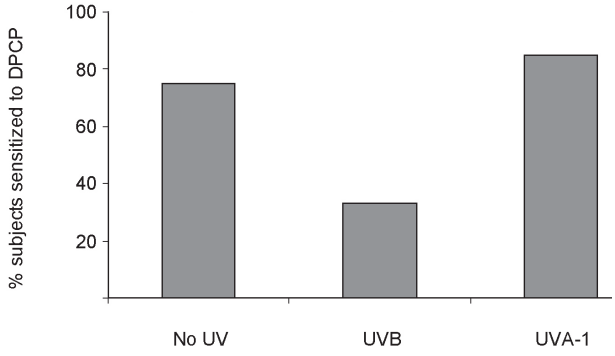

Fig. 2. Percentage of volunteers with a positive contact sensitivity reaction to DPCP judged by both a positive clinical score and a positive sum increase in skin thickness. The groups were sensitized with DPCP on non-exposed skin (No UV, n=12), skin exposed to 3 MED UVB (UVB, n=15), skin exposed to UVA-1 (UVA-1, n=13).

No other studies have looked at UVA-I radiation and induction of contact hypersensitivity in humans or mice. In humans, one other study has looked at the effect of UVA-II radiation on induction of contact hypersensitivity (61). It was found that the exposure to UVA-II radiation resulted in a similarly decreased immunization rate compared with UVB irradiation. In that study the authors used the same sensitive sensitizations protocol as ours but compared to our study they used 4 MED UVA irradiation instead of 3 MED and dinitrochlorobenzene (DNCB) instead of DPCP. The two studies are comparable and indicate that the effects on induction of contact hypersensitivity were similar for UVB and UVA-II irradiation but differed for UVA-I irradiation. The mechanisms behind the differences are not elucidated but may involve altered antigen presenting capacity and release of epidermal cytokines.

POSSIBLE MECHANISMS BEHIND WAVELENGTH-SPECIFIC UV EFFECT ON THE INDUCTION OF CONTACT HYPERSENSITIVITY

Antigen presenting cells

In normal epidermis the only antigen presenting cells are the Langerhans cells. UVB exposure of human skin deletes the function of Langerhans cells and induces infiltration of epidermal macrophages (49, 50). UVA-II like UVB radiation decreases the number of Langerhans cells and induces epidermal macrophages (61). *In vivo* UVA-I exposure of the skin, like UVA-II and UVB exposure, decreases the number of Langerhans cells but does not induce infiltration of epidermal macrophages (66). Immediately after UVB irradiation the epidermal antigen presenting capacity is blocked, probably because UVB exposure interferes with the activation-dependent upregulation of B7 molecules on Langerhans cells (68). At day 3 after UVB irradiation, the antigen presenting capacity is recovered due to infiltration of epidermal macrophages 60. The lack of Langerhans cells in epidermis at day 3 after irradiation may be due to UV-induced iC3b (69). Complement receptor type 3 (CD11b) and one of the ligands iC3b are critical for UV-induced immunosuppression (70) and a recent study has shown that iC3b transiently arrests monocytic cell differentiation into CD1-expressing dendritic cell precursors (71). At day 3 after UVA-I irradiation, the time point where we attempted to sensitize the volunteers, the antigen presenting capacity has recovered due to recovery of

the Langerhans cells (60). Thus, the different effect of UVB and UVA-I exposure on induction of contact hypersensitivity may in part be explained by differential effect on the antigen presenting cells in the epidermis. However, other changes in the micro milieu e.g. an altered cytokine balance may also be involved.

TNF-α (Study II)

In mice, TNF-α is involved in UVB-induced suppression of contact hypersensitivity (39, 72, 73). TNF-α injected into the dermis of mice mimics the effect of UVB irradiation and, furthermore, a neutralizing TNF-α antibody abrogates the UVB-induced effect on contact hypersensitivity. To determine whether different regulation of TNF-α could be one of the explanations for the different effects of UVB and UVA-I irradiation on the induction of contact hypersensitivity, we studied the effect of *in vivo* UVB and UVA-I exposure on induction of TNF-α in human skin. Volunteers were irradiated with 3 MED of UVB and UVA-I. At different time points after irradiation, suction blisters were raised from irradiated and non-irradiated skin. TNF-α was measured in suction blister fluid. UVB irradiation of human skin led to a rapid and significant increase in TNF-α concentration in suction blister fluid, with maximal values 6h after irradiation. In contrast, UVA-I irradiation led to a decrease in TNF-α concentration, with the lowest values at 6h after irradiation, (Fig. 3). A recent study in humans using solar simulated radiation confirms the release of TNF-α immediately following UV irradiation (74). Only one other group has looked at UVA and TNF-α in human keratinocytes, using a broad-band UVA light source and in agreement with our findings they did not find TNF-α mRNA or secretion (75). The main source of TNF-α in human skin remains unknown. Keratinocytes are one of the candidates. UVB irradiation of keratinocytes *in vitro* leads to upregulation of TNF-α mRNA and TNF-α release (76) and one study has shown that UVB exposure of human skin induces upregulation of TNF-α mRNA in epidermis (40). However, other cells may be important, Langerhans cells can secrete TNF-α, at least under pathological conditions 77. Dermal mast cells contain TNF-α (78, 79) and both *in vivo* and *in vitro* experiments have shown that UVB irradiation can trigger the release of TNF-α from them (80). Our finding that UVA-I irradiation decreases the TNF-α concentration in suction blister fluid may be explained by a decrease in dermal mast cells. High-dose UVA-I treatment has been shown to decrease the number of dermal mast cells (81) which also may be part of the explanation for the beneficial effect of UVA-I in atopic dermatitis and urticaria pigmentosa (82, 83).

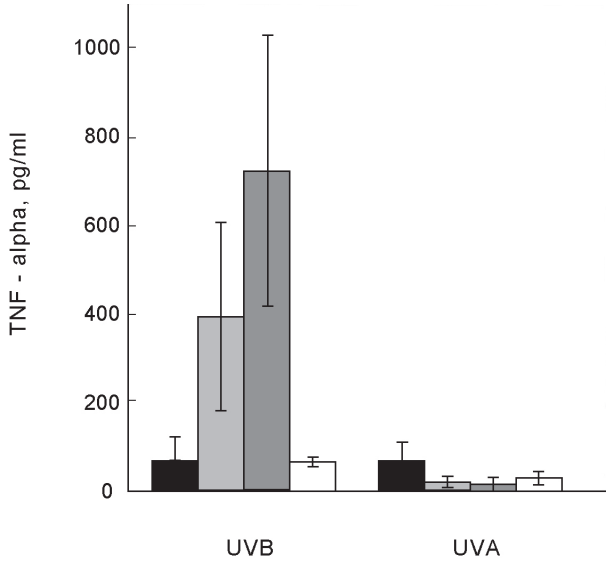

Fig. 3. TNF-α concentration in suction blister fluid. Control subjects were irradiated with 3 MED UVB (n=6) or UVA-1 (n=6) at three spots immediately (0h:), 6h (dark grey bars), and 24h (white bars) before suction blisters were raised at the irradiated sites, and at a control site (solid bars). The TNF-α protein level in the blister fluid was determined using a commercial ELISA kit and expressed as mean the mean (±SD). Reproduced with permission from Br J Dermatol (Skov et al 1998).

Interleukin 10 (Study II)

IL-10 is an important cytokine for UVB-induced immunosuppression. In mice, IL-10 injected intra peritoneally mimics the effect of UVB irradiation and inhibits the delayed-type hypersensitivity reactions whereas administration of neutralizing antibodies to IL-10 largely blocks the inhibitory effect of UVB radiation (54). UVB and UVA-I irradiation of human keratinocytes *in vitro* induces IL-10 mRNA production and protein release (84). One study has shown upregulation of IL-10 mRNA after UVB irradiation of human skin *in vivo* (85). We therefore determined IL-10 concentration in suction blister fluid using the method mentioned above. Three MED UVB irradiation of human skin resulted in a slight but significant increase in IL-10 concentration in suction blister fluid, with maximal values at 24 h after irradiation. Like UVB irradiation, 3 MED UVA-I irradiation of human skin led to an increase in IL-10 concentration in blister fluid, but this was not significant. Thus IL-10 release seems not to be responsible for the differential effect of UVB and UVA-I exposure on induction of contact hypersensitivity. In agreement with these findings studies using an IL-10 deficient mouse showed that UV-induced IL-10 seems mainly to be involved in UV-induced suppression of delayed-type hypersensitivity and not contact hypersensitivity (86). In murine skin, the keratinocytes are the major source of UV-induced IL-10. In human, in addition to keratinocytes, dermal macrophages that infiltrate the skin after UVB exposure secrete IL-10 (51). IL-10 release in the skin may lead to inhibition of Th1 cells and activation of Th2 cells and thus inhibit delayed type hypersensitivity reactions. In support, IL-12 which stimulates Th1 cells antagonized the immunosuppression effects of UV leading to UV-induced inhibition of the delayed type hypersensitivity response (56).

Urocanic acid (Study II)

DNA is the major UVB-absorbing chromophore in the skin but in addition UCA has been identified as an important chromophore for UV-induced immunosuppression (87). In normal epidermis UCA is found in high concentration as trans-UCA, which on UV exposure is photoisomerized to cis-UCA. Cis-UCA suppresses immune responses in a variety of experimental systems including delayed type hypersensitivity responses to herpes simplex virus (32). We determined the percentage of cis-UCA in suction blister fluid following irradiation with 3 MED UVB or UVA-I. We found a significant increase in the percentage of cis-UCA following both UVB and UVA-I irradiation of human skin. This is in agreement with another study showing photoisomerization of trans-UCA after both UVB, UVA-I and UVA-II irradiation (88).

UVA-I PROTECTION AGAINST UVB-INDUCED IMMUNOSUPPRESSION (STUDY III)

The contrasting effect of UVA-I and UVB exposure of human skin on induction of cell mediated immunity to contact allergens led us next to determine whether pretreatment with UVA-I due to different immunoregulation or pigmentation offers protection against UVB-induced immunosuppression. Volunteers were pretreated with UVA-I irradiation three times during one week. After another week the UVA-I pretreated volunteers were separated into two groups; one control group sensitized with DPCP on UVA-I pretreated skin and one group irradiated with 3 MED UVB on the UVA-I pretreated skin and thereafter sensitized. As a further control volunteers not pretreated with UVA-I but sensitized on non-irradiated or UVB irradiated skin were included. The immunization rate in the group of volunteers sensitized on skin pretreated with UVA-I before UVB irradiation was significantly higher than the immunization rate in the group of volunteers sensitized on UVB irradiated skin alone. There was no significant difference between volunteers sensitized on normal skin, UVA-I pretreated skin or UVA-I pretreated and UVB irradiated skin, (Fig. 4). These results indicate that pretreatment with UVA-I under certain conditions offers partial protection against the UVB-induced reduction in the immunization rates to epicutaneous allergens.

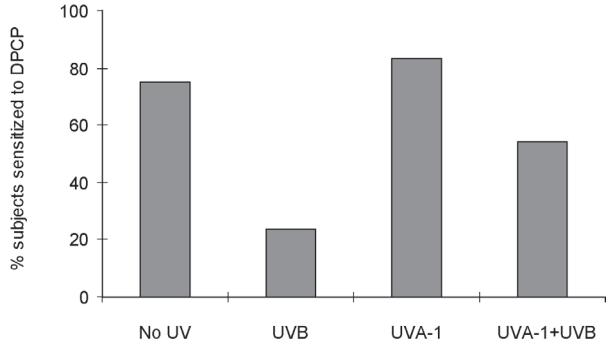

Fig. 4. Percentage of volunteers with a positive contact sensitivity reaction to DPCP judged by both a positive clinical score and a positive sum increase in skin thickness. The groups were sensitized with DPCP on non-exposed skin (No UV, n=12), skin exposed to 3 MED UVB (UVB, n=21), skin exposed to UVA-1 (UVA-1, n=12) and skin exposed to UVA-1 and UVB (UVA-1+UVB, n=22). Reproduced with permission from APMIS (Skov el al 2000).

The possible mechanisms for the protection induced by UVA-I irradiation are unknown but may include tanning or an altered balance of immunological mediators. While normal tanning is a combination of melanogenesis and epidermal thickening, a protective effect of the latter cannot explain the present results since UVB but not UVA exposure induces significant thickening of the epidermal layer (89). Melanin has a photoprotective function in the skin directly absorbing ultraviolet photons and reactive oxygen species. Consistent with this, tanned skin is less sensitive to UVB-induced erythema compared to poorly tanned skin (90–92). Individuals with constitutively pigmented skin are less susceptible to sunburn and skin cancer than fair-skinned individuals (92, 93). Tanning caused by UVA, but not UVB irradiation, has been shown to be less protective against UVB-induced erythema but equally protective against UVB-induced DNA damage (94).

Two human studies have investigated the role of pigmentation and UVB-induced immunosuppression (63, 95). Using a contact hypersensitivity model, constitutively pigmented human skin seems not to play a major role in protection against UVB-induced cutaneous immunosuppression. In contrast, sensitivity to sunburn has been shown to be associated with susceptibility to UV-induced suppression of contact hypersensitivity in human skin (96). Another potentially important factor explaining the protective effect of UVA-I irradiation is the differential immunological effect of UVB and UVA-I irradiation on the skin. Broad-band UVA and UVA-I have been shown to inhibit the UVB-induced suppression of delayed type hypersensitivity in mice (97). In another mouse model of systemic contact hypersensitivity, UVA irradiation was shown to protect against UVB-induced suppression of contact hypersensitivity (65). This immunoprotection by UVA irradiation seems partly to be mediated by heme oxygenase-1 (98) which is induced by UVA irradiation and not UVB irradiation (99) and Th1 cytokines (100). Especially the Th1 cytokine interferon-gamma (IFN-gamma) may be involved since UVA irradiation induces upregulation of IFN-gamma in the skin (100) and UVA irradiation did not offer protection in IFN-gamma knock-out mice (101). Other possible mechanisms are the altered antigen presenting capacity and the altered balance of immunological mediators following *in vivo* UVA-I irradiation in contrast to UVB irradiation.

CONCLUSION I

UVB irradiation of human skin is known to interfere with the generation of cell-mediated immunity to contact allergens presented on UVB irradiated skin. Also short-wave UVA-II irradiation and solar simulated light have been shown to have a similar effect on cell-mediated immunity. In contrast, UVA-I irradiation does not reduce the immunization rates to epicutaneous allergens and offers partial protection against subsequently UVB-induced suppression of contact hypersensitivity. Several mechanisms may be involved in the dissimilar effect of UVB and UVA-I irradiation on cell-mediated immunity. In contrast to UVA-I, UVB irradiation of human skin induces release of TNF-α, induction of epidermal macrophages, and perturbs the antigen presenting function of Langerhans cells. However, UVB and UVA-I irradiation of human skin also have similar effects including release of IL-10 and photoisomerization of trans-urocanic acid to cis-urocanic acid.

Table 1: *Differential effects of UVB and UVA-I irradiation of human skin.*

	UVA-1	UVB
Immunosuppression	-	+
Depletion of Langerhans cells	+	+
Changed function of Langerhans cells	-	+
Induction of epidermal macrophages	-	+
Induction of TNF-α	-	+
Induction of IL-10	(+)	+
Induction of *cis*-UCA	+	+
Inhibition of immunosupp. by UVA-1	NA	+

UVB-INDUCED SUPPRESSION OF THE INDUCTION OF CONTACT HYPERSENSITIVITY IN HUMANS

SUSCEPTIBILITY TO EFFECTS OF UVB IRRADIATION ON INDUCTION OF CONTACT HYPERSENSITIVITY (STUDY IV)

In mice, local immunosuppression seems to be genetically determined as suppression of contact hypersensitivity was only observed in some strains called UVB susceptible (UVB-S) and not in others called UVB resistant (UVB-R) (37). The susceptibility to the immunosuppression induced by UVB irradiation is polygenetically inherited and the TNF-α and Lps loci seem to be involved (39). In humans, sensitization on skin exposed to low dose UVB radiation also seems to separate normal humans into two phenotypically distinct groups, one group following sensitization on UVB irradiated skin develops contact hypersensitivity designated UVB-R and the second group following sensitization on UVB irradiated skin fails to develop contact hypersensitivity designated UVB-S (23, 24). The relevance of this finding has been demonstrated in one study where UVB susceptibility was found to a significantly higher degree among patients with previous non-melanoma skin cancer indicating that UVB susceptibility may be a risk factor for the development of skin cancer (24).

To determine the UVB-induced immunosuppression in the skin volunteers were sensitized with DPCP on skin irradiated with 3 MED UVB. Assessed by the clinical challenge reaction only 44% of the volunteers showed a positive reaction after sensitization on UVB-exposed skin compared to 88% in a control group sensitized on non-exposed skin. The UVB-exposed volunteers in our study could clearly be divided into two subgroups, one resistant and one susceptible group. The UVB

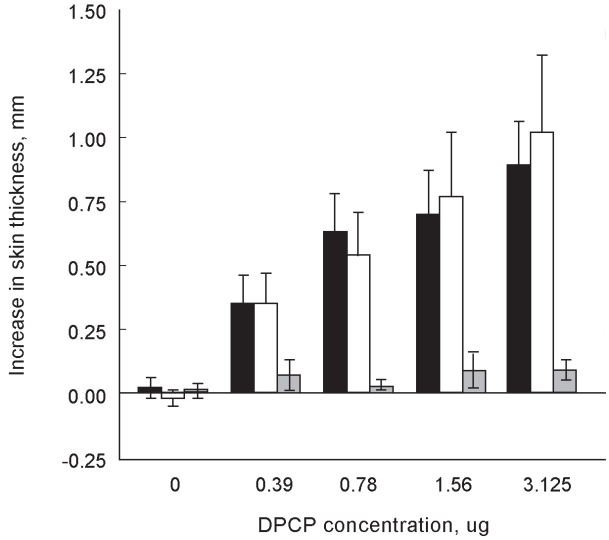

Fig. 5. Response to various challenge concentrations of DPCP assessed by increase in skin thickness at the upper inner arm 21 days after sensitization. Solid bars, group mean ± SEM of 24 non-UVB-exposed subjects sensitized on normal buttock skin. White and grey bars, 23 subjects all sensitized on skin exposed to 3 MED UVB, and separated into two groups. One group (mean ± SEM, n=11) developed contact hypersensitivity (UVB-R, white bars), and one group (mean ± SEM, n=12) failed to develop contact hypersensitivity (UVB-S, grey bars). Reproduced from Skov, Photochemistry and Photobiology, 1998, 67(6) with the permission of the American Society for Photobiology.

resistant group had the same degree of challenge reactions as the non-radiated control group, (Fig. 5). In other studies it has not been possible to separate the volunteers into two groups since the volunteers had different degrees of sensitization (25). This is probably depending on the method used and the number of volunteers. In mice it has been shown that the time between UV exposure and sensitization and the dose of hapten used for sensitization are involved (102, 103).

Among the 14 volunteers out of 25 in our study who were not immunized after primary sensitization on UVB exposed skin, nine accepted to undergo resensitization on non-irradiated skin. In eight subjects, repeated application of DPCP resulted in immunization and only one developed antigen specific tolerance. This volunteer did not have a generally decreased cellular immunity because he responded with contact sensitivity to another unrelated allergen after application on non-irradiated skin. Thus, using DPCP as allergen, only one subject out of 25 may have developed tolerance to the allergen when applied on UVB-irradiated skin. In UVB-susceptible mice sensitization on UVB-irradiated skin led to antigen specific tolerance (14). The results in human studies concerning UVB-induced tolerance have been conflicting probably due to different irradiation schedules and allergens. In one study 31% of the volunteers initially sensitized on UVB-irradiated skin developed tolerance to the allergen (23). We have used the same low sensitizing dose of the allergen and nearly the same UVB dose regimen but another allergen (DPCP instead of DNCB). Others have failed to demonstrate both local and systemic immunosuppression in humans using DPCP, suggesting that DPCP may not be the ideal sensitizer in this type of studies (104).

POSSIBLE MECHANISMS BEHIND SUSCEPTIBILITY TO UVB-INDUCED SUPPRESSION OF CONTACT HYPERSENSITIVITY (STUDY IV)

As for the difference between UVB and UVA-I induced immunosuppression many different factors may be involved in the susceptibility to UVB induced immunosuppression. To determine in humans whether the number or function of antigen presenting cells in the skin was involved in UVB susceptibility, normal human volunteers were separated into UVB-R and UVB-S as mentioned above. Single cell suspensions of epidermal cells from control skin and skin exposed to 3 MED of UVB irradiation three days previously were stained for Langerhans cells and epidermal macrophages. UVB exposure of the skin significantly decreased the percentage of Langerhans cells and increased the percentage of epidermal macrophages, however to the same degree in both the UVB-S and UVB-R group. One other group has determined the effect of UVB irradiation on the number of Langerhans cells in UVB-susceptible and resistant mice and found in agreement with us a comparable reduction in Langerhans cell number and no correlation to UVB susceptibility (37).

Langerhans cell alloreactivity was tested in epidermal cells harvested immediately after UVB irradiation and was blocked to the same degree in both groups. Epidermal cells harvested 3 days after UVB irradiation from both UVB-R and UVB-S subjects demonstrated a strong antigen presenting capacity compared to epidermal cells from control skin. The T cells activated by the epidermal cells were mainly T cells which secret IFN-γ and not IL-4. This study is the only study comparing the function of antigen presenting cells in both UVB-R and UVB-S. In this system we were not able to detect IL-4 and a shift of the immune responses towards activation of Th2 type T cells. In mice, UVB exposure of antigen presenting cells leads to impaired activation of Th1 cells (47). In human using Th0, Th1 and Th2 specific T cell clones, UVB-irradiated monocytes also demonstrated an impaired activation of Th1 cells probably due to reduced IL-12 production (55). The same authors have recently shown that UVB irradiation of human skin induces a transient appearance of IL-4 positive neutrophils immediately after irradiation (52). These IL-4 producing neutrophils may favour the Th2 type T cell activation by inducing IL-10 production and thereby reducing IL-12 production.

In humans, the UVB susceptibility was not correlated to the number or function of the antigen presenting cells in epidermis. However, many different factors may be involved including susceptibility to UVB-induced DNA damage, capacity to repair DNA damage and the cytokine milieu. The Fas/Fas-ligand system seems also to be involved in UV-induced immunosuppression, since mice deficient in these molecules do not develop UV-induced tolerance (105, 106). *In vivo* UVB irradiation of human skin leads to increased expression and activation of Fas (107, 108).

CYTOKINE POLYMORPHISM AND SUSCEPTIBILITY TO UVB-INDUCED SUPPRESSION OF CONTACT HYPERSENSITIVITY (STUDY V)

TNF-α polymorphism

Studies in mice have shown that UVB susceptibility is a genetically determined trait under the control of up to three independent loci (38). Two of these have been identified as the TNF and LPS loci (39). In humans, a G to A transition polymorphism has been identified at position -308 within the promoter region of the TNF-α gene, with the G form of the polymorphism being most common (109). Individuals carrying a haplotype that includes the A allele are known to be high secretors of TNF-α 110-112. In addition, a correlation between the genotype and the outcome of diseases such as cerebral malaria and non-Hodgkin´s lymphoma has been observed (113, 114) implying a potential biological significance of the polymorphism. We were therefore interested to see whether susceptibility to UV-induced immunosuppression in humans was associated with the TNF-α polymorphism at po-

sition -308. We identified UVB susceptible and UVB resistant volunteers as mentioned above. A total of 42 volunteers were included in the study (18 UVB-R and 24 UVB-S). However, no association was found between allele frequencies of the TNF-308 polymorphism and the phenotype in terms of UVB-induced immunosuppression. Our result may not exclude the role of TNF-α in UVB-induced immunosuppression of contact hypersensitivity but may be explained by the size of our study group, by the fact that the susceptibility is not related to one single polymorphism but may involve other TNF-α polymorphisms or to the fact that the susceptibility is a complex interaction of several different polymorphisms. Two other groups have looked at TNF polymorphisms in humans in relation to UVB-susceptibility (115, 116). The first group studied a small group of volunteers separated into UVB-R and UVB-S as in our study but used microsatellite analyses and found that TNFa2 associated with high TNF-α release was found more often in UVB-S subjects and that TNFd3 associated with low TNF-α was found more often among UVB-R subjects (115). These data support the role of TNF in UVB-induced immunosuppression but need to be confirmed in a larger group. The other group looked at different cytokine polymorphisms including TNF -308 and TNF -238 and the antibody responses to hepatitis B vaccination following UVB irradiation (116). In agreement with our findings, they found no correlation between UVB-induced immunomodulation and the two TNF polymorphisms.

IL-10 polymorphism

In addition to TNF-α, IL-10 seems to be a central cytokine in UV-induced immunosuppression. IL-10 inhibits antigen presenting cell function (117) and may thus suppress delayed-type hypersensitivity responses. Several IL-10 polymorphisms have been identified (118). We decided to look at one of the IL-10 polymorphism, a CA repeat promoter polymorphism (119) and the association with UVB-induced immunosuppression of contact hypersensitivity. The IL-10 polymorphism we looked at has been shown to be associated with IL-10 secretion (120). The same group of volunteers as mentioned above was included and a total of 22 different alleles was found. Among the six alleles that occurred most frequently there was no difference in the frequency of the alleles in the UVB-R and the UVB-S groups. The lack of association between the IL-10 polymorphism and UVB susceptibility is in agreement with the finding that IL-10 is not critically involved in UVB-induced suppression of contact hypersensitivity, but only in the suppression of delayed-type hypersensitivity (86). Another explanation for the lack of association may be related to the number of volunteers included in the study which requires a strong association to be discovered. No other studies have been published on IL-10 polymorphism and UV-induced immunosuppression in healthy volunteers. Recently a group determined the association between another IL-10 polymorphism and susceptibility to squamous cell carcinoma in kidney transplant patients including 70 renal transplant recipients and 70 healthy matched controls (121). Squamous cell carcinoma after renal transplantation may be a consequence of drug induced immunosuppression, viral infection and UV-induced DNA damage, local and systemic immunosuppression. Interestingly, the authors found that an IL-10 polymorphism associated with high IL-10 secretion was associated with an increased number of squamous cell carcinomas in kidney transplants. This finding supports the assumption that the immune system including IL-10 plays a role in skin cancer.

CONCLUSION II

In agreement with several other groups we found that low dose UVB irradiation in humans reduces the immunization rate to epicutaneous antigens. Using our method we were able to divide the volunteers onto two separate groups, one resistant and another susceptible to UVB-induced immunosuppression. However, among the UVB susceptible subjects only one subject developed antigen specific tolerance, whereas others have found that up to 30% developed antigen specific tolerance (23). The discrepancy is probably related to the UV dose and the dose and choice of allergen used.

The susceptibility to UVB-induced immunosuppression was found to be related neither to the number or function of the antigen presenting cells in epidermis nor to the capacity of epidermal cells to stimulate Th2 type T cells in vitro. TNF-α and IL-10 are important cytokines for UVB-induced immunosuppression and therefore functionally important TNF-α and IL-10 polymorphism were studied but were in this small study not demonstrated to be related to UVB susceptibility.

TNF-α AND SKIN CANCER

TNF-α POLYMORPHISM (STUDY VI)

UVB irradiation led to immunomodulation in the skin and studies in mice demonstrated that UVB-induced skin tumours are immunogenic. There is no direct evidence for the role of UV-induced immunosuppression and induction of non-melanoma skin cancer in humans. However, the increased number of non-melanoma skin cancers seen in patients undergoing transplantation is a strong evidence for the role of immunosuppression (7, 62). Since both mice and humans have a differential susceptibility to UVB-induced immunosuppression a genetic predisposition may be involved. In Study V we investigated the TNF-308 polymorphism in relation to UVB susceptibility and UVB resistance but found no correlation. This may be due to the small number of participants in the study. Next we therefore looked at the same polymorphism but in a large patient group and in a group where we expected UVB susceptibility to be important, namely patients with previous BCC. Another study has shown association with the TNF -308 polymorphism and non-Hodgkin's lymphoma, another immunogenic tumour (114). In our study 191 patients with previous BCC and 107 age-matched healthy controls were included. For the TNF-308 polymorphism there was no significant association between the genotype or allele frequencies and having a BCC, (Table 2).

Hajeer et al. (122) have previously found that the GG genotype of the TNF -308 polymorphism was significantly associated with an increased number of BCCs. Despite separating the patients according to the number of BCCs we were not able to confirm their result in a larger group of patients. The direct association between the GG genotype of the TNF -308 polymorphism and the risk of BCC was not found in two later studies by the same group (123, 124).

Several TNF-α polymorphisms and microsatellite polymorphisms are known and some related to a high TNF-α production (125), we have only looked at one TNF polymorphism. As mentioned earlier Niizeki et al (115) found an association with a TNF microsatellite polymorphism and susceptibility to UVB-induced immunosuppression in healthy volunteers and Hajeer et al. (122) found an association in BCC patients. Furthermore, the TNF-α gene cluster is located within the highly polymorphic major histocompatibility complex region on chromosome 6p21.3 and many of the TNF-α polymorphisms are found to be in linkage disequilibrium with HLA-I and II alleles. A few studies have shown evidence for an association of certain HLA-DR, including HLA-DR1 and BCC (126, 127). HLA-A11 has been associated with resistance to skin cancer (128). HLA-DR1 and DR3 have been associated with high TNF-α production (129, 130). Linkage disequilibrium between HLA-DR and TNF-α polymorphisms may be one of the explanations for this finding. Further studies on high production TNF-α polymorphisms and BCC are needed. However, it is unlikely that one single polymorphism is the major susceptibility factor in tumour formation and more likely that a combination of cytokine polymorphisms, possibly together with HLA alleles, constitutes a risk factor for tumour susceptibility.

Table 2 *Genotypes of patients with BCC and controls.*

Group	Genotype			Allele frequency	
	GG	*GA*	*AA*	*TNF1(G)*	*TNF2(A)*
All BCC, n=191	133	49	9	315	67
Controls, n=107	68	37	2	173	41

TNF-α SECRETION (STUDY VI)

Human studies have shown differential capacity to release TNF-α from mononuclear cells upon stimulation with lipopolysaccharide (LPS) (110–112, 129). Fatal cerebral malaria is associated with high TNF-α levels in the blood (131). We were not able to show an association between the TNF-308 polymorphism and having had a BCC. Others have found an association between TNF microsatelite polymorphism and susceptibility for UVB-induced immunomodulation and BCC (123). To determine whether patients with previous BCC have an increased capacity to secrete TNF-α, mononuclear cells were isolated from blood. Whole body UVB irradiation has previously been shown to lead to an increased level of

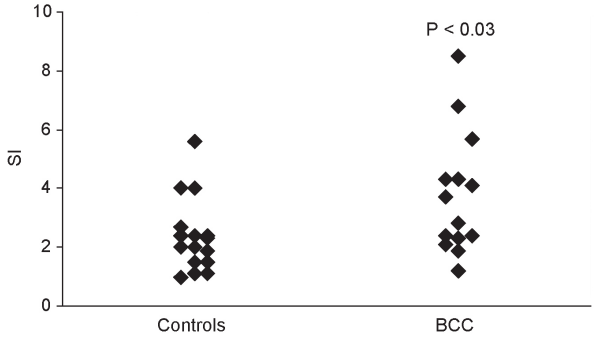

Fig. 6. TNF-α released from mononuclear cells (MNC) from healthy controls (n=16) and patients with previous BCC (n=15). MNC were stimulated with and without lipopolysaccharide (LPS) 1ng/ml for 24 h. The TNF-α concentration in the supernatant was measured by ELISA and expressed as stimulations indices. Reproduced with permission from Experimental Dermatology (Skov el al 2003).

TNF-α in the blood (76). However, we were not able to detect TNF-α following UVB irradiation of mononuclear cells in vitro. The mononuclear cells were therefore stimulated with LPS. Mononuclear cells from patients with previous BCC (n=15) demonstrated significantly increased release of TNF-α upon stimulation with LPS, $p<0.03$, compared to mononuclear cells from age matched control subjects (n=16), (Fig. 6). TNF release in BCC patients has not been studied by others. Data from our small study suggest that the capacity to release TNF-α varies among humans and that patients with previous BCC have an increased capacity to release TNF-α upon stimulation. TNF-α is involved in UVB-induced immunosuppression and the finding may support that patients with BCC have an increased susceptibility to UVB-induced immunosuppression.

CONCLUSION III

UVB irradiation induces immunomodulation of the skin which may be involved in tumour induction and promotion. TNF-α seems to be critically involved in UVB-induced immunomodulation and may be involved in skin cancer induction. In a small study we found that patients with previous BCC had an increased capacity to release TNF-α. However, in a larger group we did not find any correlation between the A allele of the TNF-308 which has been shown to be associated with the high TNF-α release and having had a BCC. We have only studied one TNF-α polymorphism. Several TNF-α polymorphisms and microsatellite polymorphisms are known and some related to a high TNF-α production. Therefore, further studies on high production TNF-α polymorphisms and BCC are needed.

CONCLUSIONS AND PERSPECTIVES

The main conclusions derived from the studies on which this thesis is based are:

1. In contrast to UVB irradiation long-wave UVA irradiation does not interfere with the induction of contact hypersensitivity in human skin.
2. In contrast to UVB irradiation long-wave UVA irradiation does not induce TNF-α release in human skin. Both long-wave UVA irradiation and UVB irradiation induces photoisomerisation of UCA.
3. Long-wave UVA irradiation inhibits UVB-induced suppression of contact hypersensitivity in human skin.
4. Susceptibility to UVB-induced suppression of contact hypersensitivity is neither correlated to the number of Langerhans cells or epidermal macrophages, nor to their capacity to activate autoreactive or Tetanus toxoid specific T cells.
5. Susceptibility to UVB-induced suppression of the induction of contact hypersensitivity is not correlated to TNF-308 or IL-10 CA repeat polymorphism.
6. In patients, previous basal cell carcinomas are associated with high TNF-α release but not with TNF polymorphism at position -308.

We were the first group to show that long-wave UVA and UVB have differential effect on induction of contact hypersensitivity in human skin (Study I). The result was not unexpected since long-wave UVA does not have the same chromophores in the skin as UVB and previous studies have shown differential effect of UVB and long-wave UVA on the antigen presenting cells in the skin. The difference is further supported by our finding that only UVB and not long-wave UVA exposure of the skin induces release of TNF-α (Study II). We have only investigated the effect of UVA-I radiation in a local immunosuppression and contact hypersensitivity model. We might have found suppression of immune responses in the delayed type hypersensitivity model. Suppression of delayed type hypersensitivity in contrast to contact hypersensitivity is critically dependent on IL-10 and UVA-I irradiation of human keratinocytes induces IL-10 release.

The finding that UVA irradiation does not suppress induction of contact hypersensitivity is in contrast to the findings by other groups. However, it is important to notice that none of these studies have used long-wave UVA radiation. Instead, they have used short-wave UVA, broad-band UVA or solar simulated light. Our results may be of high relevance for the increasing use of UVA-I phototherapy. Patients with erythrodermic atopic dermatitis in the acute phase normally do not tolerate phototherapy, especially not UVB treatment due to aggravation. The aggravation may be due to release of the proinflammatory cytokine such as TNF-α. In contrast, the patients often tolerate UVA-I phototherapy; this may probably be due to the lack of TNF-α release following UVA- I irradiation. Therefore, in addition to apoptosis of T cells in the skin and decreased number of IgE binding cells in dermis (81, 132), lack of TNF-α release may be responsible for the beneficial effect of UVA-I therapy in atopic dermatitis. Another disease where the differential effect of UVB and UVA-I irradiation on TNF-α release may be involved is lupus erythematosus. No TNF-α release together with less DNA damage following UVA-I irradiation may be important for the beneficial effect of UVA-I treatment in patients with lupus erythematosus (133).

In humans long-wave UVA seems to have a protective effect against UVB-induced immunosuppression (Study III). In mice UVA exposure immediately before UVB exposure

also had an immunoprotective effect against UVB irradiation which seems to be mediated by heme oxygenase-1. It would be interesting to study in humans the effect of UVA exposure immediately before UVB exposure and, if protection is found, to determine whether heme oxygenase-1 is also involved in humans. However, UVA irradiation as protection before UVB irradiation could not be generally recommended. UVA irradiation has other side effects such as photoaging and the role of UVA irradiation in the development of malignant melanoma is not clarified. Epidemiological and animal studies have indicated a role of UVA irradiation in malignant melanoma induction (134). In a recent human study the basal cells in SCC and actinic keratosis were shown to harbour more UVA than UVB fingerprint mutations, indicating a role for UVA also in non-melanoma skin cancer (135).

Low dose UVB irradiation of human skin interferes with the induction of contact hypersensitivity (study IV) and does at least in some studies lead to induction of antigen specific tolerance (23). The mechanism for the UVB-induced suppression of contact hypersensitivity and induction of tolerance is unknown. Murine and human studies have demonstrated that several factors seem to be involved including a change in number and function of Langerhans cells, induction of epidermal macrophages and neutrophils, changes in cytokine environment with increased TNF-α, IL-10 and IL-4 and decreased concentration of IL-12. The mechanisms involved in the induction of suppression of contact hypersensitivity and in the induction of tolerance seem not to be the same. Sensitization immediately after UVB irradiation leads to suppression of contact hypersensitivity but not to tolerance, and injection of anti-TNF-α or anti-cis-UCA inhibits UVB-induced inhibition of contact hypersensitivity but not induction of tolerance. The above mentioned mechanism may in case of tolerance lead to activation of antigen specific regulator T cells. For years it has been known that tolerance could be transferred by T cells, but the exact nature of the T cells has not been known. Recently, however, these cells have been further characterized in mice. Hopefully these data will lead to further insight into tolerance in photoimmunology but also into tolerance in general.

The role of immunosuppression for development of skin cancer is generally accepted; however, there is no direct evidence for the role of UVB-induced immunosuppression in the development of skin cancer. One study in humans showed that patients with previous non-melanoma skin cancer were particularly susceptible to UVB-induced suppression of contact hypersensitivity and induction of tolerance and strongly supports this hypothesis. However, the study is small, including only 12 patients with basal cell carcinoma or squamous cell carcinoma, and needs to be confirmed in a larger study. Also one should notice that experimental induction of contact hypersensitivity on non-irradiated skin has been shown to be weaker in patients with squamous cell carcinomas (136).

In mice it has recently been shown that genetic differences in susceptibility to UV-induced immunosuppression seem to be a risk factor for skin cancer (137) and the same may be the case in humans. Genotypes related to susceptibility to UVB-induced immunosuppression may therefore be found more often among skin cancer patients. We have studied a group of patients with previous basal cell carcinoma and found that mononuclear cells from these patients release more TNF-α upon stimulation than mononuclear cells from age-match controls (Study VI). There was no association between one single functionally relevant TNF-α polymorphism and having had basal cell carcinoma. However, other functionally relevant TNF polymorphisms may be involved. Most likely not only one polymorphism is involved and, looking at skin cancer patients, several other factors may be important, such as the degree of sun exposure, DNA damage and capacity of DNA repair. Squamous cell carcinoma among patients after organ transplantation has been shown to be associated with a specific IL-10 genotype and high IL-10 production (121). Whether these findings also count for non-transplanted patients is not known.

Increased understanding of immunosuppression for the development of skin cancer is important not only in relation to our increased exposure to sunlight but also in relation to the use of therapeutic immunosuppression. An increasing number of patients is treated with immunosuppressive drugs and a lot of new immunosuppressive drugs, with more selective immunosuppressive effects, are introduced. These selective immunosuppressive drugs will increase our understanding of the mechanisms involved in the induction of non-melanoma skin cancer.

SUMMERY

Non-melanoma skin cancer is the most frequent malignant condition in the white population and epidemiologic studies indicate that exposure to ultraviolet (UV) radiation from sunlight is the principal cause of skin cancer. The main carcinogenic effect of the solar UV radiation is direct DNA damage. However, besides DNA damage, UV radiation of the skin induces immunosuppression and activation of regulatory T cells. The exact phenotype of the activated regulatory T cells is not known, but the activation seems to involve altered antigen presenting capacity in the skin and altered cytokine balance leading to activation of Th2-type T cells.

To study the effect of local UV exposure in humans and rodents, an immunological in vivo model has been used by others. This model employs the induction of contact hypersensitivity to epicutaneously applied antigens. A strong antigen is applied on irradiated skin and on non-irradiated skin in control subjects. The degree of sensitization is evaluated by antigen challenge on non-irradiated skin. In this model, UVB irradiation has been shown to decrease the induction of sensitization resulting in antigen specific tolerance, which in mice can be transferred from mouse to mouse by T cells.

The aims of this thesis were to study in humans wavelength dependencies of UV-induced immunosuppression in a contact hypersensitivity model, possible mechanisms for UVB-induced immunosuppression, and a possible relation between UV-induced immunosuppression and non-melanoma skin cancer.

UVB (280–320 nm) exposure of the skin causes both DNA damage and immunosuppression and is the most important cause of skin cancer. Only few studies have looked at UVA (320–400 nm) irradiation and immunosuppression. The effect of long-wave UVA (UVA-I, 340–400 nm) on induction of contact hypersensitivity in humans was therefore examined. We showed that UVA-I irradiation in contrast to UVB irradiation did not affect the induction of contact hypersensitivity on irradiated skin. There are several possible explanations for the differential effect of UVB and UVA-I irradiation including altered antigen presenting capacity and release of epidermal cytokines. In the following study we found that only UVB and not UVA-I irradiation leads to secretion of TNF-α in the skin. Other studies have shown a differential effect of UVB and UVA-I irradiation on the antigen presenting capacity in irradiated skin.

Since UVA-I irradiation did not affect the induction of contact hypersensitivity, it was therefore determined whether UVA-I irradiation could protect against UVB-induced immunosuppression, which was found to be the case in human volunteers. The mechanism for this protection is not known but may include tanning of skin or immunological changes as supported by murine studies.

In humans, sensitization on skin exposed to low dose UVB radiation separates normal humans into two phenotypically distinct groups. One group develops contact hypersensitivity following sensitization on UVB-irradiated skin and is designated UVB resistant, and the second group fails to develop contact hypersensitivity following sensitization on UVB-irradiated skin and is designated UVB susceptible. In a small group of subjects we were not able to correlate UVB susceptibility to the number of antigen presenting cells in epidermis or to the capacity of the antigen presenting cells to activate Th2 cells.

In mice, the susceptibility to UVB-induced immunosuppression is correlated to polymorphisms at the TNF gene. TNF-α and IL-10 seem to play a central role in UV-induced immunosuppression. We were therefore interested to see whether susceptibility to UV-induced immunosuppression in humans was associated with the TNF polymorphism at position -308 and/or the IL-10 CA repeat polymorphism. However, in a small group of UVB-susceptible and UVB-resistant subjects we did not find such an association.

The high prevalence of non-melanoma skin cancer seen in patients undergoing organ transplantation is a strong evidence for the role of immunosuppression in induction of non-melanoma skin. One study has found that the number of UVB-susceptible subjects was increased in patients with previous non-melanoma skin cancer. The TNF-308 polymorphism was therefore determined in 191 patients with previous basal cell carcinoma and in 107 age-match controls. However, we did not find any association between basal cell carcinoma and TNF-308 genotype. Instead, a statistically significant association between having had a basal cell carcinoma and an increased capacity to release TNF-α from the patient's mononuclear cells upon stimulation was found. This indicates that other functional TNF polymorphisms may be associated with the induction of non-melanoma skin cancer.

DANSK RESUMÉ

Non-melanom hudkræft er den hyppigste kræftform i Danmark og skyldes hovedsagelig ultraviolette stråler (UV) i sollys. UV-bestråling af huden medfører, ud over ændringer i cellernes DNA, også en ændret funktion af hudens immunsystem, hvor der aktiveres T lymfocytter, som påvirker det naturlige immunrespons. Den nøjagtige fænotype af de aktiverede regulatoriske T lymfocytter er ikke kendt, men aktiveringen af disse celler menes at involvere ændret antigen præsenterende kapacitet i huden og ændret cytokin balance som medfører aktivering af Th2 type T lymfocytter. Både hos mus og mennesket har flere modeller været anvendt til at studere den UV-inducerede immunosuppression. En af disse modeller er induktion af kontakt hypersensitivitet på bestrålet hud. Et lille hudområde bestråles og efterfølgende forsøges sensibilisering med et potent kontaktallergen på det bestrålede område. Graden af sensibilisering vurderes efterfølgende ved ”challenge” på et ubestrålet hudområde. UVB bestråling har i denne model vist sig at medføre nedsat sensibilisering samt udvikling af antigen specifik tolerance, der hos mus kan overføres via T lymfocytter.

Formålet med denne afhandling var hos mennesket at undersøge: hvilke bølgelængder der medfører UV-induceret immunosuppression, mulige mekanismer for UVB-induceret immunosuppression og en mulig relation mellem UV-induceret immunosuppression og non-melanom hudkræft.

Ultraviolet B stråler (280–320 nm) menes at være hovedårsagen til udvikling af non-melanom hudkræft, idet disse stråler medfører direkte DNA skade og immunosuppression både hos mus og mennesker. Betydningen af de ultraviolette A (320–400nm) stråler for induktion af immunosuppression er kun sparsomt undersøgt. Effekten af langbølget ultraviolet A stråler (UVA-I, 340–400 nm) for udviklingen af kontakt hypersensitivitet i human hud blev derfor undersøgt. Vi fandt at UVA-I i modsætning til UVB ikke påvirker evnen til at udvikle kontakt hypersensitivitet. Der er flere mulige forklaringer på den forskellige effekt af UVB og UVA-I bestråling på induktion af kontakt hypersensitivitet. UVB og UVA-I bestråling er tidligere vist at have forskellig effekt på de antigen præsenterende celler i bestrålet hud, men vi fandt også i de efterfølgende undersøgelser at kun UVB og ikke UVA-I bestråling medfører frigivelse af TNF-α i human hud.

Da UVA-I bestråling ikke medfører hæmning af induktionen af kontakt hypersensitivitet var det oplagt efterfølgende at undersøge om UVA-I bestråling før UVB bestråling beskytter mod UVB induceret immunosuppression. UVA-I bestråling viste sig at hæmme UVB-induceret immunosuppression. Mekanismen for hæmningen er ikke undersøgt hos mennesket, men kan skyldes pigmentering eller en immunologisk ændring. Sidstnævnte støttes af murine studier.

Vores efterfølgende studier bekræftede, at alt efter forsøgspersonernes respons på sensibilisering på UVB-bestrålet hud, er det muligt at opdele de raske forsøgspersoner i 2 grupper. Forsøgspersoner som er følsomme overfor den UVB-inducerede immunosuppression og forsøgspersoner som er resistente overfor den UVB-inducerede immunosuppression. I en lille gruppe af forsøgspersoner var det dog ikke muligt at korrelere UVB følsomheden til antallet af antigen præsenterende celler i epidermis. Ej heller de epidermale antigen præsenterende cellers evne til at aktivere Th2 type T lymfocytter var korreleret til UVB følsomheden. Hos mus er følsomheden for UVB-induceret immunosuppression korreleret til polymorfisme på TNF genet og både TNF-α og IL-10 menes at være vigtige for UV-induceret immunosuppression. De to funktionelt betydende polymorfismer ”TNF-308” og ”IL-10 CA repeated” blev derfor undersøgt hos raske UVB-følsomme og raske UVB-resistente forsøgspersoner uden at der blev fundet en association mellem genotyper og UVB følsomheden.

At immunsystemet også spiller en rolle for udvikling af non-melanom hudkræft hos mennesket understøttes af den betydeligt øgede risiko for non-melanom hudkræft, der er observeret hos organtransplanterede patienter i immunsupprimerende behandling. I et tidligere studie er det fundet at hyppigheden af UVB-følsomme var øget blandt patienter med tidligere non-melanom hudkræft. TNF-308 polymorfismen blev derfor efterfølgende undersøgt blandt en stor gruppe af patienter med tidligere basocellulært karcinom og alders-matchede kontroller. Vi fandt ingen association mellem TNF-308 genotype og tidligere basocellulært karcinom. I stedet fandtes en statistisk sammenhæng mellem at have haft basocellulært karcinom og en øget kapacitet til at frigive TNF-α efter stimulation af patientens mononuklære celler. Dette tyder på at andre funktionelt betydende TNF polymorfismer kan være associeret med udvikling af non-melanom hudkræft.

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