Melanocytes, Moles and Melanoma

A Study on UV effects

by

Ulrika Stierner



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"a good accountant of beauty marks
is worth more in the defense of a kingdom
than half a dozen choice regiments."

Jacques Fontaine
Henry IV´s doctor

To the memory of Nevado,
my father.

ABSTRACT

Melanocytes, Moles and Melanoma - a Study on UV effects.

Ulrika Stierner, Department of Oncology, Sahlgrenska Hospital, University of Göteborg, S-413 45 Göteborg, Sweden.

To investigate the UV effect on epidermal melanocytes, 21 volunteers and 11 patients with dysplastic nevus syndrome (DNS) received UVB irradiation three times weekly during 17 days. Skin biopsies were taken before and three weeks after the last irradiation (on day 37) from exposed and covered buttock skin. The epidermal melanocyte population density was estimated in dopa-stained split skin preparations. The biopsies taken on day 37 revealed that repeated UVB irradiation induces an increase in the number of melanocytes not only in exposed but also in covered skin. This increased mitotic activity might be a link between sun exposure and melanoma development in covered skin. The size of the proliferative response was inversely correlated to the basal melanocyte number. The larger population increase in skin with few melanocytes might amplify the propagation of DNA damage and increase the likelihood of tumor development.
The pigment metabolite 5-S-cysteinyldopa (5-S-CD) was measured in urine before the irradiation and twice weekly until day 38. No correlation was found between the basal 5-S-CD excretion and the size or activity of the melanocyte organ, suggesting that the basal 5-S-CD excretion is mainly of non-melanocytic origin. Despite numerous nevi, DNS-patients did not differ from controls in their 5-S-CD excretion. The normal upper range for the tumor marker 5-S-CD is therefore valid in these melanoma-prone subjects. During the irradiation, subjects with a low tanning ability developed a more pronounced erythema and excreted more 5-S-CD than those with a good tanning ability. This suggests that the UVB-induced 5-S-CD excretion is rather due to melanocyte damage than to an increased melanin synthesis.

To investigate the influence of sun exposure on the development of nevi and melanoma (CMM), the distribution over the body surface of CMM, common nevi (CN) ≥2 mm and dysplastic nevi (DN) was registered in 121 melanoma patients and 310 controls. Four times as many nevi were found in a sun-exposed area than in a comparable sun-protected area, demonstrating that sun exposure plays an important role in nevus development. Subjects with DN had a larger difference in nevus counts between the two areas than subjects without DN, indicating a different UV-dose and/or a higher sensitivity to the "nevogenic" effect of UV-light than subjects without DN. The number of both CN and CMM per unit surface area was higher in intermittently exposed skin than in rarely or chronically exposed skin. Thus, intermittent UV exposure seems to have the most potent "nevogenic" and carcinogenic effect on the melanocytes. The probability of having a CMM in a specific area increased with the relative clustering of both CN and DN in that area. This site-specific melanoma risk might be the result of local insults of UV-light to the melanocyte system and/or be due to nevi being precursor lesions to CMM.

Key words: UV-light, melanocyte, nevus, melanoma, dysplastic nevus syndrome, 5-S-cysteinyldopa.

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This thesis is based on studies reported in the following papers:

I. Stierner U, Rosdahl I, Augustsson A, Kågedal B. UVB irradiation induces melanocyte increase in both exposed and shielded human skin. J Invest Dermatol 1989;92:561-564.

II. Stierner U, Rosdahl I, Augustsson A, Kågedal B. Urinary excretion of 5-S-cysteinyldopa in relation to skin type, UVB-induced erythema, and melanocyte proliferation in human skin. J Invest Dermatol 1988;91:506-510.

III. Stierner U, Augustsson A, Rosdahl I, Kågedal B, Suurküla M. UVB-induced melanocyte proliferation and 5-S-cysteinyldopa excretion in dysplastic nevus syndrome. Photodermatology 1988;5:218-223.

IV. Augustsson A, Stierner U, Rosdahl I, Suurküla M. Melanocytic naevi in sun-exposed and protected skin in melanoma patients and controls. Acta Derm Venereol 1991 (Accepted).

V. Stierner U, Augustsson A, Rosdahl I, Suurküla M. Regional distribution of common and dysplastic naevi in relation to melanoma site and sun exposure: a case-control study. Submitted.

The papers will be referred to in the text by their Roman numerals.

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LIST OF ABBREVIATIONS

CN	common melanocytic nevus
DN	dysplastic nevus
DNS	dysplastic nevus syndrome
CMM	cutaneous malignant melanoma
dopa	L-3,4-dihydroxyphenylalanine
5-S-CD	5-S-cysteinyldopa
5,6DHI	5,6-dihydroxyindole
5,6DHI2C	5,6-dihydroxyindole-2-carboxylic acid
bFGF	basic fibroblast growth factor
UVA	ultraviolet light 320-400 nm
UVB	ultraviolet light 290-320 nm
PUVA	8-methoxy-psoralen and UVA-light
AAU	arbitrary area units
Ex-Pr	exposed-protected

INTRODUCTION

Melanocytes

The melanocytes are highly differentiated dendritic cells of neural crest origin. During embryonic life, the majority of the melanocytes migrate into the epidermis to settle along the basal lamina, interspersed between the keratinocytes. Only a few per cent of the total melanocyte population will be found elsewhere, for example in the eyes, the mucous membranes, the meninges and the hair follicles (1). Each epidermal melanocyte and its surrounding keratinocytes form a structural and functional unit, the epidermal melanin unit (2). The main function of this unit is to provide photoprotection. The UV-absorbent pigment, melanin, is synthesized in specialized melanocytic organelles, the melanosomes. Via the melanocytic dendrites, which constitute a network throughout the epidermis, the melanosomes are transferred into the keratinocytes. During the transport of the keratinocytes towards the surface of the skin the melanosomes are slowly broken down to a melanin dust in the cytoplasm. This design of the epidermal melanin unit results in an effective UV-filter protecting the underlying structures from the harmful effects of UV irradiation (3).

The melanocytes are rather evenly distributed over the body surface, but with some variation in number in different skin regions. For example, there is a higher population density in the skin of the face and genitals than on the trunk and extremities. The mean melanocyte population density of the body surface has been calculated to 1000 -1500 melanocytes/mm^2 (4). If all epidermal melanocytes were assembled into a compact organ they would, in an adult not recently exposed to sunlight, form a tissue volume of 1.0 - 1.5 cm^3 (1).

The racial differences in skin color do not depend on the melanocyte population density. Instead, the skin color is determined by the amount and type of melanin within the melanosomes and the form, size and distribution pattern of the melanosomes in the keratinocytes (3).

Photoprotection and pigment

The solar irradiation that reaches the surface of the earth contains ultraviolet light in the wavelengths 290-400 nm, visible and infrared light. The ultraviolet light region is divided into 290-320 nm (UVB) and 320-400 nm (UVA). Both UVA and UVB influence the melanocytes, but UVB is considered the more harmful as it is more potent in causing DNA damage and erythema. In the skin, melanin and keratin are the most important absorbers of UV irradiation. In response to UV exposure the skin protects itself by increasing both the amount of melanin in the epidermis and the thickness of the keratin layer. In addition, repeated UV irradiation induces an increase in the number of melanocytes. This seems rational in order to increase melanin production beyond the genetic potentials for each melanocyte (3,5).

UV-induced melanocyte proliferation

It was once believed that melanocytes were non-dividing cells, because of their neural crest origin and high degree of differentiation. The increase in dopa-positive epidermal melanocytes following repeated sun exposure was then interpreted as the result of an activation of "dormant" melanocytes (6,7). It has now been demonstrated that normal human melanocytes enter mitosis (8) but there are great difficulties connected with investigations on the mitotic cycle of human melanocytes in vivo. However, it has been shown that there is a continuous mitotic turnover of melanocytes in the epidermis of the mouse and the UVB-induced increase in the number can be fully accounted for by an increased mitotic rate (9,10). Although detailed quantitative data are still lacking, it seems most likely that proliferation is the major cause of the UVB-induced

melanocyte increase also in man (11).
In the mouse, UVB-irradiation induces an increase in the melanocyte population not only in irradiated skin areas but also in covered skin (12). It was suggested that this increase is initiated by a systemic mitogenic factor released from the UVB-irradiated skin. The presence of such a mechanism is rational in view of photoprotection, since it creates a readiness of the total skin surface for future UV irradiation.

Melanin and 5-S-cysteinyldopa

There are two types of melanin, the brown-black eumelanin and the yellow-red phaeomelanin. The human melanins are mixtures of different proportions of these two melanins (13). Melanogenesis starts in the melanocyte with the hydroxylation of tyrosine to dopa and the oxidation of dopa to dopaquinone catalyzed by the enzyme tyrosinase. The addition of cysteine or glutathione to dopaquinone gives the phaeomelanin precursor 5-S-cysteinyldopa (5-S-CD). In the absence of these SH-compounds dopaquinone spontaneously forms 5,6-dihydroxyindole-2-carboxylic acid (5,6DHI2C) and 5,6-dihydroxyindole (5,6DHI), indoles which are precursors to eumelanin (14,15). These phaeomelanin and eumelanin precursors are then further converted to human melanin but the exact steps are not yet fully known. The precursors (5-S-CD, 5,6DHI and 5,6DHI2C) are to some extent released into the circulation and are finally excreted in the urine (16,17,18).

For several years, 5-S-CD has been used as a tumor marker for malignant melanoma (19,20). The analytical methods for measuring the indolic metabolites are rather recent and the usefulness of these compounds as tumor markers is under current investigation. The excretion of 5-S-CD into urine has also been used to study pigment formation and metabolism in the normal melanocyte population. It is, for instance, well known that sun exposure increases the 5-S-CD excretion in urine. This increase has been attributed to an increased synthesis of melanin. The highest excretion values of 5-S-CD have, however, been found in subjects with a tendency to burn easily and tan poorly. It was therefore suggested that the UV-induced 5-S-CD excretion instead reflects melanocyte injury and not the level of pigment production (21).
Some studies have, however, revealed unexpected data not in keeping with the general view that the 5-S-CD excretion mainly reflects melanocyte activity. For instance, the basal 5-S-CD excretion has been shown to be independent of skin color (18,22). Furthermore, normal values of 5-S-CD in urine have been found in both tyrosinase-positive and negative human albinos (23,24). Free as well as protein-bound 5-S-CD have also been found in several other organs in rodents (25,26). Based on these findings, the melanocyte specificity of 5-S-CD has lately been questioned.

Moles - common nevi (CN)

A pigmented nevus is a benign tumor derived from the melanocytes (27). A large number of common nevi (CN) indicates an increased risk for developing cutaneous malignant melanoma (CMM) (28,29). CN are also potential precursor lesions to CMM (27,30). The number of CN that turn malignant is, however, very low. Nevertheless, CN can be looked upon as intermediate lesions between the normal melanocytes and the melanoma cells. As such, they are of great interest to study when one is trying to understand the different steps leading to malignant transformation.
Common nevi are frequently and incorrectly called birthmarks. Instead, CN become visible during the first years of life and new CN continue to emerge up to adulthood. The number of nevi then starts to decrease and very few are found in old people. From cross-sectional data on the prevalence of nevi in different age-groups, the following life-cycle of nevi has been suggested. When first visible, CN are a

few mm large, dark, homogeneously pigmented lesions which grow horizontally along the dermo-epidermal junction. As they become larger, usually not more than 5 mm, a vertical growth phase starts. The lesions then become slightly raised and somewhat less pigmented. Nests of nevus cells are now also present in the dermis. Next the nevus develops into a fully intradermal lesion, a slightly pigmented or skin-colored papule, which is followed by a total involution. Recent reports indicate that today there is an increase in the frequency of CN compared to what was found in the fifties (31). The finding of few nevi in old age is therefore most probably due to both the maturation and disappearance of nevi and a cohort effect.

Very little is known about the etiology of CN (29). Epidemiological studies on the prevalence of CN have shown great geographic as well as racial differences. The nevus counts also differ between the sexes but the male to female ratio varies greatly between different populations. In a case-control study, we have recently shown that Swedes (30-50 years of age) have very high numbers of nevi in comparison with other populations studied. The mean number of nevi in the control population was 67 (range 1-305), compared to 115 (range 13-355) in the melanoma group (32,33).

Dysplastic nevus syndrome (DNS)

Dysplastic nevi (DN) were first described in 1978 as a form of atypical large nevi appearing in subjects with hereditary melanoma (34). The disorder was named B-K mole syndrome after the first two kindreds studied, but is now generally called dysplastic nevus syndrome type D-2. Subjects with DN belonging to a DNS type D-2 kindred have a cumulative lifetime melanoma risk of 100% (35,36). The disorder has an autosomal dominant mode of inheritance. Strong evidence for linkage of a single-gene locus on chromosome 1p with the DNS type D-2 has been found in American kindreds but a similar study on Dutch kindreds has not confirmed this linkage (37,38,39). Greene and Clark reported that 70% of the melanomas in their DNS-kindreds arose in connection with a dysplastic nevus. Still, 15% of these patients developed de novo melanomas (without a discernible pre-existing mole), indicating an increased melanoma susceptibility affecting the entire melanocyte system (36). In many countries DNS-kindreds are now being traced, and those with melanoma and/or dysplastic nevi are carefully followed in order to diagnose their melanomas at an early, curable stage. This hereditary form of melanoma, however, only accounts for approximately 5% of all CMM (35).

Dysplastic nevus syndrome (DNS) and UV exposure

UV-light is considered an important etiological factor for melanoma (40,41). In the hereditary disorder, xeroderma pigmentosum, the patients have a markedly increased risk of melanoma due to a deficiency in the repair of the UV-induced DNA damage. It has therefore been interesting to study the UV sensitivity also in the dysplastic nevus syndrome. These tests, performed on fibroblasts and lymphoid cells, revealed an enhanced killing effect and hypermutability in response to UV irradiation (42,43,44,45,46).

Sporadic dysplastic nevi (DN)

Once attention was drawn to the existence of dysplastic nevi in the families with hereditary melanoma, these nevi were also recognized in patients with non-hereditary melanoma and even in subjects with neither a personal nor a family history of melanoma (47,48). In our Swedish case-control study, where 8% of the patients and 3% of the controls reported heredity for CMM, one or more clinically diagnosed DN were present in 56% of the melanoma patients and 18% of the controls (33). Case-control studies from other countries have

reported a corresponding prevalence of 34-55% for melanoma patients and 5-17% for controls (49,50,51). That is, dysplastic nevi are also markers for an increased risk for non-hereditary melanoma. We found a crude relative risk of developing CMM of 3.8 for subjects having one or two DN and 12.0 for those having three of more. The relative risks adjusted for confounding factors were 2.5 and 5.6 respectively (33). This decrease in risk was mainly due to the fact that subjects with DN also had a large number of common nevi. Apart from being a marker, about one-fourth of the sporadic melanomas are reported to arise in connection with a dysplastic nevus (52).

Nevi and sun exposure

If nevi are markers for and precursors of CMM, it is reasonable to assume that both tumors have at least one common etiological factor. Nicholls (1973) suggested that sun exposure induces the formation of nevi (53). He studied the distribution of nevi over the body surface and found that nevi developed earlier in sun-exposed skin. Kopf et al compared the number of raised nevi on the lateral and medial aspects of the arms and found significantly more nevi on the more sun-exposed lateral than on the less frequently exposed medial aspect of the arm (54). This difference in nevus counts was not found in blacks. Furthermore, total body counts of nevi are lower in blacks than in whites, indicating a protective effect of pigment on nevus formation. In subjects with DNS, Kopf et al compared nevus counts from sun-exposed and relatively unexposed skin and came to the conclusion that sun exposure promotes the development of nevi also in subjects with the dysplastic nevus syndrome (55,56).

Tumor progression model

With increasing knowledge of the biological properties of normal melanocytes, nevus cells and melanoma cells at various stages, the model of carcinogenesis in the melanocyte system has become more detailed. From this more biological point of view, Clark and Elder classified CMM into radial growth phase or vertical growth phase melanoma (57). Tumors in the radial growth phase (melanoma in situ and microinvasive melanoma) have the capacity for autonomous growth but lack tumorigenicity and capacity for metastasis. Fully developed malignant properties are first seen in the vertical growth phase. In this model of tumor progression, starting with a normal melanocyte that evolves to a common nevus, Elder ranked the dysplastic nevus in between the common nevus and the radial growth phase melanoma (52). To start moving and to proceed on this pathway, both initiation and promotion are needed. Theoretically, UVB-light is capable of both initiation, by its mutagenic effect, and promotion, by its mitogenic effect on the melanocytes.

Melanoma (CMM) and sun exposure

A substantial amount of epidemiological and clinical data support the hypothesis that sun exposure is a major cause of CMM (40,41). For instance, melanoma is more common in whites than in blacks, and among whites more common in those with fair than in those with darker skin. This indicates a protective effect of pigment. When comparing populations with equivalent pigmentation, the melanoma incidence increases the closer one comes to the equator. Patients with a deficiency in enzyme repair of UV-induced DNA damage have a highly increased risk of CMM. Subjects with a tendency to burn easily or with a history of many burns also have an increased risk of CMM. In addition, the sex differences in melanoma site distribution are consistent with differences between the sexes in clothing habits and skin areas exposed to the sun. Together, all these findings speak in favor of an association between sun exposure and the development of CMM.

Still, there are some facts that create uncertainty about such a relationship (40,41). One argument

against UV-light as an etiological agent is the occurrence of CMM in skin that never or very rarely has been exposed to sunlight. Furthermore, while there is a clear dose-response relationship between sun exposure and non-melanoma skin cancers, this is not the case for melanoma. It seems instead that infrequent but intense sun exposure has a more carcinogenic effect on the melanocytes than more constant sun exposure (58). For instance, the majority of CMM occur in skin areas vigorously but infrequently exposed to UV-light and with a relatively low cumulative UV dose. In addition, melanoma is more common in indoor than in outdoor workers. This has been attributed to the fact that indoor workers without prior pigment protection are intensely UV-exposed during weekends and vacation periods while outdoor workers do not burn due to a protective tan achieved during more regular exposure (59,60).

AIMS OF THE STUDY

1. To investigate if UVB irradiation induces an increase in the number of melanocytes not only in exposed but also in shielded human skin.

2. To investigate the origin of the pigment metabolite 5-S-cysteinyldopa by studying the urinary excretion of this compound in relation to skin type, basal epidermal melanocyte population density, UVB-induced erythema and melanocyte proliferation in exposed and shielded skin.

3. To study the effect of UVB irradiation on melanocytes in patients with dysplastic nevus syndrome, by measuring the melanocyte population increase in exposed and shielded skin as well as the UV-induced increase in urinary 5-S-CD excretion.

4. To determine if the upper normal range for the tumor marker 5-S-CD is valid for subjects with the dysplastic nevus syndrome.

5. To study the influence of sun exposure on nevus and melanoma development, by analyzing the melanoma site and the distribution of common and dysplastic nevi in relation to the amount and frequency of sun exposure in different body areas.

MATERIALS AND METHODS

All examinations, in the experimental as well as in the clinical study, were performed during the winter season in order to avoid influence of recent sun exposure on the pigmentary system. The skin type was classified according to Melski´s scheme (61). This classification is based on the reaction of the skin to the first hour of sun exposure in early summer.

Experimental study (papers I-III)

Subjects

Twenty-two healthy volunteers, 13 women and 9 men (mean age 37, range 26-49 years), received UVB irradiation. Eleven subjects had skin type II (always burn, sometimes tan), 9 had skin type III (sometimes burn, always tan) and two had skin type IV (never burn, always tan). Of these, two subjects failed to complete the urinary sampling and the skin biopsy from one subject was lost in preparation.

An additional 10 healthy volunteers were subjected to skin biopsies only (5 women and 5 men, mean age 33, range 23-49 years, with skin types II-IV).

DNS patients (paper III)

Two women and 9 men (mean age 39, range 21-61 years) received UVB irradiation. Three subjects had skin type II and 8 skin type III. One patient belonged to a D-2 family (at least two blood relatives with CMM and two or more subjects with DN) and the remaining ten belonged to 7 different D-1 families (one blood relative with CMM and two or more subjects with DN) (35). None of the participants had been exposed to sunlight or any artificial UV-light during a period of at least three months preceding the study.

UVB irradiation

The light source was a Philips TL12 UVB-box with an emission spectrum of 270-380 nm (peak 305 nm). The light intensity in the UVB region was measured with an IL 1350 photometer (International Light, Inc.) with a sensitivity of 245-355 nm and a maximum at 291 nm.

All subjects received total body UVB irradiation three times weekly during 17 days. The dose was the same for all individuals and was increased to give a slight erythema for skin type II at all exposures. The eight doses were 24, 32, 32, 40, 56, 72, 88 and 104 mJ/cm^2.

During irradiation, a 15 x 15 cm area on the left buttock was shielded with black plastic tested to be non-transmittent for the UV-light used. For fixation, non-traumatizing tape was used. After each irradiation, the erythema of the skin was graded by the subjects as none, slight, moderate or severe.

Skin biopsies

Before the first irradiation, a 3 mm punch biopsy was taken from the left buttock skin. Three weeks after the last UV exposure, another biopsy was taken from the shielded left buttock area and a third one from the exposed right buttock. The two biopsies from the left buttock were taken 5 cm apart and 5 cm from the tape to avoid influence of wound healing or mechanical stimulation from tape stripping. In the ten non-irradiated control subjects, the skin biopsies were taken from the same three locations, but all three biopsies were taken on one occasion. Care was taken to avoid freckles and nevi.

NaBr was used to split the skin along the dermo-epidermal junction and the melanocytes were visualized using the dopa-reaction (62). The epidermal melanocyte population was then estimated in the split skin preparations using a random dot procedure and a x40 objective (1). The ocular plate contained 25 randomly distributed spots. If a spot was superimposed on a melanocyte cell body, the melanocyte was counted. The number of marked cells measures the relative skin surface covered by melanocyte cell bodies. In each biopsy, 40 to 60 ocular fields were counted on coded slides and the mean number of marked melanocytes per ocular field was calculated. Correction was made for the change in size of the skin biopsy during preparation. The corrected mean value will be referred to as the melanocyte population density. Biopsy counts times body surface area were used as a relative measure of the total epidermal melanocyte mass (63).

Urine sampling and analysis of 5-S-CD

Before entry to the study and twice weekly until day 38, the 24-hour urine production was collected in plastic bottles containing 5 ml of thymol (0.7 mol/l) in isopropanol and stored at -20^{o}C until analysis. The 5-S-CD concentration was determined by high-performance liquid chromatography (64).

The UV-induced increase of 5-S-CD excretion over a period of time was measured as the area between the baseline and the excretion curve with standardized scales on the x-axis (24 h) and y-axis (umol/24 h). This area was expressed in arbitrary area units (AAU). All values were normalized for body surface area.

Statistical analysis

Fisher's test for pair comparison was used for the analysis of the change in melanocyte population density, and Pitman's test was used for correlation analysis (65). When the correlation between the melanocyte increase in covered and exposed skin was analyzed, Mantel's test was used to eliminate the influence of the initial melanocyte population (66). When the relative UVB-induced melanocyte increase was tested, correction was made for the regression to the mean phenomenon.

Clinical study (papers IV-V)

Cases

All Caucasian patients, 30-50 years of age, with a history of CMM and living in Göteborg were selected from the Regional Cancer Register, n=197. Of these, 78% (n=154) were still alive. Permission to contact the patient was obtained from his or her physician in all but two cases. In a letter, each patient was offered a free skin examination by a dermatologist and an oncologist. Ten patients declined participation and an additional 5 could not be reached. The remaining 137/152 (90%) were examined. Of these, 16 patients were excluded from analysis, 6 because tumor material could not be found for histological re-evaluation and 10 because of an incorrect diagnosis. The histological re-examination was performed by one pathologist. Altogether 121 patients (52 men, 69 women, mean age 43.5 years) were included for analysis.

Controls

Five hundred subjects within the same age-span (30-50 years) were randomly selected from the census file in Göteborg. Like the melanoma patients, they were offered a free skin examination. The participation rate was 82%. Of these, 310 consecutive subjects (152 men, 158 women, mean age 41.4 years) were included in these two specific studies.

Skin examination

The total body skin examination included skinfolds, palms, soles, scalp and genital area. All brown macular or raised lesions ≥ 2 mm considered to be melanocytic nevi were counted and the distribution of nevi over the body surface was plotted on a schematic figure. It was sometimes difficult to discriminate between nevi, freckles and lentigines. When in doubt, the lesion was not counted in order to avoid overestimation. Scars after previously excised nevi (n=310) were also plotted and all histologically confirmed nevi were included in the counts.

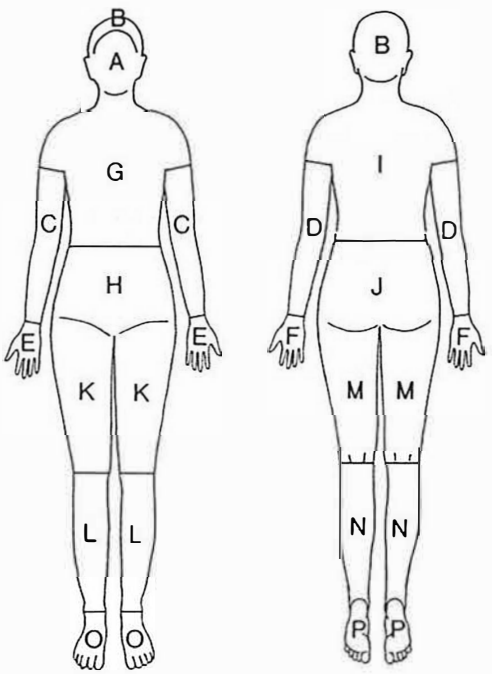


Fig. 1 Schematic figure illustrating the 16 areas studied (A-P).

To compare the distribution pattern statistically, the body surface was later divided on the plot into 16 separate areas (A-P) (Fig. 1). To facilitate comparisons between areas exposed to different amounts and frequencies of UV-light, the areas were defined taking not only the anatomy but also general clothing habits and behavior in the sun into account. The areas A and F were considered chronically and the areas C, H and J rarely UV exposed. With the ex-

ception of the areas B, E and P (scalp, palms and soles), the remaining areas were classified as intermittently exposed. The sizes of the areas studied are those calculated by Lund and Browder (67) with minor modifications. Four per cent was subtracted from the area of the arms and added to the trunk area, due to our subdivisions, as indicated on the schematic figure. Similarly, we have estimated the part of the lower trunk (areas H and J) usually covered to be 14% of the body surface area.

In addition, the number of melanocytic nevi was counted separately in two "extreme areas", 14 x 28 cm large, one sun-protected on the buttocks and one adjacent sun-exposed on the lower back (Fig. 2). The individual difference in nevus counts between the exposed and the protected area was calculated and will be referred to as the Ex-Pr difference.

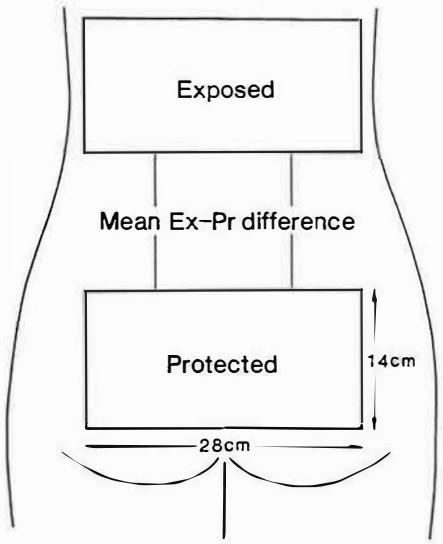


Fig. 2 Schematic figure illustrating the size and location of the sun-exposed and the sun-protected area.

Clinically diagnosed dysplastic nevi were registered separately. The generally accepted clinical criteria were used. The main criterion was a diameter ≥ 5 mm. In addition, at least two of the following criteria were required: an ill-defined border, speckled pigmentation, erythema or a pebbled surface (68). Of the 310 previously excised nevi, 286 were re-examined histologically to find possible DN excised before this entity was described. The histological criteria for DN were those defined by Elder (69) and Sagebiel (70). Nevi fulfilling the histological criteria for DN (n=44) were added to the counts of clinically diagnosed DN.

The melanoma location was registered as situated in the center of the melanoma scar.

Statistical methods

Spearman´s rank test was used for the correlation analyses. For comparisons between groups, Wilcoxon´s two-sample test was used. For comparisons of proportions between groups, Fisher´s exact test was used. Trends in contingency tables were analyzed using the Mantel-Haenszel chi square test (71). Wilcoxon´s test for paired observations was used when nevus counts between the two "extreme areas" were compared, and when the number of CMM per unit surface area was compared between areas of different UV-exposure categories. In order to assess the correlation between clustering of nevi and presence of CMM within a small skin area, we applied a special procedure which is not affected by the correlation between the total body count of nevi and CMM. For each area in each individual (cases and controls) a score was calculated, measuring the relative clustering of nevi within the area, (P-E)/S, where P is the quotient between the number of nevi in the area and the total body count, E is the mean proportion of nevi in the same area for all individuals, and S is the corresponding standard deviation. By logistic regression analysis the probability of CMM was estimated as a function of the score. Two-sided tests were used.

RESULTS

Basal melanocyte population density (papers I and III)

The individual mean melanocyte counts in buttock skin ranged from 1.3 to 5.3 with a mean of 2.9 (0.2 SEM) in the 21 biopsies from the subjects participating in the UV experiment. This estimates the relative skin surface covered by melanocyte cell bodies and corresponds to 700 - 2.700 melanocytes per mm^2 with a mean of 1.450 (1).

A similar very wide range in the melanocyte population density was seen in the 10 non-irradiated control subjects, range 1.0 - 5.3. Although the interindividual difference in melanocyte population density was large, the estimated intraindividual variance between the three non-irradiated control biopsies was low, 0.11. The basal melanocyte population density was not influenced by skin type.

Furthermore, the 11 subjects with DNS did not differ from the controls in their mean basal melanocyte density, 2.5 (0.4 SEM).

Basal urinary 5-S-CD excretion (papers II and III)

The basal 5-S-CD excretion varied between 0.07 and 0.26, mean 0.19 (0.01 SEM) µmol/24h/m^2 body surface area. The excretion was not influenced by sex. Subjects with skin type III-IV had, despite their greater capacity to produce pigment, the same basal excretion of 5-S-CD as those with skin type II. Neither was there any correlation between the basal 5-S-CD excretion and an individual's total epidermal melanocyte mass or number of nevi. Although 5-S-CD has been demonstrated in dysplastic nevi, no increased basal urinary excretion of 5-S-CD was found in the subjects with DNS.

In summary, no correlation was found between the basal 5-S-CD excretion and the size and level of activity of the melanocyte organ.

UV-induced melanocyte population increase (papers I and III)

After the irradiation period (on day 37), the mean melanocyte population density in the exposed buttock skin was twice as high 5.5 (0.3 SEM) as in the initial biopsy 2.9 (0.2 SEM), $p<0.001$. When individual counts were analyzed 18 out of 21 subjects showed a significant increase in the number of melanocytes. Of these 18, 10 subjects had a significantly increased melanocyte population density in covered skin also. The mean value in covered skin for all the 21 subjects was 3.7 (0.2 SEM), $p<0.05$.

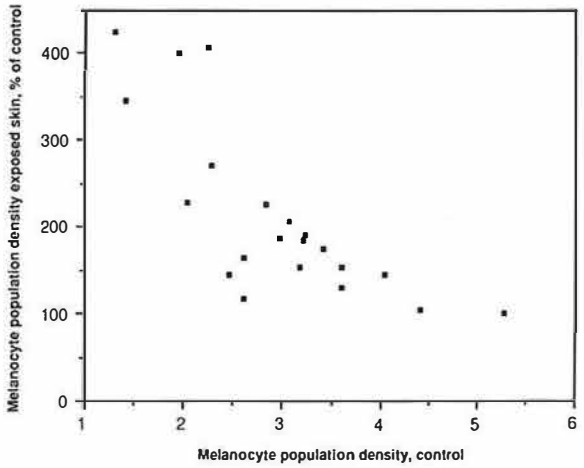


Fig. 3 Individual melanocyte population density in UVB irradiated skin plotted in percentage of the initial population density in the control biopsy.

There was a great individual variation in the UV-induced melanocyte population increase. In the exposed skin, the individual increase in melanocyte counts per ocular field ranged from 0.1 to 6.9. This variation was not correlated to skin type or erythemal reaction. In both exposed and covered skin there was, however, an inverse correlation between the size of the melanocyte population increase and the basal melanocyte population density, i.e. those with a low initial population density responded to the irradiation with a higher increase (Fig. 3). This inverse correlation could not be explained by the regression to the mean phenomenon. There was also a correlation between the size of the UV-induced melanocyte increase in exposed and covered skin, $p<0.01$.

When the influence of the basal melanocyte population density was eliminated by using Mantel´s test, this correlation was no longer statistically significant but the p-value was still <0.07. The UV effect on the melanocyte population in exposed and covered skin did not differ significantly between the DNS-patients and the healthy controls.

In summary, we have now for the first time in humans demonstrated that repeated UVB exposure induces an increase in the melanocyte population density not only in exposed but also in covered skin. The size of this increase is inversely correlated to the basal melanocyte population density.

UV-induced increase in urinary 5-S-CD excretion (papers II and III)

During the irradiation period, the mean urinary 5-S-CD excretion increased continuously to reach a maximum of 0.45 (0.04 SEM) μmol/24h/m^2 at day 17, approximately twice the basal value of 0.19 (0.01 SEM) μmol/24h/m^2. After the irradiation period, the excretion values decreased but did not return to the basal level during the three-week follow-up period. To analyze the acute and delayed effect of UVB irradiation on the 5-S-CD excretion separately, the excretion curve was divided into phase I and phase II at day 24 (Fig. 4). During phase I subjects with skin type II had a significantly larger increase in 5-S-CD excretion than subjects with skin type III-IV. The mean (SEM) area under the curve was 115 (18) AAU for skin type II and 53 (8) AAU for skin type III-IV, p<0.01.

As expected, subjects with skin type II developed a more pronounced erythemal reaction than those with skin type III-IV. The accumulated mean (SEM) erythemal score was 9.0 (1.3) for skin type II and 2.8 (0.9) for skin type III-IV, p<0.01. Neither the UV-induced increase in 5-S-CD excretion nor the erythemal reaction was correlated to the basal melanocyte population density. During phase II the mean 5-S-CD excretion level remained slightly above the basal level. The mean (SEM) area under the curve was now similar in subjects with skin type II [20 (6) AAU] and subjects with skin type III-IV [19 (8) AAU]. Seven out of twenty subjects developed a significant peak in 5-S-CD excretion during this follow-up period. In some subjects, this second peak was even higher than the first one registered during the UVB irradiation period. This second peak was of short duration, which means that we may have failed to register this event in additional subjects. The UV-induced 5-S-CD excretion during phase I and II was not correlated to the melanocyte population increase in exposed or covered skin.

In summary, the UV-induced increase in 5-S-CD excretion was not correlated to a good tanning ability, nor to the increase in melanocyte population. Instead, the highest UV-induced urinary 5-S-CD excretion was seen in subjects with skin type II, with less capacity to tan and a higher accumulated erythemal score than subjects with skin type III-IV.

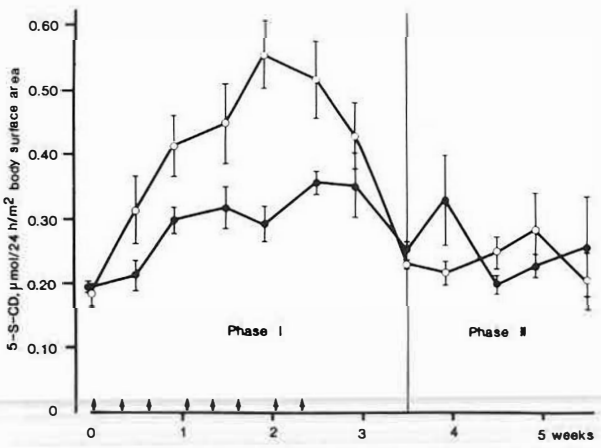


Fig. 4 UVB-induced increase of 5-S-CD excretion in subjects with skin type II (○) (n=11) and skin type III-IV (●) (n=9), mean ± SEM. Arrows indicate UVB exposure.

Influence of UV exposure on nevus formation (paper IV)

The melanoma patients had almost twice as many nevi as the controls. The mean (Md) total body count of nevi was 115 (89) in the patients (n = 121) and 66 (53) in the controls (n = 310), $p<0.001$. Subjects with DN, melanoma patients as well as controls, had significantly more nevi than those without DN. The mean (Md) total body count of nevi in patients with DN was 144 (117), compared to 78 (59) in patients without DN, $p<0.001$. The corresponding mean counts in the control group were 102 (95) and 57 (45) respectively, $p<0.001$. The number of nevi in a sun-exposed area on the back was compared with the counts in a sun-protected area of equal size on the buttocks. The difference in nevus counts between the exposed and the protected area was termed the Ex-Pr difference. Both patients and controls had more nevi in the exposed than in the protected area, $p<0.001$. The mean (Md) nevus count in the exposed area was in patients 6.5 (5) and in controls 3.7 (3). The corresponding counts in the protected area were in patients 1.4 (1) and in controls 0.9 (1). These figures demonstrate that the melanoma patients had significantly more nevi than the controls in both the exposed ($p<0.001$) and the protected ($p<0.01$) areas. More important, the patients had a significantly larger mean Ex-Pr difference, 5.1, compared to 2.8 in the controls, $p<0.001$.

Men in both groups had significantly more nevi in the exposed area on the back than women. In neither group did age or skin type have any influence on the nevus counts.

When patients and controls with and without DN were studied separately, it was found that both patients and controls with DN had a significantly larger mean Ex-Pr difference than patients and controls without DN, $p<0.001$. Melanoma patients with DN (n = 68) had a mean Ex-Pr difference of 7.1, and the corresponding figure for controls with DN (n = 59) was 4.9. Melanoma patients without DN (n = 53) had a mean Ex-Pr difference of 2.6, and the corresponding figure for controls without DN (n = 251) was 2.3. The mean Ex-Pr difference did not differ significantly between patients and controls with DN, or between patients and controls without DN.

Influence of amount and frequency of UV exposure on nevus formation (paper V)

The melanoma patients had more CN than the controls in all subareas studied, except on the dorsum of the hands and the soles. After adjustment for the total body counts of CN, this difference remained only for the lower legs and the dorsum of the feet. In neither group did skin type have any influence on total body counts or distribution of CN over the body surface.

To be able to compare nevus counts in areas of different size, the number of nevi per unit surface area was calculated.

The mean number of nevi per unit surface area was significantly larger in intermittently exposed areas than in those considered rarely or chronically exposed. In intermittently exposed skin, the mean number of CN per unit surface area was 1.40 in patients and 0.80 in controls. The corresponding figures for rarely exposed skin were 0.87 and 0.53 respectively. The difference in nevus concentration between intermittently and rarely exposed skin was twice as large in the melanoma group as in the controls, but it was still highly significant in both groups, $p<0.001$.

The mean number of CN per unit surface area was even lower in chronically exposed than in rarely exposed skin. In the melanoma patients the mean number of nevi per unit surface area was 0.62 and in the controls 0.41. Intermittent UV exposure thus seems to have a more potent "nevogenic" effect than rare or chronic UV exposure.

The prevalence of DN was 56% in the melanoma patients and 19% in the controls. The presence of DN was not influenced by age or sex, but in the

control group subjects with DN had a more sun-sensitive skin type, $p<0.001$. The distribution pattern of DN was clearly different from that of CN, with very few DN on the arms and upper chest and a large number in rarely exposed areas such as the abdomen and buttocks.
Subjects with DN had more CN in all areas except on the scalp, the hands and the soles. After adjustment for the total number of nevi in each individual, the nevus distribution was, however, very similar in subjects with and without DN. The difference in mean number of CN between intermittently and rarely exposed skin areas was larger in both patients ($p<0.01$) and controls ($p<0.05$) with DN than in those without DN.

Influence of amount and frequency of UV exposure on melanoma development (paper V)

The areas with the highest concentration of CMM were the back and the chest. There was a male predominance for CMM on the trunk ($p<0.01$), while CMM on the upper and lower extremities were more common in females, $p<0.05$. Skin type had no influence on the site distribution of CMM.
The number of CMM per unit surface area was significantly larger in intermittently exposed skin (1.6) than in rarely (0.7) or chronically exposed skin (1.0), $p<0.001$.

Melanoma location in relation to nevus distribution and site-specific melanoma risk (paper V)

The distribution pattern of CMM showed a marked resemblance to that of CN. The regional distribution of DN was, however, less strikingly correlated to melanoma location. The most obvious lack of conformity between DN and CMM was seen on the upper chest, in the head and neck region, and on the buttocks.

When analyzing if the proportion of nevi (CN or DN) was correlated to a site-specific melanoma risk, we found that the probability of CMM increased with the concentration of CN ($p<0.05$) as well as that of DN ($p<0.01$). This demonstrates that a large number of nevi is not just a general melanoma marker but also indicates a site-specific melanoma risk.

DISCUSSION

UV effects on melanocytes

In theory, UV-light might exert its carcinogenic effects on the melanocytes in several ways (30,40,52). The most obvious way is by damaging the DNA. UV-light is capable of inducing a variety of DNA lesions and an insufficient repair of any of these might lead to tumor initiation. An additional proliferative stimulus is, however, required in order to establish a tumor (72). As UV-light stimulates melanocyte division, it may also act as a tumor promotor. This study is a detailed analysis of the UV-induced proliferation of melanocytes in exposed and covered human skin.

In the UV-exposed skin we found a two-fold increase in the mean number of melanocytes. For the first time in humans, we were able to demonstrate that repeated UVB irradiation induces an increase in the number of melanocytes in covered skin also. This increase was about one-third of that in irradiated skin. In previous mouse experiments, the increase in melanocyte number in covered skin was delayed compared to that in exposed skin (12). A significant increase was not noted until 17 days after starting the UV exposure but the increase continued for another nine weeks. Here, we waited until day 37 after the start of the irradiation to take the biopsies, in order to have a safe margin for the possible delayed effect in covered skin. At this time we were able to detect a significant increase in the number of

melanocytes in covered skin but the maximal increase might have been reached later. One might ask why this indirect UV effect has not been detected by previous investigators. One explanation may be that biopsies were taken too soon after the irradiation period (5,11,73). Furthermore, covered skin was often used as control for the irradiation effect in exposed skin, and no control biopsies were taken prior to the irradiation period.

When the melanocyte increase in covered mouse skin was first documented, a number of control experiments demonstrated that this population increase could not be explained by light leakage, mechanical stimulation or stress effects (12). Accordingly, it was suggested that the melanocyte increase was initiated by a systemic mitogenic factor released from the UVB-irradiated skin. We postulate a similar mechanism in humans.

A covariation was found between the increase in melanocyte population in exposed and covered skin. When the influence of the individual basal melanocyte population was eliminated, the p-value for this covariance was still less than 0.07. Such a covariance as well as the delayed response in covered skin make it plausible that the mitosis-stimulating factor is derived from the proliferating melanocytes in the exposed skin. It is interesting to note in this context that melanocyte-conditioned medium stimulates melanocyte growth in vitro (74). That is, melanocytes release at least one autocrine growth-stimulating factor. Another alternative is that the systemic mitogenic factor is derived from the irradiated keratinocytes. Halaban et al have recently demonstrated in vitro that keratinocyte-derived basic fibroblast growth factor (bFGF), an important mitogen for melanocytes, increases after UVB irradiation (75). Furthermore, two melanocyte growth factors different from bFGF have been detected in keratinocyte-conditioned medium from unirradiated cells (76). It remains to be shown which if any of these growth factors are responsible for the observed increase in the melanocyte population in covered skin.

The functional role of a circulating mitogenic factor acting on the melanocyte might be to prepare the total skin surface for future sun exposure. By the same mechanism, UV-light act as a tumor promotor by stimulating melanocyte proliferation not only in exposed but also in covered skin. The existence of a "solar circulating factor" responsible for melanoma development in unexposed skin was in fact postulated by Lee and Merrill as long ago as in 1970 (77).

In both exposed and covered skin there was a negative correlation between the basal melanocyte population density and the UV-induced increase in melanocyte population. Subjects with a high initial melanocyte count responded with no or little increase in population density, suggesting that the melanocyte population may reach a saturation level. Such a saturation level seems likely in view of the observation that individual melanocytes respect each other´s dendritic domains and melanocytes appear to repel each other if too close (78). On the other hand, subjects with a low initial melanocyte population responded with a high increase in melanocyte number. That is, in an epidermis with few melanocytes, a single melanocyte may give rise to a larger clone than does a melanocyte in an epidermis with an already high population density. This difference in proliferative response might be of great importance for the likelihood of tumor development, since cells that give rise to large clones have a greater chance of propagating their damaged DNA. Subjects with a low basal melanocyte population density might therefore constitute a risk group for the development of CMM upon UV exposure.

A low melanocyte population density could be constitutional or due to long intervals between UV exposure. Data are still lacking for humans on the decay time for the UV-induced increase in

melanocyte number. Anamnestic data on sun exposure habits from case-control studies indicate that short UV-irradiation periods with long intervals are more harmful with respect to melanoma development than more chronic sun exposure (59,60). Apart from the skin being less protected by pigment, the higher "potency" for melanoma development of intermittent sun exposure might be a consequence of a low melanocyte population density, amplifying the propagation of UV-induced DNA damage.

Subjects with DNS are prone to form many common and dysplastic nevi not only in UV-exposed but also in covered skin (55,56,79). The lentiginous hyperplasia of the melanocytes in nevi might be the expression of a disturbed contact inhibition, which might be general for all epidermal melanocytes. This could lead to an increased melanocyte population all over the integument, or to an uneven distribution with an aggregation of melanocytes in the nevi and only a few in the surrounding skin. Our findings gave no support for a general disturbance of the distribution control of the melanocytes in the DNS-patients. The epidermal melanocytes appeared structurally normal and were uniformly distributed and the population density did not differ from that of the controls. Neither could any significant difference be found in their UVB-induced melanocyte population increase in exposed or covered skin compared to the control group. Further investigations are, however, needed to exclude the possibility that DNS-patients have a different response later in the postirradiation period.

Basal and UV-induced excretion of 5-S-CD in urine

Because of their numerous nevi, subjects with DNS have a large melanocyte organ. Furthermore, DN have been demonstrated to contain 5-S-CD (80). If the basal urinary 5-S-CD excretion is related to the size of the melanocyte organ, these subjects should therefore have a higher urinary excretion of 5-S-CD than the controls. Such a difference would be important to document, since 5-S-CD is used as a tumor marker for CMM and the DNS-patients are melanoma-prone subjects. In contrast to what we expected, there was no significant difference in the mean urinary excretion of 5-S-CD between DNS-patients and controls. That is, the normal upper range for the tumor marker 5-S-CD is also valid for this group of patients.

Neither in DNS-patients nor in controls were we able to document any correlation between an individual´s basal 5-S-CD excretion and his or her epidermal melanocyte mass or number of nevi. Our results suggest that the basal 5-S-CD excretion in humans is mainly of non-melanocytic origin. 5-S-CD has been found in non-melanocytic tissue in rodents (25,26) but similar investigations have so far not been carried out in humans. The origin of the basal 5-S-CD excretion remains to be established.

On the other hand, the large increase in urinary 5-S-CD excretion after repeated UV exposure is generally considered to be derived from the melanocytes. High excretion levels have been found in subjects with a tendency to burn easily and with a low tanning ability (21). This has led to the hypothesis that the UV-induced increase in 5-S-CD excretion is not due to an increased synthesis of melanin, but instead reflects UV-induced melanocyte injury. In-vitro studies on fibroblasts and lymphoid cells from DNS-patients have revealed an increased UV sensitivity (42,43,44,45,46). It was therefore of interest to study the urinary 5-S-CD excretion, during UVB irradiation, in these patients. The UV-induced excretion pattern and levels of 5-S-CD were, however, similar in DNS-patients and controls of the same skin type.

Both in DNS-patients and controls, subjects with skin type II had a significantly higher 5-S-CD excretion during the irradiation period than subjects with skin type III or IV (Fig. 4). One explanation might

be that subjects with skin type II, compared to those with skin type III or IV, received a higher UV-dose to their melanocytes during the irradiation due to less pigment protection. Another alternative is that the melanocytes leak more 5-S-CD in these subjects. Red or blond hair color and freckling is associated with skin type II, and subjects with this phenotype have a greater proportion of phaeomelanin in their melanin pigment than darker subjects. The melanogenesis, and especially phaeomelanogenesis, generates radical oxygen, which can produce membrane damage leading to an increased leakage of 5-S-CD to the cytoplasm and the extracellular space. Thus, the high urinary 5-S-CD excretion in subjects with skin type II might be due to a more pronounced UV-induced melanocyte injury and/or a higher content of phaeomelanin and its precursors in the epidermis.

During the follow-up period a second high peak of urinary 5-S-CD excretion was registered in some subjects. The occurrence of this peak was not limited to subjects with skin type II. The late high peaks were of short duration and we may have failed to register this event in additional subjects. Similar second peaks of urinary 5-S-CD excretion have been registered during total body PUVA (8-methoxypsoralen and UVA-light) treatment (81,82). Even during PUVA treatment of very limited skin areas (palms and soles), second high peaks of 5-S-CD in serum have been noted (83). These late peaks occurred despite continuation of the PUVA treatment period. In our study, the second peaks were noted at the same point of time as for PUVA treatment, measured from the start of the irradiation, even though our irradiation period was terminated. These second peaks might reflect a synchronized melanocyte proliferation or cell death. To elucidate this it will be necessary to make more frequent 5-S-CD analyses and melanocyte counts. This finding also underlines that a single increased 5-S-CD value must be interpreted with caution when using 5-S-CD as a tumor marker.

UV effects on nevus development

To study if sun exposure promotes nevus development, the number of nevi in a sun-exposed area on the back was compared with that in a sun-protected area of equal size on the buttocks. The areas were chosen to avoid traumatized skin and freckles. Most likely, this approach gives a more objective estimation of the UV effects than trying to correlate nevus counts to anamnestic data on lifetime UV exposure. In a previous study, a similar experimental design was used to compare nevus counts from the lateral and medial aspects of the arms in a group of volunteers (54). In two separate studies on DNS-patients, nevi were counted from photos and the number of nevi was compared between differently exposed areas on the thorax and in the lumbo-sacral region (55,56). For the first time, our study makes it possible to compare the UV effect on nevus development between melanoma patients and controls and between subjects with and without DN.

In agreement with previous investigators, we found significantly more nevi in the sun-exposed than in the protected skin. This was true for both melanoma patients and controls. It seems reasonable to conclude that this difference in nevus counts is UV-dependent. Other possible nevus-inducing factors such as hormones or medication may contribute to the interindividual variability but it seems unlikely that they should influence two neighboring areas differently (29).

The mean total body counts of nevi were compared with the mean nevus counts from the protected area extrapolated to the total body surface. This gave a rough estimate of at least 40% of the nevi being UV-dependent. The UV effect of nevus development is most probably larger than this since the buttock skin has been exposed to some sunlight. Furthermore, an indirect UV effect mediated by the systemic mitogenic factor may affect nevus development as it does melanocyte proliferation.

When the difference in nevus counts between sun-exposed and protected skin (the Ex-Pr-difference) was compared between melanoma patients and controls with and without DN, the largest mean Ex-Pr-differences were found in melanoma patients with DN and in controls with DN. This is in line with our finding that subjects with DN have the highest total body nevus counts and indicates that these high nevus counts can at least partly be explained by a more pronounced UV response. The stronger response may reflect different habits of sun exposure and/or an increased sensitivity to the "nevogenic" effect of UV-light. An important objective for future studies will be to differentiate between these alternatives. For an increased UV sensitivity speaks the fact that controls with DN had a more sun-sensitive skin type than those without DN (32).

To investigate further to what extent external factors influence the number of nevi, it will be necessary to estimate the importance of the genetic component. A start would be to compare nevus counts in pairs of mono and dizygotic twins. Until young adulthood twins usually have an almost identical lifestyle, but mono and dizygotic twins differ in their genetic conformity.

To study what type of UV exposure pattern has the most potent "nevogenic" effect, the body surface areas A-P were classified as rarely, intermittently or chronically exposed. Intermittently exposed skin had the highest nevus counts per unit surface area, followed by rarely exposed skin areas. Chronically exposed skin had the lowest nevus counts. The same ranking was found in both patients and controls. That is, intermittent UV exposure seems to have a more potent "nevogenic" effect than both rare and chronic UV exposure. The low nevus counts in chronically exposed skin even suggest that chronic exposure may have a protective effect against nevus formation or that chronic exposure enhances the maturation process of the nevi (53).

The mean difference in nevus counts between intermittently and rarely exposed skin was twice as large in the melanoma group as in the controls. Similar to what was found for the Ex-Pr difference, the difference in nevus counts between intermittently and rarely exposed skin was larger in subjects with DN, patients as well as controls, than in subjects without DN. Again, this indicates an increased UV sensitivity or a history of more intense UV exposure in subjects with DN. The fact that the melanoma group as a whole had a larger difference in nevus counts between intermittently and rarely exposed skin than controls may mainly be due to a greater proportion of subjects with DN in the melanoma group.

The distribution of DN over the body surface differed from that of CN, with no or few DN in the head and neck region, upper chest and arms. This distribution pattern indicates that UV-light does not play an essential role in the development of DN. This conclusion does not imply that the melanocytes that give rise to DN are insensitive of UV-light. Instead, local factors in the skin might have a stronger influence on the development of DN than external stimuli and/or certain melanocytes may be predetermined to form DN independent of UV-light.

UV effects on melanoma development

Based on anamnestic data on sun exposure, the concept that intermittent UV exposure is more harmful to the melanocytes than regular exposure has developed. To study what pattern of UV exposure has the most carcinogenic effect, the number of CMM per unit surface area was compared between rarely, intermittently and chronically exposed skin. The concentration of CMM was significantly higher in intermittently exposed than in both rarely and chronically exposed skin. There are few detailed reports on melanoma distribution over the body surface (84,53,85) and only one study, by Elwood and Gallagher, has investigated the distribution of CMM in relation to areas of different UV exposure

categories (86). Our data confirm their finding that the risk of developing CMM is greater in intermittently UV-exposed areas than in regularly exposed areas. This is in line with what would be expected if our hypothesis holds true that skin with a low melanocyte population density is at higher risk of developing CMM than skin with a high population density. Gilchrest et al have reported that the melanocyte population density in chronically exposed skin is constantly high (87) and melanoma in these areas is being diagnosed in older age-groups than melanoma elsewhere (86). One explanation why it takes longer for a melanoma to develop in chronically than in intermittently UV-exposed skin might be that DNA damage has less chance of being propagated in skin with a high melanocyte population density.

The distribution over the body surface of CMM as well as CN is consistent with sun exposure being an important etiological factor, even though a linear dose-response relationship does not exist. The good agreement between the distribution of CMM and CN strengthens the concept that UV-light is a common causative agent for both tumors. The number of CN in a given area might represent a cumulative record of the UV-response, whether caused by high dose or high sensitivity.

It is now well established that having a large number of nevi is a marker of an increased overall risk for melanoma (28,88,50,51,33). As nevi are potential precursors of CMM, one would expect that a large number of nevi in a specific area would also be associated with a high local melanoma risk. Previous investigators have not been able to document such a site-specific risk (88,89) but their reports do not allow an in-depth penetration of their statistical analyses. Using a statistical method which is not affected by the correlation between the total body nevus counts and risk of CMM, we have been able to demonstrate an increasing site-specific melanoma risk with increasing concentrations of CN as well as DN. The probability of developing CMM in an area increased by 26% and 15% for DN and CN, respectively, when the score (see statistical methods) increased by 1 unit. This site-specific melanoma risk might be the result of local insults of UV-light to the melanocyte system and/or be due to the fact that nevi are precursor lesions to CMM.

These investigations add important knowledge of the UV effects on the melanocyte system. Our results have revealed interesting new aspects on melanocyte tumor development and have furthermore generated new ideas for future studies on the interaction between UV-light, melanocytes, moles and melanoma.

CONCLUSIONS

1. For the first time in humans, it has been demonstrated that repeated UVB irradiation induces an increase in the number of melanocytes not only in the exposed but also in the covered skin. It is suggested that the proliferative response in covered skin is mediated by a circulating mitogenic factor. An increased mitotic activity might promote tumor development. UV exposure might therefore play a role in melanoma development not only in exposed but also in covered skin.

2. Subjects with an initially low melanocyte population density responded to the UVB irradiation with a larger increase in the population than those with a high basal population density. This finding might be of great importance for the likelihood of tumor development, since a cell that gives rise to a large clone has a greater chance of propagating its damaged DNA. Subjects with a low basal melanocyte population density might therefore constitute a risk group for the development of CMM.

3. No correlation was found between the basal urinary excretion of the pigment metabolite 5-S-CD and the size or activity of the melanocyte organ. This suggests that the basal 5-S-CD in urine is mainly of non-melanocytic origin.

4. Despite numerous nevi, subjects with DNS did not differ from controls in basal urinary excretion of 5-S-CD. That is, the normal upper range for 5-S-CD, when used as a tumor marker, is valid for this group of melanoma-prone subjects.

5. The UVB-induced increase in the urinary 5-S-CD excretion was not correlated to the proliferative response of the melanocytes. During the irradiation, subjects with skin type II developed a more pronounced erythema and responded with a higher increase in 5-S-CD excretion than those with skin type III or IV. These findings support the idea that the UVB-induced increase in 5-S-CD excretion is due to UV damage to the melanocyte.

6. In a case-control study, we investigated the influence of UV-light on nevus formation by comparing the number of nevi in a sun-exposed and sun-protected area. Both patients and controls had a four-fold increase in the number of nevi in the exposed compared to the protected area. This finding strongly supports the idea that UV-light plays an important role in nevus development.

7. Subjects with DN, melanoma patients as well as controls, had a larger difference in nevus counts between the exposed and the protected area than subjects without DN. This might reflect different habits of sun exposure and/or an increased sensitivity to the "nevogenic" effect of UV-light.

8. This study demonstrated that both CMM and CN were more common in intermittently than in rarely or chronically sun-exposed skin areas. This favors the idea that intermittent sun exposure is more harmful than regular exposure with respect to melanoma and nevus development and concords with our hypothesis that skin with a low melanocyte population is at higher risk of developing CMM than skin with a high population density. The distribution pattern of CMM and CN over the body surface is in line with the concept that UV-light is a common etiological factor for both tumors.

9. The probability of having a CMM in a specific area increased with the concentration of both CN and DN in that area. This demonstrates that a large number of nevi is not just a general melanoma marker but also indicates a site-specific melanoma risk. This site-specific melanoma risk might be the result of local insults of UV-light to the melanocyte system and/or be due to the fact that nevi are precursor lesions to CMM.

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