HIGH RISK GROUPS IN CUTANEOUS ONCOLOGY: BASAL CELL NEVUS SYNDROME, XERODERMA PIGMENTOSUM AND EPIDERMODYSPLASIA VERRUCIFORMIS

Immune surveillance and Retinoid treatment

P.C. VAN VOORST VADER

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STELLINGEN

I

Bij patienten met multipele cutane carcinomen is preventie van tumorgroei door orale retinoid therapie mogelijk.

II

Retinoiden, met name etretinaat en vitamine A-zuur, kunnen non-cirrhotische portale hypertensie veroorzaken. De graad van hypertensie is echter zo gering, dat het niet noodzakelijk is daaraan consequenties te verbinden.

III

De cutane symptomen van het basaal cel nevus syndroom worden veroorzaakt door een differentiatie stoornis van de epidermale keratinocyt, die zich zowel in het ontstaan van multipele basaal cel carcinomen kan uiten, als mogelijk ook in een verminderde immuun capaciteit van de huid.

IV

De immuun respons van patienten met xeroderma pigmentosum kan gestoord zijn, ook in de winter bij minimale zonlicht expositie. Het al of niet gestoord zijn van de immuum respons is mogelijk geliëerd aan de mate van gevoeligheid van de cel voor beschadiging door licht, waarbij waarschijnlijk ook binnen een complementatiegroep verschillen kunnen optreden.

V

Bepaalde humane papilloma virus (HPV) typen, o.a. HPV5, 8, 14, 16, 17 en 18, zijn potentieel oncogeen.

VI

Iedere vrouw, die (mogelijk) besmet is met de wratvirus (HPV) typen, die genitale wratten kunnen veroorzaken, doet er verstandig aan jaarlijks een uitstrijk van de baarmoederhals te laten maken.

VII

De Nederlandse volksgezondheid is gebaat bij Chlamydia trachomatis en ,,Pelvic Inflammatory Disease'' diagnostiek bij vrouwen met risico op een sexueel overdraagbare aandoening gezien de emotionele en financiële problemen, die kunnen voortvloeien uit fertiliteits problematiek teweeg gebracht door bovengenoemde bekken ontsteking.

VIII

Het ontmoedigingsbeleid wat betreft serologische HTLV-III diagnostiek bij mensen uit risicogroepen zonder objectieve ziekteverschijnselen, die de diagnose AIDS suggereren, is onvoldoende aanvaard in medische kringen.

IX

Dysplastische nevi nevocellulares bestaan. Anamnestische gegevens en de graad van dysplasie bepalen de consequenties van de diagnose.

X

De benamingen roos en rooseczeem weerspiegelen op realistische wijze het gegeven, dat er bij pityriasis capitis en exzema seborrhoicum sprake is van een gemeenschappelijke causale factor: relatieve overgroei van Pityrosporon orbiculare.

XI

Talgproductie is een voorwaarde voor het ontstaan van acne, maar niet de oorzaak, noch van comedonen acne, noch van inflammatoire acne.

XII

Acne tarda inguinalis et/aut axillaris is, met name bij vrouwen, geen zeldzame diagnose.

XIII

Jeuk ten gevolge van uitdroging en onvetting van de huid, veelal bij atopie, is een frequent voorkomende klacht in Nederland, waarvan de oorzaak vaak miskend wordt.

XIV

Activering van de inflammatoire component van constitutioneel eczeem kan veroorzaakt worden door direct contact van de huid met atopische allergenen.

XV

Het functioneren van het Academisch Ziekenhuis te Groningen is gebaat bij goed overleg met de medische staf, waarbij, voorlopig althans, het Vertegenwoordigend Overleg Medische Specialisten (VOMS) naast het Convent, waarin uitsluitend afdelingshoofden zitting hebben, voorziet in een behoefte.

XVI

Principes dienen er voornamelijk toe, datgene te kunnen nalaten, waar men geen zin in heeft.
(naar Multatuli)

XVII

Het doel van het leven is het leven zelf. Niet meer, maar ook niet minder.
(naar A. Herzen)

XVIII

"Se non è vero, è ben trovato" is geen goed motto voor een proefschrift, hetgeen óók niet waar is.

Stellingen behorende bij het proefschrift van P.C. van Voorst Vader, getiteld "High risk groups in cutaneous oncology: basal cell nevus syndrome, xeroderma pigmentosum and epidermodysplasia verruciformis. Immune surveillance and retinoid treatment".

Groningen, 4 juni 1986.

RIJKSUNIVERSITEIT TE GRONINGEN

HIGH RISK GROUPS IN CUTANEOUS ONCOLOGY: BASAL CELL NEVUS SYNDROME, XERODERMA PIGMENTOSUM AND EPIDERMODYSPLASIA VERRUCIFORMIS

Immune surveillance and Retinoid treatment

PROEFSCHRIFT

ter verkrijging van het doctoraat in de Geneeskunde
aan de Rijksuniversiteit te Groningen
op gezag van de Rector Magnificus Dr. E. Bleumink
in het openbaar te verdedigen op
woensdag 4 juni 1986
des namiddags te 2.45 uur precies
door

PIETER CORNELIS VAN VOORST VADER

geboren te Deventer

1986
DRUKKERIJ VAN DENDEREN B.V.
GRONINGEN

Promotores:	Prof. Dr. E. Bleumink
	Prof. Dr. T.H. The
	Prof. Dr. J. Oldhoff
Referenten:	Dr. J.P. Nater
	Dr. M.C.J.M. de Jong
	Dr. C.G.M. Kallenberg

VOORWOORD

Dit onderzoek werd verricht in de Dermatologische Kliniek (waarnemend hoofd: Dr.H.M.G. Doeglas) in samenwerking met de afdelingen Klinische Immunologie (hoofd: Prof.Dr.T.H. The) en Hepatologie (hoofd: Prof.Dr.C.H. Gips) van de Kliniek voor Inwendige Geneeskunde, de afdeling Pathologische Anatomie (hoofd: Prof.Dr.J.D. Elema), de Oogheelkundige Kliniek (hoofd: Prof.Dr. N.M.J. Schweitzer) en de afdeling Oncologie (hoofd: Prof.Dr.J. Oldhoff) van de Chirurgische Kliniek, Academisch Ziekenhuis te Groningen. Tevens werd samengewerkt met de afdeling Celbiologie en Genetica (hoofd: Prof.Dr.D. Bootsma), Erasmus Universiteit te Rotterdam, de afdeling Cytogenetica (hoofd: Dr.T.W.J. Hustinx) van het Anthropogetisch Instituut, Katholieke Universiteit te Nijmegen en de Unité des Papillomavirus (hoofd: Dr.G. Orth), Institut Pasteur, Parijs, Frankrijk.

Bij het verschijnen van dit proefschrift gaat mijn dank allereerst uit naar mijn promotoren, met name mijn eerste promotor Prof.Dr.E. Bleumink, voorzitter van de Vakgroep Dermatologie en Rector Magnificus der Universiteit, voor hun bereidheid mijn promotie te willen begeleiden en bevorderen, en in het bijzonder naar de referenten, die mij steeds weer met raad en daad ter zijde stonden.

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Dr.M.C.J.M.(Marcel) de Jong, hoofd van het immunologisch laboratorium van de Dermatologische Kliniek, heeft mij door zijn zorgvuldige en creatieve manier van werken als basiswetenschapper ("dan heb je niet goed gekeken") een inspirerend voorbeeld gegeven. Zijn kritische opmerkingen tilden het geschrevene naar een hoger plan en schetsten bovendien een sierlijke lijn voor de opzet van de verschillende teksten, hetgeen artistieke meningsverschillen over de invulling van zijn schetsen niet uitsloot.

Dr.C.G.M.(Cees) Kallenberg is als klinisch immunoloog talloze malen bereid geweest mij op zeer plezierige wijze binnen de grote lijnen van zijn vak te houden. Veelvuldig waren de besprekingen van steeds weer nieuwe versies van steeds weer andere manuscripten ("schrijven is herschrijven"). Dat was een natuurlijk gevolg van het feit dat veel onderzoek plaats vond in het laboratorium van de afdeling Klinische Immunologie. Hier wil ik met nadruk mijn grote erkentelijkheid jegens mijn tweede promotor Prof.Dr.T.H. The uitspreken, die de omstandigheden schiep, waardoor dergelijk onderzoek mogelijk was.

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Het hepatologisch onderzoek werd mogelijk gemaakt door het bestaan van de afdeling Hepatologie van Prof.Dr.C.H. Gips en de pathologisch anatomische expertise van Prof.Dr.H.J. Houthoff, die op grond van zijn histologische bevindingen het onderzoek initieerde naar portale hypertensie bij langdurig retinoid gebruik. De samenwerking met deze vakman, toentertijd verbonden aan de Groningse Universiteit, is voor mij van grote waarde geweest. Dr.H.(Hans) Verweij realiseerde binnen het Centraal Laboratorium voor Klinische Chemie (hoofd:Prof.Dr.W. van der Slik) een quantitatieve bepaling van retinoiden en was zo vriendelijk mij toe te staan de aldus verkregen gegevens te publiceren in mijn proefschrift. Aan ons optreden op en om het retinoiden symposion in Geneve in september 1984 denk ik met genoegen terug. Het onderzoek aangaande het humaan papilloma virus (wratvirus) was niet mogelijk geweest zonder de stimulerende samenwerking met Dr.G.(Gerard) Orth, hoofd van de Unité des Papillomavirus, Institut Pasteur te Parijs. Dit onderzoek vond deels zijn oorsprong in onderzoek, dat indertijd door Prof.Dr.M. Ruiter, hoogleraar Dermatologie te Groningen, is verricht. Het kwam mijn kennis van de virologie en de Franse taal ten goede.

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Voorst Vader PC van, Jong MCJM de, Blanken R, Kallenberg CGM, Vermey A, Scheres JMJC. Epidermodysplasia verruciformis: Langerhans cells, immunologic effect of retinoid treatment and cytogenetics. Submitted.

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Voorst Vader PC van, Houthoff HJ, Eggink HF, Gips CH. Etretinate (Tigason) hepatitis in 2 patients. Dermatologica 1984;168:41-6.*

Voorst Vader PC van, Houthoff HJ, Gips CH, Verweij H. Hepatologic side effects during long-term retinoid therapy: Ito cells and portal hypertension. In: Saurat J, ed. Retinoids: new trends in research and therapy. Basel: Karger, 1985:498-500.*

Verweij H, Voorst Vader PC van, Houthoff HJ, Gips CH. Quantitative analysis of retinoids in human serum and tissue samples using high performance liquid chromatography. In: Saurat J, ed. Retinoids: new trends in research and therapy. Basel: Karger, 1985:301-4.*

CONTENTS

LIST OF ABBREVIATIONS

BBC	: basal cell carcinoma
BCNS	: basal cell nevus syndrome
CMI	: cell mediated immunity
Con A	: concanavalin A
DNCB	: 2,4-dinitrochlorobenzene
DPS	: desintegrations per second
EV	: epidermodysplasia verruciformis
HLA	: human leukocyte antigen
HPH	: helix pomatia haemocyanin
HPLC	: high performance liquid chromatography
HPV	: human papilloma virus
LC	: Langerhans cell
MLC	: mixed lymphocyte culture
PBL	: peripheral blood lymphocyte
PHA	: phytohaemagglutinin
PWM	: pokeweed mitogen
RA	: retinoic acid
SCC	: squamous cell carcinoma
UDS	: unscheduled DNA synthesis
UV	: ultraviolet
XP	: xeroderma pigmentosum

INTRODUCTION

1. Cutaneous carcinomas: epidemiology and basic data

Cutaneous neoplasms represent 10-50% of all malignancies occurring in Caucasians. The percentage varies with the distance of the geographic area of habitation from the equator.[1-4] About 90% of these cutaneous neoplasms are non-melanoma skin cancers: basal cell carcinoma (BCC) or squamous cell carcinoma (SCC). In the United States the annual age-adjusted incidence rate for non-melanoma skin cancer among whites was 232.6 per 100.000 population in 1977-1978.[3] Compared to the 1971-1972 survey a 15-20% increase of incidence rate was noted, primarily due to the increased number of BCCs. According to the incidence rate of the 1977-1978 survey a new BCC or SCC of the skin develops in the United States in 400.000-500.000 individuals each year. In the Netherlands this figure is assumed to be about 10.000 (Leun JC van der, personal communication). BCCs constitute 75-90% of non-melanoma skin cancers in Caucasians. In people with a pigmented skin cutaneous carcinomas are rare. In the United States the annual age-adjusted incidence rate for non-melanoma skin cancer among blacks was 3.4 per 100.000 population in 1977-1978.[3] When they do occur, the most frequent carcinoma is SCC.[5] The mortality caused by non-melanoma skin cancers is low and is mainly due to SCC.

Basal cell carcinomas (BCCs) generally are slowly growing skin tumors, originating from the stratum basale of the epidermis, with a very low metastatic potential.[6-8] Locally invasive and/or destructive growth however does occur and is correlated with the site of origin of the neoplasm, such as the nasolabial fold, the medial canthus, the postauricular area and the scalp, and the histological features of the tumor.[6-10] A close interaction between the epithelial cells and the surrounding mesenchymal stroma is assumed.[11-12]

Squamous cell carcinomas (SCCs) are epithelial tumors with metastatic potential, originating from cells in the stratum spinosum, which is situated above the stratum basale. SCCs localized in the skin frequently develop from actinic keratoses, UV light induced premalignant skin lesions, and are generally detected at an early stage. The clinical course is highly variable, with metastasis rates varying from 0.1-50%, and is largely determined by the size of the tumor. On the whole however the prognosis of SCC of the skin is good with a metastasis rate of primary cutaneous SCCs of about 3%.[13]

2. Cutaneous carcinomas: high risk groups

In the 1977-1978 non-melanoma skin cancer survey in the United States 10%

of the cases had skin cancer at multiple sites.[3] In 2.5% of all cases both BCC and SCC were diagnosed. The risk of developing a new tumor is associated with the number of previous skin cancers. One study showed that 5% of the patients who had a history of only 1 skin cancer developed a new tumor within one year. But 21% of the patients with 2 previous tumors, 39% of the patients with 3-7 previous tumors and 100% of the patients with ›8 previous tumors developed a new skin cancer within one year.[14]

Three hereditary afflictions (Basal cell nevus syndrome, Xeroderma pigmentosum, Epidermodysplasia verruciformis) are known in which the number of cutaneous carcinomas frequently ranges from ›10 to ›100. This creates problems in management of patient care. As this thesis comprises reports of studies performed in patients with these particular disorders, their main characteristics are presented in Table 1.

3. Carcinogenesis in the skin

The most important environmental factor involved in cutaneous carcinogenesis in Caucasians is sunlight, specially UVB irradiation.[15,16] Another important factor is the capacity for cutaneous pigmentation after sunlight exposure. Caucasians who never tan are more at risk. The amount of exposure to sunlight, which is generally related to age, largely determines the incidence of BCC and SCC. With increased sun exposure the increase in SCC formation is relatively greater than the increase in BCC formation.[17] On the other hand the ageing process itself may play an additive role in the development of cutaneous cancers. Other factors are physical stimuli like heat, wind, cold and trauma, chronic ulceration, cicatrization, exposure to ionizing radiation and chemical carcinogens, certain congenital malformations like Nevus sebaceus, a persistent infection with potentially oncogenic human papillomaviruses, impairment of cell mediated immune functions as in renal allograft recipients and genetic disorders, that predispose to neoplasia.

Examples of the latter are (1) Xeroderma pigmentosum, a cell hypersensitivity syndrome,[18] (2) the Basal cell nevus syndrome, a syndrome of unknown aetiology with abnormal epithelial cell differentiation and the spontaneous development of multiple BCCs which can become agressive, especially at sunlight or ionizing irradiation exposed skin sites,[19] (3) the Bazex syndrome, which is rather similar to the Basal cell nevus syndrome,[20] and (4) the Werner syndrome, a premature ageing syndrome.[21] The Basal cell nevus syndrome is regarded as an example supporting the two-mutational-event or "two hit" theory of carcinogenesis of Knudson.[22-23] In this case the first hit is prezygotic (a germinal mutation), the second hit is thought to consist of different kinds

of irradiation which promote tumor growth.

In UV-carcinogenesis as well as in carcinogenesis in general the concept of tumorigenesis as at least a two-stage process (initiation and promotion) is held by many investigators. UV light acts as initiator and possibly as promotor. Also a pseudo-promotor effect of UV light irradiation has been postulated, due to UV light induced suppression of the immune response facilitating tumor growth.[24-25] This is further elaborated upon in section 5 of the introduction.

Thus, carcinogenesis in the Basal cell nevus syndrome, an autosomal dominant hereditary disorder, appears to be a genetically determined event related to abnormal epithelial cell differentiation, sunlight having a deleterious influence on carcinogenesis and tumor growth.[19] Carcinogenesis in Xeroderma pigmentosum, an autosomal recessive hereditary disorder, is also genetically determined and is thought to be due, through a mechanism not yet understood, to UV light induced DNA damage not properly repaired.[18] Carcinogenesis in Epidermodysplasia verruciformis, thought to be an autosomal recessive or possibly X-linked hereditary disorder,[26] is caused by a persistent infection with certain, supposedly oncogenic, human papillomaviruses, generally in association with an impaired cellular immune response and sunlight exposition.[27]

4. Cutaneous carcinomas and immune surveillance

The immune system has been implicated as playing a role in preventing and limiting tumor growth. This concept of immune surveillance has first been suggested in 1909 by Paul Ehrlich and has been modified in 1970 by Burnet, who incorporated contemporary findings stressing the functional role of T-lymphocytes as effector mechanism in antitumor resistance.[28,29] Recently Herberman proposed a broader concept which encompasses the possibility of a variety of effector mechanisms involved in host resistance, reviewing the possible role of macrophages and natural killer cells.[29] A critical role for T-lymphocytes appears to be restricted to tumors with strong tumor-associated antigens, particularly virus-induced tumors.[29] These tumors are rejected after transplantation in syngeneic recipients.[30] Evidence from animal studies has been presented suggesting that a distinction should be made between spontaneous tumors and virus- or carcinogen-induced tumors.[30] Spontaneous tumors generally occur at a relatively advanced age and are thought to involve development of a tumor clone which has escaped immune rejection, apparent from the fact that these tumors generally do not provoke a rejection response. Tumors induced by viruses and carcinogens, particularly UV light, do pro-

voke a rejection response after transplantation in syngeneic recipients. Their development, especially of virus-induced tumors, appears to be facilitated by a deficient immune response.[15,29,30.]

Impairment of cell mediated immunity, as determined by the DNCB sensitization test in vivo and the lymphoproliferative response in vitro, was found in patients suffering from SCC of the skin.[31-34] The degree of immunosuppression appears to be related to the amount of tumor load. The data for BCC are less convincing. The few studies performed report a normal DNCB sensitization test response and suggest the existence of a decreased mitogen-induced lymphoproliferative response in vitro.[31,32,35,36] Immunomorphologic studies demonstrated an active local immunologic response to BCC involving activated T-lymphocytes, Langerhans cells and indeterminate cells.[37] Despite this immunologic response, tumor growth continues however. The existence of soluble factors extracted from BCCs has been postulated, which inhibit the lymphoproliferative response to mitogens in vitro.[7,38] Especially extracts from BCCs showing agressive growth, appeared to exert this inhibitory capacity.[7] Another study suggested the presence of a plasma factor inhibiting the lymphoproliferative response to BCC-associated antigens.[39]

Immunologic studies concerning the Basal cell nevus syndrome have not been published, except for a Russian study reportedly showing a decreased lymphoproliferative response in vitro of two patients.[40] In Xeroderma pigmentosum the results of the few immunologic studies performed have been conflicting.[41] Normal and abnormal results were found using DNCB sensitization and lymphocyte proliferation tests (Table 1). In Epidermodysplasia verruciformis a persistent cutaneous infection with certain human papillomaviruses with frequent development of multiple SCCs of the skin is associated with impairment of the cell mediated immune response, although exceptions have been reported.[27] In the majority of patients the DNCB sensitization test in vivo as well as mitogen induced lymphoproliferative tests in vitro gave abnormal results (Table 1).

The Langerhans cell has been a focus of attention since the discovery of monoclonal antibodies against cell surface markers of this cell facilitated its identification.[42] Recent in vitro studies demonstrated the vital role of the Langerhans cell in antigen presentation and in the generation of cell-mediated cytotoxicity reactions against epidermal cells.[43-45] A change in the number of epidermal Langerhans cells was reported in cutaneous T-cell lymphoma, malignant melanoma, the acquired immunodeficiency syndrome and in various benign skin disorders.[46-48] Age, which affects the cell mediated immune response in vitro and in vivo,[49,50] has been said to affect the number of epidermal Langerhans cells.[51] Quantitative and morphologic in situ studies of epidermal Langerhans cells in patients with the Basal cell nevus syndrome and Epider-

modysplasia verruciformis have not been published, while a quantitative study has been performed only once in patients with Xeroderma pigmentosum.[52]

5. Photoimmunology and immune surveillance

A substantial body of evidence collected in animal as well as in clinical studies and partly reviewed in two books published in 1983,[53,54] indicates that UV light induces immunologic alterations.[16,24,55] That UV-induced skin tumors can escape immune destruction because of an UV-irradiation-induced suppression of the immune response was first suggested in 1976 by Kripke and Fisher, who found that prolonged UV exposure resulted in failure of mice to reject highly antigenic UV-induced transplanted skin tumors.[56]

Already in 1963 it had been shown that UV-irradiation interferes with the antigen presenting function of the skin, i.e. with DNCB sensitization.[57] UV-irradiation produces a local effect in the skin resulting in loss of cell surface markers and functional capacity of epidermal Langerhans cells.[25,42,58,59] Increased sensitivity to UV-irradiation of an epidermal Langerhans cell surface marker was observed in Xeroderma pigmentosum patients compared to controls.[52] On the other hand UV-irradiation appears to stimulate the production of cytokines such as ETAF (Epidermal cell derived Thymocyte Activating Factor), which has interleukin-1-like activity, by epidermal keratinocytes.[60,61] Cytokines act as mediators in mounting an inflammatory response.

The cellular immune response in mice as measured by the mitogen- induced lymphoproliferative response in vitro was not affected by UV-irradiation.[24,55,62] However, partial pre-irradiation of a mouse with UV-B facilitated the induction by UV-B of primary tumors in other skin areas of the animal, which suggests a systemic effect of UV-irradiation.[16] Testing the immune response in mice in a contact hypersensitivity reaction, sensitization through UV-irradiated skin resulted in the induction of specific immunologic tolerance and the appearance of antigen-specific T-suppressor cells.[55] It has been suggested that activation of the T-suppressor cell pathway may result from a direct effect of UV-irradiation on the cells involved in antigen presentation, there being an UV-sensitive antigen presenting cell (Langerhans cell) and possibly also an UV-resistant antigen presenting cell activating the T-suppressor cell pathway.[55] UV light irradiation has been shown to induce increased activity of T-suppressor cells in man, as assayed in a non-antigen-specific system.[63] The effect of UV light on the immune response associated with experimental photocarcinogenesis in mice can be characterized as follows: it is systemic, selective and suppressive.[24]

Dynamic studies involving the effect of light on the immune response do not form part of the studies reported in this thesis. The effect of light was especially reckoned with, however, in the study of the patient with xeroderma pigmentosum. In this disorder light is of primary importance, the cells of patients with this disorder being hypersensitive to light.[18]

6. Retinoid therapy in cutaneous oncology

Vitamin A and its derivatives, retinoids, are essential to the growth, differentiation and function of epithelial cells.[64] Vitamin A deficiency enhances the susceptibility of animals to cancer.[65] Dietary intake of vitamin A may also influence the incidence of human cancer.[66,67] In 1968 a research program was initiated to develop synthetic analogues of vitamin A, because all-trans-retinoic acid, the acid derivative of vitamin A, had been shown to possess antineoplastic and antikeratinising properties.[68] In contrast to cytotoxic agents retinoic acid caused a regression of chemically induced skin papillomas and carcinomas in an animal model. A preventive effect, later shown to be the most relevant aspect, was also noted. New compounds were selected for clinical use in both oncology and dermatology because of a favorable therapeutic index, which was calculated on the basis of two criteria: efficacy in the mouse papilloma test model and dose needed to induce signs of hypervitaminosis A in mice.

Since 1978 studies have been published, which focused on the therapeutic and preventive effect of systemic treatment with synthetic retinoids on neoplasia in man.[69-87] The vast activities in the field of retinoid research have resulted in four books so far,[69-72] all containing chapters on the subject of retinoids in oncology. In the majority of clinical studies iso-tretinoin (13-cis-retinoic acid) and etretinate (Ro 10-9359) were used. Most of these studies were concerned with the clinical effect on epithelial (pre)malignant lesions. The best therapeutic results as regards the skin were observed in the treatment of actinic keratoses and keratoacanthomas.[68,77,82-87] A preventive effect of retinoid treatment was seen in patients with superficial bladder tumors,[78] in patients with actinic keratoses,[77] in two studies of patients with multiple BCCs (among whom five patients with the Basal cell nevus syndrome; Table 1),[78,79] in patients with keratoacanthomas,[82-87] and in patients with Xeroderma pigmentosum (Table 1).[79]

The precise mechanism of action of retinoids in patients with oncologic disorders remains a matter of speculation, as a large diversity of biologic effects has been noted.[65,71,88] In general, retinoids can be said to stimulate normal differentiation. They reverse the process of malignant transformation of epithe-

lial cells induced by certain carcinogens in experimental models. Chemically induced cancer in animals is inhibited in the promotor (tumor growth) phase by retinoids, but the initiation phase is not influenced.[89-91] The protective effect of retinoic acid on skin carcinogenesis is not universal. It may be limited to certain carcinogens and particularly to the action of promoting agents.[91] The results of animal studies on the effect of topical retinoic acid on photocarcinogenesis, UV light being the carcinogen, are ambiguous.[89] Two studies showed enhancement of UV light induced carcinogenesis by topical retinoic acid. One study showed inhibition of photocarcinogenesis and tumor growth by topical retinoic acid. The results of clinical studies in man do suggest that oral retinoid medication is effective in the prevention of growth of light-induced skin tumors, such as actinic keratoses and BCCs.

Retinoids also affect the immune system in a variety of ways dependent on the test system and retinoid used.[92-95] Conflicting and equivocal results thwart a concise summary of the data. Furthermore, effects observed in an in vitro assay may not necessarily be found in vivo. It is clear, however, that timing, dose and mode of administration of the different retinoids are crucial in causing either stimulatory or inhibitory effects on immune responses. In two clinico-immunologic studies of patients with multiple keratoacanthomas and epidermodysplasia verruciformis a stimulatory effect of retinoid treatment with etretinate on the impaired mitogen-induced in vitro lymphoproliferative response has been suggested.[87,96]

Dose dependent side effects limit the usefulness of retinoid treatment. Cheilitis, desquamation, xerosis, pruritus, alopecia, onychodystrophy, facial dermatitis, conjunctivitis, arthralgia, myalgia and some other side effects are reversible, but do interfere with patient acceptance of retinoid treatment.[97] More serious side effects can affect reproductive function, the skeletal system, blood lipids and the liver.[71] Especially the teratogenic effect and retinoid related disturbed bone growth in children do have serious consequences.[98-101] Evidence has been presented suggesting that retinoid therapy does not interact adversely with oral contraceptive steroids.[102-103] The potentially serious hepatologic side effects of retinoid treatment were investigated in studies included in this thesis.

7. Scope and outline of the study

This thesis is a compilation of reports of patient-oriented studies concerned with high risk groups in cutaneous oncology and their management. Attention was focused on three hereditary afflictions in which multiple cutaneous carcinomas occur: the Basal cell nevus syndrome (BCNS), Xeroderma pig-

mentosum (XP) and Epidermodysplasia verruciformis (EV). The main characteristics of these disorders are shown in Table 1.

The investigations were centred around two themes: a) impairment of immune surveillance, possibly implicating enhancement of tumor growth and b) treatment and prevention of tumor growth by systemic retinoid medication.

The following questions were studied:

1. Immune surveillance

1.1 Is the immune response impaired in patients with BCNS and XP, possibly facilitating the development of the multiple cutaneous carcinomas occurring in these patients?

1.2.1 Is the quantity and in situ morphology of epidermal Langerhans cells abnormal in EV, contributing to the impaired cellular immune response, the persistence of the cutaneous human papillomavirus infection and probably also carcinogenesis being favoured by this impairment?

1.2.2 Is human papilloma virus DNA also present in invasive squamous cell carcinoma of our immuno-compromised EV patient, providing further circumstantial evidence for the oncogenic potential of the virus in EV?

1.2.3 Is EV a chromosomal instability disease, chromosomal instability leading to a propensity for cutaneous carcinomas and possibly an impaired immune response?

This theme was dealt with in the following chapters:

The immune response was evaluated by the DNCB sensitization test in vivo, the antigen-specific humoral immune response in vivo, lymphoproliferation tests in vitro and the number and morphology of epidermal Langerhans cells in a number of BCNS patients (Chapter 1), one XP patient (Chapter 2.1) and one EV patient (Chapter 3.1). Additionally an immuno-compromised EV patient is described, who simultaneously developed two different virus-associated carcinomas (Chapter 3.1).

An invasive squamous cell carcinoma of the immuno-compromised EV patient was investigated for the presence of human papillomavirus DNA (Chapter 3.2) and a cytogenetic study was performed in another immuno-compromised EV patient (Chapter 3.1).

2. Retinoid treatment

2.1 What is the value of systemic retinoid medication for the therapy and prevention of multiple cutaneous carcinomas of patients with BCNS and XP?

2.2 Does systemic retinoid treatment of patients with BCNS, XP and EV and

Table 1. Characteristics of three hereditary disorders with a propensity for the development of multiple cutaneous carcinomas.

	Basic abnormality	Type of skin carcinoma	Suggested role of UV light	CMI[x] status		Retinoid treatment presence/absence of positive effect		
				in vivo	in vitro	CMI status in vitro	cutaneous carcinomas therapy	prevention
Basal cell nevus syndrome	cel differentiation disorder	basal cell carcinoma	promotor pseudo-promotor	?	?	?	±	+
Xeroderma pigmentosum	UV light induced DNA repair disorder	squamous cell carcinoma, basal cell carcinoma	initiator promotor pseudo-promotor	↓-N[§]	↓-N	?	?	+
Epidermodysplasia verruciformis	persistent infection with oncogenic viruses (HPVs)*	squamous cell carcinoma	co-initiator co-promotor pseudo-promotor	↓ **	↓ **	+	±	?

[x] CMI: Cell Mediated Immunity (non-HPV-specific).
[§] N: Normal.
* HPV: Human Papilloma Virus.
** In 2 patients from the literature, one with and one without cutaneous carcinomas, the CMI response in vivo and in vitro was normal.

multiple cutaneous carcinomas affect the immune response?

2.3 Do retinoids affect the defective UV light induced DNA excision repair in XP?

2.4 Is systemic retinoid treatment, particularly long-term treatment, safe regarding (potential) hepatologic side effects?

This, second, theme was dealt with in the following chapters:

The value of systemic retinoid medication for therapy and prevention of cutaneous carcinomas was evaluated in an open longitudinal clinical study of three BCNS patients (Chapter 1) and one XP patient (Chapter 2.1), who were treated with etretinate.

The immunologic effect of systemic retinoid treatment was evaluated by assessing changes in the immunologic parameters of a number of BCNS patients (Chapter 1), one XP patient (Chapter 2.1) and one EV patient (Chapter 3.1).

Whether retinoids affect the defective UV light induced DNA excision repair in XP, was investigated in vitro (Chapter 2.2).

Hepatologic side effects of short-term systemic retinoid treatment were observed in two patients with an etretinate-induced hepatitis (Chapter 4.1). Hepatologic side effects of long-term systemic retinoid treatment were evaluated by light microscopic studies of liver tissue of thirteen patients without liver test disturbances treated with etretinate, all-trans-retinoic acid and isotretinoin (13-cis-retinoic acid) and thin needle measurement of splenic pressure in two etretinate treated patients, the latter in order to exclude portal hypertension (Chapter 4.2). To support the light microscopic studies quantitative analysis was performed on serum, liver tissue, subcutaneous fat and epidermis-dermis samples of twenty patients treated with these retinoids (Chapter 4.3).

REFERENCES

1. Sanderson KV, Mackie R. Tumours of the skin. In: Rook A, Wilkinson DS, Ebling FJG, eds. Textbook of dermatology. 3rd ed. Oxford: Blackwell, 1979:2129-231.
2. Helm F. Cancer dermatology. Philadelphia: Lea & Febiger, 1979.
3. Scotto J, Fears TR, Fraumeni JF. Incidence of nonmelanoma skin cancer in the United States. NIH publication No.83-2433. U.S. Department of health and human services. Bethesda, Md.: National Cancer Institute, 1983.
4. Slaper H, Leun JC van der. Ultraviolette straling op de menselijke huid. Publicatie van het Ministerie van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer. Den Haag: Staatsdrukkerij, 1985.
5. Mora RG, Perniciaro C. Cancer of the skin in blacks. I. A review of 163 black patients with cutaneous squamous cell carcinoma. J Am Acad Dermatol 1981;5:535-43.
6. Eichmann F, Schnyder UW. Das Basaliom. Der haufigste Tumor der Haut. Berlin: Springer Verlag, 1981.

7. Pollack SV, Goslen JB, Sheretz, Jegasothy BV. The biology of basal cell carcinoma: a review. J Am Acad Dermatol 1982;7:569-77.
8. Domarus H von, Stevens PJ. Metastatic basal cell carcinoma. Report of 5 cases and review of 170 cases in the literature. J Am Acad Dermatol 1984;10:1043-60.
9. Lang PG, Maize JC. Histologic evolution of recurrent basal cell carcinoma and treatment implications. J Am Acad Dermatol 1986;14:186-96.
10. Franchimont C, Pierard GE, Cauwenberge D van, Damseaux M, Lapiere ChM. Episodic progression and regression of basal cell carcinomas. Br J Dermatol 1982;106:305-10.
11. Goslen JB, Eisen AZ, Bauer EA. Stimulation of skin fibroblast collagenase production by a cytokine derived from basal cell carcinomas. J Invest Dermatol 1985;85:161-4.
12. Delpech A, Delpech B, Girard N, Boullie MC, Lauret P. Hyaluronectin in normal human skin and in basal cell carcinoma. Br J Dermatol 1982;106:561-8.
13. Moller R, Reymann F, Hou-Jensen K. Metastases in dermatological patients with squamous cell carcinoma. Arch Dermatol 1979;115:703-5.
14. Peck GL. Therapy and prevention of skin cancer. In: Saurat JH, ed. Retinoids. Basel: Karger, 1985:345-54.
15. Epstein JE. Photocarcinogenesis, skin cancer and aging. J Am Acad Dermatol 1983;9:487-502.
16. Leun JC van der. UV-carcinogenesis. Yearly review. Photochem Photobiol 1984;39:861-8.
17. Vitaliano PP, Urbach F. The relative importance of risk factors in nonmelanoma carcinoma. Arch Dermatol 1980;116:454-6.
18. Kraemer KH. Heritable diseases with increased sensitivity to cellular injury. In: Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF, eds. Update: Dermatology in General Medicine. New York: McGraw-Hill, 1983:113-40.
19. Howell JB, Anderson DE. The nevoid basal cell carcinoma syndrome. Arch Dermatol 1982;118:824-6.
20. Plosila M, Kiistala R, Niemi KM. The Bazex syndrome: follicular atrofoderma with multiple basal cell carcinomas, hypotrichosis and hypohidrosis. Clin Exp Dermatol 1981;6:31-41.
21. Hrabko RP, Milgrom H, Schwartz RA. Werner's syndrome with associated malignant neoplasms. Arch Dermatol 1982;118:106-8.
22. Howell JB. Nevoid basal cell carcinoma syndrome. Profile of genetic and environmental factors in oncogenesis. J Am Acad Dermatol 1984;11:98-104.
23. Bolande RP, Vekemans JJ. Genetic models of carcinogenesis. Hum Pathol 1983;14:658-62.
24. Kripke ML. Immunology and photocarcinogenesis. J Am Acad Dermatol 1986;14:149-55.
25. Friedman PS. Comment: ultraviolet carcinogenesis in mice and men. Br J Dermatol 1983;109:683-6.
26. Androphy EJ, Dvoretzky I, Lowy DR. X-linked inheritance of epidermodysplasia verruciformis. Arch Dermatol 1985;121:864-8.
27. Lutzner MA, Blanchet-Bardon C, Orth G. Clinical observations, virologic studies and treatment trials in patients with epidermodysplasia verruciformis, a disease induced by specific human papillomaviruses. J Invest Dermatol 1984;83:18s-25s.
28. Miller DG. On the nature of susceptibility to cancer. Cancer 1980;46:1307-18.
29. Herberman RB. Possible role of natural killer cells and other effector cells in immune surveillance against cancer. J Invest Dermatol 1984;83:137s-40s.
30. Klein G, Klein E. Immune surveillance against virus-induced tumors and non-rejectability of spontaneous tumors: contrasting sequences of host versus tumor evolution. Proc Natl Acad Sci USA 1977;74:2121-5.
31. Bleumink E, Nater JP. DNCB reactivity in patients with skin carcinoma. Dermatologica 1974;148:44-6.
32. Weimar VM, Ceilly RI, Goeken RA. Cell-mediated immunity in patients with basal and squamous cell skin cancer. J Am Acad Dermatol 1980;2:143-7.
33. Angelini G, Vena GA, Ovidio R de, Lospalluti M, Meneghini CI. T-cell subsets and solu-

ble immune response suppressor factor in skin squamous cell carcinoma. Acta Dermatovener (Stockh) 1983;63:109-14.
34. Eskinazi DP, Helman J, Ershow AG, Perna JJ, Mihail R. Nonspecific immunity and head and neck cancer: blastogenesis reviewed and revisited. Oral Surg Oral Med Oral Pathol 1985;60:642-7.
35. Myskowski PL, Safai B, Good RA. Decreased lymphocyte blastogenic responses in patients with multiple basal cell carcinoma. J Am Acad Dermatol 1981;4:711-4.
36. Vena GA, Angelini G, Ovidio R de, Lospalluti M, Meneghini CL. L'immunita cellulo-mediata nei carcinomi squamocellulare e basocellulare. G Ital Dermatol Venereol 1982;117:263-7.
37. Guillen FJ, Day CL, Murphy GF. Expression of activation by T cells infiltrating basal cell carcinomas. J Invest Dermatol 1985;85:203-6.
38. Raffle EJ, MacLeod TM, Hutchinson F. Cell-mediated immune response to basal cell carcinoma. Acta Dermatovener (Stockh) 1981;61:66-8.
39. McGabe M, Nowak M, Maquire D, Robertson P. Immunosuppression by human skin cancers. Austr J Exp Biol Med Sci 1984;62:539-45.
40. Kalamkarian AA, Kapkin VV, Bogatyreva II, Vavilov AN, Mikhailovski AV, Zidgenidze MS, Stenina MA. Sindrom Gorlina. Vestn Dermatol Venereol 1983; (1):4-9.
41. Kraemer KH. Progressive degenerative diseases associated with defective DNA repair: xeroderma pigmentosum and ataxia teleangiectasia. In: Nichols WW, Murphy DG, eds. DNA repair processes. Miami: Medical Books, 1977:37-71.
42. Wolff K, Stingl G. The Langerhans cell. J Invest Dermatol 1983;80:17s-21s.
43. Katz SI, Cooper KD, Iijima M, Tsuchida T. The role of Langerhans cells in antigen presentation. J Invest Dermatol 1985;85:96s-8s.
44. Bagot M, Heslan M, Dubertret L, Roujeau JC, Touraine R, Levy JP. Antigen presenting properties of human epidermal cells compared with peripheral blood mononuclear cells. Br J Dermatol 1985;113:suppl 28:55-60.
45. Faure M, Dezutter-Dambuyant C, Schmitt D, Gaucherand M, Thivolet J. Langerhans cell induced cytotoxic T-cell responses against normal human epidermal cell targets: in vitro studies. Br J Dermatol 1985;113:suppl 28:114-7.
46. MacKie RM, Turbitt ML. Quantitation of dendritic cells in normal and abnormal human epidermis using monoclonal antibodies directed against Ia and HTA antigens. J Invest Dermatol 1983;81:216-20.
47. Facchetti F, Wolf-Peeters C de, Greef H de, Desmet VJ. Langerhans cells in various benign and malignant pigment-cell lesions of the skin. Arch Dermatol Res 1984;276:283-7.
48. Belsito DV, Sanchez MR, Baer RL, Valentine F, Thorbecke GJ. Reduced Langerhans' cell Ia antigen and ATPase activity in patients with the acquired immunodeficiency syndrome. N Engl J Med 1984;310:1279-82.
49. Mascart-Lemone F, Delepesse G, Servais G, Kunstler M. Characterization of immunoregulatory T lymphocytes during ageing by monoclonal antibodies. Clin Exp Immunol 1982;48:148-54.
50. Bleumink E, Nater JP, Schraffordt Koops H, The TH. A standard method for DNCB sensitization testing in patients with neoplasms. Cancer 1974;33:911-5.
51. Thiers BH, Maize JC, Spicer SS, Cantor AB. The effect of aging and chronic sun exposure on human Langerhans cell populations. J Invest Dermatol 1984;82:223-6.
52. Koulu LM, Jansen CT. Langerhans cells in xeroderma pigmentosum. J Invest Dermatol 1983;80:374.
53. Daynes RA, Spikes JD, Krueger G, eds. Experimental and Clinical Photoimmunology. Vol. 1 & 2. Boca Raton: CRC Press, 1983.
54. Parrish JA, Kripke ML, Morison WL, eds. Photoimmunology. New York: Plenum Medical Book Company, 1983.
55. Kripke ML, Morison WL. Modulation of immune function by UV radiation. J Invest Dermatol 1985;85:62s-6s.
56. Kripke ML, Fischer MS. Immunologic parameters of ultraviolet carcinogenesis. J Natl

Cancer Inst 1976;57:211-5.
57. Greene MI, Sy MS, Kripke ML, Benacerraf B. Impairment of antigen-presenting cell function by ultraviolet radiation. Proc Natl Acad Sci USA 1979;76:6591-5.
58. Hanau D, Fabre M, Lepoittevin JP, Stampf JL, Grosshans E, Benezra C. ATPase and morphologic changes induced by UVB on Langerhans cells in guinea pigs. J Invest Dermatol 1985;85:135-8.
59. Czernielewski J, Vaigot P, Asselineau D, Prunieras M. In vitro effect of UV radiation on immune function and membrane markers of human Langerhans cells. J Invest Dermatol 1984;83:62-5.
60. Sauder DN, Monick MM, Hunninghake GW. Epidermal cell-derived thymocyte activating factor (ETAF) is a potent T-cell chemoattractant. J Invest Dermatol 1985;85:431-3.
61. Luger TA, Kock A, Danner M, Colot M, Micksche M. Production of distinct cytokines by epidermal cells. Br J Dermatol 1985:suppl 28:145-56.
62. Bergstresser PR, Streilein JW, Kripke ML. Effects of UV radiation on immune responses in animals. In: Parrish JA, Kripke ML, Morison WL, eds. Photoimmunology. New York: Plenum Medical Book Company, 1983:175-204.
63. Hersey P, Bradley M, Hasic E, Haran G, Edwards A, McGarthy WH. Immunological effects of solarium exposure. Lancet 1983;i:545-8.
64. Goodman DS. Vitamin A and retinoids in health and disease. N Engl J Med 1984;310:1023-31.
65. Lotan R. Effects of vitamin A and is analogues (retinoids) on normal and neoplastic cells. Biochim Biophys Acta 1980;605:33-91.
66. Willett WC, Polk BF, Underwood BA, Stampfer MJ, Pressel S, Rosner B, Taylor JO, Schneider K, Hames CG. Relation of serum vitamins A and E and carotenoids to the risk of cancer. N Engl J Med 1984;310:430-4.
67. Salonen JT, Salonen R, Lappetelainen R, Maenpaa PH, Alfthan G, Puska P. Risk of cancer in relation to serum concentrations of selenium and vitamins A and E: matched case-control analysis of prospective data. Br Med J 1985;290:417-20.
68. Bollag W. Vitamin A and retinoids: from nutrition to pharmacotherapy in dermatology and oncology. Lancet 1983;i:860-3.
69. Orfanos CE, Braun-Falco O, Farber EM, Grupper Ch, Polano MK, Schuppli R, eds. Retinoids. Advances in basic research and therapy. Berlin: Springer Verlag, 1981.
70. Cunliffe WJ, Miller AJ, eds. Retinoid therapy. A review of clinical and laboratory research. Lancaster: MTP Press Ltd., 1984.
71. Saurat JH, ed. Retinoids: new trends in research and therapy. Basel: Karger, 1985.
72. Sporn MB, Roberts AB, Goodman DS, eds. The retinoids. Vol. 1 & 2. New York: Academic Press Inc., 1984.
73. Elias PM,Williams ML. Retinoids, cancer and the skin. Arch Dermatol 1981;117:160-80.
74. Ward A, Brogden RN, Heel RC, Speight TM, Avery GS. Etretinate. A review of its pharmacological properties and therapeutic efficacy in psoriasis and other skin disorders. Drugs 1983;26:9-43.
75. Dicken ChH. Retinoids: a review. J Am Acad Dermatol 1984;11:541-52.
76. Kingston T, Marks R. Cutaneous neoplasia and the retinoids. In: Culiffe WJ, Miller AJ, eds. Retinoid therapy. Lancaster: MTP Press Ltd., 1984:195-9.
77. Berretti B, Grupper Ch. Cutaneous neoplasia and etretinate. In: Cunliffe WJ, Miller AJ, eds. Retinoid therapy. Lancaster: MTP Press Ltd., 1984:187-94.
78. Peck GL. Therapy and prevention of skin cancer. In: Saurat JH, ed. Retinoids. Basel: Karger, 1985:345-54.
79. Verret JL, Schnitzler L, Avenel M, Smulevici A. Etretinate and skin cancer prevention. In: Saurat JH, ed. Retinoids. Basel: Karger, 1985:355-9.
80. Meyskens FL. Isotretinoin for the treatment of advanced human cancers. In: Saurat JH, ed. Retinoids. Basel: Karger, 1985:371-4.
81. Rustin GJS, Bagshawe KD. Trial of an aromatic retinoid in patients with solid tumours. Br J Cancer 1982;45:304-8.

82. Levine N, Miller RC, Meyskens FL. Oral isotretinoin therapy. Use in a patient with multiple cutaneous squamous cell carcinomas and keratoacanthomas. Arch Dermatol 1984;120:1215-7.
83. Benoldi D, Alinovi A. Multiple persistent keratoacanthomas: treatment with oral etretinate. J Am Acad Dermatol 1984;10:1035-8.
84. Cristofolini M, Piscioli F, Zumiani G, Scappini P. The role of etretinate in the management of keratoacanthoma. J Am Acad Dermatol 1985;12:633-8.
85. Yoshikawa K, Hirano S, Kato T, Mizuno N. A case of eruptive keratoacanthoma treated by oral etretinate. Br J Dermatol 1985;112:579-83.
86. Spielvogel RL, Villez RL de, Roberts LC. Oral isotretinoin therapy for familial Muir-Torre syndrome. J Am Acad Dermatol 1985;12:475-80.
87. Blitstein-Willinger E, Haas N, Nurnberger F, Stuttgen G. Immunological findings during treatment of multiple keratoacanthoma with etretinate. Br J Dermatol 1986;116:109-16.
88. Peck GL. Retinoids and cancer. J Invest Dermatol 1985;85:87-8.
89. Moon RC, Itri LM. Retinoids and cancer. In: Sporn MB, Roberts AB, Goodman DS, eds. The Retinoids. Vol. 2. New York: Academic Press Inc., 1984:327-71.
90. Yuspa SH, Lichti U. Retinoids and skin carcinogenesis. A mechanism of anticarcinogenesis by the modulation of epidermal differentiation. In: Saurat JH, ed. Retinoids. Basel: Karger, 1985:56-65.
91. Boutwell RK, Verma AK, Takigawa M, Loprinzi CL, Carbone PP. Retinoids as inhibitors of tumor promotion. In: Saurat JH, ed. Retinoids. Basel: Karger, 1985:83-96.
92. Dennert G. Retinoids and the immune system: immunostimulation by vitamin A. In: Sporn MB, Roberts AB, Goodman DS, eds. The Retinoids. Vol. 2. New York: Academic Press Inc., 1984:373-90.
93. Hercend Th, Bruley-Rosset M, Florentin I, Mathe G. In vivo immunostimulating properties of two retinoids: Ro 10-9359 and Ro 13-6298. In: Orfanos CE, Braun-Falco O, Farber EM, Grupper Ch, Polano MK, Schuppli R, eds. Retinoids. Berlin: Springer Verlag, 1981:21-30.
94. Bauer R, Orfanos CE. Effects of synthetic retinoids on human peripheral blood lymphocytes and polymorphonuclears in vitro. In: Cunliffe WJ, Miller AJ, eds. Retinoid therapy. Lancaster: MTP Press Ltd., 1984:101-18.
95. Shapiro PE, Edelson RL. Effects of retinoids on the immune system. In: Saurat JH, ed. Retinoids. Basel: Karger, 1985:225-35.
96. Guilhou JJ, Malbos S, Barneon S, Habib A, Baldet P, Meynadier J. Epidermodysplasie verruciforme. Etude immunologique. Ann Dermatol Venereol 1980;107:611-9.
97. Christiansen JV, Holm P, Reymann F, Thestrup-Pedersen K. Patients' acceptance of etretinate therapy. A retrospective survey of long-term etretinate therapy in chronic keratotic and pustular skin diseases. Dermatologica 1984;168:122-6.
98. Chen DT. Human pregnancy experience with the retinoids. In: Saurat JH, ed. Retinoids. Basel: Karger, 1985:398-406.
99. Lammer EJ, Chen DT, Hoar RM, Agnish ND, Benke PJ, Braun JT, Currey CJ, Fernhoff PM, Crix AW, Lott IT, Richard JM, Sun SC. Retinoic acid embryopathy. N Engl J Med 1985;313:837-41.
100. Grote W, Harms D, Janig U, Kietzmann H, Ravens U, Schwarze L. Malformation of fetus conceived 4 months after termination of maternal etretinate treatment. Lancet 1985;i:1276.
101. Sillevis Smitt JH, Mari Fr de. A serious side-effect of etretinate (Tigason). Clin Exp Dermatol 1984;9:554-6.
102. Orme M, Back DJ, Shaw MA, Allen WL, Tjia J, Cunliffe WJ, Jones DH. Isotretinoin and contraception. Lancet 1984;ii:752-3.
103. Goerz G, Hamm L, Bolsen K, Merk H. Influence of 13-cis retinoic acid and of aroretinoid on the cytochrome P-450 system in rat liver. Dermatologica 1984;168:117-21.

Chapter 1

Basal cell nevus syndrome
Immune response, Langerhans cells and retinoid treatment with etretinate

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SUMMARY

14 Patients with the basal cell nevus syndrome (BCNS) were screened for the presence of decreased immune responsiveness which might enhance the development of the multiple basal cell carcinomas (BCCs) occurring in 8 of these patients. In addition the clinical and immunologic effect of retinoid treatment with etretinate was assessed in 3 BCNS patients with multiple BCCs. Immunologic evaluation showed: 1) a normal DNCB skin test score in all 6 BCNS patients without multiple BCCs, but a decreased score in 4 of the 8 BCNS patients with multiple BCCs; 2) a normal number of T6+ and HLA-DR+ epidermal Langerhans cells in 6 BCNS patients with multiple BCCs; 3) a normal antigen-specific humoral immune response in vivo and a normal in vitro lymphoproliferative response and PHA-induced lymphocyte cytotoxicity in 5 BCNS patients with multiple BCCs. Etretinate treatment of 3 BCNS patients with multiple BCCs did not markedly affect the immunologic parameters in vitro. The clinical results of retinoid treatment of these patients supported the notion of a temporary preventive effect, but did not justify treatment of BCCs with etretinate alone because of the risk of recurrence. It is concluded that the immune response of the BCNS patients tested was normal except for a possible defect of the cellular immune response in vivo in some patients with multiple BCCs and that retinoid treatment with etretinate was of preventive value.

INTRODUCTION

The Basal Cell Nevus Syndrome (BCNS) is an autosomal dominant hereditary disorder with high penetrance and variable expression. Its main features are multiple basal cell carcinomas (BCCs), milia, palmar and plantar pits, jaw cysts (keratocysts), skeletal abnormalities and ectopic calcification,[1-5] suggesting that BCNS can be partly defined as a genetically determined epidermal cell differentiation disorder. In about half of the BCNS patients of 20 years or older BCCs are seen. These usually appear between the ages of 14-35 years, but may be present in large quantities already in the first decade, both in sunlight exposed and unexposed skin. Agressive growth generally does not occur until after puberty and is mostly restricted to sunlight or ionizing radiation ex-

posed areas.[3-6] BCCs in BCNS patients are microscopically indistinguishable from BCCs in non-BCNS patients. [7,8] Genetic studies revealed slight and not very convincing abnormalities in UV-induced DNA repair and sister chromatid exchange rates.[9,10] Immunologic studies of BCNS patients have not yet been performed to our knowledge, except for one study reportedly mentioning a decreased lymphoproliferative response in vitro of two patients.[11] Studies of non-BCNS patients with BCC reported a normal in vivo cellular immune response of patients with a varying number of small and large BCCs as tested by the DNCB sensitization test,[12,13] but suggested a decreased mitogen-induced in vitro lymphoproliferative response.[14,15]

Since 1978 studies of the therapeutic and preventive effect of systemic treatment with synthetic retinoids, mainly iso-tretinoin (13-cis-retinoic acid) and etretinate (Ro 10-9359), on cutaneous neoplasia have been published.[16-29] Assessment of immunologic changes was rarely included,[29] although in vitro studies have demonstrated that retinoids may enhance T-cell cytotoxicity.[16] The best therapeutic results were attained in the treatment of actinic keratoses[23] and keratoacanthoma.[25-29] In 1982 Peck et al. published a detailed clinical study of the therapeutic and prophylactic value of isotretinoin treatment in 3 patients with multiple BCCs. A positive prophylactic and a limited therapeutic effect was noted.[19] A more extended study of 12 patients (5 with BCNS) corroborated these findings.[23] A prophylactic effect has also been reported in two BCNS patients with multiple BCCs treated with etretinate (Ro 10-9359).[24] Berretti and Grupper also described a limited therapeutic effect of etretinate treatment in 40 patients with BCC.[22]They found frequent recurrence of BCCs after cessation of treatment.

The purpose of our study was twofold: a) assessment of the therapeutic and prophylactic value of treatment with etretinate (Ro 10-9359) of BCNS patients with multiple BCCs; b) assessment of the immune response of BCNS patients, both with and without multiple BCCs, in order to evaluate whether decreased immune responsiveness might enhance the development of the BCCs. In addition we assessed the effect of etretinate treatment on the immune response in order to evaluate whether the supposed beneficial clinical effect of etretinate was (in part) based on its effect on the immune system.[16,30,31] For the assessment of the immune response analysis was made of: 1) the cellular immune response in vivo of 14 BCNS patients, 8 with and 6 without multiple BCCs, using a semiquantitative DNCB sensitization test;[32] 2) the lymphoproliferative response in vitro after stimulation of peripheral blood lymphocytes with mitogens and allogeneic cells, the humoral in vivo and cellular in vitro immune response after immunization with the primary immunogen Helix pomatia Haemocyanin,[33] and the phytohaemagglutinin-induced T-cell cytotoxicity in vitro of BCNS patients with multiple BCCs; 3) the number and

morphology of T6+ and HLA-DR+ epidermal Langerhans cells (LCs) in punch biopsies from clinically normal skin of BCNS patients with multiple BCCs, as LCs are critically needed for the cutaneous immune response and appear to be involved in the local immune response to BCCs.[34-35]

MATERIAL AND METHODS

Patients

14 Caucasian BCNS patients of Dutch descent were included in the study, 8 with and 6 without multiple BCCs (Table 1). Patients I and J did not have multiple BCCs, but two to three non-agressive BCCs on the trunk during their teen-age years without any de novo BCC since then. Informed consent was obtained from each patient.

Retinoid treatment: clinical study

Three female patients (patients D, G, and H;Table 1) with BCNS and multiple BCCs volunteered for a longitudinal clinical study of the effect of treatment with the retinoid etretinate (Ro 10-9359, Tigason). The number, location and size of the cutaneous lesions suspected of being a BCC were noted at the start of retinoid treatment, every 2 weeks for the first 12 weeks of treatment and every 4 weeks for the remainder of the course. During the post- treatment follow-up period intervals of 4-8 weeks were taken. Clinical doubts about a lesion being a BCC were allayed by biopsy as much as possible. Photographs were taken at the start of retinoid treatment, every 2-4 weeks during the course and later on whenever considered relevant. The total control period amounted to about 4 years in all patients.

One etretinate course of circa 32 weeks was meant to be given. Treatment was started at doses of 0.9-1.0 mg/kg/day. The doses were adapted to patient tolerance of the drug and toxicity. Haematologic, hepatic and renal tests as well as serum lipid values were monitored regularly before, during and after retinoid treatment. Levels of etretinate and its main metabolite (Ro 10-1670) were measured in serum samples obtained 2, 4 and 12 weeks after starting treatment using high performance liquid chromatography.[36]

Immunologic studies

Cellular immune response in vivo. In all patients a semiquantitative DNCB sensitization test was performed according to methods previously described.[32] Age-matched controls were used.[32]
Cellular immune response in vitro. The lymphoproliferative response in vitro of 5 patients with multiple BCCs was measured, i.e. of patients A, C, D, G and H. Triplicate cultures of peripheral blood lymphocytes were performed with phytohaemagglutinin (PHA) 1, 5 and 25 μg/ml, concanavalin A (ConA) 1, 3 and 10 μg/ml, pokeweed mitogen (PWM) 10 μg/ml, Helix pomatia Haemocyanin (HPH) 15 μg/ml (3 weeks after immunization) and with allogeneic irradiated lymphocytes in the mixed lymphocyte culture (MLC), according to previously described methods.[33,37] Healthy controls were used for the tests with mitogens and allogeneic lymphocytes (n=60, mean age 30 yrs, range 11-53) and for the test with HPH (n=17, mean age 40 yrs, range 29-53).

PHA-induced lymphocyte cytotoxicity of 2 patients with multiple BCCs (patients G and H) was measured according to previously described methods.[38] Sex and age-matched controls were used.

The effect of retinoid treatment on the lymphoproliferative response was assessed 2, 4 and 12 weeks after starting etretinate treatment of patients D, G and H. The results were compared to pre-treatment responses of these patients and to the untreated controls. The effect of retinoid

Table 1. Symptoms and signs of the Basal Cell Nevus Syndrome in 14 patients, 8 with and 6 without multiple basal cell carcinomas (BCCs).

Patient	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Sex, age (years)*	♂,4	♂,14	♀,31	♀,38	♀,46	♀,52	♀,67	♀,70	♀,18	♂,19	♀,22	♀,23	♀,26	♂,28
Skin														
Multiple BCCs	+	+	+	+	+	+	+	+	−	−	−	−	−	−
Number of BCCs	›50	›100	›100	›50	›25	›50	›50	›100	4	2	0	0	0	0
Palmoplantar pits	+	+	+	±	−	+	+	+	+	+	+	−	+	+
Milia	+	+	+	+	+	+	+	+	+	+	+	−	+	−
Epithelial cysts	−	−	+	+	−	+	−	+	−	+	−	−	+	−
Skeletal system														
Caput quadratum	+	+	+	+	+	+	+	−	+	+	+	+	+	−
Broad nasal root	+	+	+	+	±	+	+	−	+	+	+	+	+	−
Mandibular prognathism	+	+	+	+	±	?	+	+	−	+	+	+	+	+
Palatum excavatum	+	+	+	+	−	?	+	+	+	+	+	+	+	+
Keratocysts of the jaw	−	+	+	+	−	+	+	+	+	+	+	+	+	+
Costal abnormalities	+	+	−	−	−	−	+	+	?	+	−	+	−	?
Vertebral abnormalities	+	+	−	−	−	+	−	+	?	+	−	+	+	?
Bridging of sella	−	+	−	+	−	+	+	+	+	+	+	+	+	+
Central nervous system														
Calcified falx cerebri	−	+	+	+	+	+	+	+	+	−	+	+	+	+
Genetics														
Familial involvement	−	+	+	+	+	+	−	+	+	−	+	+	+	+

* The age at the time of the DNCB sensitization test.

treatment on PHA-induced T-cell cytotoxicity was measured 2 and 4 weeks after starting etretinate treatment of patient G and 2 and 6 weeks after starting treatment of patient H.

Humoral immune response in vivo. The humoral immune response to Helix pomatia Haemocyanin (HPH) was assessed in patient D, G and H by measuring the antigen specific IgG-, IgM- and IgA-class antibody response 3 and 5 weeks after subcutaneous immunization with the primary test immunogen.[33] Healthy controls (n=23, mean age 49 yrs, range 26-79) were used. Retinoid treatment was started 3 weeks after immunization with HPH.

Immunohistology of epidermal Langerhans cells (LCs). Punch biopsies were taken after local anaesthesia (lidocain 1% w.o. adrenaline) from clinically normal skin on the forehead and back of 6 BCNS patients with multiple BCCs (patients A, B, D, E, G and H; Table 1). The specimens were snap-frozen in liquid nitrogen and stored at -70°C until further processing. Biopsies were taken before retinoid treatment and 7 weeks after starting etretinate treatment of patient D. Epidermal LCs were identified in 6 μm cryostat sections by a two-step immunoperoxidase technique using monoclonal anti-T6 (OKT-6) and anti-HLA-DR (OKIa-1) antibodies (Ortho Diagnostic Systems Inc., Raritan, NJ, USA) and peroxidase- conjugated rabbit anti-mouse Ig (Dakopatts, Denmark) as described elsewhere.[39] Quantitative in situ analysis of T6+ and HLA-DR+ epidermal LCs was carried out according to recently developed criteria.[39] Briefly, an arbitrary distinction was made between three types of T6+ LC profiles: (1) definite LC bodies, (2) doubtful LC bodies, representing cross sectioned profiles of indistinct origin, and (3) profiles of dendritic origin. These LC profiles, except for type 3, were counted in 6-10 epidermal test areas (Vobj x Voc=400) for each biopsy specimen. The epidermal height of a given test area was defined as the distance between the stratum corneum and the dermo-epidermal junction or an imaginary line halfway the rete ridges if present. The mean number of reactive LC profiles was calculated and expressed per linear mm and per mm^2 of cross sectioned epidermis for each biopsy specimen.

In addition we assessed the state of dendritic reactivity of LCs as described before.[39] To this end three types of T6+ epidermal dendritic patterns were defined: a network pattern of interconnecting dendrites (type 1), a discontinuous intercellular dendritic pattern (type 2) and a pattern showing sparsely distributed dendritic fragments (type 3). The percentual distribution of these patterns was also estimated in 6-10 epidermal test areas for each biopsy sample.

Biopsy specimens from the forehead of 8 healthy individuals (mean age 32 yrs, range 26-38) and the back of 10 other healthy individuals (mean age 31 yrs, range 21-39) served as controls for patients ‹50 years of age (A, B and D). For the patients ›50 years of age (E, G and H) control biopsy specimens were taken from the forehead of 8 healthy individuals (mean age 68 yrs, range 54-82) and the back of 11 other healthy individuals (mean age 62 yrs, range 51-70). All controls were Caucasian. Care was taken to avoid sampling of biopsy specimens after sunlight exposure causing erythema or a tan.

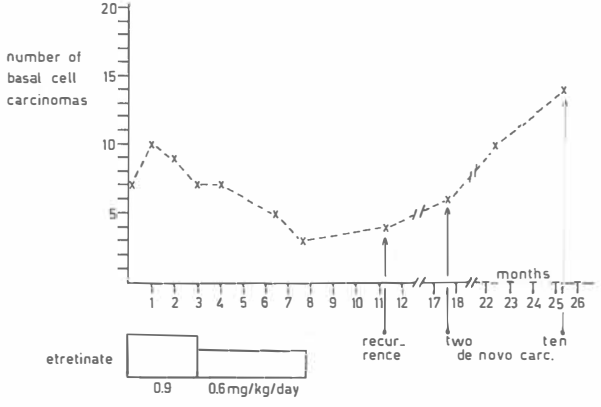
Statistical analysis

The numbers obtained by immunohistologic in situ quantification of epidermal LCs were subjected to statistical analysis. For the number of T6+ and HLA-DR+ LC bodies per mm and per mm^2 of epidermis the student t-test was used and for the grade of dendritic reactivity of T6+ LCs the Mann-Whitney test. Multiple regression analysis of the data of our controls (n=37, mean age 48 yrs, range 21-82) did not reveal a significant influence of sex, age and anatomic site on the number of T6+ and HLA-DR+ LCs per mm^2 of epidermis and the grade of dendritic reactivity of T6+ LCs in the biopsies taken from the forehead and the back (de Jong MCJM, et al.: in preparation). The level of significance was α=0.05 in all tests.

RESULTS

Retinoid treatment: clinical study

The results are represented in Fig. 1 and in the following case reports. A limited therapeutic effect of etretinate treatment was seen in all three patients. The number of BCCs decreased during treatment, but within four months after discontinuation of retinoid treatment recurrences of clinically healed lesions were noted in all three patients. During etretinate treatment of all three patients and until about nine months after discontinuation of treatment of patients D and G, no de novo BCCs were observed, suggesting a temporary preventive effect of etretinate treatment. Patient H developed an hepatitis, probably etretinate-induced,[40] which interfered with the therapeutic and preventive effect of etretinate treatment. Isotretinoin treatment was also not effective in this patient.



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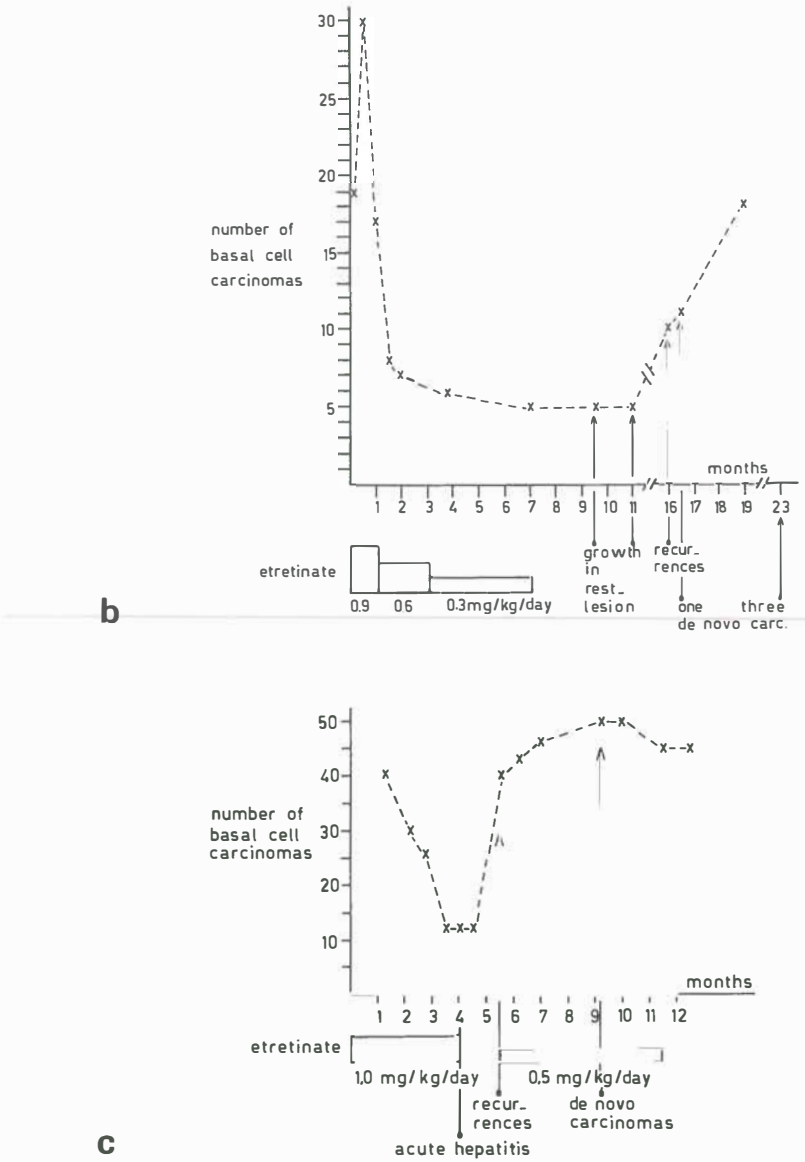

Fig. 1. Number of clinically visible basal cell carcinomas, histologically verified whenever feasible, before, during and after treatment with the retinoid etretinate (Ro 10-9359) of 3 Basal cell nevus syndrome patients (see Table 1). a) Patient D, 38 years of age. b) Patient G, 67 years of age. c) Patient H, 70 years of age. Retinoid treatment of patient H was arrested after 4 months because of an hepatitis, probably etretinate-induced.[40] Slight liver test disturbances persisted during low-dose etretinate treatment, given in an attempt to prevent further explosive growth of basal cell carcinomas.

Case reports

Patient D

This 38-year old female patient, height 177 cm, weight 82 kg, had a history of multiple BCCs, mainly on the face, but also on the trunk and in the inguinal area, since the age of 23. During the 5 years preceding the study 5-12 BCCs a year were treated. Vasectomy of her husband enabled us to start retinoid treatment without teratogenic risk.

Etretinate was given during 34 weeks, starting at 0.9 mg/kg/day divided in 3 doses of 25 mg during the first 13 weeks. Serum levels of etretinate and its main metabolite after 2 and 12 weeks were: 0.03 and 0.1 μmol/l. From week 14-34 the dosage was decreased to 0.6 mg/kg/day divided in 2 doses of 25 mg because of cutaneous side effects (cheilitis, xerosis, desquamation and fissuring). In the eighth week of the retinoid course she was treated for a maxillary sinusitis and in the twentieth week for otitis externa.

The effect of etretinate treatment on the number of BCCs is shown in Fig. 1a. At the start of the study the number was 7 (5 on the face , 2 on the trunk), of which 5 were histologically confirmed. Two lesions on the face were ›10 mm in diameter. All other lesions were ‹10 mm in diameter. After 4 weeks the number was 10 because several more lesions became clinically visible due to inflammation of these lesions. Thereafter the number of clinically detectable lesions suspected of being a BCC gradually decreased to 3 (on the face) at the end of the treatment period. New lesions were not observed during etretinate treatment. The 2 histologically confirmed lesions ›10 mm in diameter had largely remained unchanged and the third clinically visible lesion was a rest-lesion of 3x4 mm on the left temple, histologically confirmed, which showed inflammation but did not disappear. One histologically confirmed lesion on the right upper lip, 3x4 mm in diameter, clinically disappeared during treatment, but 3 1/2 months after arrest of treatment a histologically confirmed BCC, 2x3 mm in diameter, was visible again at exactly the same location. The 3 rest-lesions and the recurrence were treated with cryosurgery. About 10 months after arrest of treatment 2 de novo BCCs were seen and 8 months later 8 de novo BCCs, all on the face. All these lesions were treated conventionally.

A second, well tolerated course of etretinate at 0.6 mg/kg/day was given during 37 weeks at the request of the patient. No BCCs developed until 3 1/2 months after arrest of the second course, when one de novo BCC was discovered. Five months after arrest of treatment two de novo BCCs were noted and one more after seven months. Altogether 21 cutaneous punch biopsies were taken to confirm the clinical suspicion of BCC of which 19 were positive. Laboratory abnormalities were not found during etretinate treatment.

Patient G

This 67-year old female patient, height 167 cm, weight 85 kg, developed BCCs, mainly on the back, but also on the face, the buttocks and in the inguinal area, from the age of 37. During the 5 years preceding the study 3-7 BCCs a year were treated. Other diagnoses: slight hypercholesterolaemia (Fredrickson type IV), arthrosis of the right knee and status after mastoidectomy.

Etretinate was given during 31 weeks, starting at 0.9 mg/kg/day divided in 3 doses of 25 mg during the first 4 weeks. After 2 weeks serum levels of etretinate and its main metabolite were: 0.09 and 0.3 μmol/l; after 4 weeks 0.08 and 0.3 μmol/l. Because of side-effects (cheilitis, desquamation, xerosis, fissuring, pruritus, alopecia, conjunctival irritation and inflammation of the mastoid cavity) the dosage was decreased to 0.6 mg/kg/day divided in 2 doses of 25 mg during the next 9 weeks (week 5-14). After 12 weeks serum levels of etretinate and its main metabolite were: 0.04 and 0.3 μmol/l. Week 15-31 the dosage was further decreased to 0.3 mg/kg/day taken in 1 dose of 25 mg, mainly because of arthralgias in the right knee. During this period nail dystrophy was seen as a side-effect, besides intermittent activation of the inflammation of the mastoid cavity.

The effect of etretinate treatment on the number of BCCs is shown in Fig. 1b. At the start of the study the number was 19 (11 on the back, 4 on the face, 4 elsewhere), of which 4 were histologically confirmed. All were ‹1 cm in diameter. After 2 weeks the number had increased to 30 because many erythematous lesions of a few mm in diameter had appeared on the lower legs, which

did contain BCC tissue plus a cellular infiltrate histologically. After 4 weeks the inflammatory reaction had largely disappeared clinically. The number of clinically visible BCCs fairly rapidly decreased, finally to a minimum of 5. No new lesions were seen during retinoid treatment. Growth in a rest-lesion was noted 2 1/2 and 4 months after arrest of treatment on the back and face respectively. After 9 months 5 recurrences were noted on the back, a little while later one de novo BCC on the face and more recurrences on the back. After 14 months 3 more de novo BCCs were seen on the face. All these lesions were conventionally treated. A second retinoid treatment course was refused by the patient because of her previous experience with side-effects. Altogether 12 biopsies were taken to confirm the clinical suspicion of BCC of which 10 were positive. No new laboratory abnormalities were found during etretinate treatment except for slight initial liver test disturbances and a slight elevation of triglycerides.

Patient H

This 70-year old female patient, height 160 cm, weight 53 kg, had a history of large numbers of BCCs on the face and trunk and in the anogenital area since the age of 39. During the 5 years preceding the study at least 10 BCCs a year were treated.

Etretinate treatment was given at a dose of 1 mg/kg/day divided in two doses of 25 mg. Serum levels of etretinate and its main metabolite were: after 2 weeks 0.12 and 0.3 μmol/l; after 7 weeks 0.10 and 0.2 μmol/l; after 12 weeks 0.09 and 0.2 μmol/l. After 17 weeks etretinate treatment was discontinued because of a clinically inapparent but histologically confirmed hepatitis, probably induced by etretinate itself, as described elsewhere.[40] No other side-effects except cheilitis were noted.

The effect of etretinate treatment on the number of BCCs is shown in Fig. 1c. During treatment the number decreased from about 40 to 12. Lesions ›1 cm in diameter did not change. However, 7 weeks after discontinuation of treatment because of the hepatitis, the number of BCCs had increased to about 40 again. Most of these lesions were recurrences, but de novo BCCs were observed as well. Altogether 15 biopsies were taken to confirm the clinical suspicion of BCC of which 14 were positive.

During the follow-up period another course of etretinate treatment was given at a dose of 0.5 mg/kg/day after almost complete normalization of liver tests in an attempt to prevent further explosive growth of BCCs. After 23 weeks of this low-dose course of etretinate treatment, during which slight abnormalities of the liver tests persisted and the number of BCCs remained between 40 and 50, serum levels of etretinate and its main metabolite were 0.14 and 0.25 μmol/L. After 26 weeks etretinate treatment was discontinued with subsequent complete normalization of the liver tests.

Isotretinoin treatment at a dose of 1 mg/kg/day was started one year later after complete histologic resolution of the hepatitis. Before isotretinoin treatment all BCCs were treated by cryosurgery and other conventional methods. After 5 weeks of isotretinoin treatment a large BCC of 3x5 cm in diameter developed on the scalp. Despite continued isotretinoin treatment the number of BCCs gradually increased from 0 to about 50. Laboratory abnormalities were not found during this course. Isotretinoin treatment was discontinued after 57 weeks and therapy was restricted to agressive BCCs and BCCs at critical sites. The number of BCCs appeared to stabilize at about 70 during the year after isotretinoin treatment. Serum levels of isotretinoin after 17, 39 and 57 weeks of treatment were 0.5 μmol/l, 0.5 μmol/l and 0.8 μmol/l respectively, rapidly becoming undetectable after arrest of treatment.

Immunologic studies

Cellular immune response in vivo. The results are shown in Table 2. The

Table 2. The semiquantitative DNCB test score in vivo in 14 patients with the Basal Cell Nevus Syndrome (BCNS), 8 with and 6 without a history of multiple basal call carcinomas (BCCs).

BCNS patients with multiple BCCs (≥25)			BCNS patients without multiple BCCs			Controls	
Patient	Age (yrs)	DNCB score*	Patient	Age (yrs)	DNCB score*	Age (yrs)	DNCB score
A	4	8	I	18	12	17-30	10.4 ± 1.6
B	14	6**	J	19	12	(n=19)	
C	31	9	K	22	10	30-50	10.4 ± 1.6
D	38	4**	L	23	12	(n=10)	
E	46	3**	M	26	11	50-70	6.8 ± 2.7
F	52	9	N	28	8	(n=9)	
G	67	2					
H	70	1**					

* Range DNCB score 0-12 on challenge with 3, 10 and 30 μg DNCB.
** Decreased DNCB score compared to controls (‹mean±2xSD).

DNCB test score was decreased (‹mean±2xSD) in 4 out of 8 patients with multiple BCCs compared to age-matched controls. None of the 6 patients without multiple BCCs had a decreased score.

Cellular immune response in vitro. The lymphoproliferative response in vitro of the 5 BCNS patients tested is represented in Table 3. The response was in the normal range after stimulation with the mitogens PHA, Con A and PWM and in the MLC. The response of patient C, D, G and H after stimulation with Con A 1 μg/ml was below the normal range. The response after stimulation with HPH 15 μg/ml was in the normal range.

Retinoid treatment of patient D, G and H did not affect the lymphoproliferative response in any consistent way. All responses remained in the normal range except for the response to Con A 1 μg/ml, which remained below the normal range.

PHA-induced lymphocyte cytotoxicity of PBLs of patient G and H did not differ from controls and was not affected by retinoid treatment (Table 4).

Humoral immune response in vivo. The HPH specific humoral immune response of patient D, G and H is shown in Table 5. The IgM- and Ig A-class antibody responses were within the normal range after 3 and 5 weeks. Also the IgG-class antibody response of patient D was within the normal range after 3 and 5 weeks, but the responses of patient G and H were below the normal range.

Immunohistology of epidermal Langerhans cells (LCs). No statistically significant differences were found between the number of T6+and HLA-DR+ epidermal LCs per linear mm and per mm^2 of epidermis and between the

Table 3. Lymphoproliferative response in vitro of 5 patients with the Basal Cell Nevus Syndrome and multiple basal cell carcinomas after stimulation of peripheral blood lymphocytes with PHA, Con A, PWM, irradiated allogeneic lymphocytes (mixed lymphocyte culture = MLC) and Helix pomatia Haemocyanin (HPH) three weeks after immunization with this primary immunogen. The results are expressed as mean number of desintegrations per second of triplicate cultures.

Patient	Age (yrs)	PHA 1 μg/ml	PHA 5 μg/ml	PHA 25 μg/ml	Con A 1 μg/ml	Con A 3 μg/ml	Con A 10 μg/ml	PWM 10 μg/ml	MLC	HPH 15 μg/ml
A	4	86 (52)*	221 (133)*	–	–	64 (48)*	98 (31)*	320 (221)*	579 (427)*	–
C	31	229	799	795	7	17	39	670	719	432
D	38	158	312	616	5	26	50	131	246	575
G	67	171	325	309	7	7	31	262	439	240
H	70	300	1018	466	11	39	117	664	218	134
Controls**		350 (30-1000)	800 (300-1600)	600 (250-1000)	70 (15-600)	50 (10-250)	120 (30-400)	450 (35-900)	400 (35-1200)	400 (116-1330)

* Sex- and age-matched control.

**Controls: median value (range); n=60, mean age 30 yrs, range 11-53. Controls for HPH test: n=17, mean age 40 yrs, range 29-53.

Table 4. Non-specific PHA-induced lymphocyte cytotoxicity against Hela cells of 2 patients with the Basal Cell Nevus Syndrome (patient H, aged 70 yrs; patient G, aged 67 yrs) and multiple basal cell carcinomas before and during treatment with the retinoid etretinate (Ro 10-9359) at a dose of 0.9-1.0 mg/kg/day, compared to age- and sex-matched controls.

Patient	Retinoid treatment, duration (days)	Effector: target cell ratio*					
		2.5:1		1.25:1		0.60:1	
		Patient	Control	Patient	Control	Patient	Control
H	0	0.68	0.61	0.46	0.43	0.39	0.08
	14	0.79	0.78	0.73	0.64	0.68	0.59
	42	0.70	0.89	0.62	0.81	0.61	0.66
G	0	0.87	0.90	0.73	0.76	0.64	0.71
	14	0.88	–	0.82	–	0.66	–
	28	0.86	–	0.81	–	0.66	–

* PHA-induced cytotoxicity is expressed in the cytotoxicity index:
% PHA-induced cytotoxicity - % spontaneous cytotoxicity
100 - % spontaneous cytoxicity
The rate of cytotoxicity is measured by liquid scintillation of radioactive thymidine uptake of remaining target cells.

Table 5. Class-specific antibody response of 3 patients with the Basal Cell Nevus Syndrome and multiple basal cell carcinomas after immunization with the primary immunogen Helix pomatia Haemocyanin (HPH).

	IgM	class (units)	anti-HPH	IgG	class (units)	anti-HPH	IgA	class (units)	anti-HPH
Days after immunization	0	21	35	0	21	35	0	21	35
Patients									
D	15	153	151	≤2	23	21	≤2	47	31
G	≤4	50	46	≤2	10.8	9.6	≤2	32	21
H	≤4	27	30	≤2	11	10	≤2	25	16
Controls*	≤4	70 (17-190)	75 (20-185)	≤2	35 (15-135)	36 (15-140)	≤2	63 (20-130)	36 (16-95)

* Controls: n=23, mean age 48.8 years, range 26-79 years; HPH antibody levels are given in arbitrary units as the median value and the range (in parentheses).

grade of dendritic staining reactivity of T6+ LCs of BCNS patients and controls. During retinoid treatment of patient D epidermal thickness was increased and the number of T6+ and HLA-DR+ LC/mm^2 of epidermis was decreased with a loss of dendritic staining reactivity of T6+ LCs compared to pre-retinoid treatment values. Table 6 shows the results of quantitative in situ analysis of T6+ and HLA-DR+ LC/mm^2 of epidermis of the total group of BCNS patients and of patient D compared to the controls.

Table 6. Quantitative in situ analysis of OKT-6 and OKIa-1 (HLA-DR) positive Langerhans cell (LC) structures in the epidermis of normal skin from forehead and back of 6 patients with the Basal Cell Nevus Syndrome (BCNS) and multiple basal cell carcinomas. One BCNS patient was treated with the retinoid etretinate (Ro 10-9359).

Biopsy site			Forehead		Back	
Subjects	Controls*	BCNS**	Patient D[x]		Patient D[x]	
Epidermal height (μm)	66±10	60±16	52	(92)	71	(109)
$T6^+$ profiles/mm^2						
definite LC bodies	255±55	261±53	229	(153)	192	(169)
doubtful LC bodies	93±37	95±44	173	(42)	78	(42)
$T6^+$ dendritic pattern[§]						
% type 1	14±15	15±17	58	(0)	12	(0)
% type2	39±16	47±14	38	(19)	44	(33)
% type 3	47±23	38±22	4	(81)	44	(67)
HLA-DR$^+$ LC/mm^2	229±47	259±42	237	(154)	226	(165)

All values were obtained in frozen skin sections and are expressed as the mean ± SD.

§ Type 1: fully expressed intercellular dendrites; type 2: discontinuous intercellular pattern; type 3: sparsely distributed fragments.

* Controls: n=37; mean age 48 years, range 21-82.

** BNCS patients (n=6) A, B, D, E, G and H (see Table 1); mean age 44 years, range 4-70.

x Patient D was treated with etritinate: in between parentheses the values are given which were found after 7 weeks of retinoid treatment.

Buds of BCC tissue were detected in biopsies from clinically normal skin on the forehead of patient A and G. These buds did not contain any T6+ and HLA-DR+ cells in contrast to adjacent normal epidermal tissue.

DISCUSSION

We investigated whether decreased immune responsiveness might enhance the development of BCCs in 5 patients with BCNS and multiple BCCs. The in vitro lymphoproliferative response to mitogens, allogeneic lymphocytes and the primary immunogen Helix pomatia Haemocyanin (HPH), PHA-induced lymphocyte cytotoxicity and HPH-specific humoral immune responses in vivo of the BCNS patients tested were found to be normal. The only exception was a decreased lymphoproliferative response to Con A 1 μg/ml in the four patients tested and a decreased HPH-specific IgG-class antibody response of two out of three patients tested. Our data contrast with the data concerning non-BCNS patients with BCCs, in whom the existence of a decreased in vitro lymphoproliferative response has been suggested by others.[14,15] The cellular immune response in vivo as tested by the DNCB skin test was normal in all 6 BCNS patients without multiple BCCs, but decreased in 4 out of 8 BCNS pa-

tients with multiple BCCs. Our data again contrast with the normal DNCB test response found in non-BCNS patients with BCCs.[12,13] The grade of aggressiveness of growth of BCCs in these 4 BCNS patients with a decreased DNCB test score did not appear to be markedly increased compared to the other patients with a normal score, but the small number of patients and the lack of objective parameters in two of these patients do not permit a definite conclusion. As keratinocytes are thought to be involved in the cutaneous immune response, producing an interleukin-1-like substance which has been shown to be a potent T-cell chemoattractant,[41] one could speculate whether the epidermal cell differentiation disorder of BCNS also results in impairment of the immunologic function of the keratinocyte, causing a decreased DNCB test score in some of the patients with multiple BCCs. On the whole however the immune response of BCNS patients does not appear to be grossly disturbed.

The number of T6+ and HLA-DR+ LCs was found to be normal per linear mm and per mm^2 of epidermis of 6 BCNS patients with multiple BCCs and 1 BCNS patient without multiple BCC (patient L; data not shown) compared to controls. This does not exclude an abnormal function of these cells as (part of) the cause of the decreased DNCB test score of some BCNS patients with multiple BCCs. The increase in height of the epidermis after 7 weeks of etretinate treatment of patient D, also seen by others as an effect of etretinate therapy,[42,43] in our view could explain the concomitant decrease in number of T6+ and HLA-DR+ LCs per mm^2 of epidermis. No such effect was seen on the number of LCs per linear mm of epidermis (data not shown).

The clinical results of our study are similar to those of others, who have used etretinate[22,24] or isotretinoin[23] in the treatment of patients with multiple BCCs. In these studies complete clinical regression was observed in 16% of BCCs in patients treated with a mean dose of 4.6 mg/kg/day of isotretinoin and in 7% of BCCs in patients treated with 1 mg/kg/day or less of etretinate. The results of our study also do not justify therapy of BCCs with oral retinoid treatment alone, as recurrences after complete clinical regression of BCCs (25% recurrences in patient D, 35% in patient G, almost 100% in patient H) were observed in all patients treated. Especially in patient H numerous recurrences were seen following an etretinate-induced hepatitis.[40] Photoradiation therapy might be a useful alternative.[44] The results of our study do support however the notion of a temporary preventive effect of retinoid treatment on the growth of BCCs, even at doses of etretinate of ‹1 mg/kg/day. A similar effect was seen of low-dose isotretinoin treatment.[23] We advocate intermittent treatment with yearly retinoid free intervals of at least 1-2 months, allowing rapidly and agressively growing BCCs to become clinically detectable in that period.

Side effects can hamper the feasibility of etretinate treatment,[45] as of retinoid treatment in general. Minor but disturbing side-effects can demotivate a patient, as our patient G. Nail dystrophy, seen in this patient, is one of these minor side-effects.[46] The lack of effect of etretinate and isotretinoin treatment of patient H after recuperation of an acute etretinate-induced hepatitis should be noted.[40] As the serum level of albumin, the predominant transport protein of the main metabolite of etretinate and isotretinoin, and the retinoid serum levels were not abnormal, the possibility of dysfunction of the epidermal cell receptor should be considered.[47] Provided the liver tests remain normal, long-term etretinate treatment appears to be safe hepatologically.[48] Retinoid treatment can cause invalidating bone abnormalities in children,[49] but growth of BCCs warranting retinoid treatment generally does not occur in BCNS children before puberty. Long-term isotretinoin treatment of adults is associated with an ossifying diathesis, often asymptomatic.[50] The disadvantages of minor side-effects and the risks of major side-effects should be carefully weighed against the benefits of preventive retinoid treatment.

The mechanism by which retinoids operate to prevent growth of carcinomas remains a matter of speculation.[16-19,23] Generally normalization of cell differentiation is regarded as a probably important mechanism. In vitro the main metabolite of etretinate exerts a distinct modulatory effect on the lymphoproliferative response, particularly on mitogen-induced T-cell stimulation,[31] but no such effect was seen on in vitro tests in our study where the retinoid was given to the patient and not added to the cell culture. In contrast to others who treated mice we found no evidence of an increased T-cell cytotoxic effect after retinoid treatment.[15,30] This can also be explained by the different set-up of the investigations. The inflammatory reaction immediately after starting retinoid treatment in some but not in all BCCs that later disappeared clinically, was transient. This reaction did however reveal the existence of subclinical BCCs in BCNS patients, which was demonstrated again by the finding of BCC tissue in biopsies from clinically normal skin of 2 BCNS patients.

It is concluded that the immune response of the BCNS patients tested was normal except for a possible defect of the cellular immune response in vivo in some patients with multiple BCCs and that retinoid treatment with etretinate was of preventive value in BCNS patients with multiple BCCs without affecting immune responsiveness.

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REFERENCES

1. Howell JB, Anderson DE. Commentary: The Nevoid Basal Cell Carcinoma Syndrome. Arch Dermatol 1982;118:824-6.
2. Southwick GJ, Schwartz RA. The Basal Cell Nevus Syndrome. Cancer 1979;44:2294-305.
3. Gorlin RJ, Sedano HO. The Multiple Nevoid Basal Cell Carcinoma Syndrome Revisited. Birth Defects 1971;7:140-8.
4. Clendenning WE, Block JB, Radde IC. Basal Cell Nevus Syndrome. Arch Dermatol 1964;90:38-53.
5. Rittersma J, Het basocellulaire nevus syndroom. Thesis. University of Groningen, the Netherlands. 1972.
6. Howell JB, Nevoid basal cell carcinoma syndrome. J Am Acad Dermatol 1984;11:98-104.
7. Howell JB, The roots of the naevoid basal cell carcinoma syndrome. Clin Exp Dermatol 1980;5:339-48.
8. Lindeberg H, Jepsen FL. The nevoid basal cell carcinoma syndrome. Histopathology of the basal cell tumors. J Cut Pathol 1983;10:68-73.
9. Ringborg U, Lambert B, Landegren J, Lewensohn R. Decreased UV-induced DNA repair synthesis in peripheral leukocytes from patients with the nevoid basal cell carcinoma syndrome. J Invest Dermatol 1981;76:268-70.
10. Romke C, Godde-Salz E, Grote W. Investigations of chromosomal stability in the Gorlin-Goltz syndrome. Arch Dermatol Res 1985;277:370-2.
11. Kalamkarian AA, Kapkin VV, Bogatyreva II, Vavilov AM, Mikhailovski AV, Zidgenidze AS, Stenina MA. Sindrom Gorlina. Vestn Dermatol Venereol 1983;(1):4-9.
12. Bleumink E, Nater JP. DNCB reactivity in patients with skin carcinoma. Dermatologica 1974;148:44-6.
13. Weimar VM, Ceilley RI, Goeken JA. Cell-mediated immunity in patients with basal and squamous cell skin cancer. J Am Acad Dermatol 1980;2:143-7.
14. Myskowski PL, Safai B, Good RA. Decreased lymphocyte blastogenic responses in patients with multiple basal cell carcinoma. J Am Acad Dermatol 1981;4:711-4.
15. Vena GA, Angelini G, Ovidio R de, Lospalluti M, Meneghini CL. L'immunita cellulo-mediata nei carcinomi squamocellulare e basocellulare. G Ital Dermatol Venereol 1982;117:263-7.
16. Lotan R. Effects of vitamin A and its analogues (retinoids) on normal and neoplastic cells. Biochim Biophys Acta 1980;605:33-91.
17. Elias PM, Williams ML: Retinoids, cancer and the skin. Arch Dermatol 1981;117:160-80.
18. Bollag W. Vitamin A and retinoids: from nutrition to pharmacotherapy in dermatology and oncology. Lancet 1983;i:860-3.
19. Peck GL, Gross EG, Butkus D, DiGiovanna JJ. Chemoprevention of basal cell carcinoma with isotretinoin. J Am Acad Dermatol 1982;6:815-23.
20. Dicken ChH. Retinoids: a review. J Am Acad Dermatol 1984;11:541-52.
21. Kingston T, Marks R. Cutaneous neoplasia and the retinoids. In: Cunliffe WJ, Miller AJ, eds. Retinoid therapy. Lancaster: MTP Press Limited, 1984:195-9.
22. Berretti B, Grupper Ch. Cutaneous neoplasia and etretinate. In: Cunliffe WJ, Miller AJ, eds. Retinoid therapy. Lancaster: MTP Press Limited, 1984:187-94.
23. Peck GL. Therapy and prevention of skin cancer. In: Saurat JH, ed. Retinoids. Basel: Karger, 1985:345-54.
24. Verret JL, Schnitzler L, Avenel M, Smulevici A. Etretinate and skin cancer prevention. In: Saurat JH, ed. Retinoids. Basel: Karger, 1985:355-9.
25. Levine N, Miller RC, Meyskens FL. Oral isotretinoin therapy. Use in a patient with multiple cutaneous squamous cell carcinomas and keratoacanthomas. Arch Dermatol 1984;120:1215-7.
26. Benoldi D, Alinovi A. Multiple persistent keratoacanthomas: treatment with oral etretinate. J Am Acad Dermatol 1984;10:1035-8.
27. Cristofolini M, Piscioli F, Zumiani G, Scappini P. The role of etretinate in the management of

keratoacanthoma. J Am Acad Dermatol 1985;12:633-8.
28. Yoshikawa K, Hirano S, KatoT, Mizuno N. A case of eruptive keratoacanthoma treated by oral etretinate. Br J Dermatol 1985;112:579-83.
29. Blitstein-Willinger E, Haas N, Nurnberger F, Stuttgen G. Immunological findings during treatment of multiple keratoacanthomas with etretinate. Br J Dermatol 1986;114:109-16.
30. HercendTh, Bruley-Rosset M, Florentin I, Mathe G. In vivo immunostimulating properties of two retinoids: Ro 10-9359 and Ro 13-6298. In: Orfanos CE, Braun-Falco O, Farber EM, Grupper Ch, Polano MK, Schuppli R, eds. Retinoids. Berlin: Springer Verlag, 1981:21-30.
31. Bauer R, Orfanos CE. Effects of synthetic retinoids on human peripheral blood lymphocytes and polymorphonuclears in vitro. In: Cunliffe WJ, Miller AJ, eds. Retinoid therapy. Lancaster: MTP Press Limited, 1984:101-18.
32. Bleumink E, Nater JP, Schraffordt Koops H, TheTH. A standard method for DNCB sensitization testing in patients with neoplasms. Cancer 1974;33:911-5.
33. Kallenberg CGM, Torensma R, TheTH. The immune response to primary immunogens in man. In: Reeves WG, ed. Recent developments in clinical immunology. Amsterdam: Elsevier Science Publishers B.V., 1984:1-26.
34. Katz SI, Cooper KD, Iijima M, TsuchidaT. The role of Langerhans cells in antigen presentation. J Invest Dermatol 1985;85:96s-8s.
35. Murphy GF, Krusinski PA, Myzak LA, Ershler WB. Local immune response in basal cell carcinoma: characterization by transmission electron microscopy and monoclonal anti-T6 antibody. J Am Acad Dermatol 1983;8:477-85.
36. Verweij H, VoorstVader PC van, Houthoff HJ, Gips CH. Quantitative analysis of retinoids inhuman serum and tissue samples using high performance liquid chromatography. In: Saurat JH, ed. Retinoids. Basel: Karger, 1985:301-4.
37. Kallenberg CGM, Voort-Beelen JM van der, Amaro J de, TheTH. Increased frequency of B8/DR3 in scleroderma and association of the haplotype with impaired cellular immune response. Clin Exp Immunol 1981;43:478-85.
38. Huges-Law G, Gast GC de, The TH. PHA-induced cytotoxicity of human lymphocytes against adherent Hela cells. J Immunol Methods 1978;19:29-39.
39. Jong MCJM de, Blanken R, Nanninga J, VoorstVader PC van, Poppema S. Defined in situ enumeration of T6 and HLA-DR expressing epidermal Langerhans cells. I: morphological and methodological aspects. J Invest Dermatol 1986:in press.
40. VoorstVader PC van, Houthoff HJ, Eggink HF, Gips CH. Etretinate (Tigason) hepatitis in 2 patients. Dermatologica 1984;168:41-6.
41. Sauder DN. Biologic properties of Epidermal cellThymocyt Activating Factor (ETAF). J Invest Dermatol 1985;85:176s-82s.
42. Gillenberg A, Immel C, Orfanos CE. Retinoid-Einfluss auf die Zellkinetik gesunder menschlicher Epidermis. Arch Dermatol Res 1980;269:331-5.
43. Marks R, Finlay AY, Holt PJA. Severe disorders of keratinization: effects of treatment with Tigason (etretinate). Br J Dermatol 1981;104:667-73.
44. Tse DT, Kersten RC, Anderson RL. Hematoporphyrin derivative photoradiation therapy in managing nevoid basal-cell carcinoma syndrome. Arch Ophthalmol 1984;102:990-4.
45. Ward A, Brogden RN, Heel RC, Speight TM, Avery GS. Etretinate. A review of its pharmacological properties and therapeutic efficacy in psoriasis and other skin disorders. Drugs 1983;26:9-43.
46. Baran R. Action therapeutique et complications du retinoide aromatique sur l'appareil ungeal. Ann Dermatol Venereol (Paris) 1982 109:367-71.
47. Vahlquist A, Torma H, Rollman O, Berne B. Distribution of natural and synthetic retinoids in the skin. In: Saurat JH, ed. Retinoids. Basel: Karger, 1985:159-67.
48. Voorst Vader PC van, Houthoff HJ, Gips CH, Verweij H. Hepatologic side effects during long-term retinoid therapy: Ito cells and portal hypertension. In: Saurat JH, ed. Retinoids. Basel: Karger, 1985:498-500.
49. Sillevis Smitt JH, De Mari Fr. A serious side-effect of etretinate (Tigason). Clin Exp Dermatol 1984;9:554-6.

50. Gerber LH, Helfgott RK, Gross EG, Hicks JE, Ellenberg SS, Peck GL. Vertebral anomalies associated with synthetic retinoid use. J Am Acad Dermatol 1984;10:817-23.

Chapter 2

Xeroderma pigmentosum

Chapter 2.1

Xeroderma pigmentosum

Immune response, Langerhans cells and retinoid treatment

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SUMMARY

In a xeroderma pigmentosum patient belonging to complementation group C the immune response and the clinical and immunologic effect of retinoid treatment were evaluated. During a Northern winter immunologic evaluation showed: 1) a decreased number of peripheral blood lymphocytes with relatively normal T-cell subset percentages; 2) a cellular immune response in vitro in the lower range of normal; 3) a decreased antigen-specific humoral and cellular immune response in vivo; 4) a decreased number of T6a+ and HLA-DR+ Langerhans cells per mm^2 of epidermis on the forehead; 5) HLA-DR expression of epidermal keratinocytes. This indicates a suboptimal immune response of this XP patient, which might enhance the growth of neoplasms. Clinical observation during 5 years suggested that treatment with the retinoid etretinate (Ro 10-9359), 0.9 mg/kg/day, prevented the growth of cutaneous carcinomas. Retinoid treatment did not markedly affect the immunologic parameters. Spontaneous corneal perforation stressed the need for ophthalmologic follow-up.

INTRODUCTION

Xeroderma pigmentosum (XP) is a rare, autosomal recessive disease characterized clinically by cutaneous photosensitivity and pigmentary changes, photophobia and a propensity for the development of cutaneous malignancies, particularly in sun-exposed areas, and ocular tumors.[1] Cell biologic investigations have disclosed cellular hypersensitivity to UV radiation and certain chemicals in association with abnormal DNA repair. This is thought to be the mutagenic factor responsible for the development of neoplasms in XP. The DNA repair disorder in XP is due to a deficiency in nucleotide excision repair of DNA after UV damage. In the excision proficient XP variant group a post- replication repair disorder was detected.[1] Among the excision defi-

cient XP patients nine different groups are recognized at the moment: complementation groups A through I.[2] Cultured cells from different complementation groups were found to complement each other after cell fusion resulting in normal DNA repair of the fused cell. Neurological abnormalities are found in patients belonging to complementation groups A, B, D and G. In Europe and in the United States patients belonging to complementation group C are common.[1,3]

It has been postulated that the DNA-repair disorder after exposure to UV light in XP patients might also have consequences for the cellular immune response, possibly interfering with immune surveillance against neoplasms.[4-7] The immune response of XP patients has not been the subject of extensive studies thus far.[8] Regarding the cell mediated immunity in vivo a diminished response to intradermal test antigens, delayed skin graft rejection and impaired sensitization to DNCB has been observed.[9-12] Whether sunlight exposure influenced the results of these tests was not mentioned. A positive DNCB sensitization test and normal responses to intradermal test antigens have also been described.[11] Regarding the cell mediated immunity in vitro a diminished lymphoproliferative response was found after stimulation with phytohaemagglutinin [11-15] and concanavalin A [14] due to an inhibitory serum factor. A normal lymphoproliferative response to phytohaemagglutinin has also been reported.[15,16]

As the continuous development of de novo cutaneous carcinomas in XP patients constitutes a therapeutic problem, attempts have been made to prevent carcinoma growth by oral treatment with the aromatic retinoid etretinate (Ro 10-9359), with encouraging clinical results.[17-21]

The aim of our study was twofold: 1) to investigate the immune response of a XP patient in a Northern country in a seasonal period relatively free of sunlight exposure; 2) to evaluate the clinical and immunologic effect of preventive retinoid treatment. Therefore we conducted a longitudinal immunologic study of a XP patient belonging to complementation group C before and during retinoid treatment with etretinate in winter, and embarked on a longitudinal clinical study lasting 5 years during which intermittent treatment with etretinate was given. The immunologic study included analysis of 1) the number of peripheral blood leukocytes, lymphocytes and percentages of T-cell subsets, 2) the lymphoproliferative response in vitro to mitogens and allogeneic cells, 3) the humoral response in vivo after immunization with the primary immunogen Helix pomatia Haemocyanin (HPH),[22] 4) the cellular immune response in vivo using the DNCB sensitization test, and 5) the number and morphology of T6+ and HLA-DR+ epidermal Langerhans cells (LCs), as LCs appear to be involved in the cellular immune response to cutaneous carcinomas [23] and are susceptible to light exposure.[24,25]

PATIENT AND METHODS

Patient

A male XP patient of Dutch descent, age 20 years, volunteered for the study, having given informed consent. His deceased older brother, who showed similar clinical characteristics, was found to belong to XP complementation group C, with a residual level of UV-induced unscheduled (repair) DNA synthesis (UDS) of 20-30%. A similar level of residual DNA repair capacity may be assumed in our patient.[26] Dermatologic examination of the patient demonstrated xerosis and disseminated freckle-like lesions, especially on sunlight-exposed skin areas, actinic keratoses from infancy and skin carcinomas from his mid-teens. Between the ages of 18 and 20 years he developed three basal cell carcinomas (BCC), three squamous cell carcinomas (SCC), one dysplastic naevocellular naevus, about 5-10 actinic keratoses a year and multiple teleangiectatic granulomata.

Ophthalmologic examination disclosed photophobia, atrophy of the skin of the lower eyelids resulting in ectropion, and recurrent purulent conjunctivitis. The eye lashes of the lower lids were absent. The left cornea was highly vascularized, the right cornea showed only some vessels along the periphery. Abundant tear film fluid production was found bilaterally in the Schirmer test, but the tear-film break-up time was <1 second (normal: circa 60 seconds). Artificial tear fluid was continually used to prevent irritation of the corneas.

Methods

Retinoid treatment and etretinate serum levels. The patient was treated with three courses of the aromatic retinoid etretinate (Ro 10-9359; Tigason) at a dose of 0.9 mg/kg/day (weight 57 kg; 25 mg 2 dd). The first course from January 1981 to September 1982 lasted twenty months, the second from March to November 1983 eight months and the third from April to October 1984 six months. Using high performance liquid chromatography serum levels of etretinate and its main metabolite (Ro 10-1670) were measured.[27] The lowest level of detection was 0.05 μmol/L.

Immunophenotype of peripheral blood lymphocytes. The number of peripheral blood leukocytes and lymphocytes was counted in winter before (n=7) and during (n=3) retinoid treatment and in summer (n=4) during retinoid treatment. In winter, before retinoid treatment, percentages of T-cells and T-cell subsets were determined twice using monoclonal antibodies against T-cells (OKT-3), helper-inducer T-cells (Leu-3a) and cytotoxic-suppressor T-cells (OKT-8).[28] These percentages were compared- with those of healthy controls (n=21, mean age 27 yrs, range 19-45). OKT-3 and OKT-8 were purchased from Ortho Diagnostic Systems (Raritan, NJ, USA) and Leu-3a from Becton Dickinson (Mountain View, California, USA).

Cellular immune response in vitro. The lymphoproliferative response in vitro was measured according to previously described methods.[22,29] Peripheral blood lymphocytes cultured in triplicate in pooled serum were stimulated with phytohaemagglutinin (PHA) 1 and 5 μg/ml, concanavalin A (Con A) 3 and 10 μg/ml, pokeweed mitogen (PWM) 10 μg/ml, and with irradiated allogeneic lymphocytes in the mixed lymphocyte culture (MLC). Healthy controls were used (n=57, mean age 30 yrs, range 19-53). These tests were performed before the first retinoid treatment course in January 1981. They were repeated 3, 6 and 14 weeks after starting the first course.

The presence of a circulating serum factor inhibiting the lymphocyte proliferative response was investigated by culturing peripheral blood lymphocytes of both the patient and a control of the same age and sex in pooled serum, allogeneic serum and autologous serum. Proliferation was induced by PHA 5 μg/ml, Con A 3 and 10 μg/ml, PWM 10 μg/ml and irradiated allogeneic lymphocytes. Care was taken that serum of the patient did not contain detectable levels of etretinate and its main metabolite.

Humoral immune response in vivo. The humoral immune response to Helix pomatia Haemocyanin (HPH) was assessed by measuring the antigen specific IgG-, IgM- and IgA-class antibody response 3 and 6 weeks after subcutaneous immunization in the deltoid region with the primary test immunogen.[22] Healthy controls (n=23, mean age 49 yrs, range 26-79) were used. The first retinoid treatment course was started 3 weeks after immunization with HPH. Total levels of IgG, IgM, IgA and IgE were also measured.

Cellular immune response in vivo. A semiquantitative DNCB sensitization test was performed.[30] The patient was tested before the first retinoid course in January 1981. The challenge was repeated during this course in July 1981. Healthy controls were used (n=10, mean age 24 yrs, range 17-30).[27]

Immunohistology of epidermal Langerhans cells (LCs). Punch biopsies were taken after local anaesthesia (lidocain 1% w.o. adrenaline) from non carcinomatous and scar-free, normally pigmented skin on the forehead and back of the patient. The specimens were snap-frozen in liquid nitrogen and stored at -70°C until further processing. Biopsies were taken in January 1981 before the first retinoid treatment course and in April 1983 three weeks after starting the second course. Epidermal Langerhans cells (LCs) were identified in 6 μm cryostat sections by a two-step immunoperoxidase technique using monoclonal anti-T6 (OKT-6) and anti-HLA-DR (OKIa-1) antibodies (Ortho Diagnostic Systems Inc., Raritan, NJ, USA) and peroxidase-conjugated rabbit anti-mouse Ig (Dakopatts, Denmark) as described elsewhere.[31] Quantitative in situ analysis of T6+ and HLA-DR+ epidermal LCs was carried out according to recently developed criteria.[31] Briefly, an arbitrary distinction was made between three types of T6+ LC profiles: 1) "definite" LC bodies, 2) "doubtful" LC bodies, representing cross sectioned profiles of indistinct origin, and 3) profiles of dendritic origin. These LC profiles, except for type 3, were counted in 6-10 epidermal test areas (Vobj x Voc = 400) for each biopsy specimen. The epidermal height of a given test area was defined as the distance between the stratum corneum and the dermo-epidermal junction, or an imaginary line halfway the rete ridges if present. The mean number of reactive LC profiles was calculated and expressed per linear mm and per mm^2 of cross sectioned epidermis for each biopsy specimen.

In addition we assessed the state of dendritic reactivity of LCs as described before.[31] To this end three types of T6+ epidermal dendritic patterns were defined: a network pattern of interconnecting dendrites (type 1), a discontinuous intercellular dendritic pattern (type 2) and a pattern showing sparsely distributed dendritic fragments (type 3). The percentual distribution of these patterns was also estimated in 6-10 epidermal test areas for each biopsy sample.

Biopsy specimens from the forehead of 8 healthy individuals (4 males, 4 females; mean age 32 yrs, range 26-38) and the back of 10 healthy individuals (6 males, 4 females; mean age 31 yrs, range 21-39) served as controls. Care was taken to avoid sampling of biopsy specimens after sunlight exposure causing erythema or a tan.

In situ analysis of inflammatory infiltrates was performed by immunoperoxidase staining with the monoclonal antibodies Leu-3a (CD4), OKT-8 (CD8) and anti-B1 (Coulter Electronics). The latter antibody is a marker for B-lymphocytes.

RESULTS

Retinoid treatment: clinical effect. During the first, second and third course of 20, 8 and 6 months of retinoid treatment no de novo cutaneous (pre)malignant lesions were noted. No therapeutic effect was seen on 2 BCCs already present before the first treatment course and finally treated by cryosurgery. A vascularized lesion of the cornea of the left eye, after excision shown to be a squamous cell carcinoma in situ, also persisted during the first retinoid course. De novo cutaneous carcinomas were diagnosed within 3 months after arrest of the retinoid courses. One nodular BCC (3 mm in diameter) was found three months after the first course, one ulcerative BCC (8 mm in diameter, histologically nodular with some features of a sclerosing growth pattern) one month after the second course, one nodular BCC (3 mm in diameter) two months after the third course, and one nodular BCC (3 mm in diameter) and

three SCCs (2 mm in diameter) five months after the third course. No other (pre)malignant lesions except for some actinic keratoses were found in the intervals between the courses. These lasted 6 months after the first course and 5 months after the second course. Teleangiectatic granulomas continued to develop during and in between retinoid courses.

Subjectively the patient rather appreciated retinoid treatment. Besides the fact that treatment of cutaneous (pre)malignant lesions was not necessary during retinoid treatment, he especially valued the beneficial influence of retinoid treatment on his recurrent purulent conjunctivitis. In his opinion retinoid treatment prevented this conjunctivitis, which occurred especially in summer. Exsiccation cheilitis and granuloma formation at the nail corners of the first phalanx of both feet were seen as side-effects of retinoid treatment. Liver tests and serum lipid levels remained normal.

Etretinate serum levels. Serum levels of etretinate and its main metabolite were both 1.0 μmol/L when measured during the second course. Three weeks after the second course these levels were 0.05 and 0.1 μmol/L respectively. No detectable serum levels were found 9 and 23 weeks after the second course.

Ophthalmologic follow-up. A corneal perforation of the left eye developed one month after the start of the fourth retinoid treatment course in April 1985. Histologic investigation of the left corneal button showed a central defect surrounded by a sparse inflammatory infiltrate. The epithelial part of the cornea was hyperplastic and Bowman's layer was desintegrated. The thickness of the stroma was normal. An at random transplantation was performed and succceeded very well: no rejection was seen and the transplanted cornea remained clear during 8 months of follow-up. After these 8 months a tear film break-up time of ›30 seconds was found on the left cornea, which was increased compared to the pre-transplant situation. According to the patient less purulent conjunctivitis, irritation of the cornea and photophobia was experienced. Artificial tears were no longer necessary. An HLA-matched cornea was transplanted to the right eye, which showed a typical keratoconus when investigated by the slit lamp. This also gave a subjective positive result. Histologic examination of the right corneal button showed hyperplastic epithelium and an irregular and fragmented layer of Bowman. The thickness of the central stroma was reduced to about one quarter of normal.

Immunophenotype of peripheral blood lymphocytes. The mean number of peripheral blood leukocytes was $4.4x10^9$/L (range 4.1-5.4) in winter, which is near the lower limit of the normal range (4.0-11.0x10^9/L). This number did not change markedly during retinoid treatment in winter ($4.3x10^9$/L, range 3.5-5.5) and in summer ($4.1x10^9$/L, range 4.0-4.1). The mean number of peripheral blood lymphocytes was 884/mm^3 (range 330-1800) in winter, which is decreased (normal range: 1000-3300/mm^3). During retinoid treatment in win-

ter this number did not change markedly (963/mm^3, range 550-1200), but a slightly higher number was found during retinoid treatment in summer (1227/mm^3, range 680-1800).

Before retinoid treatment the percentages of OKT-3+ (68% and 77%, normal 81±7%), Leu-3a+ (48% and 50%, normal 47±7%) and OKT-8+ (23% and 27%, normal 30±4%) lymphocytes were within a range of mean±2xSD. The Leu-3a/OKT-8 cell ratio on these two occasions was moderately increased and normal respectively (2.1 and 1.8, normal 1.6±0.2).

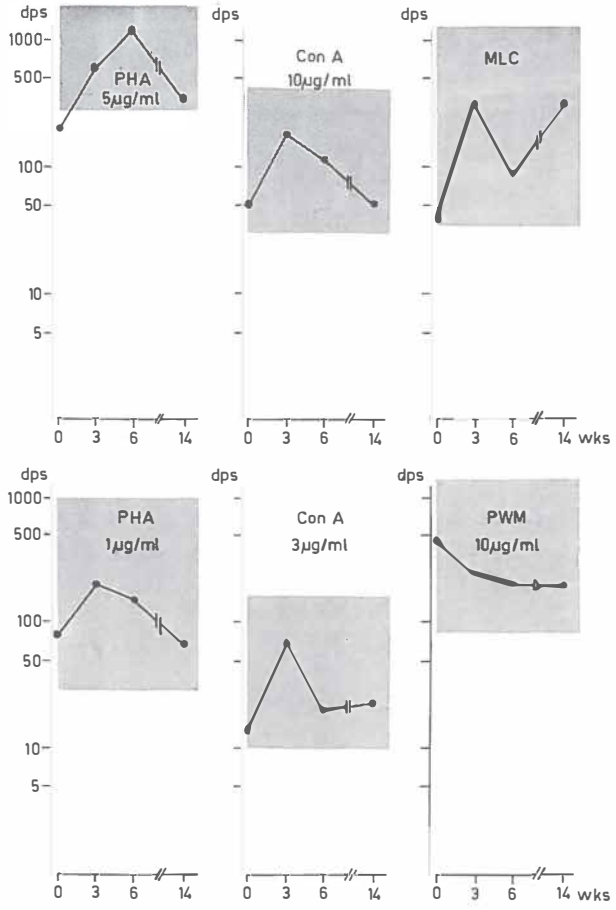

Fig. 1. In vitro lymphoproliferative response before and during treatment with the retinoid etretinate (0.9 mg/kg/day) of a patient with xeroderma pigmentosum. Cultured peripheral blood lymphocytes were stimulated with PHA 1 and 5 µg/ml, Con A 3 and 10 µg/ml, PWM 10 µg/ml and irradiated allogeneic lymphocytes (mixed lymphocyte culture = MLC). The results are expressed as mean number of desintegrations per second (dps) for triplicate cultures. The shaded area represents the normal range of the controls (n=57).

Cellular immune response in vitro. The lymphoproliferative response in vitro was in the lower range of normal after stimulation with the mitogens PHA and Con A and in the MLC (Fig 1). Only the response to PHA 5 μg/ml was below the normal range. The response to PWM was near the median value of the normal range. Neither the presence of serum of the patient nor of the control affected the lymphoproliferative response of the patient and the control compared to the response in pooled serum.

Retinoid treatment did not markedly influence the cell mediated immune response in vitro. In the tests with PHA and Con A a transient stimulatory effect was suggested (Fig 1).

Humoral immune response in vivo. The HPH specific IgG-, IgM- and IgA-class antibody response was markedly decreased compared to controls (Table 1). This impaired antibody response was not caused by retinoid treatment, as the first values three weeks after immunization at the start of retinoid treatment were also markedly decreased. The levels of total IgG, IgM and IgA were within the normal range. The level of IgE was slightly elevated (402 U/ml, normal ‹200 U/ml).

Table 1. Class-specific antibody response of a xeroderma pigmentosum patient (age 20 years) after immunization with the primary immunogen Helix pomatia Haemocyanin (HPH).

Time after immunization (days)	IgM class anti-HPH (units)		IgG class anti-HPH (units)		IgA class anti-HPH (units)	
	Patient	Controls*	Patient	Controls*	Patient	Controls*
0	≤4	≤4	≤2	≤2	≤2	≤2
21	8.5	70 (17-190)	2.5	35 (15-135)	4	65 (20-130)
42	9.5	80 (22-185)	3.4	35 (16-145)	≤2	28 (14-80)

* Controls: n=23, mean age 48.8 years, range 26-79 years; HPH antibody levels are given in arbitrary units as the median value and the range (in parentheses).

Cellular immune response in vivo. The DNCB sensitization skin test score of the patient before and during retinoid treatment was 2 and 3 respectively. These values were decreased compared to controls (10.4±1.6). No flare-up reaction was seen at the site of sensitization.

Immunohistology of epidermal Langerhans cells (LCs). The results of in situ enumeration of T6+ and HLA-DR+ LCs and the grade of dendritic staining reactivity of T6+ LCs are shown in Table 2. Epidermal thickness was increased, more on the forehead than on the back of the patient. The number of T6+ and HLA-DR+ LC/mm on forehead and back was normal. However,

Table 2. Quantitative in situ analysis of OKT-6 and OKIa-1 (HLA-DR) positive Langerhans cell (LC) structures in the epidermis of a xeroderma pigmentosum patient (age 20 years)

Biopsy site	Forehead		Back	
Subjects	Controls**	Patient	Controls***	Patient
Epidermal height (μm)	72±11	130 (128)	67±11	111 (103)
$T6^+$ profiles/mm				
definite LC bodies	19± 4	16 (17)	15± 4	20 (23)
doubtful LC bodies	6± 3	6 (6)	5± 2	7 (7)
$T6^+$ profiles/mm^2				
definite LC bodies	269±30	125 (134)	232±58	178 (219)
doubtful LC bodies	94±27	50 (52)	84±41	66 (74)
$T6^+$ dendritic pattern*				
% type 1	12± 7	6 (0)	16±21	2 (13)
% type 2	52±16	63 (13)	33±13	36 (41)
% type 3	36±18	31 (87)	51±26	62 (46)
$HLA\text{-}DR^+$ LC/mm	16± 3	17 (13)	16± 3	17 (16)
$HLA\text{-}DR^+$ LC/mm^2	225±33	137 (96)	235±53	157 (170)

All values were obtained in frozen skin sections. The values of the controls are expressed as the mean ± SD.

* Type 1: fully expressed intercellular dendrites; type 2: discontinuous intercellular pattern; type 3: sparsely distributed dendritic fragments.

** Controls: n=8, mean age 32 years, range 26-38.

*** Controls: n=10, mean age 31 years, range 21-39.

§ In between parentheses the values are indicated, which were found three weeks after starting retinoid treatment with etretinate.

the number of T6+ and HLA-DR+ LC/mm^2 on the forehead, but not on the back, was decreased (‹mean±2xSD).

During retinoid treatment no marked changes in epidermal thickness and number of T6+ and HLA-DR+ epidermal LCs were found. On the forehead, but not on the back, the grade of dendritic staining reactivity of T6+ LCs was decreased compared to the pretreatment situation.

The biopsies from the forehead and back taken before retinoid treatment showed HLA-DR expression of keratinocytes in the lower part of the epidermis, whereas control specimens were consistently negative. HLA-DR positive epidermal areas were characterized by an "intercellular" staining pattern rather than the "cell body" type of staining displayed by LCs (Fig. 2). The papillary dermis in both specimens showed perivascular inflammatory cells. Staining for subset markers showed Leu-3a (CD4) and OKT-8 (CD8) positive T-cells in a ratio of 2 to 5. Staining for B cells was consistently negative. During retinoid treatment the biopsy from the back and to a lesser degree the

biopsy from the forehead also showed HLA-DR expression of keratinocytes in the lower part of the epidermis in association with the presence of Leu-3a+ and OKT-8+ T cells in the papillary dermis.

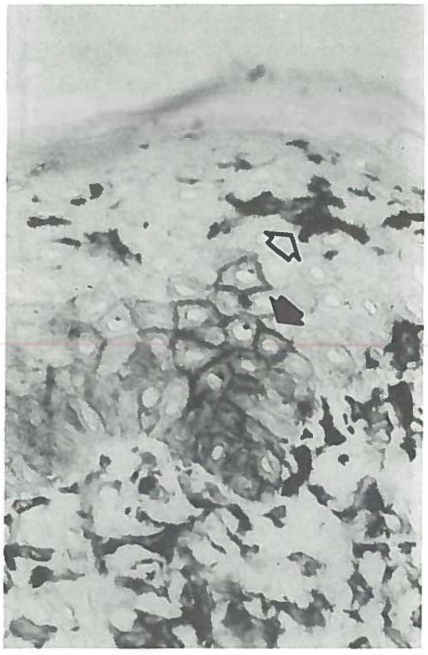

Fig. 2. Cryostat section of XP skin from the back illustrating intercellular type of OKIa-1 (anti-HLA-DR) reactivity of keratinocytes (closed arrow) and the "cell body" staining type of Langerhans cells (open arrow). Skin from the forehead showed similar staining reactivity for OKIa-1 (anti-HLA-DR). Both biopsies also contained perivascular Leu-3a and OKT-8 positive T-cells in the papillary dermis (not shown). x400

DISCUSSION

Immunologic evaluation in winter of our patient, belonging to complementation group C, revealed a normal cellular immune response in vitro after stimulation of peripheral blood lymphocytes with mitogens and allogeneic lymphocytes. In these in vitro tests however the patient tended to be a low responder.

The number of peripheral blood leukocytes and lymphocytes and the percentages of T-cells and T-cell subsets was in the lower range of normal or decreased. Although the percentage of Leu-3a+ and OKT-8+ lymphocytes was normal, the absolute number of these cells was low due to the decreased number of peripheral blood lymphocytes. The antigen specific humoral and cellular immune response in vivo, as tested by the HPH specific antibody response and the DNCB sensitization test on skin shielded from light, was impaired in our patient. In two other XP patients with a less severe clinical picture but with an amount of sunlight exposure not very different from our patient according to their history, one probably belonging to complementation group C and one to group F, we found a normal DNCB test score however (data not shown). According to the literature and our data the cellular immune response in XP apparently varies from patient to patient, possibly also within one complementation group, even if undue sunlight exposure is avoided. The presence or absence of an impaired immune response in XP may be linked with the severity of the clinical picture, i.e. to the degree of sensitivity to UV-induced cell damage.

The impairment of the HPH-specific humoral response in vivo of our patient could be due to an impairment of the regulatory function of the T-cells, but might also be due to faulty antigen presentation or impaired B-cell function. One could speculate whether the decreased cellular immune response in vivo as tested by the DNCB sensitization test was based on the same defect, e.g. faulty antigen presentation. The decreased number of peripheral T-cells of our XP patient could also be a common factor of impairment of the humoral and cellular immune response in vivo. The impairment of the immune response in vivo of our patient could enhance tumor growth. Squamous cell carcinomas are known for their association with an impaired immune response, advancing disease being related to progressive suppression.[32-36] Advanced tumors were not present in our patient.

We found a normal number of T6+ and HLA-DR+ LC/mm^2 of thickened epidermis on the back, but a decreased number of T6+ and HLA-DR+ LC/mm^2 of thickened epidermis on the forehead of our XP patient. Other investigators observed a normal number of ATPase positive LCs in epidermal sheets from the buttocks of 5 XP patients, but a stronger and longer lasting depletion of ATPase positive epidermal cells after UV irradiation of the skin compared to controls.[37] Sunlight exposure, even the relatively small amount of it in winter, may thus explain the decrease of epidermal LCs on the the forehead of our XP patient. The increase in epidermal thickness could be another explanation for the decrease of LC/mm^2 epidermis on the forehead. This appears unlikely however, because the decrease in number of epidermal T6+ and HLA-DR+ LC/mm^2 found on the forehead, but not on the back, of two other

XP patients studied by us was of the same magnitude as the decrease in the XP patient reported here, whereas the increase in epidermal thickness, also found in these two patients, was less outspoken (data not shown). One may assume that not only the number of epidermal LCs, but also the function of these cells in XP patients is decreased by light irradiation, even more than in healthy individuals.[24,25] UV irradiation did suppress the function of peripheral blood lymphocytes of a XP patient more markedly than of a control.[16] In that manner light irradiation could severely interfere with the local cellular immune response to carcinomas developing in light-exposed skin of XP patients,[23] possibly promoting tumor growth.

Epidermal keratinocytes showed positive staining with anti-HLA-DR antibody in the biopsies taken before retinoid treatment from the back and the forehead. This correlated with the presence of a perivascular mononuclear cell infiltrate in the papillary dermis. The significance of HLA-DR expression by epidermal keratinocytes is as yet poorly understood, but this phenomenon can be an in vivo indicator of the local production of γ-interferon by antigen triggered lymphocytes.[38] The antigen in this case is not clear, but might very well have been induced by light. Epidermal thickening on the back as well as the forehead of our patient, probably caused by disturbed keratinization due to light induced DNA damage of keratinocytes, supports this contention. Expression of HLA-DR antigen by epidermal keratinocytes is possibly enhanced by retinoid treatment,[39] but in our patient retinoid treatment did not result in marked changes.

Retinoid treatment with etretinate appeared to be effective in preventing skin cancer growth in our patient, which is in agreement with the experience of other investigators.[17-21] Similar results were seen in patients with multiple BCCs, treated with isotretinoin or etretinate.[40,41] Two XP patients have been described who developed cutaneous carcinomas during high-dose (1 mg/kg/day) treatment with etretinate.[18,21] Reduction of doses resulted in tumor growth in another XP patient.[19] Side effects can hamper the feasibility of retinoid treatment, but this was not the case in our patient. The rapid occurrence, one month after arrest of the second retinoid treatment course, of a fairly large ulcerative BCC suggests that growth of apparently subclinically preexistant cutaneous carcinomas may be very agressive when retinoid treatment is stopped. Agressive growth of BCCs was also noted in one basal cell nevus syndrome patient after arrest of retinoid treatment because of an etretinate-induced hepatitis.[42] For this reason we advocate intermittent use of preventive retinoid treatment with retinoid medication free periods of 2-3 months each year. This will give possibly agressive cutaneous carcinomas the opportunity to become clinically visible and available for therapy. Treatment of clinically apparent cutaneous carcinomas with retinoid medication alone cannot be re-

commended as recurrences after arrest of treatment are to be expected.[19,40-43] Nor was retinoid treatment of our patient effective for a squamous cell carcinoma in situ of the cornea. Intermittent preventive retinoid therapy however can result in long periods without clinically apparent cutaneous tumors, which have to be treated.

The preventive effect of retinoid treatment on the growth of cutaneous carcinomas in our patient was not related to any marked change in the cell mediated immune response in vivo and in vitro. Only a transient stimulatory effect on the PHA and Con A induced lymphoproliferative response was noted. Previously we have shown that the level of unscheduled DNA synthesis after UV irradiation of cultured fibroblasts of a XP patient is not influenced by the presence of a retinoid.[44] A direct effect on cell differentiation might very well be responsible for the preventive effect described.[19,40,45]

Ophthalmologic complications occur frequently in XP with potentially serious consequences.[46] Ulceration and perforation of the cornea as occurred in our patient can be the result of an exposure keratitis or a neoplasm. Our patient however was able to close his eyelids adequately and histology did not show a malignancy. His corneae were probably made more vulnerable by the abnormal tear-film break-up time. This could have been caused by atrophy of the glands of Meibom and/or an abnormal surface of the cornea itself, as the tear-film break-up time was less abnormal after the corneal transplantation. The absence of rejection of the at random corneal transplant may have been favoured by impairment of the immune response. Rejection of at random corneal transplants in XP patients has also been described.[47] A keratoconus of the non-perforated eye was detected, i.e. protrusion of the cornea in association with thinning of the corneal stroma. Using a slit-lamp keratoconus, bilateral, was also seen in an eight year old XP nephew of our patient, who had never been treated with retinoids. The corneal epithelial hyperplasia and stromal hypoplasia of our patient may both have resulted from light induced DNA damage. Thinning of corneal stroma has also been observed by others.[48] Keratoconus is generally not associated with an increased risk of perforation. In XP however this cannot be excluded. Full thickness corneal transplantation appears to have therapeutic value for corneal abnormalities causing decreased vision and irritation of the eye. The history of our XP patient stresses the importance of careful ophthalmologic follow-up of XP patients.

In summary, during a Northern winter our XP patient demonstrated a decreased number of peripheral blood lymphocytes, a cellular immune response in vitro in the lower range of normal, a decreased humoral and cellular immune response in vivo and a decrease number of LCs per mm^2 of epidermis on the forehead indicating that a suboptimal immune response might indeed interfere with immune surveillance against neoplasms in our XP patient. Reti-

noid treatment appeared to be effective in preventing growth of cutaneous carcinomas without affecting immunologic parameters. Corneal vulnerability resulting in a spontaneous perforation was successfully treated by transplantation.

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REFERENCES

1. Kraemer KH. Heritable diseases with increased sensitivity to cellular injury. In: Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF, eds. Update: Dermatology in General Medicine. New York: McGraw-Hill, 1983:113-40.
2. Fischer E, Keijzer W, Thielman HW, Popanda O, Bohnert E, Edler L, Jung EG, Bootsma D. A ninth complementation group in xeroderma pigmentosum, XP I. Mutat Res 1985;145:217-25.
3. Fischer E, Thielmann HW, Neundorfer B, Rentsch FJ, Edler L, Jung EG. Xeroderma pigmentosum patients from Germany: clinical symptoms and DNA repair characteristics. Arch Dermatol Res 1982;274:229-47.
4. Bridges BA. Some DNA-repair-deficient human syndromes and their implications for human health. Proc R Soc Lond 1981;B212:263-78.
5. Kripke ML. Immunobiology of UV-radiation-induced skin cancer. In: Parrish JA, Kripke ML, Morison WL, eds. Photoimmunology. New York: Plenum Medical Book Company, 1983:155-73.
6. Friedberg EC, Ehmann UK, Williams JI. Human diseases associated with defective DNA repair. In: Lett JT, Adler H, eds. Advances in radiation biology. New York: Academic Press Inc, 1979:86-170.
7. Morison WL, Parrish JA, Epstein JH. Photoimmunology. Arch Dermatol 1979;115:350-5.
8. Kraemer KH. Progressive degenerative diseases associated with defective DNA repair: xeroderma pigmentosum and ataxia teleangiectasia. In: Nichols WW, Murphy DG, eds. DNA repair processes. Miami: Symposia Specialists Inc, 1977:37-71.
9. Dupuy JM, Lafforet D. A defect of cellular immunity in xeroderma pigmentosum. Clin Immunol Immunopathol 1974;3:52-8.
10. Salamon T, Stojakovic M, Bogdanovic B. Delayed hypersensitivity in xeroderma pigmentosum. Arch Dermatol Res 1975;251:277-80.
11. Berkel AI, Kiran O. Immunological studies in children with xeroderma pigmentosum. Turk J Pediatr 1974;16:43-52.
12. Wijsenbeek AJ, Pick AI, Weiss H, Vana D, Atsmon A. Impaired humoral and cellular immunity in xeroderma pigmentosum. Clin Oncol 1980;6:361-5.
13. Grouchy J de, Nava C de, Feingold J, Frezal J, Lamy M. Asynchronie chromosomique dans un cas de xeroderma pigmentosum. Ann Genet (Paris) 1967;10:224-5.
14. Lafforet D, Dupuy JM. Inhibitory factors of lymphocyte proliferation in serum from patients with xeroderma pigmentosum. Clin Immunol Immunopathol 975;4:165-73.

15. Lafforet D, Dupuy JM. Photosensibilite et reparation de l'ADN. Arch Franc Pediatr (Suppl) 1978;35:65-74.
16. Agarwal SS, Brown DQ, Katz EJ, Loeb LA. Screening for deficits in DNA repair by the response of irradiated human lymphocytes to phytohemagglutinin. Cancer Res 1977;37:3594-8.
17. Verret JL, Schnitzler L, Avenel M, Smulevici A. Etretinate and skin cancer prevention. In: Saurat JH, ed. Retinoids: new trends in research and therapy. Basel: Karger, 1985:355-9.
18. Claudy AL, Soulas G, Lauras B, Freycon MT, le Petit JC, Fraisse J. Xeroderma pigmentosum et traitement au long cours par le retinoide aromatique. Ann Dermatol Venereol 1982;109:271-4.
19. Braun-Falco O, Galosi A, Dorn M, Plewig G. Tumorprophylaxe bei Xeroderma pigmentosum mit aromatischem Retinoid (Ro 10-9359). Hautarzt 1982;33:445-8.
20. Pichler E, Fritsch P. Xeroderma pigmentosum. Tumorprophylaxe mit Etretinate. Hautarzt 1984;35:159-61.
21. Guillot B, Favier C, Guilhou JJ, Meynadier J. Xeroderma pigmentosum. Ann Dermatol Venereol 1984;111:65-7.
22. Kallenberg CGM, Toresma R, The TH. The immune response to primary immunogens in man. In: Reeves WG, ed. Recent developments in clinical immunology. Amsterdam: Elsevier Biomedical Press, 1984:1-26.
23. Murphy GF, Krusinski PA, Myzak LA, Ershler WB. Local immune response in basal cell carcinoma: characterization by transmission electron microscopy and monoclonal anti-T6 antibody. J Am Acad Dermatol 1983;8:477-85.
24. Stingl G, Wolff K. The Langerhans cell. In: Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF, eds. Update: Dermatology in general medicine. New York: McGraw-Hill, 1983:1-12.
25. Czernielewski J, Vaigot P, Asselineau D, Prunieras M. In vitro effect of UV radiation on immune function and membrane markers of human Langerhans cells. J Invest Dermatol 1984;83:62-5.
26. Kato T, Akiba H, Seiji M, Tohda H, Oikawa A. Clinical and biological studies of 26 cases of xeroderma pigmentosum in Northeast District of Japan. Arch Dermatol Res 1985;277:1-7.
27. Verweij H, Voorst Vader PC van, Houthoff HJ, Gips CH. Quantitative analysis of retinoids inhuman serum and tissue samples using high performance liquid chromotagraphy. In: Saurat JH, ed. Retinoids: new trends in research and therapy Basel: Karger, 1985:301-4.
28. Giessen M van der, Postma S, The TH. Isolation of highly purified lymphocyte subsets for functional studies by means of an indirect rosette technique. Scand J Immunol 1985;22:41-9.
29. Kallenberg CGM, Voort-Beelen JM van der, Amoro J de, The TH. Increased frequency of B8/DR3 in scleroderma and association of the haplotype with impaired cellular immune response. Clin Exp Immunol 1981;43:478-85.
30. Bleumink E, Nater JP, Schraffordt Koops H, The TH. A standard method for DNCB sensitization testing in patients with neoplasms. Cancer 1974;33:911-5.
31. Jong MCJM de, Blanken R, Nanninga J, Voorst Vader PC van, Poppema S. Defined in situ enumeration of T6 and HLA-DR expressing Langerhans cells. I: morphological and methodological aspects. J Invest Dermatol 1986: in press.
32. Jansen HM, The TH, Gast GC de, Esselink MT, Pastoor G, Orie NGM. The primary immune response of patients in different stages of squamous- cell bronchial carcinoma. Thorax 1978;33:755-60.
33. Jansen HM, Esselink MT, Orie NGM, The TH. Cell-mediated immune response in patients with bronchial carcinoma. Neth J Med 1979;22:1-9.
34. Eskinazi DP, Helman J, Ershow AG, Perna JJ, Mihail R. Nonspecific immunity and head and neck cancer: blastogenesis reviewed and revisited. Oral Surg Oral Med Oral Pathol 1985;60:642-7.
35. Bleumink E, Nater JP. DNCB reactivity in patients with skin carcinoma. Dermatologica 1974;148:44-6.
36. Angelini G, Vena GA, Ovidio R de, Lospalluti M, Meneghini CI. T-cell subsets and soluble immune response suppressor factor in skin squamous cell carcinoma. Acta Dermatovener

(Stockholm) 1983;63:109-14.
37. Koulu LM, Jansen CT. Langerhans cells in xeroderma pigmentosum. J Invest Dermatol 1983;80:374.
38. Weller FR, Jong MCJM de, Kallenberg CGM, Poppema S, The TH. Epidermal expression of HLA-DR antigen in delayed-type reactions to a primary test immunogen is correlated with in vitro lymphocyte proliferation. In: Weller FR. The primary immune response in patients with chronic nonspecific lung disease. Thesis. University of Groningen, the Netherlands, 1986:31-42.
39. Walsh LJ, Seymour GJ, Powell RN. The in vitro effect of retinol on human gingival epithelium. II. Modulation of Langerhans cell markers and interleukin-1 production. J Invest Dermatol 1985;85:501-6.
40. Peck GL. Therapy and prevention of skin cancer. In: Saurat JH, ed. Retinoids: new trends in research and therapy. Basel: Karger, 1985:345-54.
41. Voorst Vader PC van, Driessen LHHM, Kallenberg CGM. Basal Cell Nevus Syndrome: immune status, etretinate therapy and etretinate hepatitis. Br J Dermatol 1983;108:109.
42. Voorst Vader PC van, Houthoff HJ, Eggink HF, Gips CH. Etretinate (Tigason) hepatitis in 2 patients. Dermatologica 1984;168:41-6.
43. Berretti B, Grupper Ch. Cutaneous neoplasia and etretinate. In: Cunliffe WL, Miller AJ, eds. Retinoid therapy. Lancaster: MTP Press Ltd., 1984:187-94.
44. Voorst Vader PC van, Jaspers NGJ, Kamp AWM van der: Retinoic acid and defective UV light induced DNA excision repair in xeroderma pigmentosum: absence of ameliorating effect. Arch Dermatol Res 1984;276:201-2.
45. Peck GL. Retinoids and cancer. J Invest Dermatol 1985;85:87-8.
46. Zabel M, Brandle I, Westkott C. Augenveranderungen beim Xeroderma pigmentosum. Hautarzt 1980;31:188-90.
47. Freedman J. Corneal transplantation with associated histopathologic description in xeroderma pigmentosum occurring in a black family. Ann Opthalmol 1979;11:445-8.
48. Stenson S. Ocular findings in xeroderma pigmentosum: report of two cases. Ann Ophthalmol 1982;14:580-5.

Chapter 2.2

Retinoic acid and defective UV light induced DNA excision repair in xeroderma pigmentosum: absence of ameliorating effect

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Retinoids may be useful in the chemoprevention of cancer in patients with multiple cutaneous carcinomas or premalignant keratotic lesions.[1-3] A tumor prophylactic effect of the retinoid etretinate (Ro 10-9359) is also suggested in xeroderma pigmentosum[4-6] and is substantiated by our own experience. Enhancement of normal differentiation may be the main factor responsible for the tumor prophylactic effect of retinoids, but other factors might be involved as well.[1,7] In xeroderma pigmentosum (XP) an alternative mechanism could be stimulation of cellular DNA repair processes. In the present communication we report the results of a study of the effect of retinoic acid on ultraviolet light induced DNA excision repair in cultured human skin fibroblasts.

The fibroblasts used were derived from a XP homozygote patient (XP16RO, complementation group C) and an obligate XP heterozygote (79RD254) from the same family. Irradiation (predominantly 254 nm) was performed with a Philips TUV lamp (15 W) at a dose rate of 0.9 J/m^2/s, maximum exposure to UV light being 20 s. A stock solution of retinoic acid (10 mM) in acetone was prepared and stored at -70°C. Cells were grown in the presence of different concentrations of retinoic acid (0 μM, 1 μM and 10 μM) for three culture passages (10-16 days). Acetone concentrations were adjusted to equal levels in all cultures. For the assay of unscheduled DNA synthesis (UDS) cells were seeded onto glass coverslips and irradiated 24 h later. After UV light exposure they were incubated for 2 h in the presence of tritiated thymidine, treated with Bouin's fixative and processed for autoradiography. The rate of UDS is expressed as the mean number of grains per nucleus. Throughout the procedure except during UV light irradiation relevant concentrations of retinoic acid were present in the culture medium, the cultures being protected from light as retinoids are light sensitive.

The results are shown in Fig. 1. In the absence of retinoic acid the fibroblasts from the XP heterozygote demonstrated UV light dose dependent le-

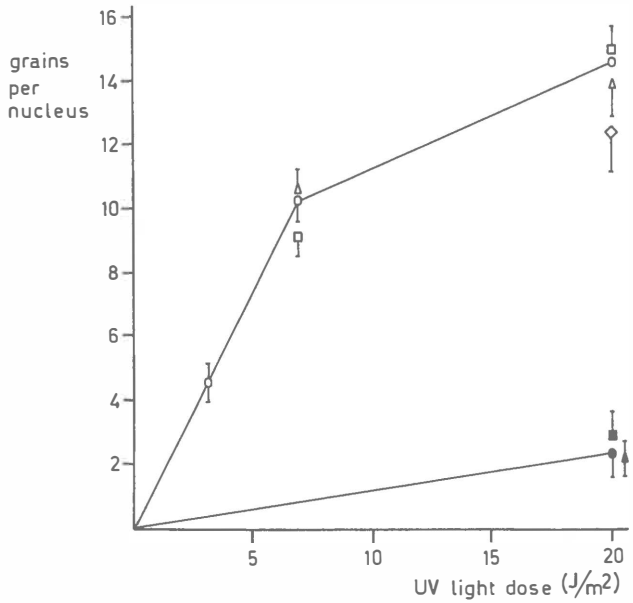

Fig. 1. The influence of retinoic acid on UV light induced DNA synthesis using autoradiography in cells from a XP-C patient (closed symbols) and a XP heterozygote (open symbols). The concentrations of retinoic acid used were 0μM (o,●), 1 μM (△,▲) and 10 μM (□,■). In one case (◇) cells cultured in the presence of 10 μM retinoic acid were assayed for unscheduled DNA synthesis with 20 μM retinoic acid. Points and bars indicate the mean and SEM respectively of 50 counted nuclei.

vels of UDS, shown not to be different from those observed in cultured cells from normal individuals.[8] The XP homozygote cells exhibited a residual rate of UDS of 15%, a level commonly found in complementation group C.[9] Cells cultured in the presence of retinoic acid showed the same characteristics. Concentrations of retinoid acid did not exceed 10μM as higher concentrations were cytotoxic and inhibited growth completely. In one instance cells pretreated with 10 μM of retinoic acid were tested for DNA repair in the presence of 20 μM retinoic acid, which also did not result in a significant alteration of the rate of UDS.

We conclude that, in vitro, long-term exposure to retinoic acid does not affect the rate of UV light induced DNA excision repair in a XP homozygote and heterozygote. These findings indicate that the prophylactic effect on tumor formation in XP patients is not likely to be due to modulation of DNA repair capacity in the skin.

REFERENCES

1. Peck GL, Gross EG, Butkus D, DiGiovanna JJ. Chemoprevention of basal cell carcinomas with isotretinoin. J Am Acad Dermatol 1982;6:815-23.
2. Voorst Vader PC van, Driessen LHHM, Kallenberg CGM. Basal cell nevus syndrome: immune status, etretinate therapy and etretinate hepatitis. Br J Dermatol 1983;108:109 (abstract).
3. Moriarty M, Dunn J, Darragh A, Lambe R, Brick I. Etretinate in treatment of actinic keratoses. Lancet 1982;i:364-5.
4. Schnitzler L, Schubert B, Verret JL. Essai de prevention des epitheliomas cutanes par le retinoide aromatique. Ann Dermatol Venereol 1980;107:657-63.
5. Claudy AL, Soulas G, Lauras B, Freycon MT, Petit JC le, Fraisse J. Xeroderma pigmentosum et traitement au long cours par le retinoide aromatique. Ann Dermatol Venreol 1982;109:271-4.
6. Braun-Falco O, Galosi A, Dorn M, Plewig G. Tumor-prophylaxe bei Xeroderma pigmentosum mit aromatischem Retinoid (Ro 10-9359). Hautarzt 1982;33:445-8.
7. Bollag W. Vitamin A and retinoids: from nutrition to pharmacotherapy in dermatology and oncology. Lancet 1983;i:860-3.
8. Bootsma D, Mulder MP, Pot F, Cohen JA. Different inherited levels of DNA repair replication in xeroderma pigmentosum cell strains after exposure to ultraviolet irradiation. Mutat Res 1970;9:507-16.
9. Friedberg EC, Ehmann UK, Williams JI. Human diseases associated with defective DNA repair. Adv Radiat Biol 1979;8:85-174.

Chapter 3

Epidermodysplasia verruciformis

Chapter 3.1

Epidermodysplasia verruciformis

Langerhans cells, immunologic effect of retinoid treatment and cytogenetics

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SUMMARY

A case study is presented of a 44 year old negroid male with epidermodysplasia verruciformis (EV), cutaneous carcinomas and impaired cell mediated immunity (CMI). Analysis was made of 1) T6+ and HLA-DR+ Langerhans cells (LCs) by immunoperoxidase staining in lesional and clinically normal skin before and during retinoid treatment, 2) the effect of retinoid treatment on CMI in vivo and in vitro, and 3) cytogenetic aspects related to chromosomal instability. The results showed the virtual absence of T6+ and HLA-DR+ LCs in koilocytic areas of involved epidermis. This may indicate a deficient role of epidermal LCs in human papillomavirus-specific CMI. It is of notable interest that light- exposed, clinically normal skin also demonstrated microscopic EV lesions largely devoid of T6+ and HLA-DR+ LCs. Retinoid treatment with etretinate (Ro 10-9359) appeared both to increase the CMI response in vitro to T-cell mitogens and to influence the in situ pattern of T6+ and HLA-DR+ LCs. The cytogenetic study did not show evidence of spontaneous or UV-induced increased chromosomal instability.

INTRODUCTION

Epidermodysplasia verruciformis (EV) is caused by a disseminated persistent cutaneous infection with Human Papillomaviruses (HPVs).[27] A remarkable phenomenon is the early development of cutaneous squamous cell carcinomas, mostly in sun-exposed areas, in about 30% of EV patients. Two phenotypes of EV are distinguished.[27] One type shows a clinical picture of disseminated flat warts due to infection with HPV3 and/or 10 and is apparently not carcinoma related. The other phenotype is characterized clinically by flat wart-like lesions, confluent hyperkeratotic plaques and pityriasis versicolor-

like lesions. The lesions in this phenotype are due to infection with a fairly large number of EV-specific HPVs. [12, 26] Of these HPV5, 8 and 14 are supposed to be potentially oncogenic, because the genomes of these HPVs have been detected in cutaneous carcinomas of EV patients.[27, 35] Actinic radiation is considered to be a cofactor in carcinogenesis in EV.[27] Black EV patients seem to be less prone to the development of cutaneous carcinomas.[18, 19]

EV is frequently associated with impaired cell mediated immunity (CMI), as evidenced by a decreased number of T-lymphocytes, a reduction of lymphocyte responsiveness to mitogens and anergy to DN CB sensitization.[17] This might facilitate HPV-related carcinogenesis in EV.[16, 27] It is unknown whether this impairment is a primary or a secondary event. Adult EV patients with normal CMI, one with and one without skin cancer, have also been reported.[8, 24] The retinoid etretinate (Ro 10-9359) has been described as capable of reducing the HPV load and of improving the clinical [8, 9, 14, 23, 27] and possibly the immunologic status.[9, 14]

A hereditary gene defect is believed to be the basic anomaly in EV.[1, 27] The nature of this defect is unknown. Approximately 25% of reported EV cases were familial with frequent parental consanguinity. About 8% of the patients was mentally retarded. Autosomal recessive and X-linked inheritance of EV have been proposed.[1] An association of EV with distinct HLA-A and -B allotypes could not be demonstrated.[37] DNA-repair after UV irradiation was found to be normal in EV.[29]

We present a case study of a negroid EV patient, infected with HPV8 and 17, with impaired non-HPV-specific CMI and cutaneous carcinomas. Focusing on immunologic and genetic factors in the aetiology of EV and the effect of retinoid therapy on CMI, the aim of our study was threefold: I) immunohistologic screening of epidermal Langerhans cells (LCs) before and during a course of retinoid treatment with etretinate (Ro 10-9359), since in vitro studies have demonstrated a critical role of LCs in the induction of antigen specific proliferative and cytotoxic T-cell responses;[3, 10] II) evaluation of the effect of retinoid treatment on CMI in vivo and in vitro; III) cytogenetic investigation of the frequency of sister chromatid exchanges and spontaneous and UV-induced chromosomal breakage, and, in addition, analysis of UV-induced unscheduled DNA synthesis, as abnormalities in these parameters would define EV as a chromosomal instability or cell hypersensitivity disease.[25]

PATIENT AND METHODS

Patient

A male, born in Surinam (formerly Dutch Guyana), son of a negro mother and a Caucasian father, first noticed skin lesions at the age of 15. He moved to the Netherlands at the age of 24. From

the age of 37 cutaneous carcinomas developed. Two invasive squamous cell carcinomas were excised, one ulcerative tumor from the posterior side of the left auricle and one large exophytic tumor from the left temporal area. Also removed were two ulcerative Bowenoid lesions from the occipital area. This patient appeared to be the only member of his family affected with EV.

Dermatologic examination at the time of his first visit to our clinic showed a male, 43 years old, of negroid appearance with a light-brown skin and black, curly hair. Flat wart-like lesions and a few lenticular erythematosquamous slightly elevated lesions were seen on the back of the hands. Disseminated flat or slightly elevated, erythematous, partially scaling, pityriasis versicolor- like lesions were observed on the trunk and on the proximal part of the extremities. No such lesions were seen on the scalp and on the face except for a lenticular elevated erythematous lesion on the exact site where a seborrhoeic keratosis had been excochleated on the left cheek. The face, neck and trunk showed multiple macular or slighty raised, non-scaling, hyperpigmented lesions of a few millimeters in diameter. The right forearm showed such lesions in a linear configuration. About 15 darkly pigmented seborrhoeic keratoses were observed on the trunk and face.

Histologic examination of biopsies from a pityriasis versicolor-like lesion on the trunk and a flat wart-like lesion on the hand showed a picture consistent with EV, i.e. epidermal acanthosis, koilocytosis in the upper part of the epidermis and prominent keratohyaline granules in koilocytic epidermal areas. EV pathology was also found in excised material from the left ear and the occiput and in a punch biopsy from a hyperpigmented lesion on the right forearm.

Routine laboratory studies - erythrocyte sedimentation rate, number of peripheral blood leukocytes, leukocyte differentiation, blood chemistry, HBsAg and anti-HBs, chest X-ray - were without abnormalities. Serologic tests for syphilis were positive (the patient had been treated in Surinam for an early syphilitic infection). Neurologic examination disclosed no abnormalities nor did a CT scan of the head and neck area.

Viral analysis was performed after extraction of DNA from scrapings of benign EV lesions on the extremities and trunk by restriction enzyme analysis and blot hybridization of viral DNA.[26] At least three different HPVs were detected, two of which were characterized as HPV 8 and 17.

The patient agreed to an investigational treatment during a period of 4 weeks with the aromatic retinoid etretinate (Ro 10-9359, Tigason), 0.9 mg/kg/day (1 capsule of 25 mg twice a day), in order to assess the immunologic effect. Sunlight exposure was avoided. On personal initiative however he discontinued treatment after 3 1/2 days and 7 capsules of 25 mg of etretinate. On day 8 he showed a mild cheilitis. He resumed treatment and took 21 capsules of 25 mg of etretinate on 10 consecutive days from day 21 to 30. Etretinate and its main metabolite (Ro 10-1670) were detected by high performance liquid chromatography in serum taken on day 30 at a level of 0.6 μmol/L and 0.5 μmol/L respectively.[34] No clinical effect on the EV lesions could be observed.

Methods

I. Immunohistology of epidermal Langerhans cells (LCs)

Punch biopsies were taken after local anaesthesia (lidocain 1% w.o. adrenalin) from clinically normal skin on the forehead and back and from a pityriasis versicolor-like lesion on the back before retinoid treatment and on the 8th consecutive day (day 28) of retinoid treatment. The specimens were snap-frozen in liquid nitrogen and stored at -70°C until further processing. Epidermal Langerhans cells (LCs) were identified in 6 μm cryostat sections by a two-step immunoperoxidase technique using monoclonal anti-T6 (OKT-6) and anti-HLA-DR (OKIa-1) antibodies (Ortho Diagnostic Systems Inc., Raritan, NJ, USA) and peroxidase-conjugated rabbit anti-mouse Ig (Dakopatts, Denmark) as described elsewhere.[20] It has been demonstrated that most T6+ and HLA-DR+ epidermal dendritic cells are LCs.[20] Quantitative in situ analysis of T6+ and HLA-DR+ epidermal LCs was carried out according to recently developed criteria.[20] Briefly, an arbitrary distinction was made between three types of T6+ LC profiles: 1) "definite" LC bodies, 2) "doubtful" LC bodies, representing cross sectioned profiles of indistinct origin, and 3) profiles of

dendritic origin. These LC profiles, except for type 3, were counted in 6-10 epidermal test areas (Vobj x Voc=400) for each biopsy specimen. The length and height of each test area was delineated with the aid of a calibrated eyepiece micrometer containing 100 scale units (1 scale unit = 2.4 μm at Vobj=x40). The epidermal height of a given test area was defined as the distance between the stratum corneum and the dermo-epidermal junction or an imaginary line halfway the rete ridges if present. From these data the mean number of reactive LC profiles was calculated, expressed per linear mm and per mm^2 of cross sectioned epidermis for each biopsy specimen.

In addition we assessed the state of dendritic reactivity of LCs as described before.[20] To this end three types of T6+ epidermal dendritic patterns were defined: a network pattern of interconnecting dendrites (type 1), a discontinuous intercellular dendritic pattern (type 2) and a pattern showing sparsely distributed dendritic fragments (type 3). The percentual distribution of these patterns was also estimated in 6-10 epidermal test areas for each biopsy sample, using the full scale (100 units) of the eyepiece micrometer as 100%. Each pattern in a test area covered by 1 scale unit of the micrometer, placed in parallel to the skin surface, was scored as 1%.

Biopsy specimens from forehead and back of 4 healthy Caucasian males (mean age 32±5 years) and from the back of 4 healthy negroid males from Surinam (mean age 33±8 years) served as control material.

II. CMI and retinoid treatment

The CMI response was assessed by: 1) the semiquantitative DNCB sensitization test;[5] 2) the percentage of E-rosette forming peripheral blood lymphocytes (PBLs); 3) the percentages of PBLs positive with the monoclonal antibodies OKT-3 (pan-T-lymphocyte marker), Leu-3a (helper-inducer T-cell marker) and OKT-8 (cytotoxic-suppressor T-cell marker);[13] 4) the proliferation rate in vitro of PBLs after stimulation with phytohaemagglutinin (PHA) 1 and 5 μg/ml, concanavalin A (Con A) 3 and 10 μg/ml, pokeweed mitogen (PWM) 10 μug/ml and irradiated allogeneic PBLs in the mixed lymphocyte culture (MLC).[21] Controls between 17-53 years of age were used for the DNCB test (n=20),[5] for the percentages of E-rosette forming PBLs (n=22) and T-cell subsets (n=21) and for the lymphocyte transformation tests (n=57).

The percentages of E-rosetting lymphocytes and T-cell subsets were assessed at the beginning and on day 15 and 30 of the retinoid treatment period. The lymphoproliferative response was measured 47 weeks before, at the start and the end of, and 13 weeks after the retinoid treatment period. The DNCB challenge test was performed before and on day 26 of the retinoid treatment period.

III. Cytogenetic study

a) Five and a half months after the retinoid treatment period PBLs were cultured in medium 199 supplemented with fetal calf serum. G-band analysis was performed [30] and 175 metaphases were studied for numerical and structural aberrations, including breaks. The frequency of sister chromatid exchanges was determined in 10 cells of the EV patient and a control grown in medium containing bromodeoxyuridine.

b) One and a half years later the chromosomal study was repeated and extended. PBLs were cultured in RPMI 1640 medium supplemented with 20% fetal calf serum and a sample of 50 cells was studied for numerical and structural aberrations. In order to determine the effect of photoirradiation on the chromosomes, PBLs of the EV patient and of a healthy control, obtained from buffy-coats and seeded onto glass coverslips, were exposed before culture to UV-C (predominantly 254 nm) at a dose rate of 0.5 $J/m^2/s$ during 8 seconds using a Philips TUV light source of 15 W. Metaphases of 50 of the cultured cells of the EV patient and the control were studied for structural aberrations.

c) The level of unscheduled DNA synthesis after photoirradiation of cultured fibroblasts of the EV patient and a control was measured according to methods previously described.[36]

RESULTS

I. Immunohistology of epidermal Langerhans cells (LCs)

The results of quantitative in situ analysis of T6+ and HLA-DR+ epidermal LCs are shown in Table 1. Light-microscopic examination of biopsy material showed EV pathology as indicated by acanthosis and the presence of koilocytosis in the upper part of the epidermis in both biopsies from the EV lesion on the back, but also at multiple sites of the epidermis in both biopsies from clinically normal skin on the forehead (Fig. 1b). For the purpose of LC quantification the epidermis was therefore divided into non-acanthotic EV- and acanthotic EV+ areas. EV+ epidermal areas consisted of a superficial part showing koilocytosis and a lower part without overt pathologic changes. The height of EV+ epidermis was about twice that of normal EV- epidermis. Before retinoid treatment the number of T6+ and HLA-DR+ LC/mm of both EV- and EV+ epidermis was within the normal range (mean±2xSD). The

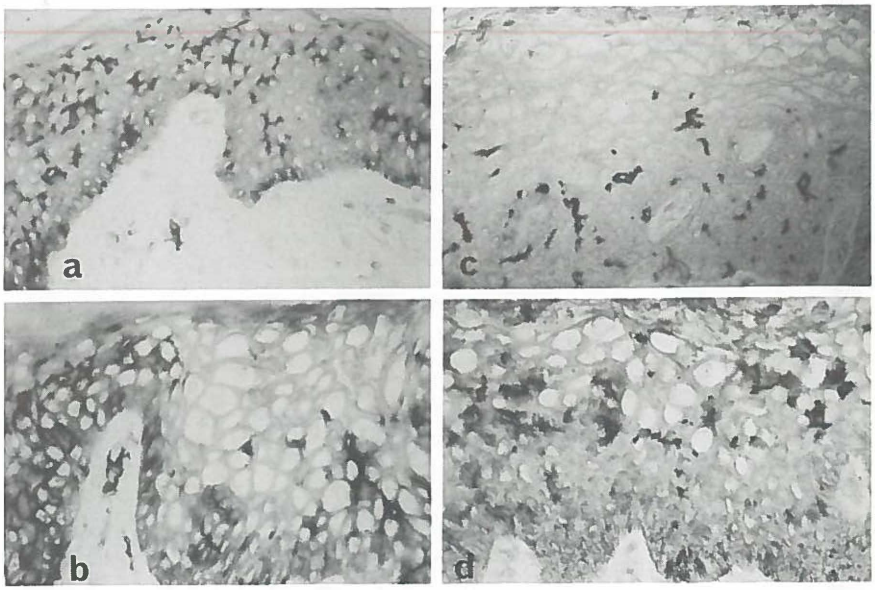

Fig. 1. Frozen skin sections from a patient with epidermodysplasia verruciformis illustrating OKT-6 reactivity of epidermal Langerhans cells (LCs). a) Clinically normal skin of the back showing darkly stained T6+ LCs in histologically EV- epidermis; b) clinically normal skin of the forehead showing an acanthotic EV+ epidermal area with relative sparsity of T6+ LCs in the koilocytic layers; c) lesional skin of the back before retinoid treatment showing T6+ LC bodies with relatively little dendritic staining reactivity underneath the koilocytic layers; d) lesional skin of the back after retinoid treatment showing relative increase of T6+ LCs at koilocytic sites and sparsity of T6+ LCs in the lower epidermal layers. x250.

Table 1. Quantitative in situ analysis of OKT-6 and OKIa-1 (HLA-DR) positive Langerhans cell (LC) structures in the epidermis of a negroid patient suffering from epidermodysplasia verruciformis (age 44 years).

Biopsy site	Forehead					Back					
	clinically normal skin					clinically normal skin				lesion	
Light microscopy	Controls**	EV–		EV+		Controls***		EV–		EV+	
Retinoid therapy	–	–	+	–	+	–	–	–	+	–	+
Epidermal height	74±10	63	77	155	143	69±11	54± 4	83	67	172	166
$T6^+$ profiles/mm											
definite LC bodies	18± 3	13	11	20	6	15± 4	11± 2	16	11	15	10
doubtful LC bodies	8± 3	7	2	7	1	6± 2	3± 2	6	7	5	4
$T6^+$ profiles/mm^2											
definite LC bodies	248±25	236	142	127	48	222±64	215±62	196	166	93	61
doubtful LC bodies	106±35	117	20	48	7	88±42	63±10	71	103	30	28
$T6^+$ dendritic pattern											
% type 1	14± 8	7	0	1	0	12±20	1± 1	8	8	0	0
% type 2	53±18	41	13	26	0	36±14	24±11	51	48	4	4
% type 3	33±22	52	87	73	100	52±26	75± 9	41	44	96	96
$HLA\text{-}DR^+$ LC/mm	18±4	17	10	13	9	16± 4	11± 4	14	14	18	15
$HLA\text{-}DR^+$ LC/mm^2	227±45	241	138	93	60	223±48	198±52	174	212	103	95

All values were obtained in frozen skin sections and are expressed as the mean ± SD.

* Type 1: fully expressed intercellular dendrites; type 2: discontinuous intercellular pattern; type 3: sparsely distributed fragments.

** Caucasian males (n=4; mean age 32±5 years)

*** Caucasian males (left column) and negroid males (n=4; mean age 33±8 years; right column).

number of T6+ and HLA-DR+ LC/mm^2 of EV- epidermis was also within the normal range. However, the number of LC/mm^2 of acanthotic EV+ epidermis was decreased (‹mean±2xSD), because LCs were very rare in the koilocytic upper part of EV- epidermis, while the normal appearing epidermis underneath the koilocytic layers contained normal numbers of T6+ and HLA-DR+ LCs. These LCs however showed considerable loss of dendritic staining reactivity for OKT-6 (Fig. 1c) compared to LCs in EV- areas (Fig. 1a).

On the 8th consecutive day of retinoid treatment T6+ and HLA-DR+ LC bodies were more frequently encountered in koilocytic areas of EV+ epidermis, especially in clinically normal skin of the forehead. But on this occasion LCs were rather sparse in the normal appearing epidermal areas underneath the koilocytic layers (Fig. 1d). This resulted in an overall decrease in number of T6+ and HLA-DR+ LC/mm and LC/mm^2 of EV+ epidermis compared to the pretreatment situation, in particular on the forehead where such a decrease was also noted in EV- epidermis (Table 1).

Mononuclear cell infiltration in the papillary dermis was sparse in the biopsy from clinically normal skin on the forehead and mild in the biopsy from lesional skin on the back. No inflammatory response was seen in EV+ epidermis. After retinoid treatment this pattern of mononuclear cell infiltration did not show marked changes.

II. CMI and retinoid treatment

A decreased DNCB test score of 1 (controls: 10.4±1.6) was found before the retinoid treatment period. On day 28 the DNCB test score was 2. The number of PBLs remained in the normal range. The percentage of E-rosette forming PBLs was low (63%) before retinoid treatment compared to controls (75± 8%). On day 15 and 30 of the retinoid treatment period normal values were found of 67% and 76% respectively. The same pattern was seen for OKT-3+ PBLs (70%, 70%, 74%; controls: 80±7%). The percentage of Leu-3a+ PBLs, decreased (30%) before retinoid treatment compared to controls (47±7%), was still decreased at day 15 and 30 (36% and 38%). The percentage of OKT-8+ PBLs was normal initially (33%) compared to controls (30±4%) and slightly higher after retinoid treatment (34% and 39%). This resulted in a decreased Leu-3a/OKT-8 ratio (0.91) compared to controls (1.6±0.2), which did not change during the retinoid treatment period (1.0 and 0.97 respectively).

The proliferation rate of PBLs in vitro before retinoid treatment was below the normal range after stimulation with PHA 1 and 5 μg/ml, around the lower limit of normal after stimulation with Con A 10 μg/ml and PWM and in the lower range of normal after stimulation with Con A 3 μg/ml (Fig. 2). In the MLC the proliferation rate was near the median value of the controls. Retinoid treatment did not result in marked changes in the proliferation rate in the MLC or after stimulation with PWM, but stimulation with PHA and Con A

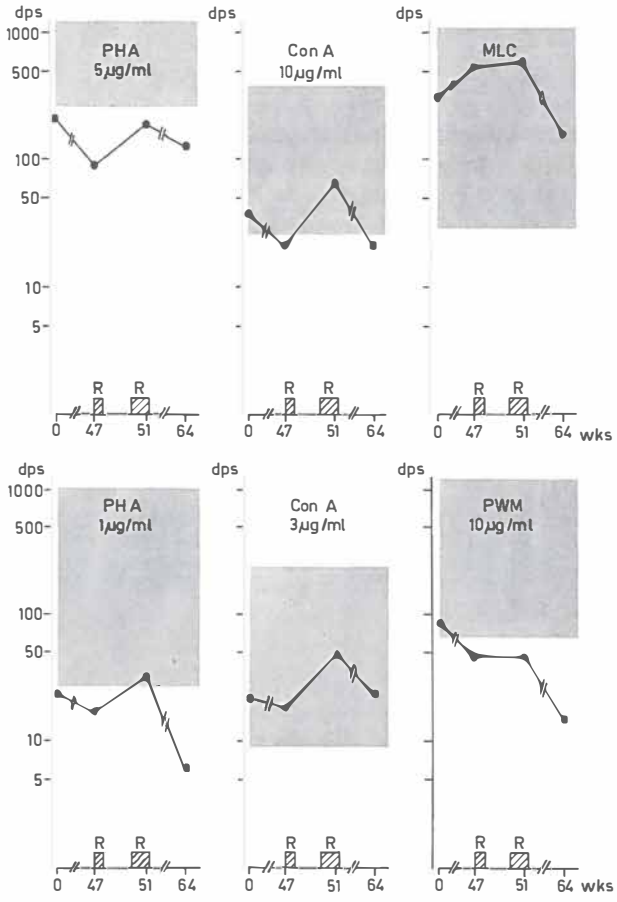

Fig. 2. In vitro lymphoproliferative response of a patient with epidermodysplasia verruciformis after stimulation with PHA (1, 5 µg/ml), Con A (3, 10 µg/ml), PWM (10 µg/ml) and irradiated allogeneic lymphocytes (mixed lymphocyte culture = MLC). The results are expressed as mean number of desintegrations per second (dps) for triplicate cultures, the shaded areas representing the normal range of the controls (n=57). Retinoid treatment with etretinate (R), 0.9 mg/kg/day, was started in the 47th week after the first assessment of the lymphoproliferative response. The patient took etretinate for 3 1/2 days, stopped doing so, and resumed retinoid treatment for 10 days until 2 days past the 51st week.

produced an increased response on day 30 of the retinoid treatment period as compared to day 0 (Fig. 2). Three months after retinoid treatment a decreased lymphoproliferative response was found after stimulation with PHA 1 and 5 µg/ml, Con A 10 µg/ml and PWM compared to controls.

a) A normal male 46, XY karyotype was found and three cells with numerical aberrations: one with trisomy 2, one with trisomies 9 and 20, and one with tetrasomy X. Two metaphases were seen with structural aberrations: one cell had an abnormal, triradial chromosome No.18 (Fig. 3a), the other cell had only one normal No.6 and two abnormal elements probably derived from its homologue (Fig. 3b). Spontaneous chromosome and chromatid breaks and gaps were seen in 14.9% of the cells, which is within normal limits (5-20%) for cells grown in medium 199. The majority of the breaks occurred in the short arm of chromosome No. 3 at 3p14 (Figs. 3c,d). The frequency of sister chro-

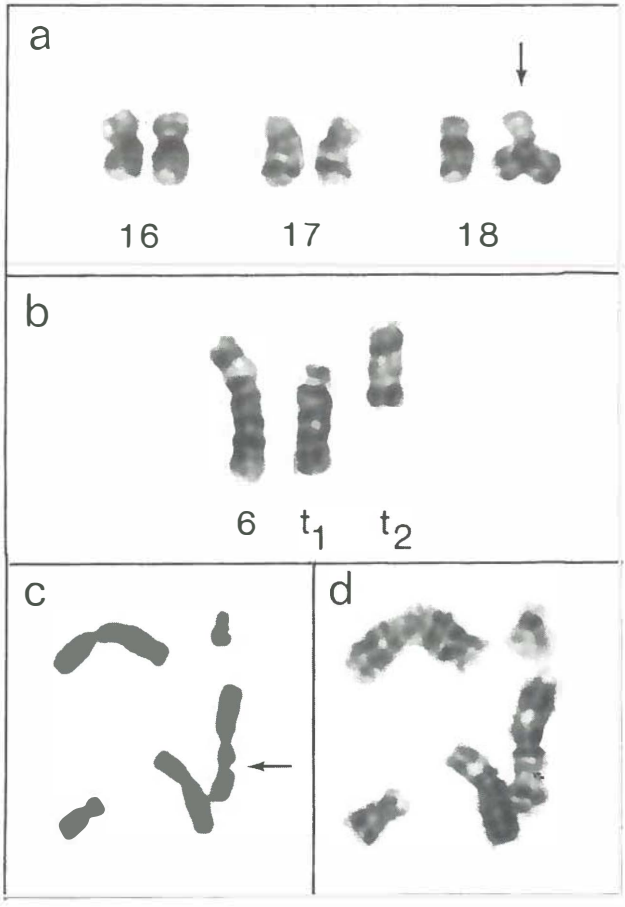

Fig. 3. Examples of the chromosomal aberrations present in cells of a patient with epidermodysplasia verruciformis, cultured in medium 199. a) Partial karyotype of a cell with a triradial chromosome No. 18 (arrow); b) normal chromosome No.6 and two abnormal elements (t1 and t2) probably derived from its homologue; c) chromosome No. 3, unbanded and d) after G banding, showing a spontaneous chromatid break in the short arm at 3p14 (arrow).

matid exchanges was 5.2/cell (normal range: 4-12/cell in this medium).
b) No aneuploidies, structural aberrations or breaks were found in cells cultured in RPMI 1640 medium. In the culture of photoirradiated cells 12% of the metaphases of the EV patient showed structural aberrations (dicentric chromosomes, ring chromosome, acentric fragment, etc.) and 14 % of the metaphases of the control.
c) The level of unscheduled DNA synthesis after photoirradiation of cultured fibroblasts was normal compared to the control.

DISCUSSION

The absence in our patient of T6+ and HLA-DR+ LCs in koilocytic areas of EV+ epidermis, koilocytosis being a HPV-specific cytopathic effect, suggests a deficient role of the LC in the HPV-specific cellular immune response. In ordinary warts, including those with an inflammatory lymphocytic reaction, the distribution of epidermal and dermal LCs is highly variable.[7] It has been postulated that a diminished density of epidermal and dermal LCs might be related to tolerance to HPV antigens.[7] The increase of epidermal thickness due to involvement with EV and the absence of T6+ and HLA-DR+ LCs in the koilocytic upper part of EV+ epidermis is reflected in the normal number of T6+ and HLA-DR+ LC/mm and the decreased number per mm^2 of EV+ epidermis. The LCs present in the lower histologically normal part of EV+ epidermis generally exhibited virtually complete loss of dendritic staining reactivity for OKT-6 compared to EV- epidermis. This suggests at least some effect, direct or indirect, of the HPV infection on the LCs. On the 8th consecutive day of retinoid treatment T6+ and HLA-DR+ LCs were observed to be present in the koilocytic areas, possibly due to this treatment. In an electron microscopic study of 3 EV patients treated with etretinate, signs of activation of epidermal LCs were also found.[23]

Non-HPV-specific CMI tests demonstrated an impaired response in our EV patient with cutaneous carcinomas, as evidenced by the decreased percentages of T-cells and helper-inducer T-cells, the diminished PHA-induced lymphoproliferative response in vitro and the abnormal DNCB sensitization test. The impairment in T-cell mediated immunity demonstrated by the in vitro test might be related to the decreased percentage of helper-inducer T-cells. Although a decreased ratio of helper/inducer to suppressor/cytotoxic T-cells was found in our patient, other EV cases showed no significant alterations.[2] Other contradictory results were found in natural killer cell activity, which was also reported to be normal by these authors,[2] while others noted an increase.[22] The role of HPV-specific CMI in EV has not yet been established. An increase concomitant with clinical regression followed by normalization of previously depressed non-HPV-specific CMI has been described.[15] Most of the presently available evidence does suggest that EV is related to impaired CMI, non-HPV-specific and/or HPV-specific.

Retinoid treatment appears to have given a temporary positive effect on the in vitro response to stimulation with the T-cell mitogens PHA and Con A. The response to stimulation with PWM, a T-cell dependent B-cell mitogen, and irradiated allogeneic lymphocytes, a basic stimulant, remained unaffected by retinoid treatment. In vitro tests, in which the main metabolite of etretinate was added to the culture medium, demonstrated an inhibitory effect on the PHA and Con A induced lymphoproliferative response.[4] Another clinico-immunologic study however of a patient with keratoacanthomas treated with etretinate also showed a positive effect on the PHA and Con A induced lymphoproliferative response.[6] Improvement of CMI may result from retinoid therapy itself, as is suggested in our case, but may also result from a decrease of HPV load.[9, 14, 15] No effect was seen of retinoid treatment on the CMI response in vivo of our patient, which concurs with the findings of others.[27] The clinical effect of retinoid therapy was found to be partial and temporary in 7 EV patients with reduction of viral content in only one patient.[23,27] Whether retinoid therapy has a preventive effect on carcinoma growth in EV patients, is not yet clear.

A remarkable and unexpected finding was the presence of microscopic EV pathology in two biopsies from clinically normal skin on the forehead of our patient. It is not understood how EV-specific HPVs, having been found only in rare EV and renal transplant patients, survive to reach their sporadic hosts. The observation that subclinical EV lesions do exist, albeit in an immunocompromised EV patient, may afford an explanation. HPV- DNA has been detected in clinically normal tissue adjacent to HPV-induced laryngeal papillomas and in clinically and histologically normal tissue adjacent to anogenital warts.[11, 32]

It has been hypothesized that EV belongs to the group of cell hypersensitivity or chromosomal instability diseases, such as xeroderma pigmentosum and ataxia teleangiectasia, because of the occurrence of multiple cutaneous carcinomas on sun-exposed areas in the phenotype infected with EV-specific HPVs associated with an impaired immune response.[25] No indication, however, has been found for an impaired DNA-repair mechanism after UV irradiation (cell hypersensitivity) - the basic abnormality in xeroderma pigmentosum - in cells of EV patients, including the patient described here.[29] Further studies in our EV patient have made it unlikely, that increased chromosomal instability is the cause of EV, as no overt signs of increased chromosomal instability, spontaneous or after UV irradiation, were observed. Medium 199 has a relatively low folate content, which usually results in a high percentage of cells with breaks and other chromosomal aberrations, as seen in the first PBL culture of our patient.[33] Site 3p14 on the short arm of chromosome No. 3, where the majority of the breaks of our patient occurred, is known to be a so-called common fragile site, probably present in almost all human beings.[31] Triradial configurations like the triradial chromosome No. 18 seen in one cell of our patient, are very rare in normal individuals, but occur more frequently

in patients with chromosome instability disorders. No breaks or structural aberrations were found, however, in cells cultured in RPMI 1640, a medium which is less permissive of spontaneous chromosome breakage.[33] Structural aberrations, the results of breakage, did occur after UV-irradiation, but in a percentage of cells similar to that of the control.

To our knowledge a total of 33 EV patients of African descent has been reported.[18, 19, 27, 28] In only two of these patients cutaneous carcinomas were observed.[18, 27] The natural protection of people with a black skin against actinic radiation may protect black EV patients against the development of cutaneous carcinomas. A relationship has been suggested between the carcinoma related phenotype in Africans and the presence of seborrhoeic keratoses,[18] also seen in our patient. Seborrhoeic keratoses, which appear to be strikingly absent in Africans, were noticed in 4 out of 6 cases of this phenotype.

In summary our investigations showed: 1) the absence of T6+ and HLA-DR+ LCs in the koilocytic areas of epidermis involved with EV; 2) microscopic EV lesions in clinically normal skin; 3) evidence of a stimulatory effect of retinoid treatment on impaired CM; 4) no evidence of increased spontaneous or UV-induced chromosomal instability and confirmation of the absence of cell hypersensitivity.

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REFERENCES

1. Androphy EJ, Dvoretzky I, Lowy DR. X-linked inheritance of epidermodysplasia verruciformis. Arch Dermatol 1985;121:864-8.
2. Androphy EJ, Dvoretzky I, Maluish AE, Wallace HJ, Lowy DR. Response of warts in epidermodysplasia verruciformis to treatment with systemic and intralesional alpha interferon. J Am Acad Dermatol 1984;11:197-202.
3. Bagot M, Heslan M, Dubertret L, Roujeau JC, Touraine R, Levy JP. Antigen-presenting properties of human epidermal cells compared with peripheral blood mononuclear cells. Br J Dermatol 1985;113:suppl 28:55-60
4. Bauer R, Orfanos CE. Effects of synthetic retinoids on human peripheral blood lymphocytes and polymorphonuclears in vitro. In: Cunliffe WJ, Miller AJ, eds. Retinoid therapy. Lancaster: MTP Press Ltd., 1984:101-18
5. Bleumink E, Nater JP, Schraffordt Koops H, The TH. A standard method for DNCB sensitization testing in patients with neoplasms. Cancer 1974;33:911-5.
6. Blitstein-Willinger E, Haas N, Nurnberger F, Stuttgen G. Immunological findings during treatment of multiple keratoacanthoma with etretinate. Br J Dermatol 1986;114:109-16.

7. Chardonnet Y, Viac J, Staquet MJ, Thivolet J. Cell-mediated immunity to human papillomavirus. Clinics in dermatology 1985;3:156-64.
8. Claudy AL, Touraine JL, Mitanne D. Epidermodysplasia verruciformis induced by a new human papillomavirus (HPV-8). Arch Dermatol Res 1982;274:213-9.
9. Craifemberghi S, Gavazzoni R, Coglio G, Pozzo G. Epidermodisplasia verruciforme di Lewandowsky-Lutz. Giorn It Derm Venereol 1982;117:191-4.
10. Faure M, Dezutter-Dambuyant C, Schmitt D, Gaucherand M, Thivolet J. Langerhans cell induced cytotoxic T-cell responses against normal human epidermal cell targets: in vitro studies. Br J Dermatol 1985;113;suppl 28:114-7.
11. Ferenczy A, Mitao M, Nagai N, Silverstein SJ, Crum CP. Latent papillomavirus and recurrent genital warts. N Engl J Med 1985;313:784-8.
12. Gassenmaier A, Lammel M, Pfister H. Molecular cloning and characterization of the DNAs of human papillomaviruses 19, 20 and 25 from a patient with epidermodysplasia verruciformis. J Virol 1984;52:1019-23.
13. Giessen M van der, Postma S, The TH. Isolation of highly purified lymphocyte subsets for functional studies by means of an indirect rosette technique. Scand J Immunol 1985;22:41-9.
14. Guilhou JJ, Malbos S, Barneon S, Habib A, Baldet P, Meynadier J. Epidermodysplasie verruciforme. Etude immunologique. Ann Dermatol Venereol 1980;107:611-9.
15. Haftek M, Jablonska S, Orth G. Specific cell-mediated immunity in patients with epidermodysplasia verruciformis and plane warts. Dermatologica 1985;170:213-20.
16. Herberman RB. Possible role of natural killer cells and other effector cells in immune surveillance against cancer. J Invest Dermatol 1984;83:137s-40s.
17. Jablonska S, Orth G, Lutzner MA. Immunopathology of human papillomavirus-induced tumors in different tissues. Springer Semin Immunopathol 1982;5:33-62.
18. Jacyk WK, Subbuswamy SG. Epidermodysplasia verruciformis in Nigerians. Dermatologica 1979;159:256-65.
19. Jacyk WK, Lechner W. Epidermodysplasia verruciformis in lepromatous leprosy. Dermatologica 1984;168:202-5.
20. Jong MCJM de, Blanken R, Nanninga J, Voorst Vader PC van, Poppema S. Defined in situ enumeration of T6 and HLA-DR expressing epidermal Langerhans cells. I: morphological and methodological aspects. J Invest Dermatol 1986:in press.
21. Kallenberg CGM, Voort-Beelen JM van der, Amaro J de, The TH. Increased frequency of B8/DR3 in scleroderma and association of the haplotype with impaired cellular immune response. Clin Exp Immunol 1981;43:478-85.
22. Kaminski M, Pawinska M, Jablonska S, Szmurlo A, Majewski S, Orth G. Increased natural killer cell activity in patients with epidermodysplasia verruciformis. Arch Dermatol 1985;121:84-6.
23. Kanerva LO, Johansson E, Niemi KM, Lauharanta J, Salo OP. Epidermodysplasia verruciformis. Clinical and light- and electron-microscopic observations during etretinate therapy. Arch Dermatol Res 1985;278:153-60.
24. Kienzler JL, Laurent R, Coppey J, Favre M, Orth G, Coupez L, Agache P. Epidermodysplasie verruciforme. Ann Dermatol Venereol 1979;106:549-3.
25. Kraemer KH. Heritable diseases with increased sensitivity to cellular injury. In: Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF, eds. Update: dermatology in general medicine. New York: McGraw-Hill, 1983:113-40.
26. Kremsdorf D, Favre M, Jablonska S, Obalek S, Rueda LA, Lutzner MA, Blanchet-Bardon C, Voorst Vader PC van, Orth G. Molecular cloning and characterization of the genomes of nine newly recognized human papillomavirus types associated with epidermodysplasia verruciformis. J Virol 1984;52:1013-8.
27. Lutzner MA, Blanchet-Bardon C, Orth G. Clinical observations, virologic studies and treatment trials in patients with epidermodysplasia verruciformis, a disease induced by specific human papillomaviruses. J Invest Dermatol 1984;83:18s-25s.
28. Pfister H, Nurnberger F, Gissmann L, Hausen H zur. Characterization of a human papillomavirus from epidermodysplasia verruciformis lesions of a patient from Upper-Volta. Int J

Cancer 1981;27:645-50.
29. Proniewska M, Jablonska S. UV-induced DNA repair synthesis in patients with epidermodysplasia verruciformis. Dermatologica 1980;160:289-96.
30. Scheres JMJC. Identification of two Robertsonian translocations with a Giemsa banding technique. Hum Genet 1972;15:253-6.
31. Smeets DFCM, Scheres JMJC, Hustinx TWJ. The fragile site on chromosome 3. Hum Genet 1984;67:351.
32. Steinberg BM, Topp WC, Schnieder PS, Abramson AL. Laryngeal papillomavirus infection during clinical remission. N Engl J Ned 1983;308:1261-4.
33. Sutherland GR, Hecht F. Fragile sites on human chromosomes. New York: Oxford University Press, 1985.
34. Verweij H, Voorst Vader PC van, Houthoff HJ, Gips CH. Quantitative analysis of retinoids in human serum and tissue samples using high performance liquid chromatography. In: Saurat JH, ed. Retinoids: new trends in research and therapy. Basel: Karger, 1985:301-4.
35. Voorst Vader PC van, Orth G, Dutronquay V, Driessen LHHM, Eggink HF, Kallenberg CGM, The TH. Epidermodysplasia verruciformis: skin carcinoma containing human papillomavirus type 5 DNA sequences and primary hepatocellular carcinoma associated with chronic hepatitis B virus infection in a patient. Acta Dermato-Venereol 1986:in press.
36. Voorst Vader PC van, Jaspers NGJ, Kamp AWM van der. Retinoic acid and defective UV light induced DNA excision repair in xeroderma pigmentosum: absence of ameliorating effect. Arch Dermatol Res 1984;276:201-2.
37. Wojtulewicz-Kurkus J, Glinski W, Jablonska S, Podobinska I, Obalek S. Identification of HLA antigens in familial and non-familial epidermodysplasia verruciformis. Dermatologica 1985;170:53-8.

Chapter 3.2

Epidermodysplasia verruciformis

Skin carcinoma containing human papillomavirus type 5 DNA sequences and primary hepatocellular carcinoma associated with chronic hepatitis B virus infection in a patient

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SUMMARY

In a case of epidermodysplasia verruciformis with impaired cell mediated immunity and multiple skin cancers human papilloma virus type 5 (HPV5) DNA sequences were demonstrated in a cutaneous squamous cell carcinoma. HPV5 and HPV8 were detected in the benign disseminated skin lesions together with three newly characterized HPVs: HPV17, HPV19 and HPV24. A chronic infection with hepatitis B virus resulting in macronodular cirrhosis asociated with a primary hepatocellular carcinoma was also acquired by this patient. This case provides an example of the circumstantial evidence, which suggests that certain types of HPV are potentially oncogenic and stresses the importance of immune surveillance in the protection against virus-associated tumors.

INTRODUCTION

Epidermodysplasia verruciformis (EV), described as a new entity in 1922, is caused by a generalized persistent cutaneous infection, usually acquired in childhood, with human papilloma viruses (HPVs).[1] The disease is associated with an impaired non-HPV-specific cell mediated immune response,[2] although exceptions have been reported.[3,4] The viral aetiology of EV was established in 1966 by electron microscopic studies.[5] Recently the association of EV with a plurality of specific HPV types has been demonstrated.[6-9] The hypothesis that certain HPV types are potentially oncogenic, a well known characteristic of several animal papillomaviruses,[10] has received a great deal of attention lately.[11-15] This hypothesis is strongly suggested by the data concerning EV. In about 30% of 147 EV patients reported cutaneous intraepithelial and invasive squamous cell carcinomas were described, usually multiple and loca-

ted on sun-exposed areas, with an average age of onset of 31 years.[1] The genome of HPV type 5 (HPV5) has been detected in primary [16-18] and metastatic [17] skin carcinomas of EV patients, suggesting that HPV5 has an oncogenic potential.

We describe here a case of EV with a persistent hepatitis B virus infection, in which the development of primary carcinomas of the skin as well as the liver was observed. This represents a follow-up study of the patient in whom the presence of papilloma virus particles in EV lesions was demonstrated for the first time. [5] We report the characterization of the HPVs in the benign skin lesions and the search for HPV DNA sequences in a cutaneous carcinoma.

PATIENT AND METHODS

Patient

A 22-year-old Caucasian male patient with EV since childhood was seen for the first time at the Department of Dermatology in 1965.[5] His skin showed multiple verruca plana-like lesions, partly giving rise to red hyperkeratotic plaques, on the extensor side of the hands and knees, disseminated red-brown macular pityriasis versicolor-like lesions on the trunk and extremities, and greyish-brown hyperkeratotic lenticular lesions on the face. He died in 1981 at the age of 38. During these 16 years the extent and morphology of the lesions did not change markedly, except on the face and scalp, where multiple malignant lesions developed from the age of 22. He was institutionalized elsewhere because of mental retardation. There was no parental consanguinity. One cousin was mentally retarded. The mother died of lung carcinoma. There is one healthy male sibling.

The histology of a lesion on the trunk was typical of EV, showing large clear cells with keratohyalin granules in the upper layers of the epidermis.[5] Intraepithelial and invasive squamous cell carcinomas and keratosis actinica-like changes were observed on face and scalp.[19] Intranuclear viral particles were demonstrated by electron microscopy in the upper layers of the epidermis in benign lesions [5] and in a carcinoma in situ,[20] but could not be detected in an invasive squamous cell carcinoma. [20]

In 1972 positive antibody responses were obtained after immunization with the primary test immunogen Helix pomatia Haemocyanin [21] and the following recall antigens: diphtheria, tetanus and typhoid O and H. Subsequent skin tests with Helix pomatia Haemocyanin, diphtheria, tetanus, tuberculin, candida and mumps were negative. A positive reaction however was obtained after intradermal injection of streptokinase-dornase. Blood chemistry and morphology were normal. In June 1980 a screening for internal disease because of ankle oedema and ascites revealed HBs antigenaemia (HBsAg+, anti HBs-), moderate elevation of the values of alkaline phosphatase, lactate dehydrogenase and γ-glutamyl transferase, normal values of transaminases and a lowered albumin level. The oedema and ascites disappeared after institution of diuretic therapy and dietary measures, but the laboratory abnormalities persisted. In December 1980, a serologic investigation for hepatitis B virus infection gave the following results: HBsAg+, anti HBs-, HBeAg+, anti HBe-, anti HBc+. The α-fetoprotein level was 266 ng/ml (normal: ≤20 ng/ml). In April 1981 ascites developed again. In May 1981 an hepatic tumor was palpable, which was confirmed by intravenous technetium colloid scintigraphy, showing a large defect in the left lobe of the liver. In November 1981, aged 38, the patient died because of hepatic insufficiency. Post mortem examination of the liver by light microscopy demonstrated a macronodular cirrhosis associated with a primary hepatocellular carcinoma. Using peroxidase conjugated anti-HBs, anti-HBc

and anti-HBe immunoglobulins (Organon, Oss, the Netherlands) HBs antigen was observed in some non-malignant cells, but not in the hepatocellular carcinoma. HBc and HBe antigens were not observed.

Methods

Scrapings were taken from the back of the hands, which showed mainly confluent verruca plana-like lesions, and from the back of the trunk, which showed pityriasis versicolor-like lesions. Seven months later, at the postmortem examination, scrapings were taken again from the hands, breast and back. A cutaneous carcinoma was excised from the forehead. The scrapings and part of the skin tumor were snap-frozen in liquid nitrogen and stored at -70°C. Part of the cutaneous carcinoma was embedded in paraffin for routine light microscopy and for immunoperoxidase studies. The skin tumor was screened for papillomavirus genus-specific antigen by the peroxidase-anti-per-oxidase method, using a rabbit antiserum against bovine papillomavirus type 1 disrupted particles.[2,12]

Viral DNA was selectively extracted from the benign skin lesions and total cellular DNA was extracted from the skin and liver carcinomas as previously described.[6-8,12,16] The ulcerated central part of the cutaneous tumor and the peripheral part were processed separately. DNAs were cleft with restriction endonucleases Pst1, BamHI, EcoRI and HindII and the fragments were separated by agarose gel electrophoresis and visualized by staining with ethidium bromide. DNA fragments were denatured in situ, transferred to "Gene Screen" hybridization transfer membrane (New England Nuclear) and hybridized with ^{32}P-labelled cloned probes specific for skin wart HPVs (HPV1,2,4)[11] and EV HPVs (HPV3,5,8,9,10,12,14,15,17,19 to 24).[6-8] All procedures have been described previously.[6-8,12,16] A search for hepatitis B virus DNA in the hepatocellular carcinoma was precluded by degradation of the liver cell DNA.

RESULTS

In DNA extracted from benign skin lesions two HPV DNAs (HPVX and Y) with Pst1 (Fig. 1a,b), BamHI, EcoRI and HindII endonuclease cleavage patterns distinct from those reported for already recognized HPVs were detected in large quantities on ethidium bromide-stained agarose gels. HPVX was found to be prominent on the hands and back (fig. 1a), HPVY on the breast (Fig. 1b). Molecular cloning of the genomes of these viruses led to their characterization as new types, HPV19 and HPV24 respectively, on the basis of less than 50% DNA sequence homology with the genomes of other HPVs, as determined by liquid phase hybridization followed by S1 nuclease analysis.[8] HPV19 shows some DNA sequence homology (6-28%) with the members of a group of related EV-specific HPV types (HPV5,8,12,14,20 to 23) and no or almost no detectable sequence homology (4%) with other HPVs, including other EV associated HPVs: HPV3 and HPV10 found also in flat warts of the general population, another group of related EV-specific HPVs (HPV9,15,17) and HPV24.[8] HPV24 shows no or almost no detectable sequence homology with any of the other 23 types of HPVs.[8]

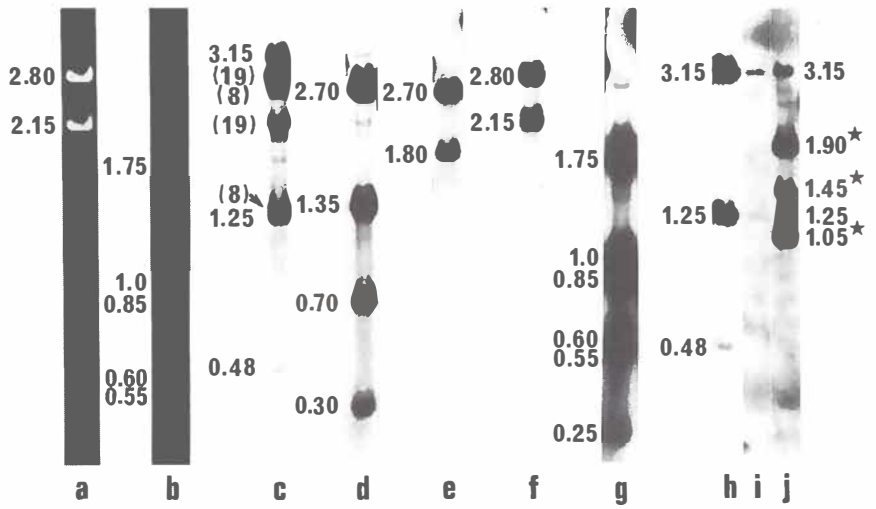

Fig. 1. Identification of HPV types associated with benign lesions and a skin cancer in an EV patient. DNAs extracted from benign lesions of the back (a), the breast (b) and both locations (c-g) and from the center (i) and the periphery (j) of a skin cancer were cleaved with Pst1 endonuclease and run into an agarose gel (1 μg per lane except for 5 μg in lane j). As a control (h) DNA extracted from the benign lesions of an EV patient infected with prototypical HPV5 [6] (0.5 ng) was run in the same gel as the skin cancer samples. Viral DNA fragments were detected after ethidium bromide staining (lanes a,b) or after transfer to a membrane (lanes c-j) and hybridization with ^{32}P-labelled cloned probes specific for HPV5 (c, h-j), HPV8 (d), HPV17 (e), HPV19 (f) and HPV25 (g). Labelled fragments were detected after exposure on a Kodak AR X-Omat film for 4 hours (f), 24 hours (c,d,g,), 3 days (e) or 6 days (h-j). Numerals indicate molecular weights of fragments expressed in megadaltons (Md) corresponding to HPV5 (c,h-j), HPV8 (d), HPV17 (e), HPV19 (a,f) and HPV24 (b,g). The smallest HPV5 Pst1 fragment (0.29 Md)[6] ran out of the gels. Numbers in brackets indicate the types of the viruses whose fragments are revealed with the HPV5 probe. Stars indicate the extra bands found in the sample from the cancer periphery.

Three other HPVs (HPV5,8,17), yielding barely detectable bands on ethidium bromide-stained gels (Fig. 1a,b), were detected together with HPV19 and 24 in pooled scrapings after blot hybridization with the different HPV cloned probes (figs. 1c to g).

The cutaneous carcinoma was shown to be an invasive squamous cell carcinoma by light microscopy. Small foci of large clear cells, i.e. with the features of the cytopathic effect associated in benign lesions with vegetative DNA replication of EV HPVs,[16] were observed in the epidermis overlying the peripheral portion of the cancer. No group specific papillomavirus antigens were detected in the tumor sections studied.

In the total DNA extracted from the central necrotic part of the tumor and cleaved with different endonucleases, bands with the mobilities expected for

HPV5 DNA fragments were detected after blot hybridization with the HPV5 probe (Fig. 1h,i). No evidence for the presence of the other HPV types found in the benign lesions, i.e. HPV8,17,19 or 24, was obtained using specific probes.

In the DNA extracted from the peripheral portion of the tumor bands with mobilities distinct from HPV5 fragments were detected with the HPV5 probe in addition to HPV5 specific DNA bands (Fig. 1j). Blot hybridization of this DNA preparation with different HPV probes revealed the specific cleavage products of HPV17 DNA and, in trace amounts, of HPV24 DNA (data not shown), originating, most probably, from benign lesions present in the epidermis covering the peripheral portion of the carcinoma. It is unlikely that the additional bands labelled by the HPVs, probe correspond to HPV17 or 24 fragments since they have distinct molecular weights (Fig. 1e,g,j) and since there is almost no cross-hybridization between HPV5, 17 and 24 DNAs.[8] They could correspond to rearranged HPV5 genomes as observed for some EV carcinomas (Orth G et al, unpublished results)[17] or to a sixth HPV, partially related to HPV5, but different from the known HPV types and overlooked in the DNA preparations obtained from pooled scrapings of benign lesions.

DISCUSSION

In 1979 two clinical phenotypes of EV were distinguished in a study of 14 patients.[22] Disseminated verrucae planae on the face and extremities caused by an infection with HPV3 or a related virus, now recognized as HPV10,[7] characterized one phenotype. In the other multiple verruca-plana like lesions on the extremities and the face together with pityriasis versicolor-like lesions on the trunk were oberved in conjunction with an infection with HPV5 or HPV5-related viruses. Only this latter phenotype, to which about 75% of EV patients belong,[1] appeared to be cancer associated. Our patient clearly belongs to this classical phenotype of EV. At least thirteen other HPVs besides HPV5 have been identified since 1979, i.e. HPV8, 9, 12, 14, 15, 17 and 19 to 25, in the benign lesions of EV patients with pityriasis versicolor-like lesions originating from different parts of the world (Orth G et al, unpublished results).[6-9] Often several HPVs were detected in one patient, as in the patient described. These HPVs have not been found so far in the general population, in contrast to HPV3 and 10, which induce flat warts.[7] HPV3-induced flat warts were also found in some EV patients with pityriasis versicolor-like lesions.[6,7,22]

The detection of HPV5 DNA sequences in a cutaneous invasive squamous cell carcinoma in our case of EV supports the hypothesis of the potential oncogenicity of some specific HPVs. HPV5 DNA has been reported to be pre-

sent in the carcinomas of three EV patients [16-18] and the DNA of HPV5, and less frequently of HPV8 and HPV14,[23] has recently been found in the DNA extracted from cutaneous carcinomas of 14 other EV patients (Orth G et al, unpublished results). HPV3-related sequences were found in a vulvar carcinoma in situ of an EV patient with disseminated verrucae planae.[24] In addition the presence of the HPV5 genome has been reported in two skin cancers of a renal allograft recipient with skin lesions resembling the pityriasis versicolor- like lesions of EV.[12] The cutaneous malignancies of that patient developed on sun-exposed skin, as is the case in the large majority of EV patients with skin carcinomas. Therefore sunlight is suggested as a co-carcinogenic factor.[1,12,19]

Mental retardation of unknown cause, as observed in the case presented, has been described in 8% of 147 EV patients reported.[1] Although sporadic cases constitute the majority of EV patients, the occurrence of familial cases and parental consanguinity and the association with mental retardation suggest a genetic factor in the pathogenesis of EV.[1,23,25] This genetic factor may be responsible for an immunologically determined persistence of HPV infection in EV patients. Impairment of the non-HPV-specific cell mediated immune response in our case of EV seems highly probable in view of the negative skin tests performed almost ten years before death. Whether this impairment, which has been frequently reported in EV patients,[2,16] is primary or secondary to the HPV infection is unknown however. When a cellular immune deficiency exists, this not only predisposes to protracted viral infections, but may also facilitate virus-induced tumor growth.[26,27] This seems illustrated in our patient, one of the rare cases of EV with long term follow-up data available,[1,25] by the simultaneous occurrence of an HPV5-associated cutaneous carcinoma and an hepatitis B virus-associated primary hepatocellular carcinoma. The occurrence of a probable Burkitt's lymphoma, an Epstein-Barr virus associated malignant lymphoma, has also been reported in another EV patient.[1]

A persistent hepatitis B virus infection, characterized by HBs antigenaemia and lack of anti HBs formation, which was also apparent in our patient, seriously aggravates the risk of the development of primary hepatocellular carcinoma.[28] Most cases of primary hepatocellular carcinoma in developed countries arise in a liver already affected with cirrhosis. Hepatitis B virus antigens are rarely found in the carcinomas, but can be seen in non carcinomatous parts of the liver, as in our case. The presence of hepatitis B virus DNA in hepatocellular carcinomas developing in HBs Ag positive and even in HBsAg negative subjects supports the association of the virus with the tumor.

Our observations support the hypothesis of a synergism between a persistent infection with specific HPV types, cellular immune deficiency and sunlight in the pathogenesis of squamous cell carcinoma of the skin in epidermo-

dysplasia verruciformis and stress the importance of immune surveillance in the protection against virus-associated tumor growth.

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REFERENCES

1. Lutzner MA. Epidermodysplasia verruciformis: an autosomal recessive disease characterized by viral warts and skin cancer. Bull Cancer 1978;65:169-82.
2. Jablonska S, Orth G, Lutzner MA. Immunopathology of papillomavirus-induced tumors in different tissues. Springer Semin Immunopathol 1982;5:33-62.
3. Kienzler JL, Laurent R, Coppey J, Favre M, Orth G, Coupez L, Agache P. Epidermodysplasie verruciforme. Donnees ultrastrucrales, virologiques et photobiologiques: a propos d'une observation. Ann Dermatol Venereol 1979;106:549-63.
4. Claudy AL, Touraine JL, Mitanne D. Epidermodysplasia verruciformis induced by a new human papillomavirus (HPV-8). Arch Dermatol Res 1982;274:213-9.
5. Ruiter M, Mullem PJ van. Demonstration by electron microscopy of an intranuclear virus in epidermodysplasia verruciformis. J Invest Dermatol 1966;47:247-52.
6. Kremsdorf D, Jablonska S, Favre M, Orth G. Biochemical characterization of two types of human papillomaviruses associated with epidermodysplasia verruciformis. J Virol 1982;43:436-47.
7. Kremsdorf D, Jablonska S, Favre M, Orth G. Human papillomaviruses associated with epidermodysplasia verruciformis. II. Molecular cloning and characterization of human papillomavirus 3a, 8, 10 and 12 genomes. J Virol 1983;48:340-51.
8. Kremsdorf D, Favre M, Jablonska S, Obalek S, Rueda LA, Lutzner MA, Blanchet-Bardon C, Voorst Vader PC van, Orth G. Molecular cloning and characterization of the genomes of nine newly recognized human papillomavirus types associated with epidermodysplasia verruciformis. J Virol 1984;52:1013-18.
9. Gassenmaier A, Lammel M, Pfister H. Molecular cloning and characterization of the DNAs of human papillomaviruses 19, 20 and 25 from a patient with epidermodysplasia verruciformis. J Virol 1984;52:1019-23.
10. Lancaster WD, Olson C. Animal papillomaviruses. Microbiol Rev 1982;46:191-207.
11. Lutzner MA. The human papillomaviruses: a review. Arch Dermatol 1983;119:631-5.
12. Lutzner MA, Orth G, Dutronquay V, Ducasse MF, Kreis H, Crosnier J. Detection of human papillomavirus type 5 DNA in skin cancers of an immunosuppressed renal allograft recipient. Lancet 1983;ii:422-4.
13. Durst M, Gissmann L, Ikenberg H, Hausen H zur. A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. Proc Natl Acad Sci USA 1983;80:3812-5.
14. Crum CP, Ikenberg H, Richart RM, Gissmann L. Human papillomavirus type 16 and early cervical neoplasia. N Engl J Med 1984;310:880-3.
15. Boshart M, Gissmann L, Ikenberg H, Kleinherz A, Scheurlen W, Hausen H zur. A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. EMBO J 1984;3:1151-7.
16. Orth G, Favre M, Breitburd F, Croissant O, Jablonska S, Obalek S, Jarzabek-Chorzelska M, Rzesa G. Epidermodysplasia verruciformis: a model for the role of papillomaviruses in human cancer. Cold Spring Harbor Conf Cell Proliferation 1980;7:259-82.

17. Ostrow RS, Bender M, Niimura M, Seki T, Kawashima M, Pass F, Faras AJ. Human papillomavirus DNA in cutaneous primary and metastasized squamous cell carcinomas from patients with epidermodysplasia verruciformis. Proc Natl Acad Sci USA 1982;79:1634-8.
18. Pfister H, Gassenmaier A, Nurnberger F, Stuttgen G. Human papilloma virus 5 DNA in carcinoma of an epidermodysplasia verruciformis patient infected with various human papillomavirus types. Cancer Res 1983;43:1436-41.
19. Ruiter M, Mullem PJ van. Further histological investigations on malignant degeneration of cutaneous lesions in epidermodysplasia verruciformis. Acta Derm Venereol (Stockh) 1970;50:205-11.
20. Ruiter M, Mullem PJ van. Behaviour of virus in malignant degeneration of skin lesions in epidermodysplasia verruciformis. J Invest Dermatol 1970;54:324-31.
21. Kallenberg CGM, Toresma R, The TH. The immune response to primary immunogens in man. In: Reevs WG, ed. Recent developments in clinical immunology. Amsterdam: Elsevier Biomedical Press, 1984:1-26.
22. Orth G, Jablonska S, Jarzabek-Chorzelska M, Obalek S, Rzesa G, Favre M, Croissant O. Characteristics of the lesions and risk of malignant conversion associated with the type of human papillomavirus involved in epidermodysplasia verruciformis. Cancer Res 1979;39:1074-82.
23. Lutzner MA, Blanchet-Bardon C, Orth G. Clinical observations, virologic studies and treatment trials in patients with epidermodysplasia verruciformis, a disease induced by specific human papillomaviruses. J Invest Dermatol 1984;82:18s-25s.
24. Green M, Brackmann KH, Sander PR, Loewenstein PM, Freel JH, Eisinger M, Switlyk SA. Isolation of a human papillomavirus from a patient with epidermodysplasia verruciformis: presence of related viral DNA genomes in human urogenital tumors. Proc Natl Acad Sci USA 1982;79:4437-41.
25. Jablonska S, Orth G, Jarzabek-Chorzelska M, Glinski W, Obalek S, Rzesa G, Croissant O, Favre M. Twenty-one years of follow-up studies of familial epidermodysplasia verruciformis. Dermatologica 1979;158:309-27.
26. Klein G, Klein E. Immune surveillance against virus-induced tumors and non-rejectability of spontaneous tumors: contrasting consequences of host versus tumor evolution. Proc Natl Acad Sci USA 1977;74:2121-5.
27. Herberman RB. Possible role of natural killer cells and other effector cells in immune surveillance against cancer. J Invest Dermatol 1984;83:137s-40s.
28. Arthur MJP, Hall AJ, Wright R. Hepatitis B, hepatocellular carcinoma and strategies for prevention. Lancet 1984;i:607-10.

Chapter 4

Hepatologic side effects of retinoid therapy

Chapter 4.1

Etretinate (Tigason) hepatitis in 2 patients

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SUMMARY

A histologically confirmed, clinically inapparent and reversible hepatitis occurred in 2 patients (1 psoriasis, 1 basal cell nevus syndrome) within the first months after introduction of etretinate therapy. Causes of hepatitis other than etretinate were not found. Reintroduction of etretinate resulted in reactivation and/or persistance of the hepatitis in both patients. These data strongly suggest that the hepatitis in both patients was caused by etretinate. Later the basal cell nevus syndrome patient was given 13-cis-retinoic acid, which caused no liver test disturbances during a follow-up period of 6 months.

INTRODUCTION

A transient, slight elevation of one or sometimes more liver tests can be observed in patients on etretinate (Tigason, Ro 10-9359) therapy [1,6] and at least in some patients appears to be provoked by etretinate itself. Very mild damage of the parenchymal tissue in the liver biopsy of such a patient has been reported.[9] A histologically confirmed hepatitis supposedly due to etretinate has been described in only 3 patients so far.[1,8]

We report 2 patients (1 psoriasis, 1 basal cell nevus syndrome) with a biopsy-proven hepatitis during etretinate therapy. The absence of other causes of hepatitis, the time course of the hepatitis in relation to etretinate administration and the results of a provocation test all suggest that the hepatitis was actually caused by etretinate itself.

In contrast with etretinate, 13-cis-retinoic acid did not provoke liver test disturbances during a follow-up period of 6 months in the basal cell nevus syndrome (BCNS) patient.

CASE REPORTS

Patient A

Patient A, female, aged 70 years, height 160 cm, weight 53 kg, with BCNS with multiple basal cell carcinomas and a slight hepato- splenomegaly without known cause, first observed in 1974 on a intravenous technetium colloid scintigraphy, was started on etretinate, 1 mg/kg/day, on August 4,

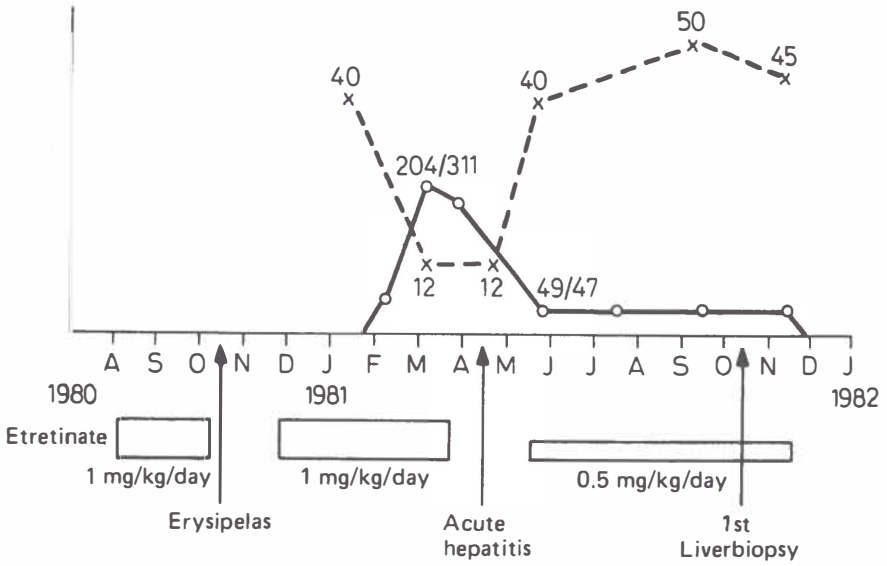

Fig. 1. Patient A, BCNS. Number of basal cell carcinomas and values of transaminases during and in between the second (high-dose) and third (low-dose) course of etretinate therapy. The first liver biopsy, performed during the third course, demonstrated signs of a late stage of acute hepatitis. o=transaminases (U/L), SGOT/SGPT; normal values at baseline. x=number of basal cell carcinomas.

1980 (Fig. 1). October 9, 1980, this first course of etretinate was discontinued because of erysipelas. November 27, 1980, etretinate was started again at the same dose and discontinued on March 25, 1981, because of rather marked liver test disturbances which first became apparent at the monthly control of liver tests on February 3, 1981. During this second course of etretinate the number of clinically apparent basal cell carcinomas diminished from about 40 to 12. However, on May 12, 7 1/2 weeks after discontinuation of the second course of etretinate, the number of clinically apparent basal cell carcinomas had increased to about 40 again. Most of these lesions were recurrences, but newly developed lesions were discerned as well. As liver tests had almost normalized except for some elevation of transaminases (SGOT49 U/L, SGPT47 U/L), a third course of etretinate was started on May 12, 1981, 0.5 mg/kg/day, to try to arrest, be it only partially, this explosive growth of basal cell carcinomas. This low dose of etretinate, however, did not influence the number of basal cell carcinomas. The number even increased to about 50. This third course of etretinate was continued from May 12 until November 13, 1981. During this period the elevation of transaminases persisted in the same range, only to become normal immediately after discontinuation of etretinate. Apart from cheilitis there were no physical complaints related to liver dysfunction or etretinate therapy at any moment during this study. Alcohol was shunned by this patient.

The disturbances of the liver tests concerned transaminases, lactic dehydrogenase, alkaline phosphatase, gamma-glutamyl transferase and anti-thrombin III. Peak values were observed on March 11, 1981: SGOT204 U/L (normal: up to 40 U/L), SGPT311 U/L (30 U/L), LDH298 U/L (235 U/L), AF166 U/L (120 U/L) and gamma-GT52 U/L (45 U/L). The values of the transaminases were the first to rise and the last to become normal. Serum albumin, bilirubin, cholesterol and

triglyceride levels were never abnormal. Coagulation studies comprising pro-thrombin time (Quick), kaoline- cephaline time (Proctor-Rappaport), fibrinogen (Leclerc) and anti- thrombin III activity demonstrated a decrease of anti-thrombin III activity on April 22: 76% (normal 80-120%), May 21: 65% and June 23: 73%, 1981. In July and October 1981 the anti-thrombin III activity was normal, to reach a level of 95% in February 1982.

May 1981 and repeatedly later on HBsAg and anti-HBs tests were negative. Serological tests excluded hepatitis A. Auto-antibodies to parietal cells, smooth muscle, mitochondria and liver membrane antigen were absent in May 1982.

A liver biopsy was performed on October 20, 1981, during the third low-dose course of etretinate 7 months after the acute hepatitis and repeated on February 16, 1982, and September 28, 1982. The first liver biopsy showed signs of a late stage of acute hepatitis consisting of an inflammatory, predominantly lymphocytic infiltrate in the portal spaces, sparse liver cell necrosis and small lymphocytic infiltrates in the parenchyma. Steatosis was not observed. The periodic acid-Schiff stain showed many macrophages in the portal spaces and some in the parenchyma. Examination of frozen sections by fluorescence microscopy demonstrated rapidly fading yellow autofluorescence similar to vitamin A fluorescence perisinusoidally. The total hepatic vitamin A level, measured by the extraction method and high performance liquid chromatography, was normal: 249 μg/g wet liver tissue (<300).

Control fluorescence microscopy was performed using etretinate, its main metabolite (Ro 10-1670) and retinoic acid, added to either water or dichloromethane under a cover glass. Selective excitation within the range of the excitation maximum of FITC (490 nm) using a XBO 75/2W light source resulted in visually different shades of yellow autofluorescence. Crystals of etretinate in water showed yellow-orange autofluorescence (++), which was also seen at the rim of an air bubble in a solution of etretinate in dichloromethane. Crystals of Ro 10-1670 in dichloromethane showed bright yellow autofluorescence (+++) and crystals of retinoic acid in dichloromethane showed pale yellow autofluorescence (+/++).

The second liver biopsy, 11 months after the acute hepatitis, showed a similar histological picture as the first biopsy, but less pronounced. Fluorescence microscopy on frozen sections of this second biopsy demonstrated some yellow autofluorescent material in the form of small perisinusoidal droplets. Histological examination of the third biopsy, 18 months after the acute hepatitis, showed no inflammatory reaction at all. All three biopsies demonstrated portal fibrosis, but no apparent increase in time was noted.

October 1, 1982, 13-cis-retinoic acid was started, 1 mg/kg/day. During a follow-up period of 6 months all liver tests remained unchanged and within normal limits.

Patient B

Patient B, female, aged 61 years, with psoriasis vulgaris, was started on etretinate, 1 mg/kg/day, on September 9, 1981. Liver test disturbances were first noted on December 12, 1981 (Fig. 2). January 28, 1982, etretinate was discontinued because of rather marked liver test disturbances. There were no physical complaints related to liver dysfunction and there was no use of alcohol. Peak values were observed on January 14: SGOT 314 U/L, SGPT 476 U/L, LDH 321 U/L, AF 147 U/L and gamma-GT 73 U/L. The values of the transaminases were the first to rise and the last to become normal. Serum albumin value was normal in December 1981, decreased in January 1982 and normal again in February. Bilirubin, cholesterol and triglyceride levels were never abnormal. Repeated coagulation studies, using the same tests as performed in patient A, were normal. Serological tests for HBsAg and anti-HBs were repeatedly negative as were tests for cytomegalovirus, Epstein- Barr virus and hepatitis A virus infection. Auto-antibodies to parietal cells and mitochondria were absent, but were present against smooth muscle and liver membrane antigen (IgM). Ja-

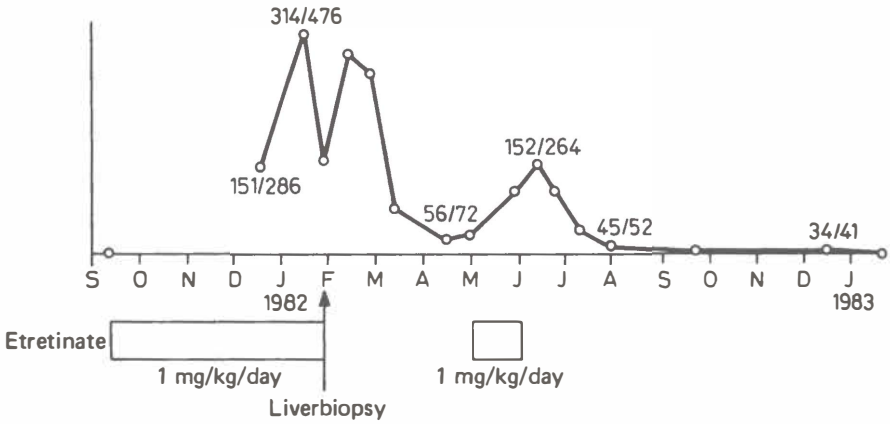

Fig. 2. Patient B, psoriasis vulgaris. Values of transaminases in relation to etretinate therapy and to a provocation test with etretinate. A liver biopsy performed at the end of the etretinate treatment course demonstrated acute hepatitis. o=transaminases (U/L), SGOT/SGPT; normal values at baseline.

nuary 1983, auto-antibodies against smooth muscle and liver membrane antigen (IgA positive, IgM and IgG negative) were still present.

A liver biopsy performed on January 28, 1982, showed an acute hepatitis with a localized inflammatory reaction, predominantly lymphocytic, in some portal spaces and at some places in the parenchyma. Sparse liver cell necrosis was observed. There was no steatosis. The PAS-stain showed many macrophages in the parenchyma and also in the portal spaces (Fig. 3). Fluorescence microscopy on frozen sections was negative.

April 28, 1982, 3 months after discontinuation of etretinate, liver tests were normal except for some elevation of SGOT(62 U/L) and SGPT(86 U/L). In order to ascertain whether the patient could tolerate etretinate or not, the drug was reintroduced at 1 mg/kg/day having obtained informed consent from the patient. As transaminase levels immediately rose again (May 12: SGOT80 U/L, SGPT152 U/L; May 26: SGOT116 U/L, SGPT201 U/L), etretinate was discontinued after 1 month on May 26, 1982. Peak values were reached on June 9: SGOT152 U/l, SGPT264 U/L. The values of the other liver tests remained unchanged and within normal limits. September 1982 liver tests were normal again except for a persistent elevation of SGPT (about 40 U/L), which became normal in January 1983.

DISCUSSION

In a histological study etretinate did not produce a consistent effect on the liver in a 6-month follow-up study of 20 patients, although one patient showed an unexpected liver cell necrosis and progressive fibrosis.[3] In 1978 chronic agressive hepatitis was mentioned as a possible complication in 2 cases treated with etretinate.[2] Three further cases of hepatitis supposedly due to etretinate

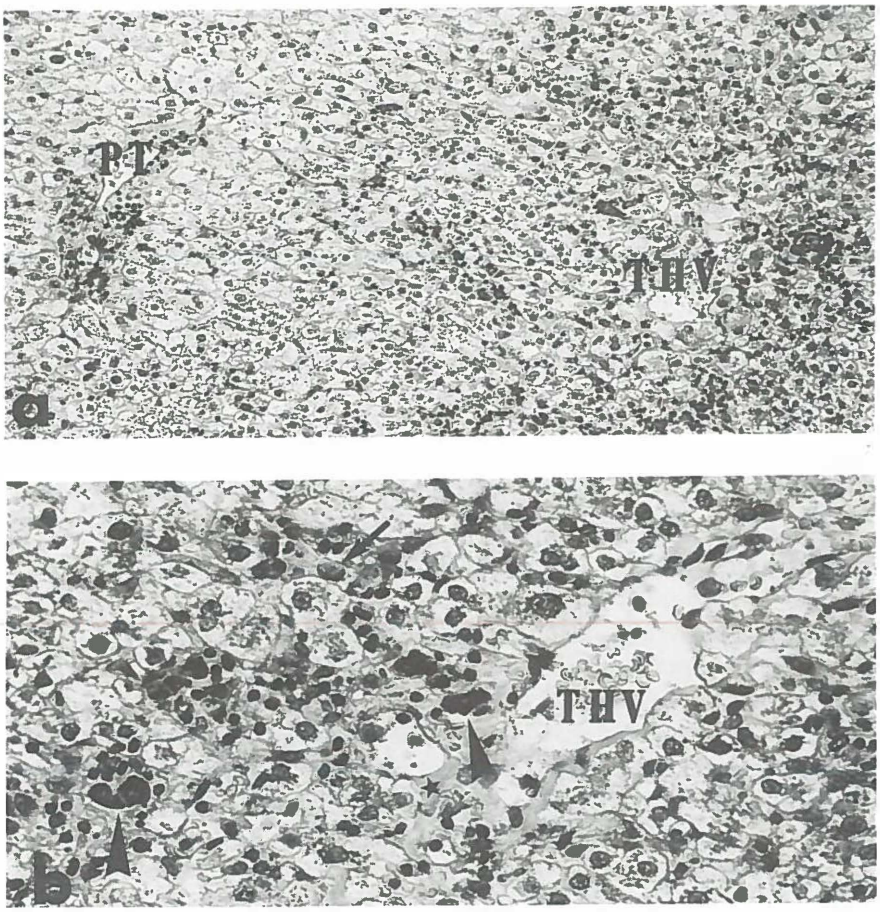

Fig. 3. Patient B. Etretinate hepatitis in a liver biopsy. Staining: PAS following diastase digestion. Overview of the liver parenchyma x160 (a) and detail of the affected area around a hepatic venule x400 (b). The portal tract (PT) and the periportal parenchyma show minor changes, whereas the area around the terminal hepatic venule (THV) is strongly affected, similar to the pathology in hypervitaminosis A. In this area acidophilic degeneration of liver cells (arrow), PAS-positive macrophages (arrowheads), an increased amount of collagen fibres asterisk) and an inflammatory infiltrate are present.

have been more extensively reported.[1,8] Thune and Mork reported that liver test disturbances were first noted about 10 weeks after introduction of etretinate therapy.[8] In their patient maximum values of transaminases amounted to SGOT1008 U/L and SGPT1260 U/L. LDH, AF, bilirubin and the thrombotest also became abnormal. There were complaints of nausea, pressure in the abdomen, increased fatigue and dark urine. Liver histology showed centrolobu-

lar necrosis. Hepatitis B was excluded. After 6 months only the transaminases were still slightly elevated.

Foged and Jacobsen reported 2 patients with liver test disturbances first noted 2 and 5 weeks after introduction of etretinate therapy.[1] Maximum values of transaminases: SGOT231 U/L, SGPT254 U/L in one patient and SGOT329 U/L, SGPT575 U/L in the other. LDH and AF also became abnormal in both patients. Bilirubin values remained within normal limits. Nothing is mentioned about coagulation studies or physical complaints due to the hepatitis. In one patient transaminase values had almost reverted to normal 2 weeks later despite continuation of etretinate therapy, although LDH elevation persisted. Liver biopsies showed focal liver cell necrosis in one patient and piecemeal necroses with bridging but a normal lobular structure in the other. Hepatitis B was excluded in both patients. It took several months for the liver tests to become normal.

In our patients abnormal liver tests were first noted about 8 and 9 weeks after starting a course of etretinate therapy. Liver test disturbances were in about the same range as in the patients of Foged and Jacobsen [1] and provided evidence for degeneration, abnormal excretion and disturbed synthesis function of the liver. Reintroduction in patient A of etretinate therapy at a lower dose resulted in persistance of elevation of transaminases. The transaminase values became normal immediately after discontinuation of the drug. Reintroduction in patient B of the same dose of the drug as previously used, instantly resulted in renewed rise of liver test values. Tests for viral hepatitis were negative in both patients. The persistance of auto-antibodies against smooth muscle and liver membrane antigen in patient B may be related to susceptibility to a drug-induced hepatitis.

The exact site of storage of etretinate and its metabolites is not known, but may well be in fat, in the liver, but possibly also elsewhere.[7] In high performance liquid chromatography different retinoids present different peaks.[4] Consequently the measurement of vitamin A and its esters in liver tissue does not include other retinoids. This probably explains the normal level of vitamin A in the hepatic tissue sample of patient A despite yellow autofluorescence of perisinusoidal liver tissue. Patient A denies any vitamin intake or unusual dietary habits. According to our control fluorescence microscopy study of etretinate and its main metabolite (Ro 10-1670), the yellow autofluorescence in the liver of patient A suggesting hypervitaminosis A may have been caused by etretinate storage. Moreover the autofluorescence pattern in the liver of patient A suggests that an important site of storage of etretinate and its metabolites could be in Ito cells, which are known to store lipid and vitamin A.[5] The increase in number of basal cell carcinomas in patient A 7 1/2 weeks after discontinuation of etretinate therapy because of the acute hepatitis and the fluores-

cence microscopy of liver tissue, which suggests the continuing presence of ample amounts of retinoid metabolite(s) 7 months after the acute hepatitis, indicate that such an hepatitis seriously interferes with the pharmacokinetics of etretinate. Such an increase in number of basal cell carcinomas immediately after discontinuation of etretinate therapy was not observed in two other patients with BCNS.[10]

Data of the 2 patients presented strongly suggest that etretinate did induce an hepatitis, clinically inapparent and reversible upon discontinuation of therapy. Although it is not possible at the moment to exclude a viral hepatitis with absolute certainty, this diagnosis seems unlikely. Regular control of liver tests, especially during the first 3-6 months after introduction of etretinate therapy, should be performed to avoid unrecognized drug-induced hepatitis.

ACKNOWLEDGMENTS

We thank Dr. H. Verweij and Mr. H. Velvis (Central Laboratory for Clinical Chemistry, University Hospital, Groningen) for determination of total vitamin A content in liver tissue and Dr. M.C.J.M. de Jong (Department of Dermatology, University Hospital, Groningen) for the control fluorescence microscopy. Pure etretinate (Tigason, Ro 10-9359) and its main metabolite (Ro 10-1670) were kindly provided for by Hoffmann-La Roche (Basel, Switzerland).

REFERENCES

1. Foged EK, Jacobsen FK. Side effects due to Ro 10-9359 (Tigason). Dermatologica 1982;164:395-403.
2. Fredrikson T. Oral treatment of psoriasis and pustulosis palmo-plantaris with Ro 10-9359. Dermatologica 1978;157:suppl 1:13-8.
3. Glazer SD, Roenigk HH, Yokoo H, Sparberg M. A study of potential hepatotoxicity of etretinate used in the treatment of psoriasis. J Am Acad Dermatol 1982;6:683-7.
4. Hanni R, Hervouet D, Busslinger A. Determination of an aromatic retinoid and its main metabolite by high performance liquid chromatography. J Chromatogr Biomed Appl 1979;162:615-21.
5. Hatoff DE, Gertler SL, Miyai K, Parker BA, Weiss JB. Hyper- vitaminosis A unmasked by viral hepatitis. Gastroenterology 1982;82:124-8.
6. Orfanos CE, Mahrle G, Goerz G, Happle R, Hofbauer M, Landes E, Schimpf A. Laboratory investigations in patients with generalized psoriasis under oral retinoid treatment. Dermatologica 1979;159:62-70.
7. Paravicini U. Pharmacokinetics and metabolism of oral aromatic retinoids. In: Orfanos CE et al, eds. Retinoids. Berlin: Springer Verlag, 1981:13-20.
8. Thune P, Mork NJ. A case of centrolobular toxic necrosis of the liver due to aromatic retinoid - Tigason (Ro 10-9359). Dermatologica 1980;160:405-8.
9. Van der Rhee HJ, Tijssen JGP, Herrmann WA, Waterman AH, Polano MK. Combined treatment of psoriasis with a new aromatic retinoid (Tigason) in low dosage orally and triamcinolone acitonide cream topically. Br J Dermatol 1980;102:203-12.
10. Van Voorst Vader PC, Driessen LHHM, Kallenberg CGM. Basal cell nevus syndrome: immune status, etretinate therapy and etretinate hepatitis. Br J Dermatol 1983;108:109.

Chapter 4.2

Hepatologic side effects during long-term retinoid therapy: Ito cells and portal hypertension

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INTRODUCTION

Non-cirrhotic portal hypertension has been described as a side effect of chronic hypervitaminosis A.[1,2] Portal hypertension in these patients was supposed to result from impediment of the sinusoidal blood flow in the liver and might have been caused by an increase in number and especially size of Ito cells and concomitantly present perisinusoidal fibrosis.[3] Ito cells are perisinusoidal hepatic cells with fat-storing and fibrogenetic capacities.[4,5] Lipid droplets of varying size can accumulate in Ito cells, as is seen in hypervitaminosis A.

We investigated 13 patients on long-term therapy with retinoids in order to evaluate the effects on the liver including portal hypertension.

MATERIAL AND METHODS

Liver biopsies of 13 patients without liver test abnormalities on long-term therapy with etretinate (Ro 10-9359; n=6), all-trans- retinoic acid (RA; n=6), and 13-cis-retinoic acid (13-cis-RA; n=1) were screened for the presence of Ito cells and other histologic abnormalities. A thin needle splenic pressure measurement was performed in 2 etretinate patients. The etretinate patients (2 women, 4 men; age 21-65 years, mean age 38 years; duration of treatment 11-49 months; daily dose 10-75 mg) and RA patients (3 women, 3 men; age 23-44 years, mean age 37 years; duration of treatment 48-84 months; daily dose 10-30 mg) suffered from ichthyosis (n=7), Darier's disease (n=3), psoriasis and pityriasis rubra pilaris. All etretinate patients had previously been treated with RA during 8-48 months. The 13-cis-RA patient (male; age 24 years; duration of treatment 30 months; daily dose 30-60 mg) suffered from acne.

Using light microscopy, Ito cells were identified in 1-μm Epon embedded liver tissue sections stained with haematoxylin-safranin by their characteristic storage of lipid droplets. A semiquantitative estimate of the number of Ito cells and of the size and number of lipid droplets in the Ito cells was made. Using this method in normal liver tissue only very few Ito cells with a few smal lipid droplets are seen.

RESULTS

In routine histological sections no abnormalities were observed except slight steatosis in some cases. Perisinusoidal fibrosis was notably absent. The number of Ito cells was increased in 6/6 etretinate and in 5/6 RA patients. Lipid droplets causing protrusion of Ito cells into the sinusoidal lumen were observed in 3/6 etretinate and in 3/6 RA patients. Thin-needle splenic pressure measurements in 2 etretinate patients with large protruding Ito cells showed elevated values of 1.2 and 1.5 kPa (normal: $\leq$0.7 kPa).[6] A normal number of Ito cells was found in the 13-cis-RA patient.

DISCUSSION

The results of this study demonstrate a reversible non cirrhotic portal hypertension in patients on prolonged etretinate and presumably also RA treatment. This hypertension appears to be due to an increase in number and especially size of Ito cells, as lipid droplets were seen to cause protrusion of Ito cells into the sinusoidal lumen, apparently impeding the sinusoidal blood flow. This finding is not likely to have serious consequences, as bleeding from oesophageal varices rarely occurs at the pressure level found in the 2 patients. Signs of fibrosis and liver cell necrosis were absent in these patients treated during 2-8 years with RA and etretinate, which seems to minimize the risk of future progressive hepatic injury as seen in hypervitaminosis A.[1,7]

REFERENCES

1. Russell RM, Boyer JL, Bagheri SA, Hruban Z. Hepatic injury from chronic hypervitaminosis A resulting in portal hypertension and ascites. N Engl J Med 1974;291:435-40.
2. Kistler HJ, Pluer S, Dickenmann W, Pirozynski W. Portale Hypertonie ohne Leberzirrhose bei chronischer Vitamin-A- Intoxikation. Schweiz med Wschr 1977;107:825-32.
3. Hruban Z, Russell RM, Boyer JL, Glagov S, Bagheri SA. Ultrastructural changes in livers of two patients with hypervitaminosis A. Am J Path 1974;76:451-68.
4. Wake K. Perisinusoidal stellate cells (fat-storing cells, interstitial cells, lipocytes), their related structure in and around the liver sinusoids and vitamin A storing cells in extrahepatic organs. Int Rev Cytol 1980;66:303-53.
5. Leeuw M de, McCarthy SP, Geerts A, Knook DL. Purified rat liver fat-storing cells in culture divide and contain collagen. Hepatology 1984;4:392-403.
6. Weits J, Sikkens H, Kruizinga K, Gips CH. Ultrasound-directed measurements of intrasplenic pressure (ISP) using the Chiba needle. The effect of vasopressin on ISP and portal vein diameter. Neth J Med 1981;24:41.
7. Jacques EA, Buschmann RJ, Layden TJ. The histopathologic progression of vitamin A-induced hepatic injury. Gastroenterology 1979;76:599-602.

Chapter 4.3

Quantitative analysis of retinoids in human serum and tissue samples using high performance liquid chromatography

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INTRODUCTION

A limited number of studies has been published concerning accumulation and metabolism of etretinate (Ro 10-9359) at different organ sites in man.[1-3] 13-cis-Retinoic acid (13-cis-RA) does not accumulate in the human body.[1] Although the metabolism of all-trans-retinoic acid (RA) has been studied,[4-6] no data were found about a possible accumulation of RA (metabolites) in man. To shed more light on this matter, sensitive and specific analytical methods are required. Previously reported methods are generally based on high performance liquid chromatography (HPLC) with spectrophotometric or spectrofluorimetric detection.[1-3,7,8] However, these methods include an evaporation step after extraction of the compounds from the biological matrix. To minimize degradation of the retinoids due to thermal and light instability, we used a simpler pretreatment which is almost uniformly applicable to different kinds of samples, including body fluids and tissues.

MATERIAL AND METHODS

20 Patients on treatment with etretinate (n=9), RA (n=9) and 13-cis-RA (n=2) and with normal biochemical liver tests were included in the study. Serum, liver tissue, subcutaneous fat and epidermis-dermis samples were stored at -20°C until analysis. Sample handlings were done in the dark as much as possible. All samples were analyzed according to the procedure scheme given (Fig. 1). Into Eppendorf centrifuge tubes serum and extraction solvent were introduced. After intense mixing for about 1 min the tubes were centrifuged. A comparable treatment was used for the tissue samples. In a Potter-Elvehjem the tissue samples were homogenized in the presence of the extraction solvent and anhydrous Na_2SO_4. After homogenization the mixtures were transferred into Eppendorf centrifuge tubes and centrifuged. To the supernatant water was added in order to obtain a comparable solvent condition as the mobile phase used for the reversed-phase HPLC.

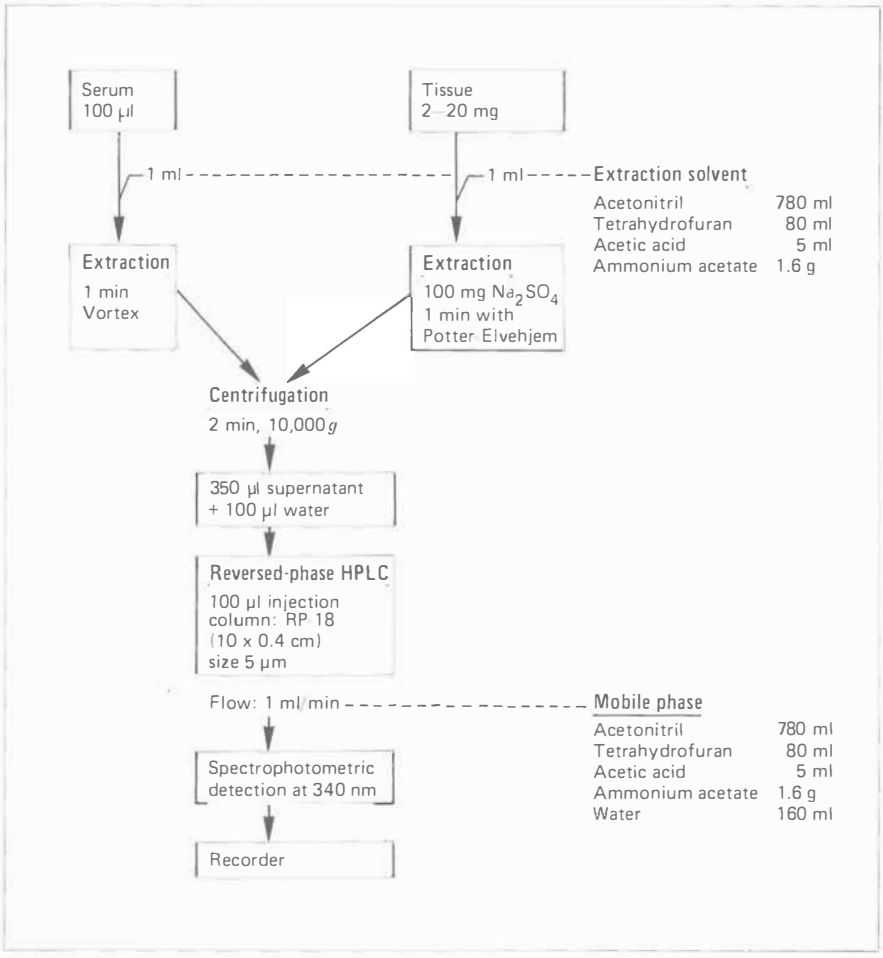

Fig. 1. Procudure used for reserved-phase high performance liquid chromatography (HPLC) analysis of retinoids in serum and tissue samples.

Calibrations were prepared by taking the standard retinoids (retinol, etretinate, Ro 10-1670, 13-cis-RA and RA) through the procedure with or without the biologic samples. Spiked samples were allowed to equilibrate for 30 min prior to the extraction treatment. The HPLC system used was connected with a Kratos spectrophotometer (Model Spectroflow 773) operated at 340 nm. The precolumn (3x0.4 cm) and the analytic column (10x0.4 cm) both were from Brown-Lee (RP-18, particle size 5 μm).

Using this approach it was found that all analysed standard retinoids were detected as separate peaks. Recoveries obtained were good as well as the linearity over a sufficiently wide concentration range. A detection limit of about 0.5 pmol injected on the column was reached for all retinoids.

RESULTS

Preliminary results found in the three patient groups are given. The etretinate treated patients (n=9) showed: about equal amounts of etretinate and the main metabolite (Ro 10-1670) in serum samples (n=15 from 8 patients); besides a low amount of etretinate itself a relatively high amount of presumably etretinate derived metabolites, including Ro 10-1670, in liver tissue samples (n=8 from 6 patients); mainly etretinate and a metabolite in subcutaneous fat samples (n=2 from 2 patients); only very small amounts of etretinate in epidermis-dermis samples (n=2 from 2 patients).

The RA-treated patients (n=9) showed: very low amounts of RA in serum samples (n=12 from 8 patients); some RA and several metabolites, presumably of RA, in liver tissue samples (n=6 from 6 patients); some RA in subcutaneous fat samples (n=2 from 2 patients); no detectable amount of retinoids in epidermis-dermis samples (n=2 from 2 patients).

The 13-cis-RA treated patients (n=2) showed: 13-cis-RA in serum samples (n=7 from 2 patients), but no detectable amount of retinoids in liver tissue, subcutaneous fat and epidermis-dermis samples obtained from 1 patient.

DISCUSSION

The findings in the etretinate treated patients concur with the findings of Vahlquist and Rollmann.[2,3] In a histological study we observed an increase in number and size of the lipid droplets seen in Ito cells in liver biopsies from etretinate and RA patients and the absence of similar changes in the liver biopsy from a 13-cis-RA patient.[9] It is suggested that these histological findings correlate with the quantitative results presented, both favouring accumulation of metabolites of etretinate and probably also RA in liver tissue, possibly mainly in Ito cells.[10]

REFERENCES

1. Paravicini U, Busslinger A. Etretinate and isotretinoin, two retinoids with different pharmacokinetic profiles. In: Cunliffe WJ, Miller AJ, eds. Retinoid therapy. Lancaster: MTP Press Ltd, 1984:11-23.
2. Vahlquist A, Rollmann O. Further observations on the pharmacology of retinoids. In: Cunliffe WJ, Miller AJ, eds. Retinoid therapy. Lancaster: MTP Press Ltd, 1984:135-43.
3. Rollmann O, Vahlquist A. Retinoid concentrations in skin, serum and adipose tissue of patients treated with etretinate. Br J Dermatol 1983;109:439-47.
4. Luca HF de. Retinoic acid metabolism. Fed Proc 1979;38:2519-23.
5. DeLuca HF, Zile M, Sietsema WK. The metabolism of retinoic acid to 5,6-epoxyretinoic acid, retinoyl-beta-glucuronide and other polar metabolites. Ann NY Acad Sci 1981;359:25-36.

6. Frolik CA. In vitro and in vivo metabolism of all-trans- and 13-cis-retinoic acid in the hamster. Ann NY Acad Sci 1981;359:37-42.
7. Paravicini U, Busslinger A. Determination of etretinate and its main metabolite in human plasma using normal-phase high performance liquid chromatography. J Chromatogr 1983;276:359-66.
8. Leenheer AP de, Lambert WE, Claeys I. All-trans-retinoic acid: measurement of reference values in human serum by high performance liquid chromatography. J Lipid Res 1982;23:1362-7.
9. Voorst Vader PC van, Houthoff HJ, Gips CH, Verweij, H. Hepatologic side effects during long-term retinoid therapy: Ito cells and portal hypertension. In: Saurat J, ed. Retinoids: new trends in research and therapy. Basel: Karger, 1985:498-500.
10. Hendriks HFJ, Brouwer A, Leeuw AM de, Knook DL. Effect of age on vitamin A storage in rat liver and rat liver fat-storing cells. In: Bezooijen CFvan, ed. Pharmacological, morphological and physiological aspects of liver aging. Rijswijk: Eurage, 1984:163-8.

SUMMARY AND CONCLUSIONS

Aim of the study

Cutaneous neoplasms represent 10-50% of all malignancies occurring in Caucasians. About 90% of these cutaneous neoplasms are non-melanoma skin cancers: basal cell carcinoma (BCC) or squamous cell carcinoma (SCC). Several factors are known to increase the risk of development of cutaneous carcinomas. In general the most important factor in Caucasians is the degree of sunlight exposure, especially UV-B irradiation, which acts as an initiator and promotor of carcinogenesis. Dysfunction of immune surveillance is thought to be another, possibly important, factor, as a deficient immune response can facilitate tumor growth (pseudo-promotor effect). Evidence in support of this concept, derived from animal and clinical studies, has been presented indicating that the risk of development of tumors induced by oncogenic viruses or physical - and possibly also chemical - carcinogens is increased by a deficient immune response (Introduction).[1-6] In patients with multiple carcinomas, in whom an impaired immune response might enhance tumor growth, oral retinoid treatment may be effective in the prevention of tumor growth (Introduction).[7]

In the studies collected in this thesis three hereditary afflictions were investigated, in which the number of cutaneous carcinomas frequently ranges from ›10 to ›100: the Basal cell nevus syndrome, Xeroderma pigmentosum and Epidermodysplasia verruciformis. The principal characteristics of these disorders are listed in Table 1.

The Basal cell nevus syndrome (BCNS) is a rare autosomal dominant hereditary disorder, which is relatively frequently encountered in the Northern provinces of the Netherlands. Its main features are multiple BCCs in about half of the patients with BCNS (sometimes already present in large numbers in the first decade of life), milia, palmoplantar pits, jaw cysts (keratocysts), skeletal abnormalities and ectopic calcification. These features suggest that BCNS can be partly defined as a genetically determined epidermal cell differentiation disorder. Agressive growth of the BCCs generally does not occur until after puberty and is mostly restricted to sunlight or ionizing radiation exposed skin areas. No other studies are known concerning the immune response of patients with BCNS, except for a Russian study which is difficult to interpret. Systemic treatment with the retinoids isotretinoin and etretinate appeared to be effective in the prevention of tumor growth in some BCNS patients, as suggested by the literature.

Xeroderma pigmentosum (XP) is a rare autosomal recessive hereditary disorder, characterized clinically by cutaneous and ocular photosensitivity, cutaneous pigmentary changes and a propensity for the development of oculocutaneous malignancies, particularly SCCs and BCCs in sun-exposed areas of the body. The basic anomaly of XP is defective UV light induced DNA excision repair. The increased frequency of UV light induced mutations, probably a result of the DNA repair defect, is thought to be responsible for the development of neoplasms in XP. The results of the few immunologic studies performed in XP are conflicting. Normal and abnormal results have been found using DNCB sensitization and lymphocyte proliferation tests. In several XP patients reported in the literature a dose-dependent preventive effect on tumor growth of systemic retinoid treatment with etretinate has been observed.

Epidermodysplasia verruciformis (EV) is caused by a persistent cutaneous infection with certain, EV-specific, human papillomaviruses (HPVs), generally in association with an impaired immune response. This rare disease is thought to be an autosomal recessive or possibly X-linked hereditary disorder. In about 30% of the EV patients reported in the literature multiple cutaneous SCCs developed, mostly on sun-exposed skin areas. DNA of EV-specific HPVs (especially HPV5 and 8) has been detected in SCCs of some EV patients. Thus, circumstantial evidence suggests that certain HPVs are potentially oncogenic. Other oncogenic factors appear to be sunlight-exposure and probably the impaired immune response. Two studies reported in the literature suggested that systemic retinoid treatment with etretinate has a positive effect on the immune response of EV patients.

The investigations reported in this thesis were centred around two themes: a) impairment of immune surveillance, possibly implicating enhancement of tumor growth; b) treatment and prevention of tumor growth by systemic retinoid medication.

a) First we investigated whether the immune response was impaired in 14 patients with BCNS (chapter 1) and 1 patient with XP (chapter 2.1), assessing 1) the in vivo cellular immune response by a semiquantitative DNCB sensitization test, 2) the in vivo humoral immune response by measuring the antigen-specific antibody response after immunization with the primary immunogen Helix pomatia Haemocyanin (HPH), 3) the in vitro lymphoproliferative response induced by mitogens, irradiated allogeneic lymphocytes and HPH, 4) phytohaemagglutinin-induced T-cell cytotoxicity in 2 BCNS patients with multiple BCCs (chapter 1). In addition, a quantitative and morphologic in situ analysis of epidermal Langerhans cells was performed in patients with BCNS (chapter 1) and XP (chapter 2.1), as these cells appear to be involved in the immunologic response against cutaneous carcinomas.

Next we investigated 2 patients with EV and an impaired immune response.

The quantity and morphology of epidermal Langerhans cells was studied in situ in one EV patient (chapter 3.1), as these cells might be involved in the antigen-specific immune response to the persistent HPV infection. Viral analysis was performed of an invasive SCC of the second EV patient (chapter 3.2), in order to provide further circumstantial evidence, that certain HPVs can be oncogenic in EV. Finally a genetic study was performed of an EV patient (chapter 3.1), in order to investigate whether EV is a chromosomal instability disease. Chromosomal instability, present in XP and ataxia teleangiectasia, probably increases the risk of cancer and can be associated with an impaired immune response.

b) The value of systemic retinoid medication for the therapy and prevention of multiple cutaneous carcinomas was investigated in 3 patients with BCNS (chapter 1) and in 1 patient with XP (chapter 2.1). The influence of systemic retinoid medication on the immune response was also investigated in these patients (chapter 1 and chapter 2.1) and in 1 EV patient (chapter 3.1). Furthermore, a study was performed in order to determine whether retinoids affect the basic anomaly of XP, i.e. defective UV light induced DNA excision repair (chapter 2.2).

Finally, (potential) hepatologic side effects of systemic retinoid treatment, particularly of long-term treatment, were evaluated (chapter 4). The occurrence of severe liver test disturbances associated with short-term retinoid treatment with etretinate was analysed in two patients (chapter 4.1). Hepatologic side effects of long-term retinoid treatment were analysed in 20 patients without liver test abnormalities (chapter 4.2 and chapter 4.3), using 1) light microscopy of liver tissue, also stained to visualize Ito cells, 2) measurement of splenic pressure in order to detect portal hypertension and 3) quantitative analysis of retinoids in serum, liver tissue, subcutaneous fat and epidermis-dermis samples of patients treated with etretinate, retinoic acid and isotretinoin.

Summary of the results

The results of the studies reported in chapter 1, 2.1 and 3.1 are summarized in Table 1. These studies focused on the immune response and the clinical and immunologic effect of systemic retinoid treatment of patients with BCNS, XP and EV.

Chapter 1 deals with the results of the studies concerning BCNS. 14 BCNS patients were screened for the presence of decreased immune responsiveness, which might enhance the development of the multiple BCCs occurring in 8 of

Table 1. Characteristics of three hereditary disorders with a propensity for the development of multiple cutaneous carcinomas. This table also represents the results of investigations related to these disorders and reported in this thesis.

	Basic abnormality	Type of skin carcinoma	Suggested role of UV light	CMI[x] status		Retinoid treatment presence/absence of positive effect		
				in vivo	in vitro	CMI status in vitro	cutaneous carcinomas therapy	cutaneous carcinomas prevention
Basal cell nevus syndrome	cel differentiation disorder	basal cell carcinoma	promotor pseudo-promotor	N[§] - ↓	N	-	⊕	⊕
Xeroderma pigmentosum	UV light induced DNA repair disorder	squamous cell carcinoma, basal cell carcinoma	initiator promotor pseudo-promotor	↓-N	↓-N	+	-	⊕
Epidermodysplasia verruciformis	persistent infection with oncogenic viruses (HPVs)*	squamous cell carcinoma	co-initiator co-promotor pseudo-promotor	↓**	↓**	⊕	±	?

Encircled data are data, which were confirmed by investigations reported in this thesis.

[x] CMI: Cell Mediated Immunity (non-HPV-specific).

[§] N: Normal.

* HPV: Human Papilloma Virus.

** In 2 patients from the literature, one with and one without cutaneous carcinomas, the CMI response in vivo and in vitro was normal.

these patients. In addition the clinical and immunologic effect of retinoid treatment with etretinate was assessed in 3 BCNS patients with multiple BCCs. Immunologic evaluation showed: 1) a normal DNCB skin test score in vivo of all 6 BCNS patients without multiple BCCs, but a decreased score of 4 of the 8 BCNS patients with multiple BCCs; 2) a normal antigen-specific humoral immune response in vivo after immunization with HPH of 3 patients with multiple BCCs, except for a decreased IgG-class antibody response of 2/3 patients; 3) a normal in vitro lymphoproliferative response after stimulation with mitogens, allogeneic lymphocytes and HPH of 5 patients with multiple BCCs; 4) normal PHA-induced lymphocyte cytotoxicity of 2 patients with multiple BCCs; 5) a normal number of T6+ and HLA-DR+ epidermal Langerhans cells in clinically normal skin from the forehead and back of 6 patients with multiple BCCs; 6) epidermal buds of BCC tissue in clinically normal skin from the forehead of 2 patients with multiple BCCs.

Retinoid treatment with etretinate of 3 patients with multiple BCCs (dosage 0.3-1.0 mg/kg/day) supported the notion of a preventive effect on the development of BCCs, which lasted until about 9 months after discontinuation of treatment. The therapeutic effect of etretinate on clinically apparent BCCs was limited, recurrences occurring in all patients. Hepatitis, probably etretinate-induced, occurred in one patient and was associated with massive recurrences of BCCs. Etretinate treatment did not markedly affect the immunologic parameters followed, i.e. the lymphoproliferative response in vitro and phytohaemagglutinin- induced T-cell cytotoxicity.

In conclusion: a) the immune response of the BCNS patients tested was normal, except for a possible defect of the cellular immune response in vivo in some patients with multiple BCCs, which might be due to the epidermal cell differentiation disturbance of BCNS, b) systemic retinoid medication with etretinate was of preventive value in the treatment of the multiple BCCs, which frequently occur in BCNS patients and did not affect the immune response.

Chapter 2.1 describes the immunologic and clinical investigations of a patient with XP, belonging to complementation group C and treated with the retinoid etretinate. During a Northern winter (in order to minimize the effect of sunlight-exposure) immunologic evaluation showed: 1) a decreased number of peripheral blood lymphocytes with relatively normal T-cell subset percentages; 2) a decreased antigen-specific humoral and cellular immune response in vitro as measured by the class-specific antibody response after immunization with HPH and the DNCB sensitization test; 3) an in vitro lymphoproliferative response after stimulation with mitogens and allogeneic lymphocytes, which was in the lower range of normal; 4) a decreased number of T6+and HLA-

DR+ Langerhans cells per mm^2 of epidermis on the forehead compared to the back, supposedly due to sunlight exposure, which might impair the local immune response to tumor growth; 5) HLA-DR expression of epidermal keratinocytes in association with the presence of Leu-3a+ and OKT-8+ T-cells in the papillary dermis, which is a common reaction pattern of unknown significance.[8]

Clinical observation during 5 years suggested that treatment with the retinoid etretinate, 0.9 mg/kg/day, prevented the growth of cutaneous carcinomas. Retinoid treatment did not markedly affect the immunologic parameters, except for a transient stimulation of the mitogen induced in vitro lymphoproliferative response. Spontaneous corneal perforation, the finding of a keratoconus and the beneficial influence of corneal transplantation stressed the need for ophthalmologic follow-up.

In conclusion: a) immunologic investigations of this XP patient during a Northern winter indicated a suboptimal immune response, which might enhance the growth of the oculocutaneous UV light induced neoplasms; b) systemic retinoid treatment with etretinate appeared to be effective in the prevention of growth of cutaneous carcinomas of this XP patient and exerted a transient effect on the immune response.

Chapter 2.2 shows the results of a study concerning the possible effect of retinoid treatment on the basic anomaly of XP, i.e. defective UV light induced DNA excision repair. The rate of unscheduled DNA synthesis after in vitro UV light exposure of fibroblasts from a XP patient and a control was not influenced by the addition of different concentrations of retinoic acid to the medium in which the fibroblasts were cultured. This suggests that the prophylactic effect of retinoid treatment on tumor growth in XP patients is not likely to be due to modulation of DNA repair capacity.

Chapter 3.1 describes an immunohistologic, immunologic and cytogenetic study of a negroid EV patient with cutaneous SCCs and an impaired immune response, as measured by the in vitro lymphoproliferative response and the DNCB sensitization test in vivo, who was treated with the retinoid etretinate. The results showed: 1) the presence of microscopic EV lesions in clinically normal skin from the forehead; 2) the virtual absence of T6+ and HLA-DR+ Langerhans cells in the koilocytic areas of epidermis histologically involved with EV, koilocytosis being a HPV-induced specific cytopathic effect; 3) a change in the in situ pattern of T6+ and HLA-DR+ epidermal Langerhans cells in epidermis histologically involved with EV, i.e. the presence of these cells in koilocytic areas, after 8 days of consecutive retinoid treatment; 4) an increase of the in vitro phytohaemagglutinin- and concanavalin A-induced

lymphoproliferative response after 10 consecutive days of retinoid treatment; 5) no abnormalities in the frequency of sister chromatid exchanges, the number of spontaneous and UV light induced chromosomal breaks and the rate of UV light induced unscheduled DNA synthesis.

These results suggest: a) a deficient role of epidermal Langerhans cells in the HPV-specific cellular immune response in EV; b) a modulatory effect of the retinoid etretinate on the immune response; c) that increased chromosomal instability is unlikely to be the cause of EV, while the absence of cell hypersensitivity, already shown by other investigators, was confirmed.

Chapter 3.2 describes a virologic study of a Caucasian EV patient with multiple SCCs and an impaired immune response, who also developed a primary hepatocellular carcinoma associated with a chronic hepatitis B virus infection. Five different HPVs were detected in the benign disseminated skin lesions of EV, i.e. HPV5, 8, 17, 19 and 24, the latter three being newly characterized HPVs. HPV5 DNA sequences were demonstrated in the central part of an invasive cutaneous SCC. This case provides an example of the circumstantial evidence which suggests that certain types of HPV are potentially oncogenic and stresses the importance of immune surveillance in the protection against virus-associated tumors.

Chapter 4.1 deals with hepatologic side effects of short-term systemic retinoid treatment with etretinate. Two patients are described in whom an histologically confirmed, clinically inapparent and reversible hepatitis occurred within the first months after introduction of etretinate therapy. Causes of hepatitis other than etretinate were not found. Reintroduction of etretinate resulted in reactivation and/or persistance of the hepatitis. These data strongly suggest that the hepatitis in both patients was caused by etretinate. No liver test disturbances were observed during treatment of one of these patients with 13-cis-retinoic acid.

Chapter 4.2 deals with hepatologic side effects of long-term systemic retinoid treatment of 13 patients without liver test abnormalities treated with etretinate (n=6), all-trans-retinoic acid (RA; n=6) and 13-cis-retinoic acid (13-cis-RA; n=1). In routine histological sections of liver tissue no abnormalities were observed apart from slight steatosis in some cases. Perisinusoidal fibrosis was notably absent. The number of Ito cells, i.e. perisinusoidal hepatic cells with fat-storing and fibrogenetic capacities, which can be visualized by light microscopy using accumulation of lipid droplets within these cells as a marker, was increased in 6/6 etretinate and in 5/6 RA patients. Lipid droplets causing protrusion of Ito cells into the sinusoidal lumen were observed in 3/6

etretinate and 3/6 RA patients. Thin-needle splenic pressure measurements in 2 etretinate patients with such protruding Ito cells showed slightly elevated values, indicative of portal hypertension. This finding is unlikely to have serious consequences, as the risk of complications of portal hypertension is very low at this pressure level. These results indicate that the risk of serious hepatologic side effects of long-term systemic retinoid treatment, such as have been seen to occur in hypervitaminosis A, i.e. fibrosis and portal hypertension, are minimal.

In chapter 4.3 the results are reported of a quantitative analysis of serum, liver tissue, subcutaneous fat and epidermis-dermis samples of 20 patients treated with etretinate (n=9), RA (n=9) and 13-cis-RA (n=2). In liver tissue of etretinate treated patients accumulation of presumably etretinate derived metabolites including the main metabolite Ro 10-1670 was found, whereas subcutaneous fat showed accumulation of etretinate. Similar findings were made in tissue samples from RA treated patients, also showing the presence of metabolites in liver tissue and the presence of RA itself in subcutaneous fat. Tissue samples did not show detectable amounts of 13-cis-RA. These quantitative data appear to correlate with the histological findings presented in chapter 4.2, both favouring accumulation of metabolites of etretinate and probably also of RA in liver tissue, possibly mainly in Ito cells.

Conclusions

The investigations reported in this thesis were centred around two themes: a) impairment of immune surveillance, possibly implicating enhancement of tumor growth; b) treatment and prevention of tumor growth by systemic retinoid medication. The conclusions drawn from the results of the investigations are presented for each theme separately.

Regarding the first theme the following is concluded:

1) The immune response of BCNS patients is not impaired except for a possible defect in the delayed type hypersensitivity response of some BCNS patients with multiple BCCs. One could speculate whether such a defect can be explained by immunologic dysfunction of the keratinocyt, as the cause of the cutaneous symptoms of BCNS appears to be an epidermal cell differentiation disorder.

2) The immune response of XP patients can be impaired and the number of T6+ and HLA-DR+ epidermal Langerhans cells in sunlight exposed skin of XP patients can be decreased, even in the winter season with a relatively low

amount of sunlight exposure. Both findings might be associated with enhancement of tumor growth. The immune response is not impaired in all XP patients, however. The presence or absence of an impaired immune response in XP may be linked with the severity of the clinical picture, i.e. to the degree of sensitivity to UV-induced cell damage.

3) Data from the literature indicating that the non-HPV-specific immune response is impaired in most EV patients, were confirmed. An immunohistologic study suggested a deficient role of the epidermal Langerhans cell in the HPV-specific cellular immune response in EV. A cytogenetic and cell biologic study appeared to exclude chromosomal instability and cell hypersensivity as the cause of EV. A virologic study demonstrated the presence of DNA of an EV-specific HPV (HPV5) in an invasive SCC of an EV patient, who also developed a primary hepatocellular carcinoma associated with a persistent hepatitis B virus infection. These data provide further circumstantial evidence for the potential oncogenicity of certain HPVs and stress the importance of immune surveillance in the protection against virus-associated tumors.

Regarding the second theme the following is concluded:

1) Systemic retinoid treatment with etretinate appeared to prevent growth of cutaneous carcinomas in patients with BCNS and XP. The limited therapeutic effect of etretinate treatment on clinically detectable BCCs and the risk of recurrence does not justify treatment of these carcinomas with systemic retinoid medication.

2) Systemic retinoid treatment with etretinate did not have any consistent effect on the immune response of BCNS patients. The suboptimal in vitro lymphoproliferative response of a XP and an EV patient appeared only transiently stimulated by etretinate treatment.

3) Retinoic acid did not affect defective UV light induced DNA excision repair in vitro, the basic disorder of XP. Enhancement of normal cell differentiation may be the main factor responsible for the tumor prophylactic effect of retinoid treatment.

4) The retinoid etretinate can induce hepatitis without the first few months after starting treatment, which appears to occur in about 1% of the patients. In patients with normal liver tests the risk of serious hepatologic side effects of long-term systemic retinoid treatment appears to be minimal.

Future investigations may answer some of the many questions left regarding the role of immune surveillance in cutaneous oncology, particularly questions concerning the immune response of patients with BCCs and questions in the field of photoimmunology. Dynamic studies may shed light on the interaction

of the immune system in man and sunlight exposure. The role of human papillomaviruses in oncogenesis is actively investigated in many centres, also in the Netherlands, especially as regards the role of these viruses in the aetiology of carcinoma of the cervix uteri. The Basal cell nevus syndrome is an interesting clinical model, in which the possible role of oncogenes can be studied.

REFERENCES

1. The TH. Kanker. In: Feltkamp TEW, ed. Klinische immunologie. Utrecht: Bohn, Scheltema en Holkema, 1980:163-92.
2. Klein G, Klein E. Immune surveillance against virus-induced tumors and non-rejectability of spontaneous tumors: contrasting sequences of host versus tumor evolution. Proc Natl Acad Sci USA 1977;74:2121-5.
3. Miller DG. On the nature of susceptibility to cancer. Cancer 1980;46:1307-18.
4. Herberman RB. Possible role of natural killer cells and other effector cells in immune surveillance against cancer. J Invest Dermatol 1984;83:137s-40s.
5. Epstein JH. Photocarcinogenesis, skin cancer and aging. J Am Acad Dermatol 1983;9:487-502.
6. Kripke ML. Immunology and photocarcinogenesis. J Am Acad Dermatol 1986;14:149-55.
7. Moon RC, Itri LM. Retinoids and cancer. In: Sporn MB, Roberts AB, Goodman DS, eds. The Retinoids. Vol. 2. New York: Academic Press Inc., 1984:327-71.
8. Aubock J, Romani N, Grubauer G, Fritsch P. HLA-DR expression on keratinocytes is a common feature of diseased skin. Br J Dermatol 1986;114:465-72.

SAMENVATTING EN CONCLUSIES

Doel van het onderzoek

Zo'n 10-50% van alle kwaardaardige gezwellen, die bij mensen van het blanke ras voorkomen, zijn gelocaliseerd in de huid. Ongeveer 90% van deze cutane maligniteiten bestaat niet uit maligne melanomen, maar uit huidkanker voortkomend uit plaveisel epitheel cellen: basaalcel carcinoom (BCC) of spinocellulair carcinoom (SCC). Er zijn verscheidene factoren bekend die het risico op het ontstaan van deze cutane carcinomen vergroten. In het algemeen is bij mensen van het blanke ras de mate van zonlicht expositie de belangrijkste factor, met name de mate van blootstelling aan bestraling met UV-B licht, dat bij de carcinogenese zowel de rol van initiator als van promotor vervult. Er is geopperd dat gebrekkig functioneren van het immunologisch bewakingsapparaat ("immune surveillance") ook een, mogelijk belangrijke, factor zou kunnen zijn, omdat een deficiente immuun respons tumorgroei kan bevorderen (pseudo-promotor effect). Bewijsmateriaal ter ondersteuning van deze opvatting, verkregen bij onderzoek met dieren en in de kliniek, geeft aan dat het risico op het ontstaan van tumoren, die geinduceerd worden door oncogene virussen of fysische - en mogelijk ook chemische - carcinogenen, vergroot is door een deficiente immuun respons (Inleiding).[1-6] Bij patienten met multipele carcinomen, bij wie een deficiente immuun respons tumorgroei zou kunnen begunstigen, is preventie van tumorgroei wellicht mogelijk door orale retinoid behandeling (Inleiding).[7]

In dit proefschrift worden de resultaten gerapporteerd van een onderzoek dat uitgevoerd werd bij drie erfelijke aandoeningen, waarbij het aantal cutane carcinomen frequent varieert van ›10 tot ›100: het Basaal cel nevus syndroom, Xeroderma pigmentosum en Epidermodysplasia verruciformis. De voor dit onderzoek meest relevante kenmerken van deze aandoeningen zijn aangegeven in Tabel 1.

Het Basaal cel nevus syndroom (BCNS) is een zeldzame, autosomaal dominant erfelijke afwijking, die relatief vaak in de Noordelijke provincies van Nederland voorkomt. De voornaamste symptomen van dit syndroom zijn multipele BCC's bij ongeveer de helft van de patienten met BCNS (soms al aanwezig op kleuterleeftijd), milien, palmoplantaire epidermale putjes, kaakcysten (keratocysten), skeletafwijkingen en ectopische calcificatie. Deze symptomen suggereren dat BCNS deels gedefinieerd kan worden als een genetisch bepaalde epidermale cel differententiatie stoornis. Agressieve groei van BCC's bij BCNS treedt over het algemeen niet op voor de puberteit en is

meestal beperkt tot gebieden van de huid die aan zonlicht of röntgen straling zijn blootgesteld. Er zijn geen onderzoekingen bekend aangaande de immuun respons van patienten met BCNS, behoudens een Russisch onderzoek dat moeilijk te intrepreteren is. Verdere literatuur gegevens suggereren dat systemische behandeling met de retinoiden isotretinoine en etretinaat van nut kan zijn als maatregel ter preventie van de tumor groei bij BCNS patienten.

Xeroderma pigmentosum (XP) is een zeldzame, autosomaal recessief erfelijke aandoening, klinisch gekenmerkt door overgevoeligheid van de huid en de ogen voor licht, cutane pigmentveranderingen en predispositie tot de ontwikkeling van oculocutane maligniteiten, met name SCC's en BCC's in gebieden van het lichaam die aan zonlicht blootgesteld zijn. XP wordt veroorzaakt door een stoornis van het DNA excisie herstel na UV licht bestraling. Men veronderstelt dat de toegenomen frequentie van mutaties geinduceerd door UV licht, waarschijnlijk een gevolg van de DNA herstel stoornis, verantwoordelijk is voor de ontwikkeling van de maligniteiten in XP. De resultaten van het beperkte aantal immunologische onderzoekingen dat verricht is, zijn tegenstrijdig. Er werden normale en abnormale resultaten gevonden door middel van DNCB sensibilisatie en lymfocyten transformatie testen. Bij meerdere in de literatuur beschreven XP patienten werd een dosis-afhankelijk preventief effect van systemische retinoid behandeling op tumor groei waargenomen.

Epidermodysplasia verruciformis (EV) wordt veroorzaakt door een persisterende cutane infectie met bepaalde, EV-specifieke, humane papillomavirussen (HPV's), in het algemeen gepaard gaand met een verminderde immuun respons. Men veronderstelt dat deze zeldzame ziekte een autosomaal recessief of mogelijk X-chromosoom gebonden erfelijke afwijking is. Multipele cutane SCC's ontwikkelden zich bij ongeveer 30% van de EV patienten, die in de literatuur beschreven zijn en waren meestal gelocaliseerd op gebieden van de huid, die aan zonlicht blootgesteld waren. In SCC's van enkele EV patienten werd DNA van EV-specifieke HPV's (met name HPV5 en 8) gevonden. Daarmee werd een indirect bewijs geleverd voor de hypothese, dat bepaalde HPV's potentieel oncogeen zijn. Andere oncogene factoren, met name zonlicht bestraling en mogelijk de deficiente immuun respons, lijken ook een rol te spelen. Twee artikelen suggereerden, dat systemische retinoid behandeling met etretinaat een positief effect heeft.

De onderzoekingen, waarvan de resultaten in dit proefschrift gepresenteerd worden, waren gegroepeerd rondom twee themas: a) dysfunctie van het immunologisch bewakingsapparaat, mogelijk leidend tot begunstiging van tumorgroei; b) behandeling en preventie van tumorgroei door systemische retinoid medicatie.

a) Allereerst werd onderzocht of de immuun respons van 14 patienten met

BCNS (hoofdstuk 1) en 1 patient met XP (hoofdstuk 2.1) verminderd was. In dat kader werd bestudeerd: 1) de cellulaire immuun respons in vivo door middel van een DNCB sensibilisatie test; 2) de humorale immuun respons in vivo door middel van de antigeen-specifieke antilichaam respons na immunisatie met het primaire immunogeen Helix pomatia Haemocyanin (HPH); 3) de lymfoproliferatieve respons in vitro geinduceerd door mitogenen, bestraalde allogene lymfocyten en HPH; 4) T-cel cytoxiciteit geïnduceerd door fyto haemagglutinine bij 2 BCNS patienten met multiple BCCs (hoofdstuk 1). Bovendien werd een quantitatieve en morfologische in situ analyse verricht van epidermale Langerhans cellen bij patienten met BCNS (hoofdstuk 1) en XP (hoofdstuk 2.1), omdat deze cellen lijken te participeren in de immunologische respons tegen cutane carcinomen.

Vervolgens werden twee patienten met EV en een verminderde immuun respons onderzocht. De quantiteit en morfologie van epidermale Langerhans cellen werd in situ in een EV patient bestudeerd (hoofdstuk 3.1), aangezien deze cellen betrokken zouden kunnen zijn bij de antigeen-specifieke immuun respons op de persisterende HPV infectie. Een invasief SCC van de tweede EV patient werd gebruikt voor virale analyse (hoofdstuk 3.2), met het doel aanvullend indirect bewijsmateriaal te leveren voor de stelling dat bepaalde HPV's oncogeen zijn in EV. Tenslotte werd bij een EV patient genetisch onderzoek verricht (hoofdstuk 3.1), met het doel te bestuderen of EV veroorzaakt wordt door toegenomen chromosomale instabiliteit. Toegenomen chromosomale instabiliteit, zoals in XP en ataxia teleangiectasia, vergroot waarschijnlijk het risico op kanker en kan geassocieerd zijn met een verminderde immuun respons.

b) De waarde van systemische retinoid medicatie voor de therapie en preventie van multipele cutane carcinomen werd onderzocht bij 3 patienten met BCNS (hoofdstuk 1) en bij 1 patient met XP (hoofdstuk 2.1). De invloed van systemische retinoid medicatie op de immuun respons werd ook onderzocht bij deze patienten (hoofdstuk 1 en hoofdstuk 2.1) en bij 1 patient met EV (hoofdstuk 3.1). Bovendien werd onderzocht of retinoiden de basisafwijking van XP, d.w.z. de DNA herstel stoornis, beïnvloeden (hoofdstuk 2.2). Tenslotte werd onderzoek gedaan naar (potentiele) hepatologische bijwerkingen van systemische retinoid medicatie, met name die na langdurige retinoid behandeling (hoofdstuk 4). Het optreden van ernstige lever functie stoornissen tijdens kortdurende retinoid behandeling met etretinaat werd geanalyseerd bij 2 patienten (hoofdstuk 4.1). Hepatologische bijwerkingen van langdurige retinoid behandeling werden geevalueerd bij 20 patienten met normale lever functies, gebruik makend van de volgende methoden: 1) licht microscopie van lever weefsel, waarbij een speciale kleuring werd toegepast om Ito cellen zichtbaar te maken; 2) meting van de miltdruk om portale hy-

Tabel 1. Karakteristieken van drie erfelijke aandoeningen gekenmerkt door het voorkomen van multiple cutane carcinomen. Deze tabel toont tevens de resultaten van het in dit proefschrift gepubliceerde onderzoek bij deze aandoeningen.

	Basale stoornis	Type huid carcinoom	Mogelijke rol UV licht	Cellulaire immuun respons		Retinoid behandeling aan- of afwezigheid positief effect		
				in vivo	in vitro	cell imm. resp. in vitro	cutane carcinomen therapie	preventie
Basaal cel nevus syndroom	cel differentiatie stoornis	basaal cel carcinoom	promotor pseudo-promotor	N§ - ↓	N	-	(±)	(+)
Xeroderma pigmentosum	UV licht geïnduceerde DNA herstel stoornis	spinocellulair carcinoom, basaal cel carcinoom	initiator promotor pseudo-promotor	(↓)-(N)	↓ -(N)	+	–	(+)
Epidermodysplasia verruciformis	chronische infectie met oncogene virussen (HPV's)*	spinocellulair carcinoom	co-initiator co-promotor pseudo-promotor	(↓)**	(↓)**	(+)	±	?

Omcirkening van een gegeven betekent bevestiging van dat gegeven door het in dit proefschrift gepubliceerde onderzoek.

§ N: Normaal.

* HPV: Humaan Papilloma Virus.

** Bij 2 patienten in de literatuur, één met en één zonder cutane carcinomen, was de non-HPV-specifieke cellulaire immuun respons in vivo en in vitro normaal.

pertensie uit te sluiten; 3) quantitatieve analyse van retinoiden in serum, lever weefsel, subcutaan vet en epidermis-dermis, welk materiaal verkregen werd bij patienten, die met etretinaat, vitamine A-zuur en isotretinoine behandeld werden.

Samenvatting van de resultaten

De resultaten van de onderzoekingen die beschreven zijn in hoofdstuk 1, 2.1 en 3.1 zijn samengevat in Tabel 1. Deze onderzoekingen waren gericht op de immuun respons en het klinische en immunologische effect van systemische retinoid behandeling van patienten met BCNS, XP en EV.

Hoofdstuk 1 bevat de resultaten van de onderzoekingen aangaande BCNS. 14 BCNS patienten werden nagekeken op de aanwezigheid van een verminderde immuun respons, die de ontwikkeling van de multipele BCC's, die bij 8 van deze patienten aanwezig waren, zou kunnen begunstigen. Bovendien werd het klinisch en immunologisch effect van retinoid behandeling met etretinaat onderzocht bij 3 BCNS patienten met multipele BCC's. Immunologische evaluatie toonde het volgende aan: 1) een normale DNCB huid test reactie in vivo bij alle 6 BCNS patienten zonder multipele BCC's, maar een verminderde reactie bij 4 van de 8 BCNS patienten met multipele BCC's; 2) een normale antigeen-specifieke humorale immuun respons in vivo na immunisatie met HPH van 3 patienten met multipele BCC's, met uitzondering van een verminderde IgG-klasse antilichaam reactie bij 2/3 patienten; 3) een normale lymfoproliferatieve immuun respons in vitro na stimulatie met mitogenen, allogene lymfocyten en HPH bij 5 patienten met multipele BCC's; 4) normale fytohaemagglutinine-geinduceerde T-cel cytotoxiciteit bij 2 patienten met multipele BCC's; 5) een normaal aantal T6+ en HLA-DR+ epidermale Langerhans cellen in klinisch normale huid van het voorhoofd en de rug van 6 patienten met multipele BCC's; 6) epidermale "knopvorming" bestaand uit BCC weefsel in klinisch normale huid van het voorhoofd van 2 patienten met multipele BCC's.

Retinoid behandeling met etretinaat van 3 patienten met multipele BCC's (dosering 0.3-1.0 mg/kg/dag) gaf steun voor de opvatting, dat retinoid behandeling een preventief effect heeft op de ontwikkeling van BCC's. Dit effect hield aan tot ongeveer 9 maanden na staken van de behandeling. Het therapeutische effect van etretinaat behandeling van klinisch waarneembare BCC's was beperkt, terwijl recidieven optraden bij alle patienten. Hepatitis, waarschijnlijk door etretinaat veroorzaakt, trad op bij 1 patient en was geassocieerd met het massaal recidiveren van BCC's. Etretinaat behandeling had geen duidelijke invloed op de immunologische parameters, die daarop nage-

keken werden, d.w.z. op de lymfoproliferatieve respons in vitro en de fytohaemagglutinine- geïnduceerde T-cel cytotoxociteit.

Concluderend: a) de immuun respons van de onderzochte BCNS patienten was normaal, met uitzondering van een mogelijk defect in de cellulaire immuun respons in vivo van sommige patienten met multipele BCC's, hetgeen een gevolg zou kunnen zijn van de epidermale cel differentiatie stoornis van BCNS; b) systemische retinoid medicatie met etretinaat was van preventieve waarde bij de behandeling van de multipele BCC's, die vaak voorkomen bij BCNS patienten, en had geen invloed op de immuun respons.

Hoofdstuk 2.1 toont het immunologisch en klinisch onderzoek van een patient met XP, die behoorde tot complementatie groep C en die behandeld werd met het retinoid etretinaat. Gedurende een Noordelijke winter (met het doel het effect van zonlicht expositie te minimaliseren) toonde immunologisch onderzoek het volgende aan: 1) een verlaagd aantal lymfocyten in het perifere bloed met een relatief normale verdeling wat betreft de percentages van de verschillende soorten T-cellen; 2) een verminderde antigeen-specifieke humorale en cellulaire immuun respons in vivo, gemeten aan de hand van de klasse-specifieke antilichaam respons na immunisatie met HPH en de DNCB sensibilisatie test; 3) een laag normale lymfoproliferatieve respons in vitro na stimulatie met mitogenen en allogene lymfocyten; 4) een verlaagd aantal T6+ en HLA-DR+ Langerhans cellen per mm^2 epidermis van het voorhoofd vergeleken met de rug, waarschijnlijk ten gevolge van zonlicht expositie, hetgeen de locale immuun respons op tumor groei negatief zou kunnen beinvloeden; 5) HLA-DR expressie van epidermale keratinocyten geassocieerd met de aanwezigheid van Leu-3a+ en OKT-8+ T-cellen in het stratum papillare van de dermis, hetgeen een veel voorkomend reactie patroon is van onbegrepen betekenis.[8]

Klinische observatie gedurende 5 jaar suggereerde, dat behandeling met het retinoid etretinaat, 0.9 mg/kg/dag, de groei van cutane carcinomen voorkwam. Retinoid behandeling had geen duidelijke invloed op de immunologische parameters, met uitzondering van een passagère stimulatie van de lymfoproliferatieve, door mitogenen geïnduceerde, immuun respons in vitro. Spontane perforatie van de cornea, het aantonen van de aanwezigheid van een keratoconus en de gunstige invloed van cornea transplantatie onderstrepen de noodzaak van oogheelkundige controle.

Concluderend: a) immunologisch onderzoek van deze XP patient gedurende een Noordelijke winter toonde een suboptimale immuun respons aan, die de groei van oculo-cutane, door UV licht geinduceerde, maligniteiten zou kunnen begunstigen; b) systemische retinoid behandeling met etretinaat leek de groei van cutane carcinomen van deze XP patient te voorkomen en had een

voorbijgaand effect op de immuun respons.

Hoofdstuk 2.2 beschrijft de resultaten van een onderzoek naar het mogelijke effect van retinoid behandeling op de basisafwijking van XP, d.w.z. de UV licht geinduceerde DNA herstel stoornis. De DNA synthese na UV licht expositie in vitro van fibroblasten van een patient met XP en een controle werd niet beinvloed door het toevoegen van verschillende concentraties vitamine A-zuur aan het medium, waarin de fibroblasten gekweekt werden. Dit suggereert dat het preventieve effect van retinoid behandeling op tumor groei bij XP patienten niet samenhangt met beinvloeding van het vermogen tot DNA herstel.

Hoofdstuk 3.1 beschrijft een immunohistologisch, immunologisch en cytogenetisch onderzoek van een negroide EV patient met cutane SCC's en een verminderde immuun respons, gemeten aan de hand van de lymfoproliferatieve respons in vitro en de DNCB sensibilisatie test in vivo, die kortdurend behandeld werd met het retinoid etretinaat. De resultaten toonden: 1) de aanwezigheid van microscopische EV lesies in klinisch normale huid van het voorhoofd; 2) de vrijwel volledige afwezigheid van T6+ en HLA-DR+ Langerhans cellen in de koilocytotische gebieden van epidermis met EV-specifieke histologische afwijkingen (koilocytosis is een HPV-specifiek cytopathisch effect); 3) een verandering van het in situ patroon van T6+ en HLA-DR+ Langerhans cellen in epidermis met EV-specifieke histologische afwijkingen, d.w.z. de aanwezigheid van deze cellen in koilocytotische gebieden, na 8 dagen continue retinoid behandeling; 4) een toename van de lymfoproliferatieve respons in vitro, geinduceerd door fytohaemagglutinine en concanavaline A, na 10 dagen continue retinoid behandeling; 5) geen afwijkingen wat betreft de frequentie van "sister chromatid exchanges", het aantal spontane en UV licht geinduceerde chromosomale breuken en de mate van DNA synthese na UV licht bestraling.

Deze resultaten suggereren: a) een deficiente rol van de epidermale Langerhans cel in de HPV-specifieke cellulaire immuun respons in EV; b) beïnvloeding van de immuun respons door het retinoid etretinaat; c) dat toegenomen chromosomale instabiliteit waarschijnlijk niet de oorzaak van EV is, terwijl de afwezigheid van cel overgevoeligheid, reeds aangetoond door andere onderzoekers, werd bevestigd.

Hoofdstuk 3.2 beschrijft een virologisch onderzoek van een blanke EV patient met multipele SCC's en een verminderde immuun respons, die bovendien een primair levercel carcinoom ontwikkelde geassocieerd met een chronische hepatitis B virus infectie. Vijf verschillende HPV's werden gevonden

in de benigne gedissemineerde huid afwijkingen veroorzaakt door EV, d.w.z. HPV5, 8, 17, 19 en 24. De laatste drie HPV's werden als gevolg van dit onderzoek voor het eerst gekarakteriseerd. DNA sequenties van HPV5 werden aangetoond in het centrale deel van een invasief SCC. Deze casus ondersteunt de hypothese, die suggereert dat bepaalde HPV typen potentieel oncogeen zijn en benadrukt het belang van immunologische afweer tegen virusgeassocieerde tumoren.

Hoofdstuk 4.1 betreft het onderzoek naar hepatologische bijwerkingen van kortdurende systemische retinoid behandeling met etretinaat, die gepaard gaan met lever functie stoornissen. Er worden twee patienten beschreven met een histologisch bevestigde, reversibele hepatitis zonder klinische symptomen, die optrad gedurende de eerste maanden na het starten van therapie met etretinaat. Andere oorzaken van hepatitis dan de behandeling met etretinaat werden niet gevonden. Het opnieuw innemen van etretinaat resulteerde in activatie en/of persisteren van de hepatitis. Deze gegevens maken het zeer waarschijnlijk, dat de hepatitis van beide patienten veroorzaakt werd door etretinaat. Tijdens behandeling van één van deze patienten met 13-cis-vitamine A-zuur werden geen lever functie stoornissen waargenomen.

Hoofdstuk 4.2 betreft het onderzoek naar hepatologische bijwerkingen van langdurige systemische retinoid behandeling van 13 patienten zonder lever functie stoornissen, die behandeld werden met etretinaat (n=6), vitamine A-zuur (VAZ; n=6) en 13-cis-vitamine A-zuur (13-cis-VAZ; n=1). In gewone histologische coupes van lever weefsel werden geen afwijkingen waargenomen afgezien van enige steatose in sommige gevallen. Perisinusoidale fibrose was niet aanwezig. Het aantal Ito cellen, d.w.z. perisinusoidale levercellen, die vet kunnen opslaan en bindweefsel kunnen vormen en die door de lichtmicroscoop gezien kunnen worden, wanneer men de accumulatie van vet druppeltjes in deze cellen als markering gebruikt, was toegenomen bij 6/6 etretinaat en bij 5/6 VAZ patienten. Vet druppeltjes, die maakten dat de Ito cellen uitpuilden in het sinusoidale lumen, werden bij 3/6 etretinaat en bij 3/6 VAZ patienten gezien. Meting van de miltdruk van 2 etretinaat patienten met zulke uitpuilende Ito cellen toonde licht verhoogde waarden aan, hetgeen wijst op portale hypertensie. Deze bevinding zal waarschijnlijk geen ernstige consequenties hebben, aangezien het risico op complicaties door portale hypertensie bij dit druk niveau zeer laag is. Deze resultaten geven aan dat het risico op ernstige hepatologische bijwerkingen van langdurige systemische retinoid behandeling, zoals die bij hypervitaminasis A gezien worden, d.w.z. fibrose en portale hypertensie, minimaal is.

Hoofdstuk 4.3 bevat de resultaten van een quantitatieve analyse van serum, lever weefsel, subcutaan vet en epidermis-dermis, welk materiaal verkregen werd bij 20 patienten, die behandeld werden met etretinaat (n=9), VAZ (n=9) en 13-cis-VAZ (n=2). In lever weefsel van patienten, die met etretinaat behandeld werden, werd accumulatie gevonden van metabolieten, die waarschijnlijk van etretinaat afkomstig waren, inclusief de voornaamste metaboliet (Ro 10-1670) van etretinaat, terwijl in subcutaan vet accumulatie van etretinaat zelf werd gevonden. Vergelijkbare bevindingen werden gedaan in weefsel van patienten, die met VAZ behandeld werden, d.w.z. dat er metabolieten in lever weefsel werden gevonden en VAZ zelf in subcutaan vet. In het onderzochte weefsel was geen 13-cis-VAZ aantoonbaar. Deze quantitatieve gegevens lijken te correleren met de histologische bevindingen, die in hoofdstuk 4.2 gepresenteerd werden, aangezien beide benaderingen accumulatie van metabolieten van etretinaat en waarschijnlijk ook van VAZ in lever weefsel suggereren, mogelijk voornamelijk in Ito cellen.

Conclusies

De onderzoekingen, welke gepresenteerd worden in dit proefschrift, zijn gegroepeerd rondom twee themas: a) dysfunctie van het immunologisch bewakingsapparaat, mogelijk leidend tot begunstiging van tumor groei; b) behandeling en preventie van tumor groei door systemische retinoid medicatie. De conclusies, voortkomend uit de resultaten van het onderzoek, worden voor elk thema apart besproken.

Aangaande het eerste thema wordt het volgende geconcludeerd:

1) De immuun respons van BCNS patienten is niet gestoord, afgezien van het feit, dat er aanwijzingen zijn voor een verminderde respons in de cutane overgevoeligheids reactie van het vertraagde type bij sommige BCNS patienten met multipele BCC's. Men kan speculeren of een dergelijk defect verklaard kan worden door immunologische dysfunctie van de keratinocyt, aangezien een epidermale cel differentiatie stoornis de oorzaak van de cutane symptomen van BCNS lijkt te zijn.

2) De immuun respons van XP patienten kan verminderd zijn en het aantal T6+ en HLA-DR+ epidermale Langerhans cellen in zonlicht geëxponeerde huid van XP patienten kan verlaagd zijn, zelfs in de winter met relatief geringe zonlicht expositie. Beide bevindingen zouden mogelijk kunnen leiden tot begunstiging van tumor groei. De immuun respons is echter niet in alle XP patienten gestoord. De aan- of afwezigheid van een verminderde immuun response in XP is wellicht gebonden aan de ernst van het klinisch ziektebeeld,

d.w.z. aan de mate van gevoeligheid voor UV licht geïnduceerde cel beschadiging.

3) Dat er bij EV veelal sprake is van een gestoorde non-HPV-specifieke immuun respons, werd door immunologisch onderzoek bevestigd. Immunohistologisch onderzoek suggereerde, dat de epidermale Langerhans cel in gebreke blijft wat betreft zijn aandeel in de HPV-specifieke cellulaire immuun respons in EV. Cytogenetisch en celbiologisch onderzoek gaf geen steun aan de veronderstelling, dat er bij EV sprake is van chromosomale instabiliteit of cel overgevoeligheid. Door virologisch onderzoek werd DNA van een EV-specifiek HPV (HPV5) aangetoond in een invasief cutaan SCC van een EV patient, die tevens een primair levercel carcinoom geassocieerd met een chronische hepatitis B virus infectie ontwikkelde. Dit ondersteunt de hypothese, dat bepaalde HPV's potentieel oncogeen zijn en benadrukt het belang van immunologische afweer tegen virus-geassocieerde tumoren.

Aangaande het tweede thema wordt het volgende geconcludeerd:

1) Systemische retinoid behandeling met etretinaat lijkt een preventief effect te hebben op de groei van cutane carcinomen bij patienten met BCNS en XP. Het beperkte therapeutische effect van etretinaat behandeling op klinisch waarneembare BCC's en het recidief risico zijn argumenten tegen de behandeling van deze carcinomen met systemische retinoid medicatie.

2) Systemische retinoid behandeling met etretinaat had geen effect op de immuun respons van patienten met BCNS. De suboptimale lymfoproliferatieve respons in vitro van een XP en een EV patient leek door etretinaat behandeling slechts tijdelijk gestimuleerd te worden.

3) Vitamine A-zuur had, in vitro, geen invloed op de UV licht geinduceerde DNA herstel stoornis van XP. Het bevorderen van normale cel differentiatie lijkt het belangrijkste mechanisme, waardoor het preventieve effect van retinoid behandeling op tumor groei verklaard kan worden.

4) Het retinoid etretinaat kan hepatitis veroorzaken in de eerste maanden na het starten van de behandeling, hetgeen het geval schijnt te zijn bij ongeveer 1% van de patienten. Bij patienten met normale lever functies, die langdurig systemisch met retinoiden behandeld worden, lijkt het risico op ernstige, bij hypervitaminosis A voorkomende, hepatologische bijwerkingen, zoals fibrose en portale hypertensie, minimaal.

Toekomstig onderzoek zal de vele nog open vragen aangaande de rol van het immuun systeem in de cutane oncologie moeten beantwoorden, met name vragen aangaande de immuun respons bij patienten met basaalcel carcinomen

en vragen vanuit het terrein van de fotoimmunologie. Dynamisch onderzoek zal mogelijk licht werpen op de invloed van zonlicht expositie op het immuun systeem van de mens. De mogelijk oncogene rol van humane papillomavirussen wordt actief bestudeerd in vele centra, ook in Nederland, in het bijzonder wat betreft de aetiologische rol van deze virussen bij carcinomen van de cervix uteri. Het Basaal cel nevus syndroom is een boeiend klinisch model, waarin de mogelijke rol van oncogenen onderzocht kan worden.

Literatuur

1. The TH. Kanker. In: Feltkamp TEW, ed. Klinische immunologie. Utrecht: Bohn, Scheltema & Holkema, 1980:163-92.
2. Klein G, Klein E. Immune surveillance against virus-induced tumors and non-rejectability of spontaneous tumors: contrasting sequences of host versus tumor evolution. Proc Natl Acad Sci USA 1977;74:2121-5.
3. Miller DG. On the nature of susceptibility to cancer. Cancer 1980;46:1307-18.
4. Herberman RB. Possible role of natural killer cells and other effector cells in immune surveillance against cancer. J Invest Dermatol 1984;83:137s-40s.
5. Epstein JH. Photocarcinogenesis, skin cancer and aging. J Am Acad Dermatol 1983;9:487-502.
6. Kripke ML. Immunology and photocarcinogenesis. J Am Acad Dermatol 1986;14:149-55.
7. Moon RC, Itri LM. Retinoids and cancer. In: Sporn MB, Roberts AB, Goodman DS, eds. The Retinoids. Vol. 2. New York: Academic Press Inc., 1984:327-71.
8. Aubock J, Romani N, Grubauer G, Fritsch P. HLA-DR expression on keratinocytes is a common feature of diseased skin. Br J Dermatol 1986;114:465-72.