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THEME ISSUE: FROM ACNE TO RETINOIDS AND LYMPHOMAS

COMPOSED BY
ANDERS VAHLQUIST, EDITOR-IN-CHIEF

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THEME ISSUE:
FROM ACNE TO RETINOIDS AND LYMPHOMAS

Composed by Anders Vahlquist, Editor-in-Chief

This theme issue of Acta Dermato-Venereologica bridges two seemingly unrelated diseases, acne and lymphoma, by including papers on retinoid therapy for both conditions. Beginning with acne vulgaris, accumulating evidence suggests that diet after all plays a role, which is ventilated in a commentary (p. 228) of a prospective study using low- and high-calorie diets in adolescents with acne (p. 241). Acne treatment regimes usually differ from one country to another; a Korean survey (p. 236) combined with a review on how to treat post-acne hyperpigmentation with hydroquinone (p. 232) add valuable information to this field. While isotretinoin is still the drug of choice for severe acne, the optimal dosage needs to be repeatedly discussed (p. 247). Interestingly, isotretinoin is also worth trying (off-label) in large condylomas (p. 249). Two newer types of retinoids, alitretinoin and bexarotene, are used in treating such diverse conditions as eczema and cutaneous lymphomas. By binding to RXR receptors, alitretinoin and bexarotene exert positive and negative effects far beyond those of pure RAR agonists. For the former drug, this is illustrated in a large trial on chronic hand eczema (p. 251) and in a pilot study of its use for congenital ichthyosis (p. 256). For bexarotene, a new Finnish study shows 10 years experience of this therapy in severe cutaneous lymphoma (p. 258). Depending on the stage of the disease, lymphomas can also be treated for instance with photodynamic therapy (p. 264) and methotrexate (p. 276). Eventually, novel therapies for cutaneous lymphoma will hopefully be developed based on the expanding knowledge about its pathogenesis, as exemplified in this issue by studies of cytokine release and receptor expression (p. 280 and 282).

In preparing this theme issue the following colleagues have provided invaluable help: Tilo Biedermann, Reinhard Dummer, Bodo Melnik, Annamari Ranki, and Kristian Thestrup-Pedersen.

Uppsala, March 5, 2012
Anders Vahlquist
Editor-in-Chief

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COMMENTARY to Kwon et al. on p. 241

Diet in Acne: Further Evidence for the Role of Nutrient Signalling in Acne Pathogenesis

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Recent evidence underlines the role of Western diet in the pathogenesis of acne. Acne is absent in populations consuming Palaeolithic diets with low glycaemic load and no consumption of milk or dairy products. Two randomized controlled studies, one of which is presented in this issue of *Acta Dermato-Venereologica*, have provided evidence for the beneficial therapeutic effects of low glycaemic load diets in acne. Epidemiological evidence confirms that milk consumption has an acne-promoting or acne-aggravating effect. Recent progress in understanding the nutrient-sensitive kinase mammalian target of rapamycin complex 1 (mTORC1) allows a new view of nutrient signalling in acne by both high glycaemic load and increased insulin-, IGF-1-, and leucine signalling due to milk protein consumption. Acne should be regarded as an mTORC1-driven disease of civilization, like obesity, type 2 diabetes and cancer induced by Western diet. Early dietary counselling of teenage acne patients is thus a great opportunity for dermatology, which will not only help to improve acne but may reduce the long-term adverse effects of Western diet on more serious mTORC1-driven diseases of civilization. *Key words: acne; diet; glycaemic load; milk; mTORC1.*

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The influence of diet on the induction and aggravation of acne has been a matter of intense debate over the last few years. The pioneering observation by Cordain et al. (1), who demonstrated that acne is a disease of Western civilization and is absent in populations consuming Palaeolithic diets without refined sugars, grains, milk and dairy products, resulted in a paradigm change. The randomized, controlled Australian study by Smith et al. (2) provided the first clinical evidence for the beneficial therapeutic effects of a low glycaemic load diet on the clinical course and intensity of acne and sebum production. The randomized controlled South Korean trial of Kwon and co-workers in this issue of *Acta Dermato-Venereologica* (3) confirmed that glycaemic load plays a substantial role in the pathogenesis and treatment of acne. Subjects within the low glycaemic group demonstrated significant clinical improvement in the number of both non-inflammatory and inflammatory acne lesions. Remarkably, Kwon et al. (3) now provide the first histopathological and immunohistochemical evidence that a low glycaemic load diet reduced the size of sebaceous glands, decreased inflammation, and diminished the expression of pro-inflammatory interleukin-8 and sterol regulatory element binding protein-1 (SREBP-1), the key transcription factor of lipid biosynthesis.

HIGH GLYCAEMIC LOAD AND MILK ACTIVATE THE NUTRIENT-SENSITIVE KINASE mTORC1

Experimental evidence has been provided for the important role of insulin/insulin-like growth factor-1 (IGF-1) signalling in SREBP-1-mediated sebaceous lipogenesis (4, 5). Although the impact of hyperglycaemic carbohydrates on enhanced insulin-/IGF-1 signalling in acne has been robustly supported by the studies of Smith et al. (2) and Kwon et al. (3), until recently only a weak association has been accepted for the role of milk and dairy products in acne pathogenesis (6). There is, however, substantial epidemiological and biochemical evidence supporting the effects of milk and dairy products as enhancers of insulin-/IGF-1 signalling and acne aggravation (7–12). In fact, milk signalling potentiates the signalling effects of hyperglycaemic carbohydrates (13) (Fig. 1).

We have to ask whether there is a unifying link connecting the nutrient signalling pathways induced by hyperglycaemic carbohydrates with those of milk consumption. We will answer this question only when we change our perception of milk and dairy as simple food. We have to appreciate that milk is a species-specific endocrine signalling system that activates a central signalling node in cellular metabolism for stimulation of growth and cell proliferation: the nutrient-sensitive kinase mammalian target of rapamycin complex 1 (mTORC1) (14). Both puberty-induced growth and milk-induced neonatal growth are driven by the same insulin/IGF-1 signal transduction pathways, which finally upregulate mTORC1 signalling. In all mammalian species, mTORC1 integrates nutrient signals, such as glucose (ATP/energy status of the cell), essential amino

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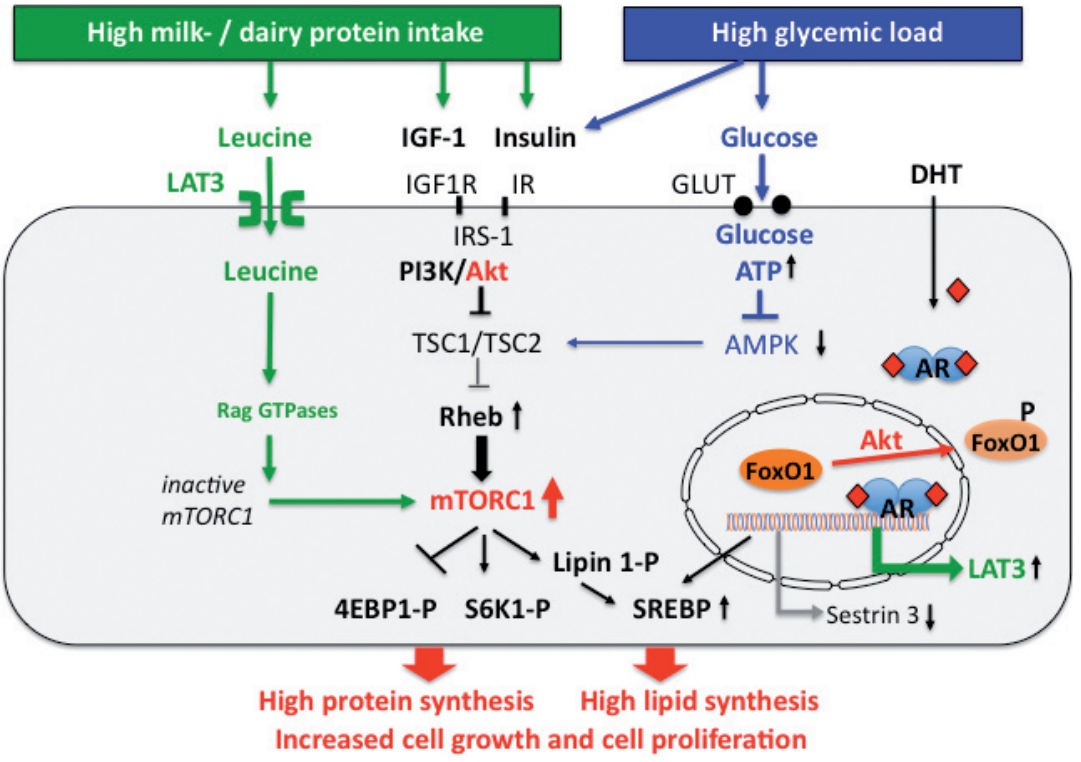

Fig. 1. Nutrient-mediated signalling pathways in acne. High glycaemic load increases cellular adenosine triphosphate (ATP) levels, which suppress AMPK. Low AMPK activity impairs the inhibitory effect of TSC2, thus promoting the activation of Rheb, the final activator of mTORC1. High insulin/IGF-1-signals activate Akt (protein kinase B), thereby reducing the inhibitory function of TSC1/TSC2 towards Rheb, thus leading to activation of mTORC1. Milk activates insulin/IGF-1 signalling towards Rheb and furthermore activates mTORC1 by increased availability of leucine. Activated Akt phosphorylates FoxO1, which is expelled from the nucleus, thereby augmenting androgen receptor (AR) signalling. LAT3 is expressed in an AR-dependent manner and activates intracellular leucine-uptake for further mTORC1 activation. Akt-mediated nuclear extrusion of FoxO1 decreases the expression of Sestrin 3, an important activator of AMPK. mTORC1 is activated by high glycaemic load diets and increased milk/dairy protein consumption. Hyperactivated mTORC1 promotes protein (via 4EBP-1, S6K1) and lipid synthesis (via SREBP-1). Abbreviations: IGF-1: insulin-like growth factor-1; IGF1R: IGF-1 receptor; IR: insulin receptor; IRS-1: insulin receptor substrate-1; PI3K: phosphoinositol-3 kinase; Akt: Akt kinase; AMPK: AMP-activated kinase; TSC1: hamartin; TSC2: tuberin; Rheb: ras homolog enriched in brain; mTORC1: mammalian target of rapamycin complex 1; 4EBP1: 4E-binding protein; S6K1: S6 kinase 1; LAT3: L-type amino acid transporter-3; GLUT; glucose transporter; SREBP: sterol regulatory binding protein; DHT: dihydrotestosterone; AR: androgen receptor; FoxO1: forkhead box class O transcription factor 1.

acids (predominantly leucine availability) and growth factor signals (insulin, IGF-1, fibroblast growth factors (FGFs)) (14) (Fig. 1). The endocrinological changes in milk signalling are thus comparable to the endocrinology of puberty. Both periods of growth, the milk-driven period of neonatal growth and growth hormone-driven puberty are associated with elevations in IGF-1, insulin and insulin resistance.

CROSS-TALK BETWEEN ANDROGEN-, FOXO1- AND mTORC1 SIGNALLING

Remarkably, insulin/IGF-1 signalling via activation of phosphoinositol-3 kinase and Akt kinase control the nuclear localization of the nutrient-sensitive transcription factor FoxO1, which has been implicated to play a major role in acne pathogenesis (15). The androgen receptor (AR) co-suppressor FoxO1 regulates AR transcriptional activity (16, 17). High insulin/IGF-1 signalling results in Akt-mediated nuclear extrusion of FoxO1 and activation of AR-mediated gene expression (16, 17). Intriguingly, AR signalling increases the expression of the L-type amino acid transporter LAT3 (18), which increases intracellular leucine levels, a most critical step for mTORC1 activation by amino acids (18, 19). An important downstream target of mTORC1 is the kinase S6K1, which induces insulin resistance in adipose tissue, liver and skeletal muscle by inhibiting insulin signalling via phosphorylation of insulin receptor substrate-1 (IRS-1) (20). The fact that most acne-associated syndromes, such as polycystic ovary syndrome, are associated with insulin resistance points to increased mTORC1-S6K1 signalling in acne (21). Although there are no randomized controlled clinical studies investigating the impact of milk and dairy products on sebaceous gland signal transduction, epidemiological data and recent biochemical concepts strongly support the acne-promoting effect of milk consumption. Milk consumption has been demonstrated to induce hyperinsulinaemia, insulin resistance, and raise serum IGF-1 levels in children and adults (8, 9, 22).

mTORC1-SREBP-1 PATHWAY

Intriguingly, the key lipogenic transcription factor SREBP-1 has recently been identified as an important downstream target of mTORC1 (23, 24). Attenuation of mTORC1 hyperactivity by a low glycaemic load diet may thus suppress the expression and activity of SREBP-1, a possible mechanism compatible with the findings of Kwon et al. (3). The intake of abundant hyperglycaemic carbohydrates and high consumption of milk and dairy protein predominantly during puberty, a period of high insulin/IGF-1 signalling, may overactivate mTORC1, which enhances sebocyte growth and proliferation and SREBP-1-mediated sebaceous lipogenesis. A lipid-enriched sebaceous gland microenvironment may than promote excessive proliferation of *Propionibacterium acnes* with resultant inflammatory reactions of the pilosebaceous follicle. A low influx of glucose due to restriction of hyperglycaemic carbohydrates thus reduces insulin/IGF-1 signalling and increases cellular AMPK levels, which finally attenuate mTORC1- and SREBP-1-activity.

HYPERACTIVATED mTORC1 AND OTHER DISEASES OF CIVILIZATION

The importance of the insulin/IGF-1 signalling axis towards mTORC1 becomes obvious in untreated short-stature individuals with Laron syndrome, who exhibit congenital insulin/IGF-1 deficiency and do not develop acne (25). Intriguingly, untreated patients with Laron syndrome are protected from common diseases of civilization, such as acne, type 2 diabetes and cancer (25, 26). In contrast, increased mTORC1 signalling has been associated with obesity, type 2 diabetes and cancer (27, 28). Dairy protein consumption in adults as well as daily milk consumption during adolescence has been related to higher risk of prostate cancer (29, 30). Moreover, the addition of commercial milk or purified casein to an AR-sensitive prostate cancer cell line significantly enhanced cancer cell growth (31). These findings shed new light on the role of milk signalling during adolescence and may explain the observed association of severe acne and increased risk of prostate cancer later in life by a common mode of signal transmission (32).

CONCLUSION

We are only beginning to understand crucial nutrient-derived signalling pathways that are integrated and further processed by mTORC1. The high glycaemic load pathway to mTORC1 in acne appears to be established, but the nutrient signalling of high milk/dairy protein consumption awaits further experimental confirmation. Acne appears to be an early clinical indicator of hyperactivated mTORC1 signalling, paving the way to other more serious late-onset mTORC1-driven Western diseases of civilization, such as obesity, type 2 diabetes and cancer. Dermatologists have the opportunity to observe and elaborate nutrient-driven skin pathology of Western diets, and should provide early dietary counselling for teenage acne patients at the beginning of their lifelong exposure to Western diets.

The author declares no conflicts of interest.

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MINI-REVIEW

Hydroquinone Therapy for Post-inflammatory Hyperpigmentation Secondary to Acne: Not Just Prescribable by Dermatologists

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Post-inflammatory hyperpigmentation after acne can be as troublesome as the acne itself. Hydroquinone, a tyrosinase inhibitor, in a 4% cream can be used safely twice daily for up to 6 months to treat post-inflammatory hyperpigmentation. The efficacy of this treatment can be enhanced by using a retinoid nightly and a mid-potent steroid, which is applied twice daily for 2 weeks, then at weekends only. Combination creams help with compliance, but often lack the strongest individual ingredients. Because steroids should not be applied to the face for prolonged periods, care should be taken when a hydroquinone cream containing a steroid is chosen. If post-inflammatory hyperpigmentation consists of a few lesions, spot therapy is useful. If post-inflammatory hyperpigmentation consists of many lesions, field therapy is favored. Safety concerns with hydroquinone consist only of occasional irritation, which can be suppressed with topical steroid or a short drug holiday. Physicians should feel comfortable to use hydroquinone without consulting a dermatologist. *Key words: hydroquinone; acne; adolescent; post-inflammatory hyperpigmentation.*

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Nearly 50 million Americans have acne vulgaris each year (1), making it one of the most commonly encountered conditions in primary-care and dermatology practices nationwide. Post-inflammatory hyperpigmentation (PIH) often develops secondary to either the acne itself or to damaged skin caused by overly aggressive treatment. Clinically, PIH presents as localized or diffuse brown macules at sites of former acne papules and pustules (Fig. 1). The skin discoloration, which is due to excess melanin, may persist for several months or even years. Many patients find the persistent PIH more psychologically disturbing than their original acne lesions (2). It is important for physicians to recognize the negative impact that acne and PIH can have on a patient's emotional health (causing anxiety and depression), as well as on his or her social interactions, self-esteem, self-confidence, and even employment opportunities (3).

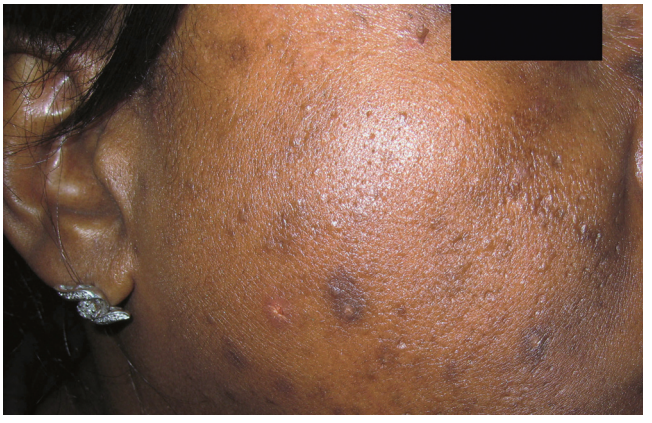

Fig. 1. Post-inflammatory hyperpigmentation due to acne in a dark skin-type.

Hydroquinone, a skin-bleaching cream, is the gold-standard for treating PIH and other disorders of hyperpigmentation, such as melasma and solar lentigines (4). It is indicated for patients age 13 years and up. Hydroquinone has been produced in various over-the-counter (OTC) and prescription formulations in the USA for over 55 years, with only exceedingly rare adverse reactions reported (5). OTC hydroquinone 2% preparations are typically less effective than the prescription 4% preparations. Adolescents are among those affected by acne, and a significant subset of these patients will experience related PIH. However, the majority of hydroquinone prescriptions written in the USA are done so by dermatologists. Indeed, from November 2009 to November 2010, a total of 470,964 hydroquinone prescriptions were written in the USA (excluding in-office dispensing), out of which 252,066 were written by dermatologists, 72,346 by primary care physicians, and another 146,552 by other specialty physicians (6). This paper aims to make the physician more comfortable with hydroquinone therapy by delineating how to use hydroquinone for PIH secondary to acne, as well as the drug's mechanism of action and safety profile.

PATHOPHYSIOLOGY OF POST-INFLAMMATORY HYPERPIGMENTATION

In normal skin, melanocytes (specialized dendritic cells located at the dermal–epidermal junction) convert

tyrosine into melanin via the enzyme tyrosinase. This process occurs within specialized intracellular vesicles called melanosomes, which are then transferred to keratinocytes and sent to the epidermal surface. The quantity, melanin content, and distribution of these melanosomes determine the various hues of human skin color (7).

Hyperpigmentation disorders usually result from an increase in melanin production, and less commonly, from an increase in the number of active melanocytes (7). The most important risk factor in the development of all hypermelanotic conditions is ultraviolet (UV) irradiation from sun exposure, although in acne, inflammation plays an equal if not more important role. Even minimal sunlight sustains melanocytic activity. Because exposure to UVA and UVB light leads to melanocytic growth and increased transfer of melanosomes to keratinocytes, broad-spectrum sun-blocks are an essential adjunct to any treatment regimen for hyperpigmentation (8).

PIH frequently develops secondary to cutaneous inflammation or injury (7). Acne vulgaris is one of the most common inflammatory skin disorders that results in hypermelanosis (2). All age groups are equally affected, and there is no difference between genders; however, PIH is more likely to develop in patients with darker skin types (7). The time of onset of hyperpigmentation relative to the inciting inflammation has never been studied rigorously, but it typically evolves over a few days. The hyperpigmentation frequently becomes apparent only after the erythema has resolved.

The skin discoloration of PIH, which is caused by excess melanin within the epidermis and/or dermis, ranges in color from either tan to dark brown (epidermal melanin) or gray-blue to gray-brown (dermal melanin). Epidermal hyperpigmentation results from increased melanin production and/or melanosome transfer to keratinocytes. This melanin will be shed with the monthly turnover of the epidermis. In contrast, dermal hyperpigmentation develops when melanin crosses the damaged basement membrane, where it is phagocytosed and retained by dermal macrophages, sometimes permanently (7).

MECHANISM OF HYDROQUINONE

Hydroquinone, or 1,4-dihydroxybenzene, is a phenolic bleaching compound that is the gold-standard therapy for PIH. The mechanisms of action of this drug include: (*i*) reversible inhibition of tyrosinase (the main enzyme involved in the conversion of tyrosine to melanin); and (*ii*) selective damage to melansomes and melanocytes (7). Therefore, the mechanism of action of topical hydroquinone is through prevention of new melanin production. As skin cells mature, the melanin-containing keratinocytes within the epidermis are shed and new keratinocytes are formed with less pigmented melanosomes (7). As depicted in Fig. 2, the epidermis effectively lightens over time. Hydroquinone is relatively ineffective against dermal hyperpigmentation because it cannot penetrate the dermal–epidermal junction and dermal melanin that is already present has less means of egress.

Because the amount of time that elapses between the initial inflammation and development of hyperpigmentation is unknown, when to begin hydroquinone therapy remains subjective. Theoretically, one would prefer initiating therapy prior to the onset of PIH (i.e., at the onset of an inflammatory acne lesion); however, that would require adding a hydroquinone cream to an often topical-intensive acne regimen. Compliance, and possibly drug incompatibility (in the case of benzoyl peroxide and hydroquinone), are limiting factors. More often, hydroquinone therapy is initiated once the acne is under control or resolved.

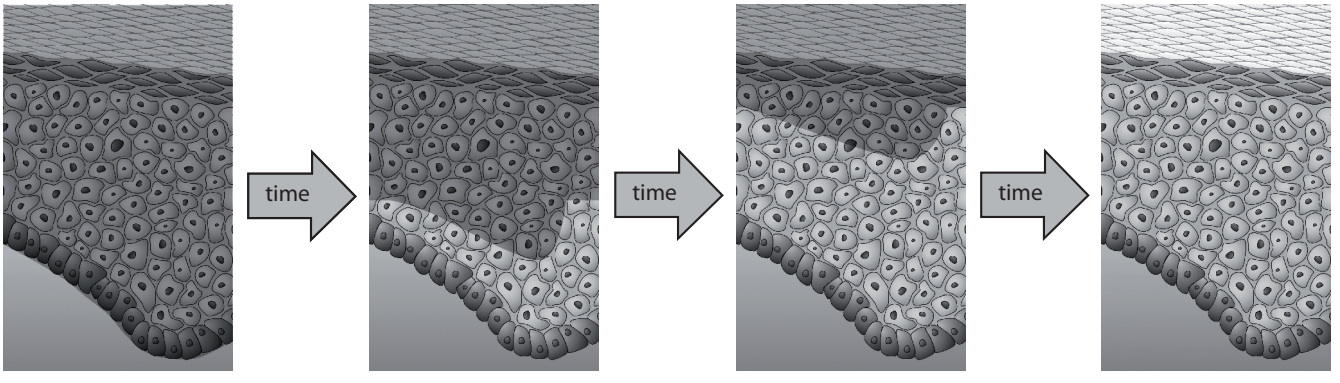

Fig. 2. Schematic depicting the progression of skin lightening that occurs as epidermal melanin is shed while preventing new epidermal melanin formation. In dyschromia, excess melanin is found in skin cells throughout the epidermis. As skin cells mature, cells containing melanin are shed. When new melanin production is inhibited, the rate of new melanin production is less than the rate melanin is shed. Thus, the skin lightens. The process occurs over 1–6 months, depending on the physician's ability to quell the inciting trigger for local excess melanin formation (i.e., in the case of acne, the ability successfully to treat the acne and/or protect the pigmented area from ultraviolet light).

HYDROQUINONE TREATMENT REGIMENS

In 1975, a new formula was introduced by Kligman & Willis (9) for the effective treatment of hyperpigmentation that consisted of hydroquinone 5%, tretinoin 0.1%, and dexamethasone 0.1%. Known today as "Kligman's formula", this combination was found to be more therapeutically effective in treating PIH, melasma, and ephelides than any of the three components independently. In fact, Kligman reported that his formulation achieved complete depigmentation in normal skin of black patients when it was applied daily for 5–7 months (9).

Given the observations of Kligman & Willis, maximal PIH therapy of the face should include hydroquinone 4% or 5% (4% is the strongest available prescription concentration available without compounding in the USA), a mid-potent steroid, a topical retinoid, and sunscreens. The hydroquinone should be applied twice daily for 2–6 months. If no results are seen after 2 months, it should be discontinued. Therapy beyond 6 months is not expected to yield additional improvement when positive results are seen. Because long-term steroid side-effects include cosmetically unappealing cutaneous atrophy, marked by striae distensae, telangiectases, and steroid acne, it should be used twice daily for 2 weeks then once to twice weekly thereafter. Signs of skin thinning or excessive skin lightening are an indication to stop using the steroid. These side-effects, and therefore use limitations, are expected of any class of steroid, whether low or medium potency. As such, a mid-potent steroid allows for maximal efficacy. A topical retinoid should be chosen on the basis of balancing skin irritation vs. efficacy. Gentle retinoids include retinol 0.3% applied twice daily, which, in combination with hydroquinone 4%, has been show to have comparable skin lightening effects to tretinoin 0.05% applied nightly (10). Stronger retinoids include tazarotene 0.1% cream (the gel formulation tends to be more irritating). Because tazarotene can be irritating if left on all night, one method for applying it is to leave it on for 2 min and then wash off, increasing the application time by 2 min every 4 days. Once 7–10 min is reached, it can be left on all night (11). Finally, sunscreen with an SPF of ≥ 15 should be applied in the morning and every 2 h thereafter if going in the sun.

Because 4 different agents are needed for the optimal treatment of PIH, combination creams become an important adjunct to therapy. The ideal combination would allow for twice daily dosing of hydroquinone and sunscreen with SPF 15 or greater (since patients may not comply with additional applications (12)), with our without a gentle retinoid that could be used twice daily without being overly irritating. Independent control over both the steroid and the retinoid is favored, allowing for limited use of a mid-potent steroid and titration of retinoid potency.

While a triple combination cream containing hydroquinone 4%, tretinoin 0.05%, and fluocinolone acetonide 0.01% is available, the presence of the steroid forces its long-term use, raising concern for telangiectases and steroid atrophy. As stated above, twice daily application of hydroquinone is favored; however, the irritation of tretinoin 0.05% limits this combination to once daily use. It also does not contain a sunscreen (13).

Because hydroquinone lightens the skin and because PIH due to acne results in discrete macules of hyperpigmentation, the question arises whether to apply the treatment as spot therapy or as field therapy. Spot therapy spares lightening of otherwise normal skin, but can, over time, leave a halo of hypopigmentation that normalizes over a period of weeks. In spite of this occasional occurrence, spot therapy is appropriate for patients with only a few macules of PIH. Those with many or clustered macules of PIH should simply apply the creams to the entire affected field. Skin lightening occurs gradually over time, such that the patient can stop applying the medications once desired lightening is achieved, the thereby maintaining excellent control over the degree of lightening.

The objective of therapy is to make the hyperpigmented macule achieve the same tone as the skin at baseline. With pure epidermal pigmentation, after successful treatment, the tone of the surrounding normal skin and the hyperpigmented macule should become identical, even if both are lighter than the baseline skin tone. Results are usually noticeable after 8–12 weeks of treatment (2). With resolution of the inciting inflammatory stimulus, skin should re-pigment evenly to the baseline skin tone in approximately 1–2 months after discontinuation of therapy. It is important for both patients and physicians to have realistic expectations for hydroquinone therapy. Although perfect skin tone will never be attained, due to hydroquinone's inability to treat dermal hypermelanosis, a softening of pigment contrasts can be achieved that results in patient satisfaction and/or easier concealment with facial cosmetic products.

SAFETY OF HYDROQUINONE

The US Food and Drug Administration (FDA) recently presented several key safety concerns regarding hydroquinone. Their primary issue was in response to reports that oral hydroquinone causes cancer in rodents fed copious amounts of the drug (14). However, oral consumption does not reflect the miniscule doses related to topical application, and no cases of human carcinogenicity have ever been reported in over 30 years of use (4). The FDA was also concerned about South African reports of exogenous ochronosis secondary to hydroquinone in black people (14). The majority of these patients used high concentrations of hydroquinone-containing products on large areas of skin, multiple times a day, with exag-

gerated overuse, for years. Often, the products contained confounding agents, such as resorcinol, that also cause exogenous ochronosis. Hydroquinone therapy in the USA differs from, and is safer than, that in Africa with respect to government regulation, formulation, concentration, amount used, and application frequency and duration (4, 15). Therefore, it is not surprising that there have been less than 25 reported cases of exogenous ochronosis in the USA in more than 50 years. Considering that 10–15 million tubes of skin-lightening products containing hydroquinone are sold per year in the USA the number of cases of exogenous ochronosis is extremely low and virtually absent in the context of prudent, supervised use (15). Mild erythema can be expected due to irritancy of hydroquinone, but this is rare and concentration-dependent, frequently occurring when concentrations greater than 4% are used (9). Such irritation can be suppressed with a topical steroid or a short drug holiday. Finally, most hydroquinone preparations contain sodium metabisulfite, a preservative that very rarely causes hives, itching, wheezing, anaphylaxis, and severe asthma attacks in susceptible persons.

CONCLUSION

In conclusion, the gold standard therapy for hyperpigmentation disorders, including PIH secondary to acne vulgaris, is hydroquinone. The drug works by inhibiting new epidermal melanin production. Hydroquinone has been proven to be safe and effective for treating hyperpigmentation in patients aged 13 years and older when used as directed. PIH associated with acne is an extremely common condition that may lead to long-term psychosocial problems for patients, especially adolescents, where cosmesis and social acceptance are major issues. Therefore, it is incumbent upon the physician to become comfortable with hydroquinone and make this therapy available to their patients who have PIH.

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ACNE, RETINOIDS AND LYMPHOMAS
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INVESTIGATIVE REPORT

A Nationwide Study of Acne Treatment Patterns in Korea: Analysis of Patient Preconceived Notions and Dermatologist Suggestion for Treatment

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Patients often have preconceived notions about acne treatments before visiting dermatologists. The aim of this study was to explore the association between patients' beliefs regarding acne and physicians' suggestion for treatment modality in dermatology clinics. A cross-sectional, nationwide multicentre study was conducted. A total of 1,370 patients completed questionnaires about beliefs about acne treatment before seeking medical care, and 101 dermatologists assessed their acne severity and proposed treatment methods. We found that patients had preconceptions in understanding disease characteristics, assessing subjective acne severity and preferring specific treatment modalities. Dermatologists' determination of topical agents as first-line treatment was affected by disease severity and patients' preferences. They were also more likely to prescribe isotretinoin even in moderate acne compared to oral antibiotics and topical agents. Selections of physical treatments and light-based therapies were affected by patients' preferences, subjective self-evaluation and dermatologists' assessments. Thus, we suggest that acne treatment strategies should incorporate both patients' subjective perceptions and objective clinical practices into a management paradigm. *Key words: acne; epidemiology; guideline; patient's preference; physician's selection; treatment modality; treatment pattern*.

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Although topical and oral treatments for acne have been widely used under well-established, global guidelines (1–4), the choice of acne treatment depends not only on the interaction of disease severity and its impact on the patient, but also upon issues concerning patient selection. Patients often have preferences for specific treatment methods based on their preconceived notions. Furthermore, with the increase in in-office procedures and new technologies, adjuvant therapies including physical treatments and light-based therapies (5–8) have also been actively introduced as acne treatment options in Korea. Although they provide a variety of therapeutic options for acne treatment, evidence is generally lacking for both objective validity and cost-effectiveness compared with well-established medications (5–8).

Therefore, the Korean Society for Acne Research (KSAR) recently decided to establish acne treatment guidelines reflecting our domestic medical practices. Before establishing these new guidelines for Korea, we conducted a nationwide postal investigation of patients' perceptions and beliefs regarding acne treatments and physicians' treatment patterns in dermatology clinics to gain basic information from both patients and dermatologists. Since patients' subjective assessment of disease severity and preconceived notions about treatment were expected to be important in selecting treatment modalities (9–11), we first investigated patients' general recognition of acne treatments, and then analysed possible causative factors contributing to physician's first-line treatment selection through multivariate analysis.

Although this study was conducted only in medical practices in Korea, we believe that the results of this research might provide valuable information to other medical communities.

MATERIALS AND METHODS

Study design

An observational, analytical, cross-sectional, multicentre study evaluating patients' general recognition and treatment patterns of acne in Korea was carried out from December 2008 to January 2009. A total of 101 board-certified dermatologists working in different regions of Korea participated in this study. To obtain a representative distribution, dermatologists were selected on the basis of geographical distribution for the country and their working places (Fig. 1). They were required to

ask their visiting patients to complete patients' questionnaires before medical examination. The survey was completed for all consecutive patients agreeing to participate in the study. In addition, dermatologists themselves were also asked to supply a detailed objective assessment and treatment plan for every patient completing a questionnaire. The patient questionnaire included information regarding sex, age, occupation, affected body parts, period of recurrence, up-to-the minute occurrence of acne lesions, acne severity self-rating, perceptions and beliefs of provoking factors, expected treatment period, favoured and unfavoured treatment modalities, attributed reasons for their choices, past treatment history, and priorities in choosing treatment options. For each patient, a dermatologist determined the objective acne severity and a primary treatment method based on clinical assessment. Acne severity was evaluated based on the Korean Acne Grading System (KAGS), as follows: Grade 1: papules ≤ 10, Grade 2: papules 11–30, Grade 3: papules ≥ 31, nodules ≤ 10, Grade 4: nodules 11–20 or mild ongoing scars, Grade 5: nodules 21–30 or moderate ongoing scars and Grade 6: nodule ≥ 31 or severe ongoing scars or sinus tracts.

Demographic data

The subjects of this survey were 1,370 consecutive new patients presenting to 22 referral hospitals and 46 private dermatology offices between December 2008 and January 2009. Full data are shown in Table I.

Statistical analysis

Pearson's χ^2 test was used to analyse categorical variables. Categorical values were denoted as frequencies and percentages. Kendall's rank correlation was used for acne severity comparisons between patients and dermatologist-assessed scores. Both univariate and multivariate logistic regression analysis were used to explore causative factors for dermatologists' determination of treatment options. A p-value of < 0.05 was considered statistically significant. The data were analysed using SPSS® (version 17.0, SPSS Inc., Chicago, IL, USA).

RESULTS

Beliefs and perceptions of patients regarding acne treatment

Only 7% of patients ($n = 102$) believed that acne was incurable. Twenty-nine percent of patients expected that acne treatment would take less than 1 month, 32% believed it would take less than 3 months, and only 13% believed treatment would take longer than 6 months. Only 24% believed that acne medication had no harmful effect on their health. Related data are shown in Table SI (available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1331).

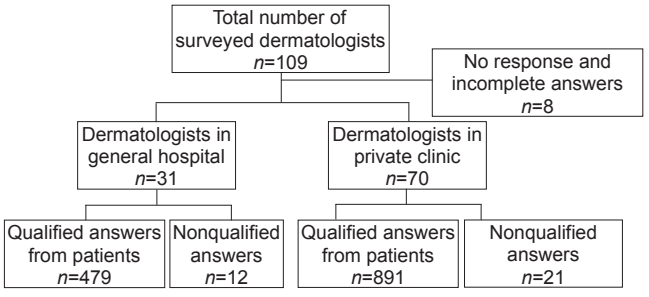

Fig. 1. Flow-chart of participating dermatologists and patients. A total of 101 board-certified dermatologists working in different regions of Korea were included in this nationwide study. These dermatologists were selected on the basis of geographical distribution and working places (general hospital and private clinic) to obtain a representative distribution. The participating dermatologists were required to ask their visiting patients to complete a patient's questionnaire before the medical encounter. A total of 1,370 patients submitted the qualified answers.

Table I. *Demographic and clinical characteristics of the subjects*

Characteristics	Value ($n = 1,370$)[a]
Age, years	
Mean ± standard deviation	23.7 ± 6.0
Range	12–56
Sex, *n* (%)	
Male	489 (35.7%)
Female	881 (64.3%)
Occupation, *n* (%)	
Student	690 (50.3%)
Non-student	680 (49.6%)
Sites of acne[b], *n* (%)	
Forehead	740 (22.7%)
Cheek	1,029 (31.5%)
Chin	624 (19.1%)
Perioral	424 (13.0%)
Chest	176 (5.4%)
Back	218 (6.7%)
Shoulder	53 (1.6%)
Duration of acne, *n* (%)	
Within 1 month	179 (13.1%)
1 ~ 6 months	264 (19.3%)
6 months~1 year	192 (14.0%)
1 ~ 5 years	429 (31.3%)
Over 5 years	306 (22.3%)

[a]Because of rounding, not all percentages total 100.
[b]Since this question was a multiple-answer question, the sum of numbers was > 1,370.

Relationship between subjective self-rating and objective assessment of acne severity

Fifty-eight percent of all patients thought that their acne severity was moderate or severe. In contrast to the self-assessment, only 13% of patients were grade 4 or more according to dermatologists' assessment. No significant correlation between patients' and dermatologists assessments of acne severity was observed (Kendall's tau-b 0.293, $p < 0.001$; Kendall's tau-c, $p < 0.001$) (Table II).

Patients' priorities for treatment modality as first-line therapy for acne

Patients were asked to select 3 favoured treatment modalities in order of preference before consulting with dermatologists, and to give reasons for each of them. Total calculated scores showed that patients preferred topical treatments, physical treatments and light-based therapies (Fig. 2A). The reasons for preferring specific treatment methods were generally different among treatment modalities ($p < 0.05$) (Fig. 2B). Ease was the main reason for the selection of topical treatments, whi-

Table II. *Comparison of acne severity between patient-reported severity score and dermatologist-assessed scores (*n = *1,326). There was no significant relationship between patient- and dermatologist-assessed acne severity (Kendall's tau-b = 0.293; Kendall's tau-c = 0.275)*

		Dermatologist-assessed severity score[a]					
Patient-reported severity score	*n* (%)	Grade 1 *n* = 256 (18.7%)	Grade 2 *n* = 463 (33.8%)	Grade 3 *n* = 434 (31.7%)	Grade 4 *n* = 107 (7.8%)	Grade 5 *n* = 57 (4.2%)	Grade 6 *n* = 9 (0.7%)
Very mild	142 (10.4)	66	33	28	4	3	1
Mild	390 (28.5)	105	158	86	20	10	1
Moderate	578 (42.2)	55	208	223	46	23	2
Severe	214 (15.6)	18	55	78	34	20	5
Unknown	46 (3.4)	12	9	19	3	1	0

[a]Korean acne grading system.

le expected clinical efficacies were the main reasons for the choice of other treatment modalities. Patients were also asked to select 3 unfavoured treatment modalities. Total calculated scores showed that patients disliked light-based therapies, oral antibiotics, and isotretinoin (Fig. S1; available from http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1331). The reasons for disliking specific treatment methods were also different between treatment modalities ($p < 0.05$). Expected cost was the main reason for disliking light-based therapies, while possible side-effects were the main reasons for avoiding the two oral medications.

Factors influencing dermatologists' acne treatment decisions

Since sex, age, occupation, acne frequency, patient-reported acne severity, patient's preference for specific treatment, and dermatologist-assessed severity were shown to affect the dermatologist's treatment modality choice in the univariate analysis (data not shown), we included these variables in a multivariate analysis (Table SII; http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1331). We also included additional variables, such as expected duration of treatment and duration of acne. According to the multivariate analysis, there was a different pattern of significant variables that affected the treatment decision between medical treatment (topical agent, oral antibiotics, oral isotretinoin) and physical treatment (chemical peeling, comedo extraction, incision and drainage and steroid intralesional injection and light-based therapy). In medical treatment, the only statistically significant factors influencing the treatment decision by dermatologists were sex, dermatologist-assessed severity, and patient's preference. The patients who preferred a more aggressive treatment (oral isotretinoin, physical treatment and light-based therapy) had a reduced chance of receiving topical therapy. However, oral antibiotic treatment had no significant association with patient's preference.

Multivariate analysis revealed that physical treatment and light-based therapies were widely used in acne patients of grade 2 or more, and physical treatment was more frequently used in female patients ($p = 0.005$).

Priority on choosing the treatment options

Patients and dermatologists showed similar patterns of rank orders in the most important factors for deciding treatment options. Efficacy and safety respectively ranked first and second in both patients and dermatologists. Intriguingly, the dermatologists took little account of patients' preferences or comfort (Table III).

DISCUSSION

In this study, we tried to reveal the underlying mechanisms of the decision-making process in the dermatology clinics and provide practical information to establish bet-

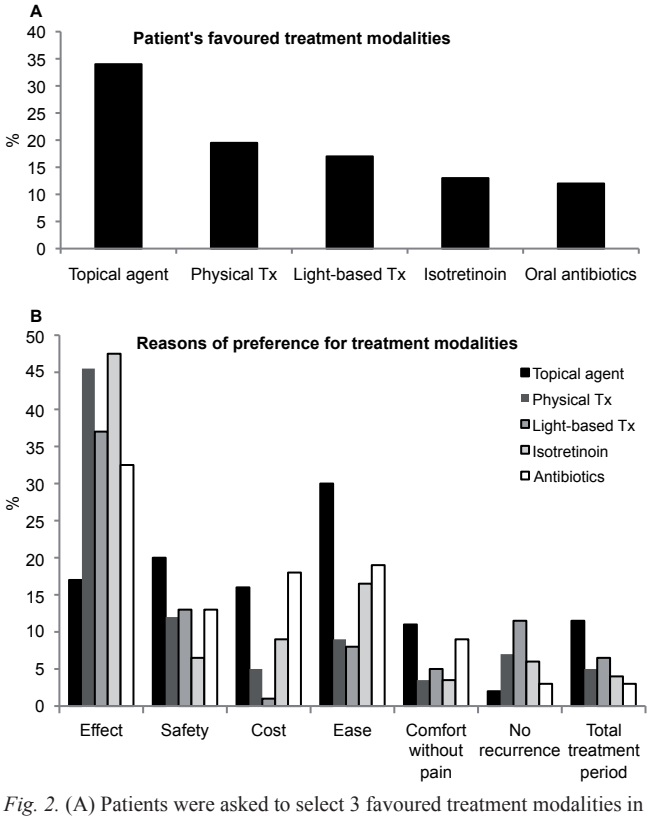

Fig. 2. (A) Patients were asked to select 3 favoured treatment modalities in order of preference before consulting with dermatologists. Total scores were calculated using weight as follows: 1st order, 3; 2nd order, 2; 3rd order, 1. (B) Reasons for preference of 3 highly scored treatment methods are demonstrated in the graph. Distribution of reasons for preferring specific treatment methods was generally different among treatment modalities ($p < 0.05$).

ter acne treatment guidelines. Since we thought a holistic approach was a more reasonable method of studying acne treatment patterns rather than focusing solely on physicians' perspectives, we analysed the contributing factors determining first-line treatment of acne in Korea from the perspectives of both patients and dermatologists.

In this context, we first analysed patients' preconceived notions about treatment of acne before initial consultation with dermatologists in the clinic. Firstly, our results showed that patients had some misinformation about clinical aspects of acne. This point is clinically important for appropriate treatments because acne is a chronic disease (12) and preconceived notions that patients have about acne are highly related to adherence with consistent treatment (13–15).

Secondly, our studies showed that there was no significant correlation between subjective acne severity measured by patients and dermatologist-assessed acne severity. Potential discordance between subjective self-satisfaction of patients and objective morphological severity are frequently observed in the treatment of acne (16, 17). Previous studies have even demonstrated that quality of life measured by Acne Quality of Life (AQOL) correlated well with patients' assessment of acne severity, while there was no association with objective acne severity (18–24).

Finally, we also found that many patients had preferred treatment modalities as a first-line therapy before visiting a dermatology clinic. Patients thought that physical treatments and light-based therapies were quite effective, while high costs prevented their common use. Additionally, patients were afraid of side-effects due to oral medication. Oral antibiotics have been recommended as a first-line therapy in moderate acne because of their efficacy and safety profile (2, 3, 25), even though antibiotic resistance is a significant concern for long-term treatment (1). Therefore, we believe that investigation of cost-effectiveness of adjuvant treatments and education on oral medications should be conducted. In addition, supplementing objective means with tools such as patients' self-rating, acne-specific quality of life scales and patient adherence, may resolve associated problems. These might be facilitated by the use of validated questionnaires, such as APSEA (19), ADI (23), SKINDEX (24), and ECOB (26).

Table III. *Patients' and dermatologists' order of the most 3 important factors in the treatment for acne. Total scores were calculated using weight as follows: 1st order, 3; 2nd order, 2; 3rd order 1*

	1st order	2nd order	3rd order	Total scores	Rank
Patients' opinion (n=1354)					
Efficacy	914	243	83	3,311	1
Safety	149	363	249	1,422	2
No recurrence	157	273	358	1,375	3
Cost	81	298	305	1,144	4
Easiness	19	70	105	302	5
Comfort without pain	24	58	111	299	6
Total treatment period	10	49	141	269	7
Dermatologists' opinion (n=131)					
Efficacy	89	18	5	308	1
Safety	11	34	33	134	2
Cost-effectiveness	17	28	22	129	3
Rapid onset of efficacy	7	24	17	86	4
Low recurrence rate	2	15	11	47	5
Preference of the patient	1	7	21	38	6
Easiness	4	4	16	36	7
Frequency of visiting	0	0	5	5	8
Relief without pain	0	1	1	3	9

We found several characteristic patterns of dermatologists' decision-making processes for first-line treatment. Firstly, physicians' prescriptions of topical agents were affected by dermatologist-assessed severity and patients' preferences. In mild to moderate acne, physicians put a higher priority on oral antibiotics, isotretinoin, physical treatments, and light-based therapies, while prescription of topical agents decreased significantly. Physicians were also less likely to prescribe topical agents for patients preferring other treatment methods. Nevertheless, the combination of a topical retinoid and antimicrobial agent remains the preferred approach for almost all patients with mild to moderate acne (2–4), demonstrating excellent efficacy and patients' satisfaction in clinical trials involving more than 16,000 patients (1, 27–30).

Secondly, many physicians prescribed isotretinoin as a first-line therapy for patients with moderate acne. Although the approved indication of oral isotretinoin is severe nodular, treatment-resistant acne, some groups have suggested that isotretinoin should be indicated for all cases of acne that are either treatment-resistant or produce physical or psychological scarring (2, 31). Others still advocate oral isotretinoin as second-line therapy (32, 33).

Finally, our study showed that physical treatments and light-based therapies are preferred by patients who desire better efficacy, quicker onset of action, and no systemic side-effects. This is despite the fact that the evidence for efficacy of these adjunctive therapies is not particularly robust (1, 5, 7, 8). Evidence from controlled clinical trials indicates short-term efficacy from lasers and various light sources for acne vulgaris, with the most consistent outcomes for blue light and photodynamic therapy (PDT) (1, 7). Scientific assessment of these adjuvant therapies is still needed however.

There are some limitations to our study. For example, since costs of physical treatments and light-based therapies are not standardized around the country, this may have affected both patients' preference and dermatologists' selection of treatment modality.

In our study, both patients and dermatologists put high priorities on efficacy and safety as the most important factors to consider in choosing treatment options. Since they share common values in selecting acne treatment methods, the establishment of a new guideline comprising updated and accessible information on various

treatment methods would address many issues found in this study. In formulating new guidelines, we suggest that acne treatment strategies should incorporate both patients' subjective evaluations and objective clinical practices into a management paradigm.

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INVESTIGATIVE REPORT

Clinical and Histological Effect of a Low Glycaemic Load Diet in Treatment of Acne Vulgaris in Korean Patients: A Randomized, Controlled Trial

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Recent studies have suggested that dietary factors, specifically glycaemic load, may be involved in the pathogenesis of acne. The aim of this study was to determine the clinical and histological effects on acne lesions of a low glycaemic load diet. A total of 32 patients with mild to moderate acne were randomly assigned to either a low glycaemic load diet or a control group diet, and completed a 10-week, parallel dietary intervention trial. Results indicate successful lowering of the glycaemic load. Subjects within the low glycaemic group demonstrated significant clinical improvement in the number of both non-inflammatory and inflammatory acne lesions. Histopathological examination of skin samples revealed several characteristics, including reduced size of sebaceous glands, decreased inflammation, and reduced expression of sterol regulatory element-binding protein-1, and interleukin-8 in the low glycaemic load group. A reduction in glycaemic load of the diet for 10 weeks resulted in improvements in acne. ***Key words: acne; epidemiology; IGF-1; diet; glycaemic load.***

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An association between diet and acne has long been postulated, and there has been an increase in research in this area in recent years (1–4). Specifically, there has been a re-evaluation of nutritional influences related to endocrine factors involved in promoting the development of acne (5–7). Current research interests have focused on the concept of glycaemic load. Glycaemic load is interpreted as the measure of the increased blood glucose and insulin-raising potential of a meal, linking acne and hyperinsulinaemia (8). Hyperinsulinaemia has been implicated in acne pathophysiology through mediation of increased androgen bioavailability and free concentrations of insulin-like growth factors-1 (IGF-1), which aggravate acne by stimulating androgen synthesis, androgen receptor signal transduction, and sebocyte lipogenesis (6, 9, 10). In fact, a high prevalence of acne in Westernized countries, where individuals consume a high glycaemic load, is observed.

Therefore, it is clinically intriguing as to whether the low glycaemic load diet (LGLD) might have a beneficial effect on acne. Reynolds et al. (11) did not find statistically significant changes in acne severity by modification of the glycaemic index and glycaemic load over a relatively short period of 8 weeks. Another study by Kaymak et al. (12) reported that no significant differences were observed between patients with acne and control subjects in serum glucose, insulin, overall glycaemic index, or dietary glycaemic load. However, Smith et al. (3) demonstrated a link between reduction in glycaemic load and severity of acne in a 12-week randomized, controlled trial.

The present study was initiated to clarify these contradictions and to further include histological examination of acne lesions before and after dietary intervention. We expected that histopathological changes may provide valuable insight into the molecular mechanisms involving a reduction in acne lesions as a direct result of dietary control.

MATERIALS AND METHODS

Subjects and study design

This study was designed as a parallel dietary intervention study with investigator-blinded dermatological assessments. A total of 32 participants (age range 20–27 years; 24 males, 8 females) with mild to moderate acne were randomly assigned to either the LGLD group ($n = 17$) or the control group ($n = 15$) at the acne clinic of Seoul National University Hospital between August and February 2011 (Table I). A blocked random allocation sequence was created by computer-generated random numbers, and allocation to specific groups was performed by a research nurse. A washout period of 6 months was required for subjects who had previously taken oral retinoids or received physical treatments, and 2 months for subjects who had taken oral antibiotics or applied topical agents. Facial acne was scored at each visit (weeks 0, 2, 5 and 10). At each visit, body weight of all subjects was measured in light clothes, and body mass index (BMI) was calculated as the weight (kg)/height squared (m^2). At the first and final visit, 2-mm punch biopsies were taken from facial acne lesions.

The primary end-points of the study were changes in the number of inflammatory lesions (papules, pustules and nodules), the number of non-inflammatory lesions (open comedones and closed comedones) and histopathological changes in the acne lesions.

Table I. *Clinical characteristics of the low glycaemic load diet (LGLD) group and the control group at baseline*

Variable	LGLD group ($n=17$)	Control group ($n=15$)	p
Males/females, *n*	13/4	11/4	0.88
Age, years, mean ± SD	23.5 ± 3.2	23.7 ± 2.6	0.34
Body weight, kg, mean (SD)	62.3 ± 8.5	65.4 ± 10.1	0.43
BMI, kg/m^2, mean ± SD	23.4 ± 4.2	24.7 ± 2.2	0.52
Inflammatory lesion counts, mean ± SD	21.3 ± 9.6	24.7 ± 6.8	0.34
Non-inflammatory lesion counts, mean ± SD	8.3 ± 7.1	8.1 ± 5.6	0.45

BMI: body mass index.

Secondary end-points included changes in patient's subjective assessments. Informed consent was obtained from each participant, and the study was approved by the Institutional Review Board (IRB) of Seoul National University Hospital (H-1007-083-323).

Dietary intervention

The LGLD was achieved mainly by modifying the type and amount of carbohydrates consumed. The LGLD group was instructed to substitute high-glycaemic index (GI) foods with foods with lower GI foods (e.g. barley, wholegrain breads, fruits, beans, vegetables and fish).

In order to maintain standard levels of energy intake, the percentage of energy lost by reduced intake of carbohydrates was partially replaced with energy from protein. The recommended LGLD consisted of 25% energy from protein, 45% from low-GI carbohydrates, and 30% energy from fats. In contrast, the control group was instructed to eat carbohydrate-rich foods daily. All participants were educated repeatedly as to food record-keeping protocol throughout the study. A qualified nutritionist was available for consultation with each participant. Her role included a review of the food diary and helpful instruction to participants in the LGLD group about how to maintain the LGLD. Nutritional information based on the submitted food diaries of all participants were calculated, and participants received individualized dietary plans. Recommended eating habits for the LGLD group were also finalized and presented to participants during each visit. The control group was not informed about GI, but was urged to maintain their regular diets. Nutrient intake was calculated from a 7-day timeframe of weighed and measured food records during each visit (2, 5 and 10 weeks) by using the Computer Aided Nutritional Analysis program version 3.0 software (The Korean Nutrition Society, Seoul, Korea). Based on this tool, the total calorie intake per day, the mean GL, GI and total amount of carbohydrates, protein and lipid were calculated. Dietary compliance was monitored periodically (2/week) via telephone interviews and e-mails from physicians participating in the study. A nutritionist regularly answered questions about LGLD via e-mail from all participants.

Calculation of dietary glycaemic index and glycaemic load

The dietary glycaemic load was calculated using the following equations: dietary GI = ∑(GI for each food item × proportion of total carbohydrate contributed by item), and the dietary glycaemic load = ∑(GI for each food item × its carbohydrate content in grams ÷ 100). The GI values related to glucose as a reference food were taken from reference data (13) and related websites (www.glycaemicindex.com, www.gitest.co.kr). The GI values of unlisted Korean foods in the database were estimated by an experienced nutritionist.

Dermatology assessment

During each visit, clinical assessments of the number of inflammatory and non-inflammatory acne lesions and the overall severity of acne were performed blind by 2 independent dermatologists using the Leeds revised acne grading system, described by O' Brien et al. (14). To ensure that all acne lesions were counted, located and graded by size and severity, standardized digital photographs were taken prior to initiation of the dietary intervention and at each follow-up visit using identical camera settings (Nikon D70, Nikon Corp., Tokyo, Japan). An independent dermatologist performed acne grading based on photographs to ensure objective clinical evaluations of the acne severity grade. Patient's subjective self-assessments of acne severity were also recorded. The disease-free state was designated as 0, and acne state at the initial visit was set as 10. If patients felt that their acne had been aggravated in relation to the first visit, they could choose scores of greater than 10 for grading to allow the recording of any acne deterioration during the period of dietary intervention.

Immunohistochemical procedures

Immunohistochemical (IHC) analysis of skin samples was performed using the streptavidin-biotin amplification method. Tissue samples were processed for IHC staining using antibodies to interleukin-8 (IL-8) (R&D systems, GA, USA), sterol regulatory element-binding protein-1 (SREBP-1) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and transforming growth factor beta 1 (TGF-β1) (Santa Cruz Biotechnology). In samples stained with haematoxylin and eosin (H&E), the severity of inflammation was ranked from 0 (no inflammation) to 4 (very severe inflammation). In immunohistochemical staining for SREBP-1, TGF-β1 and IL-8, the intensity of staining was ranked from 0 (unstained) to 4 (very intensely stained). Skin biopsies and histopathological evaluations were performed independently by 2 dermatologists.

Image analysis of sebaceous gland size

Following H&E staining of sections from each of the biopsies, image analysis was performed to calculate sebaceous gland size in all available tissue sections. Images were captured using a Spot digital camera (Leica Camera AG, Solms, Germany), and measurements were obtained with TINA (Raytest Isotopenmebgerate, Straubenhardt, Germany) software after calibration with a micrometer slide under the 10× objective. All areas of sebaceous glands were circled using a freehand measuring tool, and the mean area of a distinct sebaceous gland for each section was calculated from the baseline and 10-week biopsies.

Statistical analysis

Comparison between the 2 dietary groups was performed using the likelihood ratio test and the Mann-Whitney *U* test for categorical and continuous values, respectively. Repeated measures analysis of variance (ANOVA) was used to explore the effects of dietary intervention, the time course of the study, and the potential influence of these 2 factors. Pooling data from both groups, bivariate linear regression analysis was also conducted to explore relationships between the LGLD and decrease in acne. All statistical analyses were performed with the use of SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA), and significance was accepted for p-values < 0.05.

RESULTS

Dietary intake

Table II shows the nutritional composition of the mean diet of both the LGLD and the control groups

Table II. *Detailed information of the dietary intakes in the low-glycaemic-load diet (LGLD) group and control group at baseline and intervention periods (mean value at week 2, 5, and 10)*

Variable	LGLD group ($n=17$) Mean ± SD	Control group ($n=15$) Mean ± SD	p-value[a]		
			Group	Time	Group × time
Energy, kcal/day					
Baseline[b]	2024 ± 264.6	2182.6 ± 584.6			
Intervention period[c]	1900.3 ± 333.2	2133.9 ± 477.9	0.25	0.36	0.50
Dietary glycaemic load					
Baseline	177.2 ± 41.5	190.5 ± 33.1			
Intervention period	129.5 ± 22.2	207.2 ± 23.2	0.001	0.041	0.23
Dietary glycaemic index					
Baseline	65.9 ± 4.5	63.9 ± 5.8			
Intervention period	50.1 ± 6.3	69.5 ± 2.4	<0.001	0.001	0.19
Carbohydrate, g					
Baseline	275.9 ± 47.4	262.5 ± 79.0			
Intervention period	233.7 ± 40.0	283.2 ± 63.9	0.029	0.15	0.31
Protein, g					
Baseline	78.2 ± 17.4	88.6 ± 22.6			
Intervention period	88.1 ± 16.8	83.8 ± 24.6	0.44	0.76	0.42
Lipid, g					
Baseline	69.5 ± 7.2	76.1 ± 7.7			
Intervention period	61.9 ± 6.7	74.3 ± 6.9	0.006	0.098	0.38

[a]Repeated-measures analysis of variance (ANOVA) was performed to incorporate data from all time-points during intervention periods. We evaluated the differences between the LGLD and the control groups (main effect of group), and the change over time (main effect of time).
[b]An independent-sample t-test showed no significant differences between the LGLD and the control groups for all the listed dietary variables at baseline.
[c]Means of data collected at 2, 5 and 10 weeks.

at the baseline and final 10-week visit. No significant differences between the group or time-frame were observed in the total energy intakes of both groups. In addition, there were no significant changes in the calculated BMI for both groups throughout the research period (23.4 ± 4.2→22.7 ± 5.3 in the LGLD group and 24.6 ± 2.2→24.1 ± 2.9 in the control group). On the contrary, a significant reduction in glycaemic load was observed during dietary intervention in the LGLD group. This change was attributed mainly to the reduction in carbohydrate and lipid intake and the consumption of low GI foods ($p<0.05$).

Acne severity and lesion counts

The mean baseline acne scores for both LGLD and control groups were 2.18 and 2.08, respectively. After the 10-week dietary intervention, only the LGLD group demonstrated a significant decrease in acne grades, to 1.60 ($p=0.02$). The difference in severity between the 2 groups was also significant at the final visits ($p=0.02$) (Fig. 1). In detail, the mean non-inflammatory lesion counts for the LGLD group and the control group were significantly decreased, by 27.6% and 14.2%, respectively ($p=0.02$, $p=0.04$), at the final visit compared with the baseline. The difference between the 2 groups was evident only after the full 10 weeks of dietary intervention ($p=0.02$) (Fig. 2A). Inflammatory acne lesions were significantly decreased in the LGLD group at the earlier time-point of 5 weeks ($p=0.03$) (Fig. 2B). At the final visit, the mean number of lesions had decreased to 70.9% of baseline, while there was no significant reduction in the lesions in the control group.

Patient subjective assessments

After 5 weeks of treatment, the patients' subjective self-assessment scores started to decrease significantly for both groups (7.2 for the LGLD group and 7.5 for the control group) ($p=0.03$). At the final visit, the patients' self-assessment scores had decreased to 6.7 and 6.8, respectively ($p=0.01$) (Fig. S1; available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1346).

Correlation between changes in glycaemic load and improvement in severity of acne

Linear regression analysis showed that there was a significant correlation between the changes in total number of acne lesions and a reduction in the glycaemic load

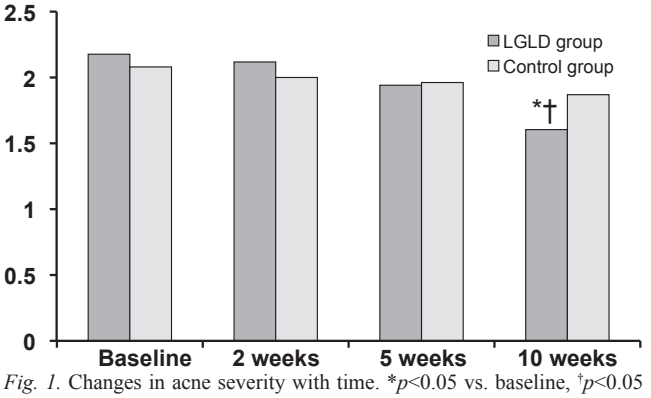

Fig. 1. Changes in acne severity with time. *$p<0.05$ vs. baseline, †$p<0.05$ between the 2 groups.

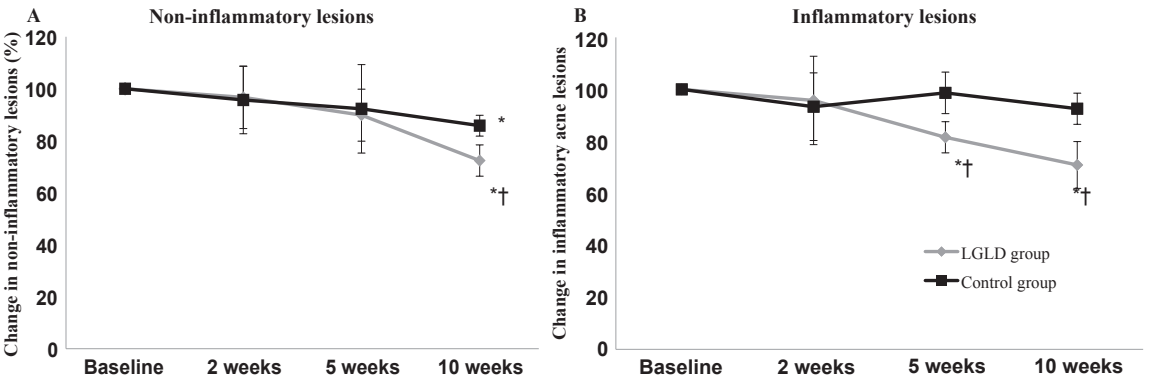

Fig. 2. Mean percentage change in (A) the non-inflammatory lesion counts and (B) the inflammatory acne lesion counts at each visit. *$p < 0.05$ vs. baseline, †$p < 0.05$ between the 2 groups.

($y = 0.1337$, $x - 7.1437$, $R^2 = 0.35$, $p < 0.01$), suggesting that lowering the glycaemic load mitigated the overall number of acne lesions (Fig. 3).

Changes in the overall size of sebaceous glands

A significant decrease in the overall size of the sebaceous glands was observed in the LGLD group compared with baseline measurements. The mean area of sebaceous glands in the baseline samples was 0.32 ± 0.03 mm^2 (mean ± standard error of the mean (SEM)), compared with 0.24 ± 0.03 mm^2 in the 10-week samples, which is a statistically significant reduction ($p = 0.03$).

Immunohistochemical findings

Mean scores for H&E, SREBP-1 and IL-8 staining demonstrated reductions after 10-week dietary intervention (H&E: 2.7→1.6, $p = 0.023$, SREBP-1: 2.6→1.3, $p = 0.03$, IL-8: 2.9→1.7, $p = 0.03$). However, there was no significant change in mean intensity of TGF-β1 at the final visit (3.5→3.6, $p = 0.83$) (Fig. S2; available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1346). In the control group, no significant changes in intensities for H&E staining and immunohistochemical staining were observed ($p > 0.05$).

DISCUSSION

Epidemiological studies have suggested that components of the Western diet are associated with the development of acne (1, 15). Previous research has also revealed that a high glycaemic load diet can induce significant hyperinsulinaemia, causing a hormonal cascade leading to androgen-induced sebum production and keratinocyte growth (5, 16, 17). In fact, endocrine disorders with increased insulin and IGF-1 serum levels, such as premature adrenarche, polycystic ovary syndrome, and acromegaly, are clinically associated with a high prevalence of acne (18–20). In addition, individuals with congenital deficiency of IGF-1 or Laron syndrome were almost free of acne (21).

In our study, the severity of acne in the LGLD group demonstrated a significant improvement after 10 weeks of dietary intervention. This observation might be important in order to understand the kinetics of acne response to dietary modifications, including the glycaemic load. In contrast to the 8-week study performed by Reynolds et al. (11), Smith et al. found a significant reduction in the level of acne lesions after a reduction in the glycaemic load over a period of 12 weeks (3). Therefore, we determined that a period of 10 weeks on the LGLD most likely did not reach the possible clinical end-point of a dietary intervention in

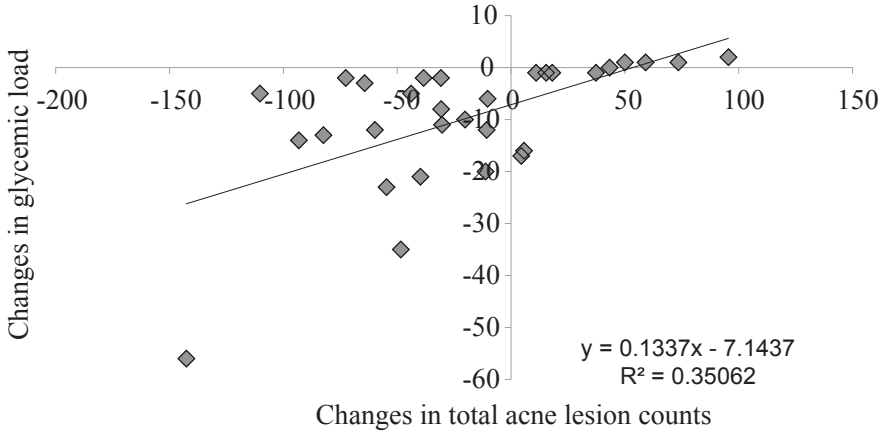

Fig. 3. Relationship between changes in dietary glycaemic load and improvement in acne. Bivariate analysis was performed with a 2-tailed Pearson's correlation.

acne. This result is of importance for further studies with longer study periods designed to assess the clinical and metabolic end-point of dietary intervention in acne. In both the LGLD group and the control group, no statistically significant changes in the BMI were observed, which is probably due to the short study period of 10 weeks. However, long-term dietary interventions (>3 months) with the LGLD generally reduced the overall BMI. It is noteworthy that the BMI has been identified as a risk factor for the development of acne (22, 23). Taken together, our data demonstrate a linear correlation between improvement in acne and reduction in glycaemic load.

However, our study did not consider other dietary components, including milk and dairy products leading to increased insulin/IGF-1 signalling (24), which have been identified as nutrient-derived acne-aggravating risk factors, as shown in our previous study (25). During puberty, there is a physiological onset of increased levels of growth hormone secretion, leading to an increase in IGF-1 serum levels, which is further enhanced by the consumption of milk (26). In this context, the epidemic incidence of adolescent acne in Western milk-consuming societies can be also explained by the increased insulin- and IGF-1-stimulation of sebaceous glands mediated by milk consumption. Therefore, we hypothesized that additional studies should not only consider the impact of the glycaemic load, but also that of milk and dairy products.

Following the 10-week LGLD, we found that the mean size of the sebaceous glands was significantly reduced, and the expression of SREBP-1 protein, master regulator of lipid synthesis, was also decreased. IGF-1 normally activates PI3K/Akt and MAPK/ERK-signal transduction pathways and induces the SREBP-1 expression, resulting in increased sebaceous lipogenesis (27–29). Since the LGLD is expected to decrease the biological activity of IGF-1, the decrease in non-inflammatory acne lesions during our dietary intervention might be partially elucidated by the proposed mechanisms. We also found that the results of H&E staining and IL-8 immunostaining of acne lesions demonstrated decreased inflammation in the LGLD group. Increased IL-8 expression in skin has been reported to be significantly associated with follicular hyperkeratosis, and acne inflammation (30). Since IGF-1 has also been identified as inducing acne inflammation through the Phospholipase C-γ pathway (29), we suggest that a LGLD might also mitigate inflammation through the modulation of related pathways. Therefore, our findings correlated well with previous dietary trials and *in vitro* research.

Interestingly, through our subjective testing, patients in the control group believed that acne lesions improved after the 5-week trial. This may indicate a placebo effect, or slight improvements in non-inflammatory acne lesions might also affect patients' satisfaction. Several methodological aspects of our study deserve mention. First, a self-reporting food diary might have prevented the accurate calculation of the nutritional composition of the food consumed during the study. Under-reporting the quantity of food eaten is a well-known source of error when evaluating adolescent diets (31). Secondly, other dietary factors, including saturated fat, fibre content, and zinc and iodine intake, might confound the relationship between diet and acne improvement.

Our results showed a beneficial effect of a LGLD in both non-inflammatory and inflammatory acne lesions, both clinically and histopathologically, in this 10-week dietary intervention study. In conclusion, these results show that a reduction in glycaemic load can result in a reduction in the level of acne lesions.

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The authors declare no conflicts of interest.

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Treatment of Severe Acne with Low-dose Isotretinoin

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Acne vulgaris is the most common skin disorder with possibly significant social consequences for those affected. Acne conglobata is a severe, inflammatory, nodulocystic form of acne (1), and acne fulminans is a severe, ulcerative form of acne with an acute onset and systemic symptoms (2). Acne has a complex pathogenesis with likely factors including increased seborrhoea, ductal cornification, and colonization of the pilosebaceous ducts by *Propionibacterium acnes* with ensuing inflammation, as well as elevated insulin-like growth factor 1 (IGF-1 levels (3) and increased signalling via the fibroblast growth factor receptor 2 pathway (4). Inflammation seems to result ultimately from an increased production of interleukin 1 beta and tumour necrosis factor-α (5). A wide array of treatment options, including systemic isotretinoin are available (6).

The aim of this study was to show that a low-dose isotretinoin therapy, 0.1–0.3 mg/kg daily, can be effective in treating severe forms of acne.

METHODS

A retrospective analysis of 4 patients with acne conglobata or acne fulminans treated with low-dose isotretinoin (0.1–0.3 mg/kg/day) was performed. The primary endpoint was the photodocumented improvement of the skin lesions during therapy, quantified using Cook's grading scale for acne (7), a method grading the severity of skin lesions on a scale from 0 (≤ 3 comedones/papules) to 8 (most severe, i.e. highly inflammatory acne, acne conglobata) using photographic standards. Secondary endpoints were the occurrence of side-effects, therapy duration and cumulative isotretinoin dose. The use of topical therapeutics or other medication for acne treatment was reviewed. Before starting treatment, pregnancy of female patients was ruled out. Patients had been informed of the strict necessity to use reliable methods of contraception until one month after treatment cessation. Patients underwent routine laboratory checks including a full blood count, liver function tests, and a serum lipid profile before treatment initiation and during treatment, as well as routine clinical examination for the monitoring of side-effects.

RESULTS

The study sample comprised 4 patients; all were between 14 and 18 years of age at the start of treatment (Table I). All patients initially had a Cook's grading scale acne score of 8 and received isotretinoin 10–20 mg daily; 3 were also treated intermittently with oral corticosteroids for 7–9 weeks. The patients were treated for a mean of 10.4 months, with a mean daily isotretinoin dose of 0.19 mg/kg, and a mean cumulative dose of 65.8 mg/kg. Three of 4 patients also recieved topical treatment (adapalene, clindamycin in combination with benzoyl peroxide or erythromycin). After treatment, one patient had a Cook's grading scale acne score of 0, two of 2 and one patient of 4 (Fig. 1). The side-effects were mild. Except for one patient with persistently elevated serum cholesterol and creatine kinase, the laboratory findings normalized during treatment without further intervention.

DISCUSSION

Isotretinoin is indicated for severe nodular acne that is unresponsive to combined oral therapy with antibiotics and topical therapy, or under special clinical circumstances as a first-line therapy in individual cases, at a dose of 0.5–1.0 mg/kg/day, with a cumulative dosage of 120–150 mg/kg over 4–6 months (8). Adverse effects include dry skin and mucosa (9), elevated liver enzymes

Table I. *Therapeutic effects of low-dose isotretinoin in 4 cases of severe acne*

Pat. no/ Sex	Diagnosis	Clinical response[a]	Mean dose (mg/kg/day)	Cumulative dose (mg/kg)	Duration (months)	Adjuvant medication	Topical therapy	Side-effects
1/M	Acne conglobata	8 to 0	0.25	60.5	8.1	None	None	Elevated serum cholesterol, creatinine kinase at last visit, mild cheilitis
2/M	Acne fulminans	8 to 2	0.18	55.5	10.0	Oral methyl-prednisolone	Adapalene gel	Transient elevation of serum TG, mild cheilitis
3/F	Acne fulminans	8 to 4	0.15	34.8	7.4	Oral predni-solone	Clindamycin/ benzoyl-peroxide	Transient elevation of serum cholesterol, γ-GT, TG, mild cheilitis
4/M	Acne conglobata	8 to 2	0.16	112.3	16.2	Oral predni-solone	Erythromycin cream	Transient elevation of cholesterol, TG, alkaline phosphatase, mild cheilitis

[a]Cook's grading scale.
TG: triglycerides; γ-GT: γ-glutamyltransferase.

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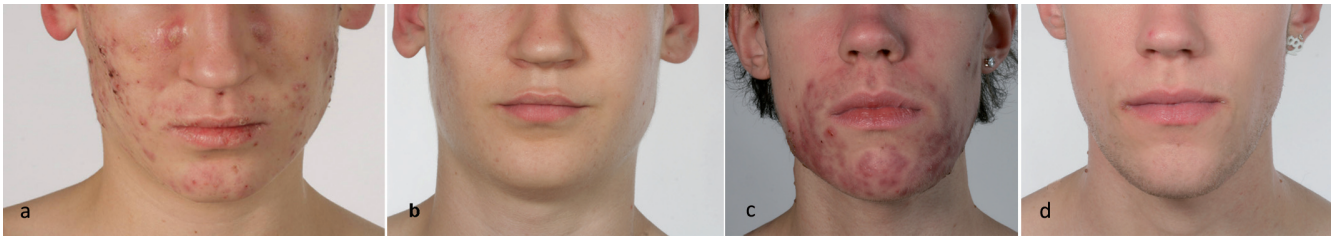

Fig. 1. Patient 2. (a) Before treatment, Cook's grading scale acne score of 8. (b) After treatment, 0.18 mg/kg/day for 10.0 months, score 2. Patient 4. (c) Before treatment, score of 8. (d) After treatment, 0.16 mg/kg/day for 16.2 months, score 2.

and an increase in serum lipids, especially triglycerides (10), most of the side-effects being dose-dependent. Isotretinoin is highly teratogenic, with a reported 40% incidence of birth defects occurring in children who have been exposed to isotretinoin during the first trimester of pregnancy (9). Most of the side effects, such as dryness of the lips and skin, are dose-dependent, although this can not be asserted for isotretinoin's teratogenicity (12, 14). Moreover, isotretinoin of less than 0.2 mg/kg is reported to reduce the risk of acne flare upon initiation of therapy (11). This study clearly shows that severe forms of acne can be treated effectively with low-dose isotretinoin (0.1–0.3 mg/kg), if necessary in combination with oral prednisolone, all 4 treated patients improving significantly. Our results are supported by the findings of Plewig et al. (12) and of Lee et al. (13), who demonstrated the effectiveness of low-dose isotretinoin in the treatment of acne conglobata and moderate acne, respectively. Karvonen et al. (14) demonstrated the effectiveness of combining long-term isotretinoin therapy with an initial course of systemic prednisolone in cases of cystic acne. Strong indirect evidence supports the mechanism of action of isotretinoin through the upregulation of FoxO transcription factors, thus dampening promotive effects in the pathogenesis of acne (15). Our patients did not experience a flare of acne upon treatment initiation, nor did any patients show signs of neuropsychiatric problems. None complained about dry eyes or skin, although all developed a mild cheilitis. The laboratory findings were mild and in all but one case resolved spontaneously. None of the patients had to stop isotretinoin therapy due to side-effects. In conclusion, the efficacy and favourable side-effect profile strongly support the use of low-dose isotretinoin treatment, 10–20 mg/day, as first-line therapy for acne conglobata, and in combination with a short-term course of oral corticosteroids, also for acne fulminans. Larger studies are necessary to validate our findings. Moreover, it will be interesting to determine the rate of recurrence of severe forms of acne treated with low-dose isotretinoin.

The authors declare no conflicts of interest.

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Large Benign Condyloma Acuminatum: Successful Treatment with Isotretinoin and Interferon Alpha

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Condylomata acuminata are fleshy exophytic cutaneous lesions most commonly occurring in the anogenital region that are caused by the mostly sexually transmitted human papilloma virus (HPV). Benign condylomata are usually caused by HPV types 6 and 11, whereas HPV types 16, 18, 31 and 33 are often found in lesions with neoplastic transformation (1). A vast variety of therapeutic approaches has been used for years in the management of condylomata acuminata; however, no form of therapy has yielded consistently effective results. In particular, in large and extensive lesions, conventional topical therapy is ineffective, whereas a wide and sometimes mutilating surgical excision is, in many cases, necessary. We describe here a case of a large benign vulvar condyloma acuminatum treated successfully with combined systemic administration of isotretinoin and interferon (IFN-α).

CASE REPORT

A 19-year-old HIV-negative woman presented with a 6-month history of multiple small condylomata acuminata on the vulva, which had been treated with electrocauterization. Three months thereafter, the patient observed the recurrence of confluent condylomatous lesions in the same region and the development of a rapidly growing large exophytic mass. Approximately one month prior to the relapse of the vulvar condylomata she had been treated with high doses of systemic glucocorticoids over a period of 2 weeks because of recurrent severe anaphylactic reactions. She had no history or evidence of systemic infectious, autoimmune or neoplastic disorders. Physical examination revealed a large, soft, cauliflower-like, pale pink mass in the vulvar region (Fig. 1A). Routine laboratory tests including a complete blood count, blood chemistry, urinalysis, immunological (immunophenotype of peripheral lymphocytic subsets and serum immunoglobulins) and serological investigations (tests for syphilis, herpes simplex virus (HSV) 1&2, HIV 1 & 2, hepatitis A, B and C, Epstein-Barr virus and cytomegalovirus) were either negative or within normal limits. Chest X-ray investigations and electrocardiography were unremarkable. Colposcopy and proctoscopy did not detect any HPV lesions. Histological examination of skin biopsy specimens obtained from 3 different sites of the lesions revealed a hyper- and parakeratotic epidermis with marked acanthosis and focal occurrence of koilocytes at the upper cell layers; there was an excessive papillomatosis, but no evidence of cellular atypia or stromal invasion. Based on these histological findings and the clinical features of the lesion, the diagnosis of large benign condyloma acuminatum was made. *In situ* hybridization using biotinylated HPV-DNA probes revealed the presence of HPV types from 6/11 group in the nuclei of keratinocytes at the upper epidermal layers.

Since the patient refused any form of surgical therapy, we decided to start a combined treatment with oral isotretinoin and subcutaneous IFN-α. The patient gave a written consent and was orally treated with 1 mg/kg/day isotretinoin (Roaccutan, Roche Hellas, Athens, Greece), whereas a dose of 3×10^6 IU IFN-α (Roferon, Roche Hellas, Athens, Greece) was subcutaneously injected three times/week. Two months after onset of treatment there was an impressive regression of the lesions (Fig. 1B), which revealed a complete remission after 4 months of continuous treatment (Fig. 1C). During the first 3 months of treatment the patient experienced occasional fever, chills, myalgias and leukopenia subsequent to the injection of IFN-α, whereas isotretinoin-associated adverse reactions, such as moderate cheilitis and dryness of mucosae, were observed throughout the treatment period. She has presently completed a 3.5-year follow-up after discontinuation of combined IFN-α and isotretinoin treatment and has experienced no recurrences of large benign condyloma acuminatum being completely free of any lesions.

DISCUSSION

Classification and nomenclature of large and extensive condylomata acuminata remain controversial. Those without atypia or locally invasive growth are classified by some authors as "giant condylomata" (2) and by others as "giant-sized condylomata" if they measure more than 2.5 cm in diameter (3). On the other hand, many authors use the term "giant condylomata" as a synonym of the so-called Buschke-Löwenstein tumours (BLT) (4). These HPV-induced tumours represent a rare maximal variant of condylomata acuminata, are mostly localized in the anogenital region, have a locally aggressive course, show a tendency to recur, and are regarded by some authors as a type of verrucous carcinoma. In our

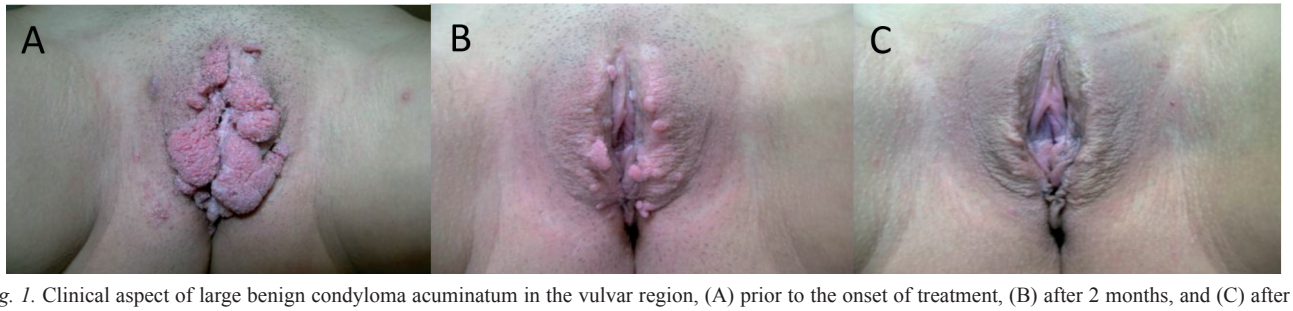

Fig. 1. Clinical aspect of large benign condyloma acuminatum in the vulvar region, (A) prior to the onset of treatment, (B) after 2 months, and (C) after 4 months of combined therapy with subcutaneously applied interferon alpha and oral isotretinoin.

opinion, the term "giant condylomata" should be used exclusively to describe BLT, whereas the term "large benign condylomata acuminata" (LBCA) should be applied only to those lacking invasion or destruction of the underlying tissues. Nevertheless, in some cases LBCA, if left untreated, can transform into BLT, which can in turn evolve into verrucous carcinomas in approximately 30–50% of cases (5).

Olsen et al. (6) evaluated the effectiveness of systemic IFN-α vs. isotretinoin in the treatment of condylomata acuminata. After 6 weeks of therapy 56% of the patients treated with IFN-α alone had an objective clinical response, whereas no patient responded to isotretinoin alone. Nevertheless, a more prolonged administration of this retinoid might have positively influenced the therapeutic response. When IFN-α-treated patients with incomplete clearing additionally received isotretinoin there was an additive therapeutic effect. Our group reported that a 12-week treatment of 56 patients with refractory condylomata acuminata with isotretinoin alone, led to a complete response in 39.6% and to a partial response in 13.2%. Interestingly, a complete resolution was seen in 77.7% of the patients who had lesions of small size and short duration (7). IFN-α alone reveals an excellent therapeutic efficacy, not only in condylomata acuminata irrespective of size and duration, but also in giant condylomata (BLT) (8, 9).

The efficacy of the combined IFN-α and isotretinoin administration vs. isotretinoin alone in the treatment of condylomata acuminata has been evaluated in two separate studies (10, 11). In the first study it was found that combination therapy led to higher remission rates, compared with isotretinoin alone, whereas in the second study the achieved remission rates were similar. In both studies the duration of treatment required for remission was significantly shorter in the group of patients receiving the combination therapy.

To the best of our knowledge, this is the first time that combined IFN-α and isotretinoin administration is successfully applied in the management of LBCA. The exact mechanisms underlying the observed impressive therapeutic response and the complete remission of LBCA in our patient within 4 months of treatment are presently unknown. IFN-α is capable of reducing viral replication and epithelial growth and exerts distinct immunomodulatory effects (12). Isotretinoin, apart from being also a potent immunomodulator, dramatically affects epithelial differentiation and proliferation and induces apoptosis in HPV-affected cells (13). Since HPV replication is related to the state of keratinocyte differentiation, it is possible that isotretinoin may inhibit the DNA replication and assembly of HPV within the affected cells (14). Furthermore, a synergistic inhibitory effect of IFN-α and isotretinoin on HPV-induced angiogenesis has been detected subsequent to a 5-day intra-peritoneal administration of both compounds to experimental animals (15). Further controlled studies are now required on the efficacy and safety of this combination therapy for LBCA.

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INVESTIGATIVE REPORT

Efficacy and Tolerability of Alitretinoin for Chronic Hand Eczema Under Daily Practice Conditions: Results of the TOCCATA Open Study Comprising 680 Patients

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This non-interventional observational open study (TOCCATA, sponsored by Basilea Pharmaceutica Germany) investigated the use of alitretinoin to treat chronic hand eczema under daily "real life" medical practice conditions in Germany. A total of 349 dermatologists throughout Germany enrolled 680 adult patients with chronic hand eczema. Patients were prescribed and treated with alitretinoin in accordance with the summary of product characteristics. The maximum observation duration was 24 weeks, with efficacy and safety parameters evaluated every 4 weeks. Efficacy was primarily evaluated by assessing disease severity according to the Physician Global Assessment. In total, 56.7% of patients achieved a Physician Global Assessment rating of "clear" or "almost clear" hands, with only small differences in patients with different morphological forms: hyperkeratotic-rhagadiform (59.2%), fingertip (52.2%) and vesicular (47.9%). This observational study demonstrates the effectiveness and tolerability of alitretinoin in everyday clinical practice in addition to the known efficacy and safety obtained by randomized controlled clinical trials. ***Key words: alitretinoin; chronic hand eczema; observational study: efficacy.***

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Hand eczema is a common skin disorder, with a one-year prevalence of up to 10%. Severe chronic hand eczema (CHE) is thought to account for up to 7% of cases of CHE (1–4). Although no single causative factor for hand eczema has been identified, genetic predisposition, an altered immune response, atopy and environmental factors, such as handling chemicals or other skin irritants, have all been suggested as contributing factors. Due to this multifactorial aetiology it is often difficult to identify and eliminate all potential causative factors (4–6).

Severe hand eczema resulting in prolonged disability is associated with a high health economic burden and significant loss of quality of life (7, 8). Although numerous treatment options are available, the management of CHE is often difficult and unsatisfactory. Therefore, guidelines for the management of hand eczema have been developed during the last 2 years in several countries (9–14). The authors of these guidelines came to the conclusion that there is a lack of, and simultaneously a need for, well-designed controlled clinical trials, especially in directly comparing available treatment modalities and demonstrating effectiveness under daily practice conditions.

It is estimated that the majority of patients with severe CHE are unresponsive to standard treatments (4), and for these patients alitretinoin (Toctino®) has recently become available as the only approved systemic treatment option. Alitretinoin (9-cis retinoic acid) is an endogenously occurring physiological vitamin A derivative (retinoid) with its main mechanism of action in CHE thought to be anti-inflammatory and immunomodulatory.

In the clinical development programme for registration, studies of up to 6 months' duration with alitretinoin have shown that it is highly effective in the treatment of severe CHE unresponsive to potent topical corticosteroids and is well tolerated with a good safety profile (15–17). Alitretinoin was initially granted approval by several European authorities, including the German Health Authorities in autumn 2008, for the treatment of patients with severe CHE unresponsive to potent topical corticosteroids.

TOCCATA was designed as a non-interventional study to further investigate the effectiveness and tolerability of alitretinoin, and to collect structured data under daily dermatological practice conditions. While clinical trials are conducted in an ideal setting, adequately designed and performed observational studies can contribute significantly to the understanding of disease and its management under real-life conditions. In healthcare research, investigations of the treatment of chronic skin diseases under everyday conditions in dermatological clinics and private practice have become more important in recent years. TOCCATA is the first such study investigating the treatment of CHE with oral alitretinoin in Germany.

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MATERIALS AND METHODS

Between November 2008 and March 2010, 680 patients were enrolled into the TOCCATA study by 349 dermatologists throughout Germany. (TOCCATA: "Toctino® bei schwerem chronischem Handekzem – Therapieverlauf in Anwendungsbobachtung" (Toctino® in severe chronic hand eczema – therapy in an observational study)).

The study was approved by the ethics committee of the University of Heidelberg. In line with the principles of a non-interventional observational study, the treating physicians documented alitretinoin treatment (30 or 10 mg daily) of adult patients with severe CHE unresponsive to potent topical corticosteroids during routine medical practice. This was done in the usual manner, in accordance with the terms of the marketing authorization and the summary of product characteristics (18) and considering the pertinent hand eczema management guideline (11). Patients with CHE treated by a dermatologist could be included in the study if the following criteria were met: (*i*) disease duration of at least 3 months or more than 2 flares within the last 12 months (according to the Guideline of the German Dermatological Society (11); (*ii*) pretreatment with topical corticosteroids; (*iii*) no long-lasting healing under adequate topical treatment including corticosteroids; and (*iv*) no other active severe skin diseases or acute skin infections dominating the clinical picture.

For female patients of child-bearing age, strict adherence to pregnancy prevention measures, including pregnancy testing, contraceptive counselling and effective contraception was required, and contraception was continued for one month after completion of therapy according to the summary of product characteristics.

The planned maximum observation period was 24 weeks, and information was documented at baseline (T0), i.e. before treatment start, at the 4-weekly follow-up visits, and at the end of observation at week 24 (or after clearance of CHE symptoms). From visit T3 onwards (week 12), treatment could be stopped if the patient showed total clearance of their CHE, as assessed by the Physician Global Assessment (PGA). The severity of hand eczema was defined according to the PGA (17), and for a standardized assessment a validated photographic guide (19) was distributed to each study investigator as an aid to evaluating the severity of CHE. The definition of subtypes of hand eczema was defined according to the German guidelines "Guideline on the management of hand eczema" (11).

The case report forms (CRFs) captured 10 major data categories (demographics, CHE diagnosis, previous therapies, working ability, Toctino® dosage, therapy completion, compliance, PGA, overall assessment of effectiveness and tolerability by the physician and patient, respectively, adverse drug reactions), amounting to 100 up to almost 200 data points for each patient, depending on the number of follow-up visits. For women of childbearing age, all activities documenting concurrence with the approved Pregnancy Prevention Programme were documented by the treating physician.

Statistical methodology

Data from all available patient CRFs were taken into account, including those from patients who discontinued treatment early. All parameters were presented descriptively; for continuous variables the descriptive parameters: number, mean value, standard deviation, minimum, 5% quantile, 25% quantile, median, 75% quantile, 95% quantile and maximum were calculated. Missing values were not considered in the calculations. For categorical variables, absolute and relative frequencies were calculated.

RESULTS

Population demographics and characteristics

Baseline patient demographics and characteristics are shown in Table I. The mean duration of disease was approximately 7 years, and over 50% of patients had ongoing disease for more than 3 years. The majority of the patients (78%) were working, although 42% of them reported sickness absence in the previous 12 months (mean 35 days); at the start of the study 15% were currently on sick leave (mean 59 days). The most frequently represented employment groups were metalworkers and electricians (17%), healthcare professionals (10%), foodstuff and gastronomy workers (9%), construction workers (6%) and cleaners (5%).

Apart from the 96% of patients presenting with CHE on both hands, 39% also had eczema present on the feet, and 19% on other body areas. Almost two-thirds of patients presented morphologically with hyperkeratotic disease and over one-third with the vesicular (dyshidrosiform) pompholyx type (Table I, multiple nominations possible).

In the 12 months prior to baseline, 669 patients (98%) had been treated with many different types of therapies for severe CHE: 665 (98%) with topical therapies, 289 (43%) with various forms of ultraviolet (UV) therapy (cream, bath or systemic therapy), and 458 (67%) with systemic treatments. Of the systemic non-symptomatic therapies, the most common were corticosteroids (45%), retinoids (15%), cyclosporine (7%), methotrexate (5%) and azathioprine (1%).

Table I. *Patient characteristics and aetiology, morphology and localization of chronic hand eczema (CHE) at baseline (including eczema in other body areas)*

Characteristics	
Total patients enrolled, *n*	680
Age, years, mean ± SD	49.9 ± 12.6
Males, *n* (%)	375 (56)
Females, *n* (%)	299 (44)
Body weight, kg, mean ± SD	79.2 ± 14.3
Duration of CHE, years, mean ± SD	6.7 ± 8.0
≤ 12 months, *n* (%)	127 (19)
> 12–36 months, *n* (%)	177 (27)
> 36–72 months, *n* (%)	115 (17)
> 72 months, *n* (%)	244 (37)
CHE subtypes[a,b]: aetiological and morphological criteria, *n* (%)	
Irritant	159 (24)
Atopic	166 (25)
Allergic	91 (14)
Vesicular/pompholyx	241 (36)
Hyperkeratotic-rhagadiform	437 (65)
Fingertip	91 (14)
Localization of eczema[a,c], *n* (%)	
Both hands	645 (96)
Feet	254 (39)
Other body areas	124 (19)

[a]Multiple nominations were possible; data available for [b]668 patients; [c]665 patients.
SD: standard deviation.

In spite of this extensive use of topical and systemic therapies, at baseline all patients presented with a CHE history of at least 3 months or more than two relapses within the previous year. The inadequacy of previous therapy was also reflected by the actual PGA-severity grade that was determined post-inclusion at baseline, showing that the overwhelming proportion of patients (99.1%) were actually PGA-severe (64.5%) or PGA-moderate (34.6%).

Efficacy

The flow of patients through the study is shown in Fig. 1. Over 91% of patients were initially prescribed alitretinoin 30 mg per day, and almost 75% of the patients received this dose unchanged throughout their treatment course. A total of 333 (49%) patients completed treatment ahead of schedule, 45% of whom stated their reason for discontinuation was "clearance of hand eczema".

According to the last observation carried forward analysis (LOCF), 57% of patients achieved a PGA rating of "clear" or "almost clear" hands (Fig. 2), with a continuous increase in response observed during the treatment course. Similar response rates (LOCF) were seen for all 3 morphological types, with a slightly higher response rate observed for the hyperkeratotic type (Fig. 2). Slightly higher response rates (61%) were observed for patients rated PGA-moderate at the beginning and slightly lower rates (53%) for patients rated PGA-severe at baseline. In addition, a trend for a reduction in the level of sickness absence was apparent during alitretinoin treatment.

The median length of treatment duration in TOCCATA was 153 days, with a tendency to be shorter for patients with a shorter pre-study duration of disease. This observation was most pronounced in patients with a hand eczema history of ≤1 year, where the median treatment duration was approximately 20% less (121 days). Other factors, such as gender, history of previous hospitalization due to CHE, or morphological type of CHE, had no influence on treatment duration.

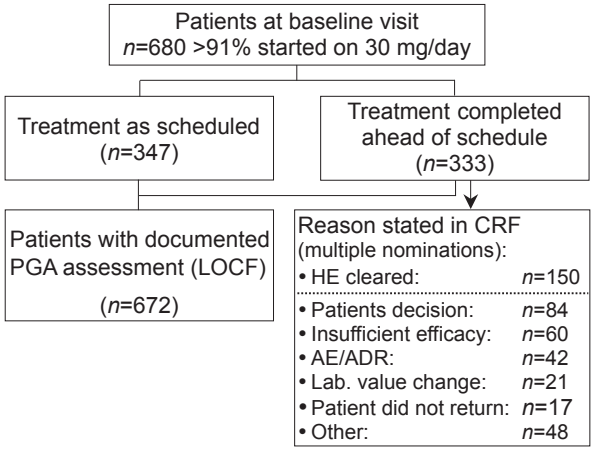

Fig. 1. Flow of study participants. PGA: Physician Global Assessment; CRF: case report form; HE: hand eczema; AE/ADR: adverse event/adverse drug reaction; LOCF: last observation carried forward.

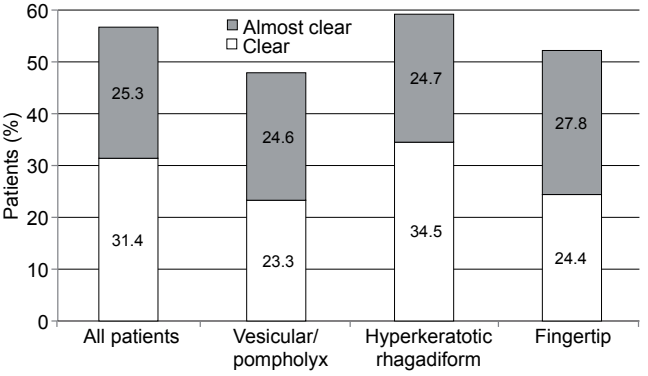

Fig. 2. Physician Global Assessment (PGA) efficacy overall and stratified by baseline morphology (last observation).

Safety

The overall safety profile of alitretinoin was consistent with the known profile of the drug. A total of 298 adverse drug reactions were recorded in 23% of the 680 patients with headache as the most frequent one (7.5%) followed by increased blood triglycerides (4.9%) and increased blood cholesterol (3.8%). Serious adverse drug reactions were documented in only four (0.6%) patients (lymphatic oedema, paranoia, recto-sigmoiditis and soft-tissue swelling). In the 42 patients where treatment was discontinued due to adverse reactions, headache was the most frequent reason (26 patients) and in the 21 patients where treatment was discontinued due to clinically relevant changes of laboratory values this was mostly due to lipid value changes (9 out of 11 patients where changes were specified).

Physician's overall assessment of efficacy and tolerability, respectively, was "good/very good" in approximately 80% of patients, which was similar to the corresponding assessments of the patients themselves (approximately 75%). Patient compliance, as judged by the treating physician at each visit, was rated as "good" in 90% throughout the observation period, and only 6% of patients discontinued treatment within the first 4 weeks for any reason.

DISCUSSION

The objective of the TOCCATA study was to gain further insight into the effectiveness and tolerability of alitretinoin in patients with severe CHE under daily medical practice conditions, and how these observations might correlate with data collected under a strict randomized clinical trial setting.

The patient demographics and characteristics of TOCCATA were very similar to those seen in the Phase III

clinical trial programme. The mean duration of disease was approximately 7 years in TOCCATA, slightly shorter than in the pivotal Phase III BACH study (9 years) (17).

Occupational exposure to skin irritants and allergens can contribute to the development of hand eczema (20, 21). Of note, a trend for a reduction of sickness absence and CHE-related productivity loss was apparent with alitretinoin treatment, and this important aspect should be further explored in the most appropriate healthcare and social system setting, e.g. an occupational health insurance framework.

Taking all dosages and different morphological/aetiological diagnoses into account, 57% of patients were assessed as having "clear" or "almost clear" hands according to PGA in the TOCCATA study. This correlates remarkably well with the 48% of patients with a similar PGA result in the alitretinoin 30 mg treatment arm of the BACH study, even though not all patients in TOCCATA were treated continuously with alitretinoin 30 mg as they were in the BACH study in the 30 mg treatment arm.

The slightly better therapeutic response in TOCCATA might be explained by the fact that patients were able to continue with some concomitant treatments, whereas all concomitant CHE medications were excluded in the alitretinoin CHE clinical trials. In addition, one-third of patients in TOCCATA had only PGA-moderate disease post-inclusion at baseline, whereas in the BACH study all patients had to be PGA-severe for inclusion in the study.

The well-documented morphological diversity of CHE was clearly demonstrated in this study and the therapeutic response broken down by morphological diagnosis showed a high level of healing ("clear" or "almost clear") in all three forms of eczema in this study.

The heterogeneity of the disease was also reflected by the fact that the majority of the patients (96%) had eczema on both hands, and 40% of patients also had eczema of the feet (not recorded in previous trials). A post-TOCCATA follow-up in nearly 200 patients with "clear" or "almost clear" PGA whose feet were also affected showed that in 60% it was indicated that the treatment response was comparable on hands and feet.

The duration of treatment with alitretinoin in TOCCATA showed a tendency to be shorter in those patients with a shorter duration of disease, especially those less than one year, but this observation requires further verification.

The safety findings in TOCCATA were consistent with those in previous clinical trials (15–17, 22), and confirmed the tolerability profile of alitretinoin. The most common treatment-emergent adverse reaction was headache (7.5%), followed by increased triglycerides and increased cholesterol. These are known to be typical retinoid class effects and are transient and well manageable, e.g. treatment of headache by commonly used analgesics or by dose reduction. However, by design in observational trials specific laboratory investigations are usually not imposed but follow common medical practice. Therefore, it might be possible that relevant changes in laboratory values, such as indicating hypothyreosis (23, 24) might have been overlooked in our study.

According to the summary of product characteristics for alitretinoin, for CHE patients with pre-existing cardiovascular risk factors treatment is recommended to be started with 10 mg. In 9% of patients, 10 mg was chosen as the initial dose and 6% of patients remained at this dose throughout treatment. The 10 mg dose also provides an option for temporary or permanent dosage reduction in case of treatment emergent side-effects or tolerability problems that might occur on the recommended starting dose of 30 mg per day. The option of titrating the alitretinoin daily dose either up or down was used in 22% of the patients, similar to data from earlier market investigations. TOCCATA has confirmed that treating physicians are taking advantage of the dosage adjustment option when using alitretinoin.

For a variety of reasons randomized clinical trials, considered as the gold standard in the clinical evidence hierarchy, cannot collect all the data relevant for use in everyday clinical practice. Time-limited drug exposure, restricted endpoints, limited sample sizes, and strict inclusion and exclusion criteria all limit the use of the results in a wider context. Non-interventional observational studies, if adequately designed and performed, can add important information from real-world medical practice despite the known inherent limitations, such as non-random assignment, unblinded assessments, and quality limitations of the collected data. Despite these potential limitations, TOCCATA has shown that data collected during routine medical practice are consistent with the data obtained during the strict clinical trial conditions of the drug development programme. These types of "real-life" studies are important in confirming the effectiveness and tolerability of newly licensed drugs.

In conclusion, this first, large, non-interventional observational open study of alitretinoin, following approval in Germany, shows that the good efficacy and safety profile obtained in the CHE clinical development registrational programme is consistent with the effectiveness and tolerability profile observed under "real life" conditions in dermatological practice.

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Conflict of interest. TL Diepgen was the principal investigator for the study and has received lecture and consultancy fees from Basilea. T. Zimmermann was the study director and is an employee of Basilea Pharmaceutica GmbH, Germany. E. Pfarr was the study statistician and is an employee of the CRO AMS Advanced Medical Services GmbH Mannheim, Germany.

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Oral Alitretinoin in Congenital Ichthyosis: A Pilot Study Shows Variable Effects and a Risk of Central Hypothyroidism

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Congenital ichthyosis is a large group of hereditary skin disorders with different aetiologies, all of which are present at birth (1). The patients have dry, widespread scaling and thickened skin (2). At present there is no cure for ichthyosis and therapy is mostly symptomatic. Life-long treatment with emollients is essential, and some patients also use systemic therapy with retinoids, especially acitretin (3). The most serious adverse effect of retinoids is teratogenicity, which is a special concern for acitretin as it is excreted from the body slowly (3).

Alitretinoin (9-cis retinoic acid) is a fairly new oral retinoid with more rapid clearance than acitretin. In contrast to acitretin and isotretinoin, alitretinoin binds to both types of nuclear retinoid receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs). It is indicated for chronic hand eczema in most European countries and has been found to be well-tolerated (4). We report here our preliminary experiences from an uncontrolled study of alitretinoin in 4 patients with congenital ichthyosis.

PATIENTS AND METHODS

Four adult patients (two women and two men, age range 32–74 years), one with ichthyosis variegata (OMIM 609165) due to a *KRT10* mutation (Danish patient no. 1) and 3 with lamellar ichthyosis (OMIM 242300) due to *TGM1* mutations (Swedish patients nos. 2–4), were invited to participate in this study after informed consent. The patients were all healthy except for the oldest man (patient no. 4) who was on warfarin for atrial fibrillation, having experienced a mild stroke several years before. The female patients used oral contraceptives. The patients' previous treatments for ichthyosis included systemic acitretin ($n = 2$; 25–50 mg/day) or isotretinoin ($n = 1$; 50 mg/day), and/or topical moisturizers and keratolytic agents 1–2 times a day ($n = 4$). Systemic retinoids were stopped at least 3 weeks before baseline. All patients continued to use their ordinary topical treatment during the study.

The trial began in December 2009. The Danish patient was started on alitretinoin (Toctino, Basilea Pharmaceuticals A/S, Denmark) at a dose of 30 mg/day, whereas the Swedish patients started with a lower dosage of 10 mg/day, which was then gradually increased to 30 mg/day during the first month. If the clinical effect was insufficient at first re-visit and no side-effects had occurred, the dose was increased to 40–60 mg/day over a planned treatment period of 3 months. Clinical and laboratory evaluations were performed before the start of therapy and at monthly intervals, and consisted of physical examination, photography, interviewing the patients about effects and side-effects of therapy, and blood sampling for analysis of haematological parameters, liver enzymes, creatinine, cholesterol, triglycerides, thyroxin (T4) and tyroid-stimulating hormone (TSH) levels.

RESULTS

All 4 patients completed the 3-month long trial, and two of them (nos 1 and 3) wished to continue alitretinoin therapy after the end of the trial. In the first month of therapy only the patient with ichthyosis variegata (no. 1), showed significant improvement. The patients with lamellar ichthyosis using lower initial dosages effects showed minimal effects; only at the higher dose (30–50 mg) did the skin become smoother and less scaly (Fig. S1; available from http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1302). After 3 months, two patients (nos 1 and 3) considered alitretinoin equally effective or better than acitretin at 25–50 mg/day.

Dry lips were recorded by all patients at the highest dose. One patient (no. 4) experienced mild myalgia. All these side-effects were reversible on dose reductions. Blood lipid and liver parameters were within the normal range during therapy, but two patients (nos 3 and 4) showed treatment-related changes in the thyroid hormone levels (Table I). Within 1 month after stopping treatment the thyroid-stimulating hormone (TSH) levels in patient no. 4 reverted to baseline values. However, in patient no. 3, who wished to continue alitretinoin therapy despite both laboratory and clinical signs of hypothyroidism (tiredness), the T4 level remained low even after dose reduction to 20 mg/day. Her T4 value was normalized only after combined cessation of alitretinoin and adding substitution therapy with T4, which also cured her tiredness. Interestingly, her TSH value was border-line high at follow-up, and a subsequent investigation revealed laboratory signs of autoimmune hypothyroidism.

The 3 Swedish patients (nos. 2–4) resumed acitretin therapy after completing the alitretinoin trial, but the Danish patient (no. 1) is still (as of September 2011) on alitretinoin 60 mg daily with maintained clinical benefit and no hair-loss similar to what she experienced during previous acitretin therapy.

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Table I. *Thyroid-stimulating hormone (TSH) and thyroxin (T4) levels before and after alitretinoin therapy in 2 patients with treatment-related hypothyreosis*

Pat. no./age, years/sex: dose	Baseline	1 month	2 months	3 months	4 months	5 months	FU
3/40/F: Alitretinoin, mg/day	0	10–30	30–40	40	20	20	0
TSH (normal: 0.4–4.0 mIU/l)	2.71	1.44	NA	2.22	2.60	2.73	3.69
T4 (normal: 12–22 pmol/l)	13.3	11.5	NA	9.2	11.3	10.5	12.0
4/74/M: Alitretinoin	0	10-30	40–50	60	–	–	0
TSH, mIU/l	2.56	1.67	1.84	1.70	–	–	2.87
T4, pmol/l	15.5	11.2	11.0	9.4	–	–	14.0

FU: follow-up 1–2 months after stopping alitretinoin. Patient no. 3 started therapy with L-thyroxine (0.1 mg/day) 2 weeks prior to FU. NA: not available.

DISCUSSION

In this pilot study, 3 patients with lamellar ichthyosis when given alitretinoin in varying doses for at least 3 months did not perform better than previously observed while on acitretin. However, the patient with ichthyosis variegata due to a *KRT10* mutation (Sommerlund et al., unpublished observation) appeared to respond better, although rather high doses of alitretinoin had to be used, which in this case resulted in headache. Side-effects were otherwise similar to those usually seen in acitretin-treated ichthyosis patients, except for the decreased TSH levels observed in two (50%) of our patients. The latter observation is not unexpected because other RXR ligands, such as bexarotene, are known to frequently produce hypothyroidism via suppression of thyrotropin secretion (5). Although Aguayo-Leiva et al. (6) recently found a case of low TSH level during alitretinoin therapy of hand eczema, and Bissonnette et al. (7) noticed a < 10% incidence of low TSH or T4 levels, this side-effect has not been highlighted in larger trials of alitretinoin in patients with chronic hand eczema (8). However, it is possible that hypothyroidism predominantly occurs in predisposed individuals and may not be noticed when lower doses of alitretinoin are used, as is often the case in patients with eczema. It is reassuring though that one of our patients (no. 1) has now been on continuous alitretinoin therapy for 2.5 years without appearance of any serious adverse effect or hypothyreosis. This patient is fertile and prefers alitretinoin therapy because of its shorter elimination time, in case she wants to get pregnant.

In conclusion, alitretinoin might be an alternative to other oral acitretin in the treatment of congenital ichthyosis, especially in women of child-bearing age who want to become pregnant after stopping retinoid therapy and who do not respond to isotretinoin, or in the rare event of hypersensitivity to aromatic retinoids. However, the risk of hypothyroidism should be considered and checked carefully, especially if there is a previous history of thyroid disease. A larger controlled study is necessary to confirm or dispute our results, especially in the case of ichthyosis variegate. Our previous studies have indicated that other types of *KRT10* mutations are particularly responsive to the down-regulatory effects of retinoid therapy (9).

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INVESTIGATIVE REPORT

Ten-year Experience of Bexarotene Therapy for Cutaneous T-cell Lymphoma in Finland

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Bexarotene is an oral retinoid shown to be active against the cutaneous manifestations of cutaneous T-cell lymphoma (CTCL). Literature on the efficacy, dosing and side-effects of bexarotene is sparse. We present here data on 37 Finnish patients with CTCL treated with bexarotene during the last 10 years. Bexarotene was equally effective as monotherapy or when combined with other treatment modalities, resulting in overall responses of approximately 75%. Early-stage CTCL responded better than advanced-stage CTCL (83% vs. 33%). The mean time to observable response was 3 months and the mean duration of the response was 21 months. The dose of bexarotene was generally lower than recommended due to side-effects. Abrupt elevation of liver transaminases, resulting in cessation of treatment, was observed in 4 (11%) patients. We conclude that the dose of bexarotene should be titrated individually to achieve optimal results. Maintenance therapy with low-dose bexarotene is a feasible alternative. ***Key words: cutaneous T-cell lymphoma; mycosis fungoides; Sézary syndrome; bexarotene; Finland.***

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Primary cutaneous T-cell lymphomas (CTCL) define a heterogeneous group of T-cell lymphoproliferative disorders originating in the skin. Mycosis fungoides (MF) is the most common form, comprising almost half of all primary cutaneous lymphomas. MF typically affects older adults (median age at diagnosis 57.5 years), and the male:female ratio is 1.8:1 (1). Sézary syndrome (SS) is the leukaemic form of CTCL with distinct molecular pathogenesis of MF (2). The treatment algorithm of CTCL is based on the stage of the disease (3, 4). The tumour-node-metastasis-blood (TNMB)-based staging criteria were revised in 2007 (5).

MF has an indolent clinical course and the disease progresses slowly. The estimated 5-year survival rate is 88% (1). However, in SS the prognosis is poor, the 5-year survival rate being only 24%. Skin-directed therapy (e.g. topical steroids) usually leads to long remissions in the early stages (IA–IIA). MF confined to the skin is treated with photo-(chemo)-therapy, ultraviolet B (UVB) and psoralen plus ultraviolet A (PUVA), whereas combination chemotherapy is recommended for systemic CTCL (stage IV). SS is treated with extracorporeal photopheresis (ECP), either alone or in combination with, e.g. interferon (IFN)-α, or with IFN-α alone or in combination with PUVA. Other novel treatment modalities for MF and SS include CD52 antibody alemtuzumab, histone deacetylase inhibitors vorinostat, romidepsin; recombinant fusion protein denileukin diftitox, and selective retinoid bexarotene, as discussed further in this article (3, 6–9). In highly selected cases of MF or SS, bone marrow transplantation may be an option (10).

Retinoids belong to the steroid hormone family of molecules. They have long been used alone or in combination with other therapies for CTCL (11, 12). The advantage of retinoids is that they do not have the side-effects of immunosuppressive drugs and can be administered orally. The biological effects of retinoids have been shown to be mediated by the retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Bexarotene is the first synthetic highly (RXR) selective retinoid "rexinoid" that has proven effective, safe and well tolerated in refractory CTCL (13). Bexarotene was approved by the Food and Drug Administration (FDA) in 1999 and was licensed in Europe in 2002 for the treatment of advanced stages of CTCL. In Finland, we have used bexarotene as a second-line therapy since 2002. The exact mechanism of action of bexarotene is unknown, but it binds to RXR and induces dose-dependent apoptosis of malignant T lymphocytes (14). It does not affect T-regulatory cells, keratinocytes or Langerhans' cells of the skin (15–17). The well-known side-effects of bexarotene include hypertriglyceridaemia, which requires individual dosing of this drug and often a preventative usage of lipid-lowering therapy and thyroid hormone replacement, followed by monitoring of laboratory parameters. There are only a few reports concerning the optimal duration and guidance for bexarotene treatment (8, 18, 19).

The aim of this study was to evaluate 10-year bexarotene treatment results and observed unexpected side-

effects in CTCL patients treated in our hospital district, to determine the optimal schedule for bexarotene treatment, tapering of the dose, and duration of therapy. Also, we report on the difference in response rates between bexarotene monotherapy and combination with other treatment modalities.

PATIENTS AND METHODS

Thirty-seven patients with CTCL treated with bexarotene in Helsinki University Central Hospital (HUCH, the Skin and Allergy Hospital for the years 2002 to 2012) were examined retrospectively. Six patients (16%) were followed up in other dermatological clinics after initiation of bexarotene therapy in HUCH. The median age of the patients was 60 years (range 23–87 years). Twenty-one patients (57%) were male and 16 (43%) female. The diagnosis of CTCL was based on the clinical picture, histopathological characteristics of multiple skin biopsies, computed tomography analysis, and T-cell receptor gene rearrangement analysis (1, 20). The patients were mainly evaluated clinically, but occasionally skin biopsy histology was also performed. All patients were classified according to the TNMB classification for CTCL staging and the International Society for Cutaneous Lymphomas (ISCL)/European Organization of Research and Treatment of Cancer (EORTC) classification (5), according to which 26 of the patients (70%) had an early-stage MF (stages IA–IIA). Of these early cases, 8 (31%) were folliculotropic type. Three MF patients (8%) had advanced stage disease (stages IIB–IVB), 4 (11%) had Sézary syndrome (SS), 1 (3%) had peripheral T-cell lymphoma and 2 (5%) had subcutaneous panniculitis-like T-cell lymphoma. The majority of patients had received other therapies before bexarotene administration. Twenty-two patients (59%) received UV therapy, mainly PUVA. Eight patients (22%) received radiation therapy, and 14 (39%) received systemic therapy, for example interferon (IFN), doxorubicin, methotrexate, acitretin, and chlorambucil. Twenty-three patients (64%) had a pre-existing chronic illness, typically a cardiovascular disease requiring regular medication. On the other hand, 13 patients (36%) were otherwise healthy with no regular medication. Ten patients (28%) developed another primary malignancy after CTCL diagnosis, mainly squamous cell carcinomas of the skin and carcinomas of the prostate, mammary glands, and lungs. These secondary malignancies were recorded randomly before or after bexarotene therapy.

Before starting therapy, the patients were evaluated carefully for past medical history, clinical examination, skin biopsy (if not taken recently), computed tomography (CT) scan (if not performed within the past 12 months), and wide laboratory examinations, including full blood cell count, Sézary cell count, renal and liver function tests, infection parameters, lipid profile and thyroid hormone levels. When starting bexarotene therapy, the patients were hospitalized for a short period and monitored for the immediate side-effects of bexarotene. The essential laboratory parameters were assessed weekly at the beginning of the treatment for 4 weeks and monthly thereafter. Clinical follow-up was every 2–3 months, and later, in a stable phase of the treatment, every 4–6 months.

The daily starting dosage of bexarotene was 300 mg/m^2 in the earliest years of our bexarotene experience. Since only a few patients tolerated it without considerable side-effects, we changed to more individual dosing, starting with approximately 150 mg/m^2 daily dose. The dosage is individually altered in relation to clinical response and manageable side-effects. In the first years of bexarotene use, the patients were followed up for laboratory side-effects and a thyroid hormone supplement and lipid-lowering agents were started only when needed. Thereafter, upon the establishment of bexarotene usage guidelines (8), all the patients have been placed on thyroid hormone supplement and 2 different lipid-lowering agents, atorvastatin and fenofibrate, simultaneously with bexarotene.

In this retrospective study it was not possible to use the tumour burden index (TBI) to determine the degree of cutaneous involvement. A complete response (CR) to bexarotene therapy was defined as no evidence of disease in the skin or extracutaneous organs for a minimum of one month. A partial response (PR) was defined as a 50% reduction in the area of skin lesions for a minimum of one month. The overall response category included both PR and CR patients. The patient had stable disease (SD), when no significant change in the skin or extracutaneous manifestations occurred. Progressive disease (PD) was defined as a 50% area increase in skin lesions, and/or appearance of lymph node, blood, or visceral involvement. The time to achieve the response was determined from the time of starting bexarotene to the first documentation of either CR or PR. The duration of response was determined from the first documentation of achieving CR or PR to the subsequent documentation of PD.

Adverse effects occurring during bexarotene therapy were determined and graded according to National Cancer Institute common terminology criteria for adverse events.

RESULTS

Management of bexarotene therapy

The mean age at starting bexarotene treatment was 65 years (range 37–88 years). All but 3 patients started bexarotene treatment as monotherapy. Those 3 patients started bexarotene therapy simultaneously with PUVA therapy. Nine patients underwent several periods of bexarotene treatment because of initial termination of the therapy due to side-effects or CR. The dose of bexarotene was first determined based on the body surface area, but as experience with the drug increased, the dosage was adjusted individually, and it was generally lower than the recommended dose. The bexarotene doses varied between 75 and 675 mg/day. Patients were treated with bexarotene from 2 to 107 months (mean 26 months). The overall experience of bexarotene treatment studied is thus 68 patient years.

Majority of cutaneous T-cell lymphoma patients respond to bexarotene

A total of 33 patients (75%) achieved an overall response (either CR or PR; Figs 1 and 2). On the other hand, 11 patients (25%) did not respond to bexarotene (stable or progressive disease). Of the early-stage patients (stages IA–IIA), 25 (83%) were responders and 5 (17%) were non-responders. Of the advanced-stage patients (stages IIB–IVB) only 1 (33%) was a responder and 2 (67%) were non-responders. All 4 patients with SS responded to bexarotene therapy completely or partially. The 2 patients with subcutaneous panniculitis-like T-cell lymphoma (SPTL) responded partially to bexarotene. The mean time to achieve the response to bexarotene

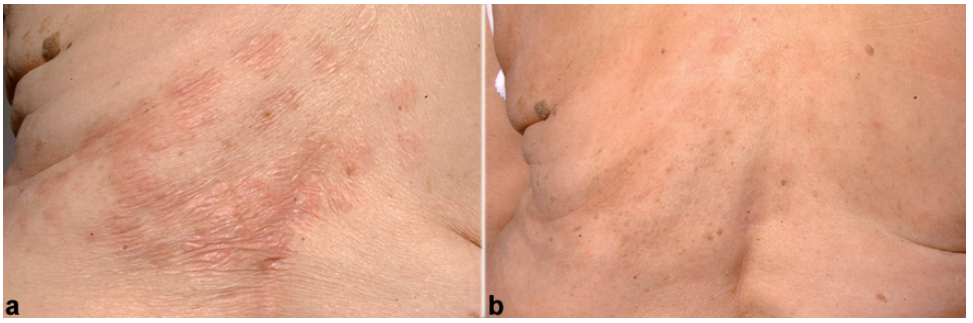

Fig. 1. Stage IA mycosis fungoides (a) before and (b) after bexarotene treatment (complete response). The time-interval between (a) and (b) is 3 months in patient No. 17.

treatment was 3 months (range 1–11 months). The mean duration of response to therapy was 21 months, ranging from 1 to 74 months. Thirteen patients (35%) remained on low-dose bexarotene maintenance therapy (Figs 3 and 4).

In total, 15 patients (41%) underwent combination therapy later in the course of bexarotene treatment. The duration of combination therapy was short compared with the overall duration of bexarotene therapy. The most common concurrent therapy was PUVA for 8 patients (53%), followed by IFN (6 patients, 40%). Local electron beam (EB) and extracorporeal photopheresis (ECP) were combined with bexarotene therapy in 2 and 1 cases, respectively. PUVA therapy was given for a 2-month period, and IFN for a period of 1 to 9 months (mean 3 months). Eleven patients (73%) receiving concurrent therapy with bexarotene responded well to the combination.

The most common reasons for withdrawal of bexarotene were inefficiency (41%) or side-effects (20%). Three patients stopped taking bexarotene after achieving CR and one patient after becoming pregnant despite precautions. Table SI (available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1359) shows in detail the different CTCL subgroups and their response, duration of response, combination therapy and percentage of maintenance therapy.

Side-effects of bexarotene therapy

In the early phases of bexarotene use in our clinic, all patients developed hypertriglyceridaemia and hypothyroidism. With increasing clinical experience, lipid-lowering therapy and thyroid supplementation were routinely administered to patients undergoing bexarotene therapy, and, thus, the incidence of these side-effects was reduced. Nine patients (24%) reported no side-effects when undergoing bexarotene therapy. The most common side-effect remained hypertriglyceridaemia, with 19 patients (51%) affected (Table SII; available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1359). The grade of hypertriglyceridaemia varied between II and IV, the highest value being 37 mmol/l. Hypertriglyceridaemia was manageable with lipid-lowering therapy and for only one patient was it the reason for cessation of bexarotene treatment. Hypercholesterolaemia was evident in 7 patients (19%), usually manageable with

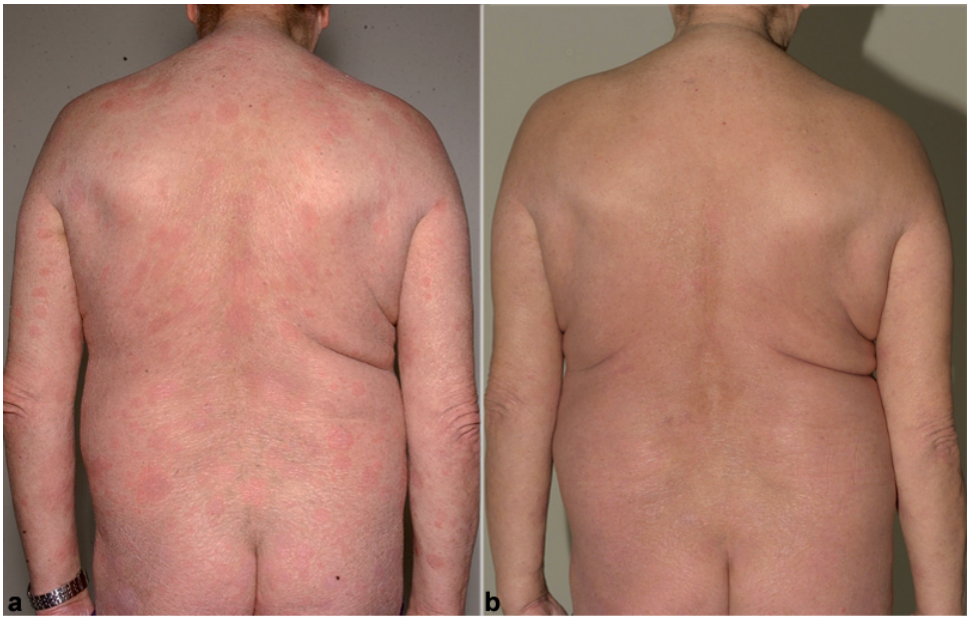

Fig. 2. Stage IIA mycosis fungoides (a) before and (b) after bexarotene treatment (partial response). The time-interval between (a) and (b) is 6 months in patient No. 26.

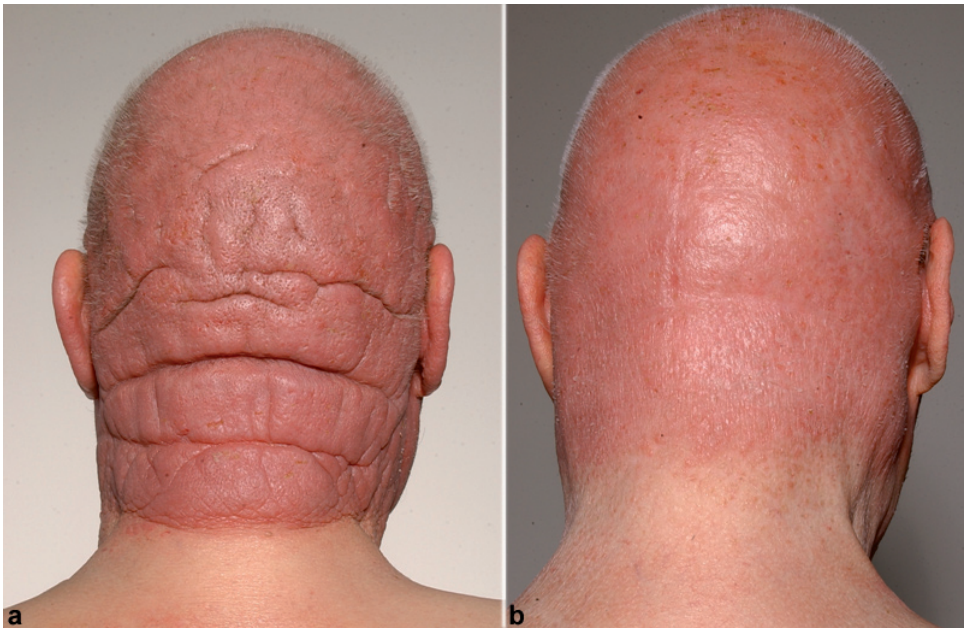

Fig. 3. Patient No. 32 with Sézary syndrome (SS) (a) before and (b) after treatment with bexarotene. The patient is now on maintenance treatment with 75 mg/day (stable disease). This patient was initially treated for ultraviolet B (UVB)-photosensitivity (and actinic reticuloid), which still persists in the neck and face area.

lipid-lowering drugs. Five patients (14%) developed reversible leucopaenia, usually neutropaenia. Four patients (11%) were diagnosed with elevated liver transaminases while undergoing bexarotene therapy. The transaminases (AST and ALT) were at least 5-fold, even over 20-fold elevated, and resulted in termination of bexarotene therapy. The levels of liver enzymes returned to normal after termination of bexarotene, and no hepatitis virus infection or any other known cause was involved. For 2 patients, bexarotene was re-administered with a lowering dose, but rapidly resulted in elevation of liver transaminases in one patient.

Since the management algorithm was different at the beginning of bexarotene treatment, hypothyroidism was diagnosed in only 4 patients (11%). Three of these patients had preventatively administered thyroid supplementation and in one patient thyroid supplementation was added after starting bexarotene. Anaemia and rash (e.g. flush reaction during the first days of bexarotene) was observed in 3 patients (8%). Hypoglycaemia (2 patients), thrombocytopaenia (1 patient) and gastrointestinal nausea (1 patient) were rare side-effects. One patient developed erysipelas of the lower leg 3 times during bexarotene treatment (Table SII).

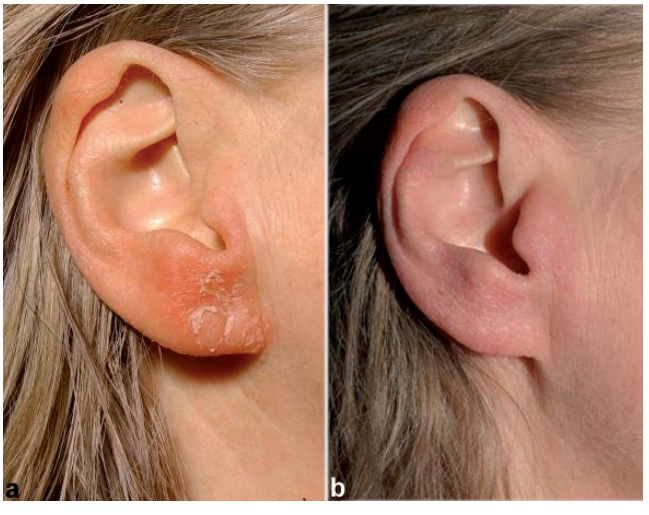

Fig. 4. Follicular mycosis fungoides (a) before and (b) after treatment. This patient was treated with bexarotene 225 mg and 300 mg on alternating days for 17 months (complete response) in patient No. 1. This patient was nickel patch test negative.

DISCUSSION

This article summarizes our 10-year experience of using bexarotene for treatment of CTCL in Finland. Bexarotene is a well-tolerated and effective systemic therapy in advanced stages of CTCL. For patients bexarotene is user-friendly, convenient and easy to administer. It can be used as monotherapy or in combination with other therapies. Our results show that bexarotene was equally effective as monotherapy or when combined with other treatment modalities. The overall response rate as monotherapy was 75% and as combination therapy 73%. The response rates are higher than previously reported (13, 21, 22), probably due to the larger number of early-stage patients included in our series. Of the early-stage CTCL, 83% responded to bexarotene, while 17% did not. Of the advanced-stage CTCL, 33% responded and 67% did not. In a previous British study (22) the response rates were higher in advanced-stage disease than in early-stage disease. The authors speculate the role-increased tolerance of bexarotene side-effects in patients with advanced disease. Interestingly, all 4 of our patients with SS responded well to bexarotene. Similarly, Abbott et al. (22) reported the best response for bexarotene therapy

among patients with SS. Bexarotene is known to inhibit malignant T-cell chemotaxis in SS, which may be a possible explanation for a better response (23). Of cases of follicular MF, 63% (5/8) responded to bexarotene, while 37% (3/8) did not. Previously, a good response to bexarotene has been reported in some cases of folliculotropic MF (24). Our 2 SPTL cases first responded partially to bexarotene. However, after 3–7 months of clinical response, the disease progressed, resulting in cessation of bexarotene therapy. Recently, 82% overall response rates have been reported for bexarotene in SPTL (25). The mean time to achieve a response to bexarotene treatment was 3 months in our CTCL patients. A large majority of the patients achieved the response to bexarotene early, 73% within 3 months. For only 27% of patients, the response was achieved after 3 months of bexarotene therapy. Similar results have been reported previously (22). The duration of the response to therapy was a mean of 21 months, ranging from 1 to 74 months. This is significantly longer than reported previously (22, 26).

CTCL is a group of diseases that has no curative treatment. The aim of different therapies is to achieve the most durable remission. CTCL patients usually survive for many decades, especially those in early-stage disease. Thus, maintenance treatment must be convenient, with minimal side-effects, and it should also prolong remission and survival. After targeted therapy, we need tools to keep the disease in a non-progressive and stable stage (27). Previous studies have shown that bexarotene is able to induce and maintain long-lasting responses (19). One of our patients with SS has been in complete remission with bexarotene monotherapy for 74 months, i.e. for longer than 6 years. Ten patients (32%) have been in remission for longer than 24 months and 22 patients (71%) for longer than 12 months. Our experience shows that, after reaching response, bexarotene should be continued for an extended time with a minimal effective dose as maintenance therapy. Our current practice is to individually titrate the dose to the lowest dose that will maintain the response. For the SS patient mentioned previously we have been able to keep the disease under control with only 75 mg of bexarotene/day (approximately 45 mg/m^2).

In our study we first used higher dosages of bexarotene (300 mg/m^2 daily), but with experience and due to side-effects, we began with approximately 150 mg/m^2 daily and aimed at individual dosing. This is in line with previous reports, in which the dose of bexarotene was titrated to 2–4 tablets (150–300 mg) per day (19). We have also found alternate dosing (e.g. 3 and 4 capsules on alternate days) to be most optimal for several patients.

The most common side-effects in the early years of using bexarotene were hypertriglyceridaemia and hypothyroidism, which were seen in all our patients. We did not pre-treat patients with fenofibrate, which may have explained the increased levels of triglycerides. Also, at the beginning thyroid supplementation was not added simultaneously with bexarotene. With increasing clinical experience, lipid-lowering therapy and thyroid supplementation were administered routinely to patients undergoing bexarotene therapy, and thus the incidence of these side-effects was lowered. Hypertriglyceridaemia, however, remained the most common side-effect (51%) of bexarotene treatment. We reported 4 patients with elevated liver transaminases during bexarotene therapy. In one patient the increase was detected 2 years after the start of therapy. We ruled out all other possible reasons (hepatitis, other drugs and toxins) for the increase. The levels of liver enzymes returned to normal level after termination of bexarotene. To our knowledge, this is the first clinical study reporting elevated liver enzymes in CTCL patients treated with bexarotene. In one previous report elevated liver transaminases were detected, but this was in combination with methotrexate (28).

Bexarotene has been used in Finland for 10 years. In our experience, it is a safe, effective and reasonably well-tolerated drug. This retrospective study revealed somewhat higher response rates than previous studies. Specific subgroups of CTCL, e.g. SS and follicular MF, responded well to bexarotene therapy.

In conclusion, the dose of bexarotene should be determined individually in order to achieve maximum benefit with manageable side-effects. Bexarotene therapy should be continued for long periods as a maintenance therapy after achieving CR. Side-effects should be monitored carefully with routine laboratory tests. We reported notable and recurrent elevation of liver enzymes resulting in cessation of therapy.

The authors declare no conflicts of interest.

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Included in the theme issue:
ACNE, RETINOIDS AND LYMPHOMAS
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INVESTIGATIVE REPORT

Photodynamic Therapy with Methyl-aminolaevulinic Acid for Mycosis Fungoides

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Mycosis fungoides is the most common type of cutaneous T-cell lymphoma. There are a wide range of treatments for early-stage and advanced-stage mycosis fungoides. Photodynamic therapy (PDT) has emerged as a new treatment modality due to its safety and efficacy. The aim of this study was to investigate the safety and efficacy of PDT with methyl-aminolaevulinic acid (MAL) for the treatment of mycosis fungoides. Ten patients with mycosis fungoides were enrolled in this study. A 16.8% MAL cream was applied under occlusive dressing for 3 h. The lesion was irradiated at 37.5 J/cm^2 with red light. The patients underwent two sessions of PDT at one-week intervals. Follow-up biopsy was performed 3 months after the last treatment. In case of partial response, treatment was repeated once a week until complete response. Seven patients had a good therapeutic response. Complete and partial responses were seen in 5 and 2 patients, respectively. During the follow-up period (8–31 months), 6 of the 7 patients remained in stable remission. The treatment was well-tolerated overall, and no patients discontinued the PDT due to pain. In conclusion, PDT with MAL is a fast, effective and well-tolerated treatment for unilesional mycosis fungoides. ***Key words: mycosis fungoides; photodynamic therapy; methyl-aminolaevulinic acid.***

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Mycosis fungoides (MF) is the most common form of cutaneous T-cell lymphoma. The course of MF is unpredictable. It often progresses through three clinical stages: patch, plaque and tumour or it slowly progresses over years or even decades while remaining confined to the skin. The majority of cases of MF present with localized skin lesions, which progress to lymph node and systemic disease in approximately 10% of cases. Patients with a localized patch (T1 <10% of body surface area) have similar survival rates to the general population, and often experience a normal life-span without progression to the plaque or tumour stages (1). Unilesional MF is a rare variant that is characterized by a single patch or plaque involving <5% of the body surface area (2–4). This type of MF has a benign course and does not tend to progress into more widespread lesions or tumours or to disseminate to internal organs. In a cohort study, however, the 10-year survival rates for those with generalized patches or plaques (T2), tumours (T3) and erythroderma (T4) were 67.4%, 39.2% and 41%, respectively (1). Current treatment methods for MF with localized lesion are topical steroids, topical chemotherapy, phototherapy and spot radiation therapy (5). These treatments produce acute side-effects or long-term toxicity, such as immunosuppression, due to psoralen and ultraviolet A phototherapy (PUVA).

Recently, photodynamic therapy (PDT) has been used in the treatment of MF. The advantages of topical PDT include minimal systemic toxicity and good cosmetic results. However, PDT is disadvantageous because it is a time-consuming procedure and often requires retreatment. The most common photosensitizer used for PDT in dermatology is ALA (5-aminolaevulinic acid). Methyl-aminolaevulinic acid (MAL) (Metvix® cream) was introduced recently as a dermatological photosensitizer. MAL is a methyl-ester derivative of ALA, but MAL is more lipophilic and selective toward tumour cells and therefore penetrates better through the epidermis and deeper into tumours than does ALA (6, 7). Additionally, MAL-PDT is less painful compared with ALA-PDT because of its selectivity for tumour cells.

Only a few studies of PDT in MF have been published, and their results are controversial because no standardized treatment exists. There is no study of PDT in Asian patients with MF. We performed MAL-PDT in 10 Korean patients with localized MF and investigated its safety and efficacy.

MATERIALS AND METHODS

Patients

Ten Korean patients with localized MF were enrolled in this study (5 males, 5 females; age range 26–68 years (mean 44.1 years)). Any patients with a history of photosensitivity disease or who were using photosensitizing medications were excluded. Nine patients had stage IA (90%) MF and one patient had stage IB (10%). All 10 patients had Fitzpatrick skin types III–V. Seven patients had a single lesion and the other three had multiple lesions. The diagnosis of MF was confirmed via routine histopathology and immunophenotyping, and the duration of disease ranged from 4 months to 14 years (mean 5.1 years).

Seven patients had received previous treatment, such as topical steroids, PUVA, ultraviolet A1 (UVA1), and oral retinoids, prior to PDT, but all previous treatments were ended at least 6 months prior to starting PDT. Patients received no other treatments for MF during the course of the study.

Therapeutic procedure

A 16.8% MAL cream (Metvix® cream, Galderma, Paris, France) was applied topically to the lesion in a 1-mm-thick layer with a 5-mm border extending to the normal skin. The lesion was then covered with an occlusive, light-shielding dressing. After 3 h, the dressings were removed, and the cream was washed off with a 0.9% saline solution. The red fluorescence of porphyrins was visualized with Wood's light before treatment. The lesions were irradiated with red light from a light-emitting diode (LED) (Aktilite CL128, PhotoCure ASA, Oslo, Norway) at a mean wavelength of 630 nm, a total light dosage of 37.5 J/cm^2 and an irradiation intensity of 75 mW/cm^2 at skin level for 8 min 20 s. The irradiance was measured with an IL-1700 photometer (International Light, Newburyport, MA, USA).

The patients received 2 MAL-PDT sessions at a 1-week intervals. Follow-up biopsy was performed 3 months after the final treatment. In case of partial response, PDT was repeated weekly until complete clearing. Any adverse effects, such as pain, erythema, hyperpigmentation, hypopigmentation, blistering, ulceration and necrosis and scarring, were recorded after PDT. The intensity of pain during the procedure was measured using a visual analogue scale (VAS). Pain was evaluated by VAS ranging from 0 to 10, where 0 = no pain and 10 = worst unendurable pain.

Assessment of therapeutic effectiveness

Therapeutic effectiveness was assessed according to the clinical and histological responses. Clinical response was evaluated by two dermatologists who were blinded to the study, at baseline, 1 month and 3 months after treatment. The clinical response was graded as either a complete response (95–100%), a partial response (50–95%) or no response (<50%). Complete and partial responses were regarded as good therapeutic responses. Photographs were taken with a digital camera (Sony, Tokyo, Japan, alpha 350, 10.0 megapixels) with the patient in the same position under controlled lighting conditions before each treatment session and 3 months after the last treatment. Histological response was evaluated 3 months after the last treatment.

RESULTS

Seven of 10 patients with MF achieved a good therapeutic response, and 3 patients had no response. Seven patients with therapeutic responses had unilesional MF, and the other 3 patients with no response had multiple lesions. Complete and partial responses were observed in 5 and 2 patients, respectively (Table I). Four of the 5 patients with a complete response received two sessions of PDT (Fig. 1 A–D). However, the one remaining patient showed a partial response after two PDT sessions and finally achieved a complete response after three additional PDT sessions (Fig. 1 E, F). Two patients who achieved a partial response did not receive a histological evaluation. On histological evaluation, 5 of 8 patients showed a complete response (Fig. 2).

During the course of treatment, none of the patients reported any adverse effects except for variable de-

Table I. *Clinical data and clinical responses of 10 patients with mycosis fungoides (MF) treated with photodynamic therapy (PDT)*

Patient	Age, years/ sex	Stage	Skin type	MF lesion	Location	Size, cm	Duration, months	Previous treatment	PDT treatments, *n*	Pain score Mean	Clinical response	Histological response	Follow-up, months	Relapse, months
1	47/M	IA	V	Patch	Face	6×3	36	UVA1 acitretin	2	3.0	CR	CR	31	–
2	49/F	IA	V	Patch	Face	1×1	4	–	2	2.5	CR	CR	11	–
3	37/F	IA	IV	Plaque	Scalp	2×1.5	24	Topical steroid	2	0.5	CR	CR	8	–
4	68/M	IA	IV	Plaque	Abdomen	4×3	60	–	5	1.8	CR	CR	19	–
5	37/F	IA	III	Patch	Face	4×2.5	48	Topical steroid	2	1.0	PR	–	22	–
6	52/M	IA	IV	Plaque	Palm	7×4	9	Topical steroid	6	4.5	PR	–	15	9
7	60/M	IA	III	Plaque	Buttock	8.5×3.5	168	Topical steroid	2	1.5	CR	CR	28	–
8	36/M	IB	III	Plaque	Buttock	11×9	120	Acitretin	2	1.0	NR	NR	22	–
9	29/F	IA	V	Plaque	Leg	8×6 6×5 1×1 0.5×0.5 3×1.5	120	Topical steroid PUVA Topical steroid	2	2.5	NR	NR	15	–
10	26/F	IA	IV	Patch	Leg	2×1 4×3 1×1	24	–	2	2.0	NR	NR	23	

UVA1: ultraviolet A1; CR: complete response; PR: partial response; NR: no response.

grees of pain and erythema. The main problem was the variable degree of pain. The reported pain scores using a 10-cm VAS ranged from 1 to 7. However, no patients discontinued PDT due to pain or received local anaesthesia during the course of the study. Serious adverse effects, such as blistering, ulceration and necrosis, were not observed.

One of the patients with a partial response relapsed 9 months after the last treatment. The relapsed patient achieved a partial response after two sessions of PDT. The patient underwent an additional 4 sessions of PDT, but did not obtain a complete response. The other 6 patients who achieved a good therapeutic response did not relapse within a mean follow-up period of 19.1 months after the last treatment.

DISCUSSION

A good therapeutic response to PDT with MAL was achieved in 7 of 10 patients with localized MF lesions in this study. Seven patients with a good therapeutic response had unilesional MF, and the other 3 patients with no response had multiple lesions. Complete and partial responses were observed in 5 and 2 patients after 2 sessions of PDT, respectively. One of patients with a partial response achieved a complete response after 3 additional treatments. One of the patients who had a partial response relapsed after 9 months. This patient received four additional PDT treatments, but only a partial response was achieved. This patient had lesions of the palms, which may have played a role in the treatment response. The stratum corneum of the palms is thicker than that of other body surface areas, so the patient might not have achieved a complete response despite receiving a total of 6 treatments, due to the thickness of the affected area. Our patients with unilesional MF beneficially responded to two sessions of PDT during the follow-up period. Edstrom et al. (8) reported that larger plaques, with a diameter of 7.5 cm or more, showed less successful responses after PDT. However, in another study, two patients with larger plaques with diameters of at least 10 cm showed complete remission after four to five treatments (9). We observed that response to the PDT was not influenced by the lesion size, but may be related to the number of lesions, the thickness of the stratum corneum, the degree of tumour-cell infiltration, and the invasion depth.

The mechanism of PDT in MF is not yet completely understood. In addition to the direct destruction of

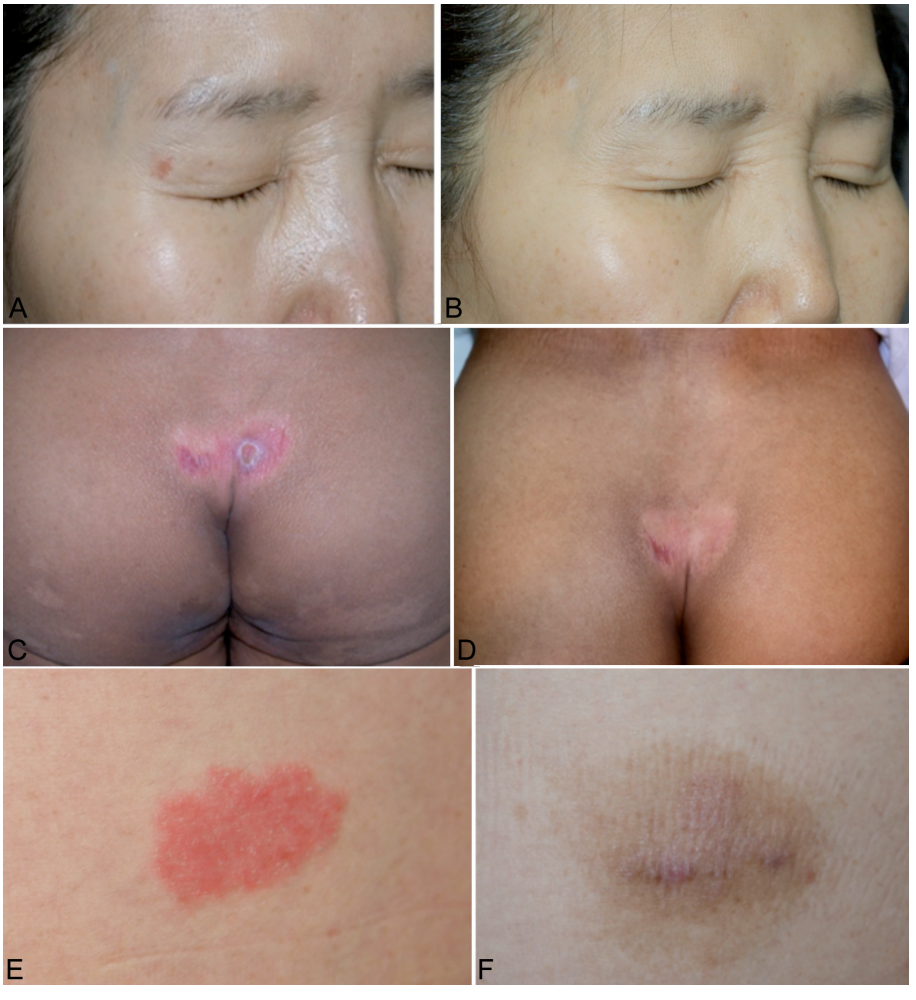

Fig. 1. (A) Clinical features of patient with unilesional mycosis fungoides (MF) before treatment. (B) Complete response was achieved after two sessions of photodynamic therapy (PDT) (case 2). (C) Clinical features of patient with unilesional MF before treatment. (D) Complete response was achieved after two sessions of PDT (case 7). (E) Clinical features of patient with unilesional MF before treatment. (F) Complete response was achieved after five sessions of PDT (case 4).

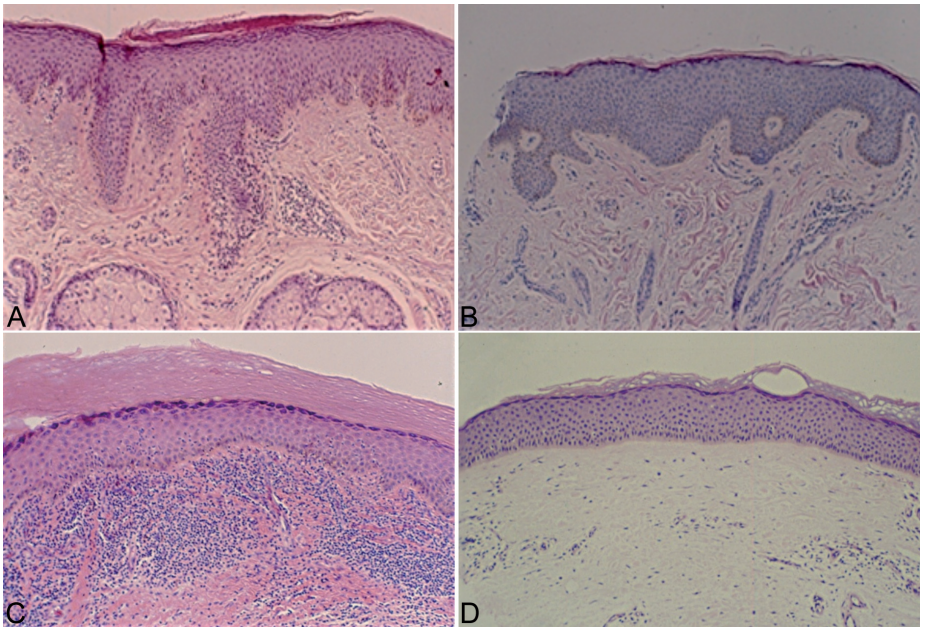

Fig. 2. (A) Histopathological features of patient with unilesional mycosis fungoides (MF) before treatment (haematoxylin and eosin (H&E)×100). (B) Complete response was observed at 3 months after two sessions of photodynamic therapy (PDT) (H&E×100) (case 1). (C) Histopathological features of patient with unilesional MF before treatment (H&E×100). (D) Complete response was observed at 3 months after two sessions of PDT (H&E×100) (case 7).

malignant lymphocytes by the generation of reactive oxygen species, there may be a contribution from the PDT-induced inflammatory reaction, although this possibility requires further confirmation. The maximal efficacy of PDT is achieved through the use of a highly selective accumulation of photosensitizers and light. MAL is a methyl-ester of ALA that has increased lipophilicity, a shorter incubation time and a higher selectivity for malignant lymphocytes compared with those of ALA (20–22).

Patients with patch- or plaque-stage MF who receive PUVA, UVA1 or narrowband UVB therapy have to receive at least 15–20 treatments to obtain a complete response (23), whereas MF patients treated with PDT require fewer treatment sessions to achieve good results (8, 9, 16, 17, 24). In this study participants showed good therapeutic responses after 2 to 6 sessions of PDT. Also, PDT is simple and convenient without systemic side-effects.

Histological therapeutic response is more important than clinical response with regard to MF disease characteristics. We suggest that a final decision about complete or partial response should depend on histological confirmation. In this study, seven patients with unilesional MF had obvious improvement. With a few exceptions, most previous studies had no histological follow-up (9, 16, 19). In this study, 8 of 10 patients received histological follow-up whereof 5 patients had identical clinical and histological complete response.

Optimal parameters for ALA-PDT or MAL-PDT have not been defined for the treatment of MF. Little is known about the optimum number of treatments, frequency of treatment, optimal dose of irradiation, or application time for ALA-PDT or MAL-PDT. According to previous reports, light dosimetry, application time of photosensitizer and frequency of treatment were not markedly different in the treatment of MF (9, 16–18, 24). However, there was great variation in the number of irradiations for ALA-PDT (1–8). The authors speculate that the variation in number of treatments needed is due to MF being a T-cell disease, unlike actinic keratosis, Bowen's disease and basal cell carcinoma, which are

Table II. *Summary of previous reports of photodynamic therapy (PDT) for mycosis fungoides*

Reference	Patients *n*	Clinical features	Topical photosensitizer	Dose J/cm^2	Mean PDT *n*	Lesions *n*	Clinical response *n*	Histological response *n*	Follow-up, months *n*
Wolf et al. (9)	2	Plaque	ALA	40	4.5	3	CR: 3	CR: 3	3–6
Svanberg et al. (24)	2	Not reported	ALA	60	1.5	4	CR: 2	Not done	6–14
Orenstein et al. (16)	2	Patch, Tumour	ALA	170, 720	1	6	CR: 6	CR: 6	24–27
Markham et al. (19)	1	Tumour	ALA	20	5	1	CR: 1	CR: 1	12
Leman et al. (17)	1	Plaque	ALA	100	4	2	CR: 2	CR: 2	12
Coors & von den Driesch (18)	2	Plaque, Tumour	ALA	96, 72–144	5	4	CR: 4	Not done	14–18
Zane et al. (6)	5	Patch	MAL	37.5	3.8	NR	CR	CR: 4, PR: 1	12–34
Recio et al. (11)	2	Plaque	ALA	8	3	2	CR: 2	CR: 2	24
Edström et al. (8)	9	Plaque, Tumour	ALA	90–180	3.9	12	CR: 7	CR: 7	6–9 years

NR: not reported; CR: complete response; PR: partial response; ALA: 5-aminolaevulinic acid; MAL: methyl-aminolaevulinic acid.

keratinocyte diseases. In addition, the difference in the number of treatments may be related to the small size of the studies and inclusion of different types of MF patients. MF has a clinical course that can be ameliorated or that can relapse for a prolonged period of follow-up. Therefore, physicians must verify whether or not a complete response has been achieved according to histological clearance as well as clinical clearance.

In conclusion, good therapeutic results were observed when using MAL-PDT to treat unilesional MF. PDT is well tolerated and provides good cosmetic outcomes. Further large-scale and long-term follow-up studies are needed to establish the optimal treatment protocol for unilesional MF.

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ACNE, RETINOIDS AND LYMPHOMAS
Acta Derm Venereol 2012; 92: 227–289

INVESTIGATIVE REPORT

Distribution and Maturation of Skin Dendritic Cell Subsets in Two Forms of Cutaneous T-cell Lymphoma: Mycosis Fungoides and Sézary Syndrome

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Dendritic cells (DCs) critically regulate immune responses and the "immune-surveillance" of tumours. This study retrospectively analysed the distribution and maturation status of DC-subsets in T-cell lymphoma of the skin. Mycosis fungoides and Sézary syndrome ($n=25$) were investigated immunohistochemically for DC subsets, based on C-type lectin receptor expression: Langerhans' cells (langerin/CD207$^+$, DEC-205/CD205$^+$), dermal DCs (DC-SIGN/CD209$^+$, CD205$^+$) and plasmacytoid DC (BDCA-2/CD303$^+$). Maturation status was assessed by double-labelling for CD83 and CD208/DC-LAMP. DCs were interspersed between the neoplastic infiltrate, and a marked increase in numbers of all three subsets was noted, DC-SIGN$^+$ dermal DCs constituting the majority. Substantial numbers of plasmacytoid DCs were consistently observed. Most DCs in epidermis and dermis were phenotypically immature. Amongst the relatively few mature DCs in the dermis, langerin$^+$ cells predominated. There was a positive correlation between the histological intensity of the tumour infiltrate and DC numbers. It is possible that mature DCs reflect ongoing anti-tumour immune responses, and immature DCs the induction of tumour tolerance. ***Key words: cutaneous T-cell lymphoma; plasmacytoid dendritic cells; Langerhans' cells; dermal dendritic cells; C-type lectins; mycosis fungoides; Sézary syndrome.***

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Mycosis fungoides (MF) is the most common type of epidermotropic primary cutaneous lymphoma. Its clinical course ranges from an indolent premalignant syndrome, through a patch and plaque stage, to an aggressive tumour stage and systemic involvement (1). The major histological findings in MF consist of a dermal, mostly helper T-cell phenotype (CD4$^+$), patchy to band-like dense infiltrate, epidermotropism of solitary lymphocytes or lymphocytes arranged in so-called Pautrier's microabscesses, and moderate to marked dermal fibrosis (2). Possible contributing stimuli to the pathogenesis of MF involve chronic inflammation and clonal abnormalities in the neoplastic T cells. Infectious agents and occupational exposure have been considered as aetiological factors, but supporting evidence is not readily available. Alterations in cellular immune responsiveness in relation to MF is possibly mediated by Langerhans' cells (LCs) (3–5). Sézary syndrome is also included in the classification of cutaneous T-cell lymphomas. It is defined historically by the triad of erythroderma, generalized lymphadenopathy, and the presence of neoplastic T cells (Sézary cells) in skin, lymph nodes and peripheral blood. The histopathological features of skin lesions are indistinguishable from those of MF.

Dendritic cells (DCs) play a pivotal role in the immunobiology of cutaneous lymphoma (6–9). They function as sentinels beneath the body surfaces. Depending on their maturation stage DCs can either: (*i*) act as professional antigen-presenting cells, which have evolved to monitor the environment, detect pathogens and trigger T-cell activation to initiate immune responses; or (*ii*) induce peripheral tolerance in steady state (10). Different subsets of DCs (11) may subserve different functions, as indicated by the differential expression of C-type lectin receptors. These surface receptors operate as components of the antigen capture and uptake machinery of DCs, including LCs (12). Epidermal LCs and the three subsets of dermal DCs, i.e. (*i*) langerin/CD207$^+$, DEC-205/CD205$^+$ DCs, (*ii*) langerin$^-$, DC-SIGN/CD209$^+$, CD205$^+$ DCs, and (*iii*) langerin$^-$, BDCA-2/CD303$^+$ plasmacytoid DCs (pDCs) have characteristic C-type lectin expression profiles. Langerin/CD207 is specifically produced by LCs in the epidermis and localizes on the cell surface and within Birbeck granules (13). Migration and transport of antigen from the skin to the lymph nodes is an important feature of LCs (14). The DC-SIGN/CD209 receptor is involved in cross-talk between DCs and T cells and binds HIV virions and other types of microbes (15). Blood DC Antigen 2 (BDCA-2) is specifically expressed by pDCs and has been reported to play a role in antigen uptake as well as in inhibition of interferon (IFN)-α/β production (16). The immune system has the potential to eliminate neoplastic cells, but tumours

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may escape immune surveillance by altering tumour immunity (17). The aim of the study was to analyse in more detail the distribution and maturation status of DCs in MF and Sézary syndrome and to characterize DCs subsets, which have as yet been incompletely studied in these disease entities. To determine DC distribution and quantity, immunolabelled cryostat sections of patients' lesions were compared with normal human skin from healthy donors. Also, morphological features were noted. Furthermore, the intra- or peritumoural localization, and the correlation between clinical stage and histological intensity of the infiltrate and DC density were analysed. Finally, staining with dendritic cell maturation markers DC-LAMP/CD208, a lysosome-associated membrane glycoprotein, expression of which increases progressively during *in vitro* DC-differentiation and upon activation with pro-inflammatory stimuli (18), and CD83, one of the most utilized cell surface markers for fully mature DCs (19) was performed to determine their state of maturation and thus to better judge their potential role in the anti-tumour response.

MATERIALS AND METHODS

Tissue specimens

Biopsies of 25 patients with MF ($n=19$) at patch, plaque or tumour stage and Sézary syndrome ($n=6$) seen in the outpatient clinic for cutaneous lymphoma from 1998 to 2003 were studied retrospectively. Clinical information and histopathological data were obtained from the medical charts. The age of the patients ranged from 34 to 91 years, and the female/male ratio was 8/17. Normal skin samples from 14 different healthy donors were included as controls. Skin was obtained, after informed consent, from reductive plastic surgery of breast or abdomen, i.e. from areas not, or rarely, exposed to sunlight. Biopsy specimens were snap-frozen and stored in liquid nitrogen at –196ºC until cryosectioning.

Immunohistochemistry

Frozen tissue sections (8–10 µm thick) were air-dried and fixed in acetone. After blocking slides with phosphate-buffered saline (PBS)/1% bovine serum albumin (BSA) for unspecific binding, the slides were incubated with the primary antibody (Table SI; available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1220). Negative control staining with an IgG isotype (DAKO, Glostrup, Denmark) of irrelevant specificity was performed concurrently. Subsequently, biotinylated polyvalent anti-mouse Ig secondary antibody (Amersham Pharmacia Biotech, UK) was applied, followed by streptavidin-horseradish peroxidase (Amersham). Finally 3´3-diaminobenzidine tetrahydrochloride (Sigma, Saint Louis, MO, USA) was added and the slides were incubated until the desired colour (brown) intensity was reached. Haematoxylin was used for nuclear counterstaining.

Double immunofluorescence

All incubation steps were performed for 1 h at room temperature. Two different protocols were employed with identical results. (A) The primary mouse monoclonal antibody was detected by a biotinylated anti-mouse Ig (Amersham Pharmacia Biotech), followed by a Streptavidin-Texas Red conjugate (Vector Laboratories, Burlingame, CA, USA). Then, an excess of mouse γ globulin (100 µg/ml; Jackson Immunoresearch, Avondale, PA, USA) was used as blocking reagent and, for double-staining, a fluorescein isothiocyanate (FITC)- or Alexa-488-conjugated mouse monoclonal antibody was applied as the last incubation step. (B) The primary mouse monoclonal antibody (Table SI) was visualized by a FITC-Alexa Fluor Signal – Amplification kit (Invitrogen-Molecular Probes, Eugene, OR, USA). Then, residual binding sites were saturated with mouse γ globulin (100 µg/ml; Jackson). In the next step a biotinylated antibody (CD83 biotin) was added and revealed by Streptavidin Texas Red (Vector Laboratories). Isotype control was done with biotinylated mouse IgG (e-Bioscience). Stainings were examined with an Olympus BX60 conventional epifluorescence microscope.

Evaluation of immunolabelled sections

Analyses were performed by a semi-quantitative comparison between the intensity of total infiltrating cells of lymphoma specimens and the numbers of DC in lymphoma compared with normal human skin from healthy donors. The arbitrary score for different classes was defined as: 0 = no infiltrate, +/– mild, + moderate and ++ dense infiltrate. Numerical comparison of DC numbers in lymphoma compared with normal human skin from healthy donors (20) was defined as follows: 0 = normal numbers of DC in healthy human skin, – mild decrease, +/– mild increase, + moderate and ++ strong increase in DC numbers in lymphoma specimens, as described in Table SII (available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1220). DC numbers were studied in the epidermis and dermis.

RESULTS

Plasmacytoid dendritic cells

Distribution. The pDC subset was characterized by the expression of BDCA-2. BDCA-2$^+$ cells in MF and Sézary syndrome were increased compared with the skin of healthy donors. The cells were irregularly distributed throughout the dermal infiltrate. A few positively stained cells were also found in the lower part of the epidermis in 14 out of 25 samples (Fig. 1 a, b).

Localization. pDCs were scattered throughout the lymphoid infiltrate, preferentially close to the basement membrane. Their number was dependent on the intensity of the infiltrate. Formation of loose aggregates was noted, but was not a common feature (Fig. 1 a, b).

Maturation stage. Co-expression of BDCA-2 and CD83 could be detected in only a few cases. Most of the pDCs showed an immature phenotype. CD83$^+$ cells were present in lower numbers compared with the numbers of pDCs (Fig. 1 c–e).

Morphology. Morphology of pDCs showed two opposite aspects. One type appeared plasma cell-like. Cells were round-to-polygonal with dendrites missing. The other type showed a much more dendritic shape. Intra-epidermal BDCA-2$^+$ DCs resembled LCs in terms of morphology (Fig. 1, insets).

Langerhans' cells/dermal langerin$^+$ cells

Distribution. LCs showed a mainly linear and regular (network-like) pattern across the epidermis. Intra-

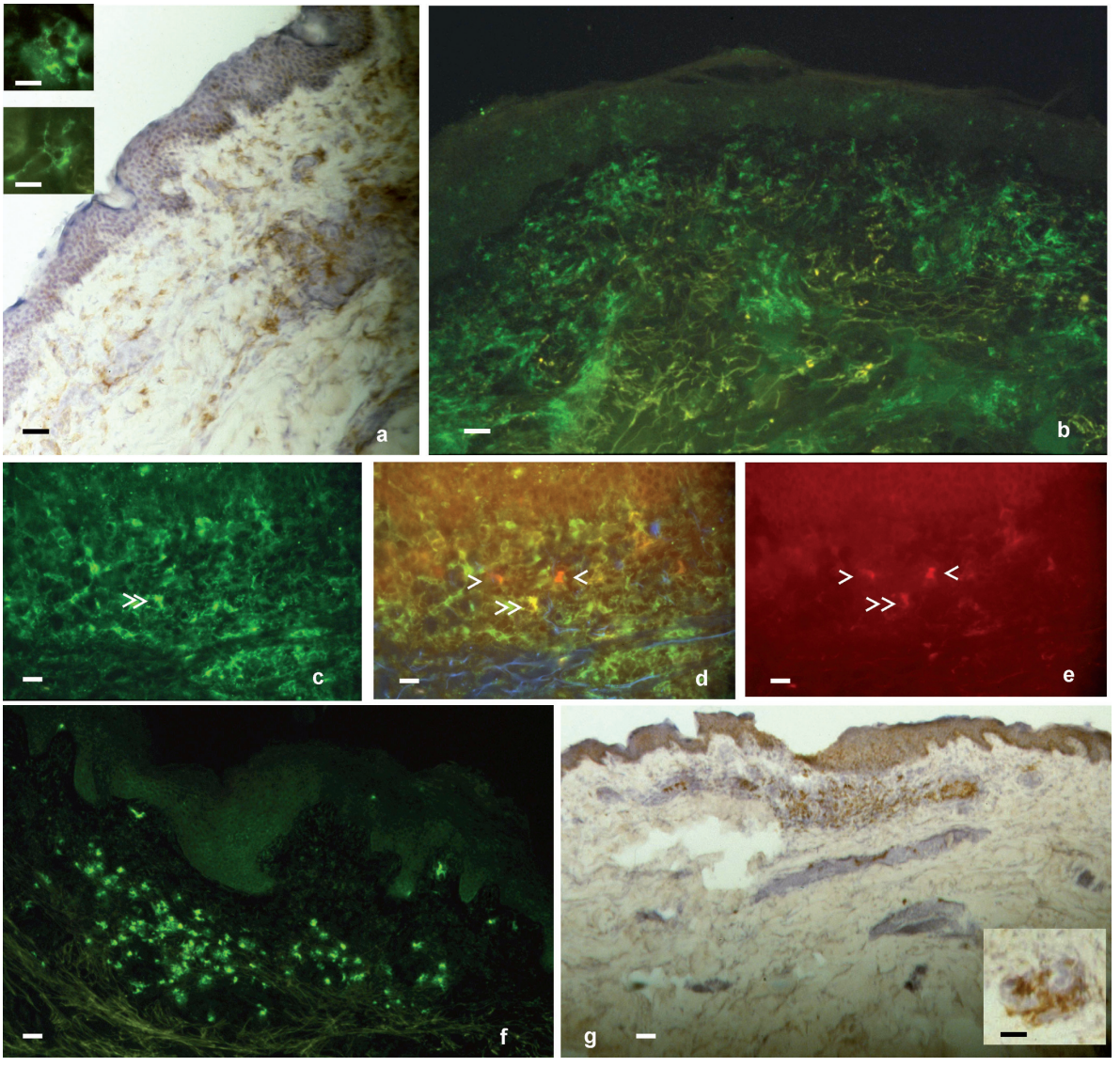

Fig. 1. Plasmacytoid dendritic cells (pDC). Mature dendritic cells. (a, b) BDCA-2$^+$ cells (brown/green) showed an increased number, with irregular distribution throughout the dermal infiltrate. A few positively stained cells were also found in the lower part of the epidermis. The morphology of pDCs showed two opposite aspects: the one looked more plasma cell-like, round and without dendrites (a, *upper inset*); the others had a much more dendritic shape (a, *lower inset*). (c–e) Co-expression of BDCA-2 (*green*) and CD83 (*red*) could just be detected in a few cases. Most of the pDCs showed an immature phenotype. (f, g) An increase in dermal DC-LAMP$^+$ DCs (*green/brown*) cells in the lesional infiltration and in DC clustering is observed. Mature DCs form small-to-large, loose-to-clustered accumulations depending on the intensity of the infiltrate. DC-LAMP$^+$ DCs are predominantly dendritic in shape. Scale bars 50 µm (c, d, e, *insets*), 100 µm (a, b, f), and 200 µm (g).

epidermal clusters of up to four cells were sometimes observed. Epidermal LCs seemed to be slightly increased in number compared with normal human skin (Fig. 2 a, b).

Localization. LCs were mainly lined up along the upper part of the epidermis, close to the stratum corneum, whereas the suprabasal layer, where LCs are typically found, was sparsely populated. Compared with normal skin a marked increase of langerin$^+$ cells was observed in the dermis. There, langerin$^+$ cells were mainly located within the infiltrate of T lymphoma cells and they tended to form clusters. However, some single langerin$^+$ cells were also seen in the infiltrate (Fig. 2 a, b).

Maturation stage. CD83 labelling showed a weaker intensity. Therefore, in general, fewer DCs were labelled with CD83 compared with DC-LAMP. Double-labelling with Langerin and CD83 revealed that there were some small clusters of mature LCs in the epidermis, probably forming Pautrier's microabscesses. The majority of LCs in the epidermis was immature, as described for skin of healthy donors, where DC-LAMP$^+$ langerin$^+$ cells can only rarely be detected (20). Most of the CD83$^+$ dermal cells co-expressed langerin. However, these mature dermal langerin$^+$ cells represented only a small portion of the dermal langerin$^+$ cells. In other words, the vast majority of dermal langerin$^+$ cells was immature (Fig. 2 f–h).

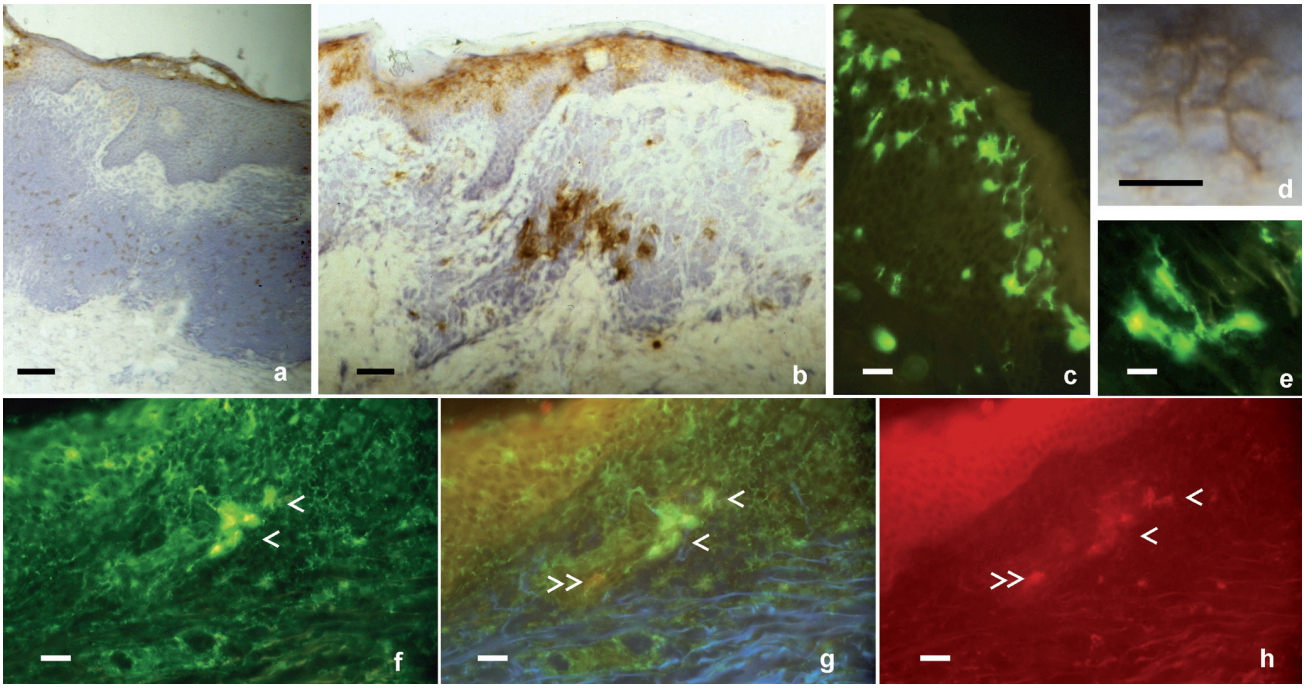

Fig. 2. Langerhans' cells (LC). (a) A marked increase in Langerin$^+$ cells (*brown*) was observed mainly within the dermal infiltrate (*blue*) of T-lymphoma cells. (b) Langerin$^+$ cells formed prominent clusters. (c) Epidermal LC (*brown/green*) cells were predominantly lined up along the upper part of the epidermis and showed a linear and regular pattern. (d, e) Most epidermal LCs and dermal langerin$^+$ cells show typical morphology: dot-like staining of the cytoplasm and dendrites. (f–h) Double-labelling with Langerin (*green*) and CD83 (*red*) revealed that the majority of CD83$^+$ dermal cells co-expressed Langerin (*single arrowheads*). Note, that these mature cells are only a small portion of the dermal langerin$^+$ cells. *Scale bars*: 20 μm (e), 40 μm (d), 50 μm (c, f, g, h), 100 μm (b), and 250 μm (a).

Morphology. Most of the langerin$^+$ cells in MF and Sézary syndrome showed typical LC morphology: dot-like staining of the cytoplasm and the dendrites with anti-langerin antibodies (Fig. 2d). This goes for epidermal and some dermal langerin$^+$ cells. In contrast, other dermal langerin$^+$ cells revealed to some extent a reduced dendritic shape: they were rounding up and had lost their typical LC morphology (Fig. 2e).

Dermal dendritic cells

Distribution. MF skin lesions, as well as skin of healthy donors, presented equal distribution patterns of dermal DCs as defined by the expression of DCSIGN/CD209. There was no specific staining of dermal DCs in the epidermis. The vast majority of these cells showed a regular distribution across the upper dermis, whereas gradually decreasing numbers of CD209$^+$ cells were observed toward the lower dermal region (stratum reticulare) (Fig. S1 a, b; available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1220).

Localization. In all samples DC-SIGN$^+$ cells were densely interspersed throughout the dermal infiltrate. Compared with normal human skin, skin lesions displayed a clearly increased number of dermal DCs. Again, it appeared that they might build a network with direct contacts with each other via dendrites. In three samples a remarkably strong immunolabelling of vessel-like formations was observed. Although not proven, it appeared as if dermal DCs would adhere to the vessel wall and prepare to migrate (Fig. S1c). It must be emphasized, that this staining was specific because isotype controls were totally negative.

Maturation stage. In contrast to the abundant DC-SIGN$^+$ cells in the infiltrate there were few dermal DCs co-expressing a weak CD83$^+$ labelling (Fig. S1 d–f).

Morphology. DC-SIGN+ cells displayed a predominant dendritic shape. Labelling was bright and uniform, with an accentuated surface-membrane staining (Fig. S1, insets).

Mature dendritic cells

As opposed to the double-immunolabelling approach described in the previous paragraphs, these analyses were single-labelling experiments using anti-DC-LAMP antibody (Fig. 1 f, g). The epidermis presented a scattered distribution-pattern of the DC-LAMP$^+$ cells. Mature DCs were very few in number, but consistent in their appearance. Their morphology mainly resembled either LCs, or they were more round-to-oval in shape. In the dermis, mature DC-LAMP$^+$ DCs displayed a predominant dendritic staining and an increased frequency of lesional infiltration and DC clustering compared with normal skin. Mature DCs formed small-to-large, loose-to-clustered accumulations depending on the

intensity of the infiltrate. The stronger the infiltrate the more DC-LAMP$^+$ cells were found.

Clinical correlation

Possible links between the clinical appearance of MF and Sézary syndrome, histological intensity of the neoplastic infiltrate and DC number were investigated. Surprisingly no correlation between clinical stage (patch-plaque-tumour-Sézary syndrome) and the density of the lymphoid infiltrate was found in the particular specimens evaluated in this study. Nor did a correlation of the staining patterns of any of the DC markers with clinical stage become evident. Results summarized in Table SII demonstrate that only a link between the histological intensity of the tumour infiltrate and DC frequencies could be established. In other words, the denser the subepidermal lymphoid infiltrate the higher were the numbers of dermal langerin$^+$ cells, dermal DCs and pDCs in the dermis (Table SII, compare shaded columns). Epidermal LC numbers only marginally exceeded LC numbers in epidermis of healthy donors. It should be noted that no correlation of sex with any of the DC markers was seen.

DISCUSSION

In this study the frequency, distribution and maturation state of DC subsets was semi-quantitatively analysed in MF and Sézary syndrome. Numbers of plasmacytoid DCs, as well as LCs and dermal DCs, were increased in lesional skin compared with healthy human skin. Most DCs were phenotypically immature.

Dendritic cell subsets and origin of malignant T cells. It has been suggested that MF is a malignancy of skin resident effector memory T cells, whereas Sezary syndrome consists of central memory T cells (21). A possible speculation that this may be a consequence of stimulation by different DC subpopulations could not be substantiated in our study: no differences in numbers and distribution of the DC subsets became apparent between MF and Sézary syndrome.

Plasmacytoid dendritic cells. Amongst other tumours, the presence of pDCs has been reported in melanoma (22). Regarding lymphomas, pDCs were found in the peripheral blood of patients with Sézary syndrome (23). Our observations confirm a very recent report showing a similar distribution pattern of pDCs in skin lesions of MF and Sézary syndrome (24). Importantly, we extend these data, in that we double-labelled pDCs with the maturation marker CD83, and demonstrate directly for the first time that the majority of pDCs is immature.

Similar to LCs and dermal DCs, pDCs are capable of stimulating antigen-specific T-cell proliferation. Upon binding of, for example, viruses and CD40L (25), pDCs mature into efficient antigen-presenting cells and, in the case of virus uptake, or ingestion of CpG DNA produce large amounts of IFN-α. Whereas this cytokine is pathogenic in autoimmune diseases, such as systemic lupus erythematosus (26), it may be beneficial for the host in neoplastic diseases by contributing to the stimulation of innate immune cells and mechanisms such as macrophages, natural killer (NK) T cells and NK cells that kill the tumour. Tumour cell bodies may subsequently be captured by immature DCs. In addition, IFN-α can inhibit tumour growth, differentiation, and vascularization through other pathways, such as induction of G0/G1 arrest, suppression of Rb phosphorylation, and down-regulation of G1 cyclins and cyclin A (27). Indeed, many genes involving cell cycle arrest and apoptosis were shown to be upregulated by IFN-α specifically in cutaneous T-cell lymphoma (CTCL) lines. When these genes become de-regulated, sensitivity of tumour cells to IFN-α is lost (28).

IFN-α production of pDCs is induced during their maturation process; our morphological observations of an increased amount of plasma cell-like (immature) plasmacytoid cells and some dendritic cell-like (mature) pDCs (29) would permit to suspect an ongoing maturation procedure with the release of IFN-α. This was not assessed, however. In a small number of samples it has been shown previously that imiquimod-induced recruitment of pDCs to lesions of CTCL correlated with an increase in the expression of IFN-inducible genes, strongly indicating secretion of this cytokine by the infiltrating pDCs (30). Therefore, it would be of great interest to investigate in more detail the cytokine environment in CTCL in order to determine the role of pDCs in the immune control of tumour proliferation.

Langerhans' cells/dermal langerin$^+$ cells. The conspicuous clustering of clonal CD4$^+$ T cells around LCs in the epidermis in "Pautrier's microabscesses" has previously suggested a dependence of the T cells on interactions with this type of DCs (31). Based on *in vitro* experiments, it has been hypothesized that T helper cells proliferate and become malignantly transformed in response to tumour antigens presented by epidermal LCs (32). The data of this study are in line with previous observations (7) suggesting that immature DCs, mainly of the epidermis, mature and migrate to the papillary dermis to become mature antigen-presenting cells. This is supported by the increased numbers of LCs in the dermis in MF compared with normal skin. In double-labelling experiments, the majority of activated CD83$^+$ dermal cells were contained within the langerin-positive subset of DCs (also shown by Lüftl et al. (7)). Inversely, and in extension of a recent report on LCs in skin lymphoma lesions (5), we show here by double-labelling that most dermal langerin$^+$ cells are immature. It remains therefore unclear whether LCs subserve immunogenic or tolerogenic functions in these lesions. An indication of an ongoing anti-tumour response may be the finding of CD8$^+$ T cells

in the infiltrate of MF (6), which, speculatively, might be activated by LCs presenting unknown lipid antigens on their Langerin receptor (33, 34).

Further studies on the migration path of LC precursors into the epidermis (e.g. staining with MIP-3alpha/CCL20 antibody) and of resident LCs from dermal lesions of MF (labelling with CCR7 and Lyve-1 antibodies) to identify lymph vessels may lead to a more precise understanding of the role of LCs in the development of this lymphoma and in an eventual anti-lymphoma immunity, and perhaps also help in the subsequent development of targeted anti-tumour therapies.

A novel aspect must be considered in this context; an unexpected, functionally active population of blood-derived langerin$^+$ dermal DCs has been described recently in mice (reviewed in (14)). The dermal langerin+ cells found in our study may in part belong to this novel population. It must be stressed, however, that at this time a likely human equivalent is still being sought (14).

DC-SIGN$^+$ dendritic cells. DC numbers in the dermis (langerin$^+$ cells, dermal DC, pDC) were found to be related only to the density of the subepidermal tumour infiltrate. Their mainly immature state, which we determined here by double-labelling experiments, in extension of a recent description of DC-SIGN$^+$ DC in skin lesions (24), might be the result of an active tumour immunosuppressive mechanism, e.g. by blocking the maturation process of dermal DCs and thus avoiding the generation of an anti-tumour immune response. Observations from cell culture experiments report the ability of CTCL cells to retard DC maturation. The prolonged survival of the DCs, their ability to proliferate and take up dying CTCL cells may drive the long-term proliferation of CTCL (9). Speculatively, the persistence of malignancy may further be promoted by the induction of tolerance through MHC-II presentation of tumour antigens by immature DC (10). This would require a close contact between those cells and explain the intra-infiltrate-localization of the DC subsets juxtaposed to the CD4$^+$ T-cell clones. A caveat is warranted with regard to CD209$^+$ cells in the dermis. This population may also contain dermal macrophages that express CD163, as shown previously (35, 36). We did not further pursue this issue by double-labelling experiments with both antibodies.

Distribution and state of maturation of dendritic cells. Presence and increased density of DCs in tumours have frequently been found associated with a better prognosis. In melanoma an increased number of mature DCs (DC-LAMP$^+$) was observed in the neoplastic infiltrate (37). They were found in close apposition to T cells (of tumour and/or infiltrate origin). Therefore, it is tempting to assume that their presence stimulates an anti-tumour immune response. This was confirmed in a more recent study that reported a significant correlation between the density of DC-LAMP$^+$ DC infiltrates in sentinel lymph node of melanoma patients with the absence of metastasis in downstream lymph nodes (38). Our observations therefore support the hypothesis of an ongoing anti-tumour immune response in MF lesions, leading to a prolonged disease course. The intensive bystander infiltrate found in MF lesions involves early stages of clonal as well as reactive cells that have the capacity to produce regulating cytokines (3).

Although an anti-tumour immune response is suggested, our findings of an immature state of most DCs are somewhat contrasting. One may speculate that the immature DCs are recruited to the site of inflammation, but their maturation may be largely suppressed by the tumour environment. One may further speculate that this suppression of DC activation may be the cause for an insufficient immune response and, as consequence, for the protracted course of the disease. In a recent study by Schlapbach et al. (24) similar patterns of DC distribution and states of maturation were observed. These authors hypothesized that the preponderance of immature DC may even tolerize against the malignant cells and thereby allow their escape from an immune attack. They substantiated this notion by showing increased numbers of FoxP3$^+$ regulatory T cells in close proximity to the immature dermal DC. On the other hand, the consistently found mature DCs (mainly of the LC type) may reflect some ongoing anti-tumour response. The balance of these pro- and anti-immunogenic observations may shape the appearance of the disease, that is the failure to eradicate the tumour cells and, on the other hand, the relative containment/control of tumour cell growth (at least over long periods). Clearly, further research is needed to better understand the immune response in MF and Sézary syndrome.

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The authors declare no conflicts of interest.

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Epstein-Barr Virus-positive Mucocutaneous Ulcers as a Manifestation of Methotrexate-associated B-cell Lymphoproliferative Disorders

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Immunosuppressive states due to immunological senescence (1) or administration of immunosuppressants (2) occasionally cause Epstein-Barr virus (EBV)-induced B-cell lymphoproliferative disorders (LPDs). While methotrexate (MTX) is an anti-metabolite and anti-foliate agent for the treatment of cancers and autoimmune disorders, it can also potentiate tumourigenesis due to its immunosuppressive effect. EBV reactivation is observed in half of such cases, suggesting that EBV contributes to the pathogenesis (3, 4). A newly described clinicopathological entity, EBV-positive mucocutaneous ulcer (EMU), occurring in immunocompromised patients, has been proposed (4). We describe here a case of EMU presenting with large deep facial ulcers in association with MTX-LPDs, which has not previously been reported in literature.

CASE REPORT

A 62-year-old woman with polymyositis was treated with low-dose prednisolone (5–10 mg/day) and MTX (5 mg twice a week) for 7 years. Four years before our initial examination, erosive lesions emerged suddenly around her lips and evolved gradually into large ulcers on the mouth, nose and right lower eyelid. Topical anti-bacterial agents, such as gentamicin sulphate, nadifloxacin, and sulfadiazine silver cream, were given by a rheumatologist, with only limited effects. The ulcers progressively enlarged to double the original size and, in November 2007, she was referred to us for clinical assessment of these lesions.

On examination, her body temperature was 36.7ºC. Since she felt intolerable pain when opening her mouth, eating was severely disturbed. Several cervical lymph nodes were palpable at a size of 1–1.5 cm. There were five facial ulcers, ranging from 1–6 cm in diameter, each located on the lower lip to jaw, neck, left nasolabial groove, philtrum, and right lower palpebra (Fig. 1A). The ulcers were sharply demarcated and raised on the skin, with mottled telangiectasia and an erythematous hue, as seen on the jaw. Scars were also noted. Laboratory investigations revealed mild elevations of liver enzymes, lactate dehydrogenase (LDH) (291 IU/ml; <208 IU/ml), aspartate transaminase (AST) (38 IU/ml; <30 IU/ml), alanine transaminase (ALT) (40 IU/ml; <30 IU/ml), leucine amino peptidase (78 IU/ml; <43 IU/ml), and a high elevation of C-reactive protein (6.67 mg/dl; <0.1 mg/dl). White blood cell counts fluctuated within the normal range during the course (5,800–8,800/μl) although mild lymphocytopenia was constantly observed (340–582/μl; 1,500–4,000/μl). Serum immunoglobulin G (IgG) levels were low (689 mg/dl; 1,200–2,120 mg/dl), while serum levels of IgA and IgM were normal. The level of soluble interleukin-2 receptor was extremely high (5,834 IU/ml; <534 IU/ml). Cytomegalovirus pp65 (C7-HRP) antigen-positive cells were detected in 94 cells/48,000 cells (normal 0) of peripheral blood mononuclear cells (PBMCs). No anti-EBV-virus capsid antigen IgM (anti-EBV-VCA IgM), anti-EBV-erythrocyte ATP/ADP ratio IgG (anti-EBV-EADR IgG) or anti-EBV-Epstein-Barr nuclear antigen IgG (anti-EBV-EBNA IgG) was detected. EBV-deoxyribonucleic acid (DNA) copy number in the peripheral blood was 1,500 copies/10^6 PBMCs (normal <20 copies/10^6 PBMCs). The anti-VCA-IgG titre was ×160. β-D glucan levels was 876 pg/ml (<20 pg/ml). These data indicated opportunistic reactivation of cytomegalovirus (CMV), EBV and not-yet-identified fungal infection, presumably due to an underlying immunocompromised status.

Skin histopathology from around the ulcer on the right cheek revealed hyperkeratosis and epidermal inclusion cysts (Fig. 1B). Lymphocytes bearing large nuclei and even Reed-Sternberg (RS) cell-like nuclei had massively infiltrated the dermis and subcutis (Fig. 1C). Large abnormal lymphocytes that clustered around the vessels (Fig. 1D). These large cells were $CD3^-$, $CD15^-$, $CD20^+$, $CD30^+$ and $CD79a^-$, and partially LMP-1^+. Because of similarity in the size and distribution, $CD20^+$ cells, but not $CD3^+$ cells or $CD56^+$ cells, are likely to be EBV-encoded RNA positive

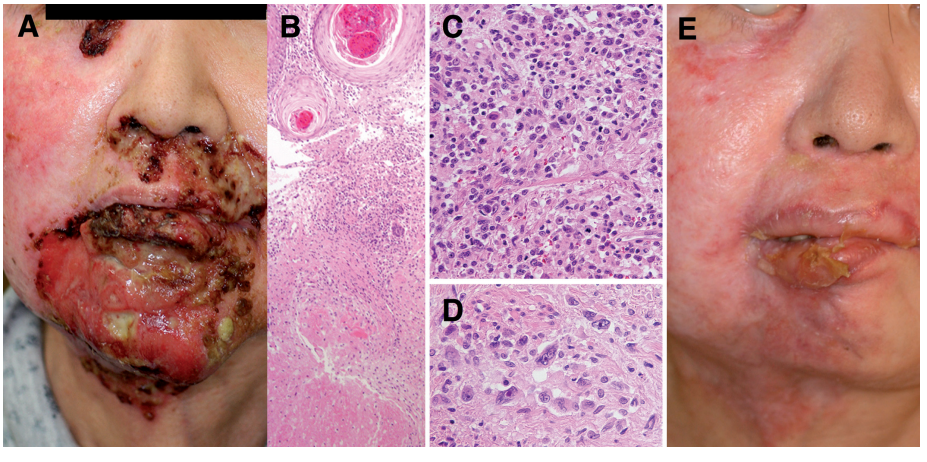

Fig. 1. Clinical and histological findings. Skin ulcers of the face, (A) before and (E) after withdrawal of methotrexate (MTX). Skin histopathology of the right cheek (haematoxylin-eosin staining). (B and C) Lymphocytic infiltration in the skin with hyperproliferative epidermal changes was noted (B: ×40; C: ×100, original magnification). (D) Reed-Sternberg cell-like, large abnormal lymphocytes were located near the dermal vessels (×400, original magnification).

(EBER$^+$), as identified by *in situ* hybridization analysis. PCR analysis of DNA extracted from the skin sample for spectrotyping assay using a LymphoTrack™ IGH TrackOne™ kit (InVivoScribe Technologies, San Diego, CA, USA) identified a monoclonal spike the size of the immunoglobulin gene D1-6 region, indicating monoclonality of the infiltrating B cells. A diagnosis of EMU, in association with MTX-LPDs, was made. MTX was discontinued and prophylactic treatments with the anti-fungal agents, ganciclovir and combined trimethoprim-sulphamethoxazole, were initiated. Ulcers reduced in size dramatically within 2 weeks and healed within one month (Fig. 1E). However, during this time, the patient's body temperature increased to 39ºC and she developed a cough. Chest roentgenograms and bronchoscopic investigation revealed interstitial pneumonitis and bronchial ulcers due to CMV and *Aspergillus* infection. Intensive treatments against these infections, including ganciclovir, valganciclovir, foscarnet and various anti-fungal agents, relieved her symptoms within 3 weeks. A skin biopsy was performed in the vicinity of the first biopsy, and showed sparse lymphocytic infiltration into the dermis. PCR analysis of the skin-derived DNA indicated two substantially lower peaks of different sizes than in the first analysis, confirming that the lymphoma cells had disappeared. EBV-DNA was not detected in the blood. To date, there has been no recurrence of the facial ulcer.

DISCUSSION

Our patient was diagnosed with EMU in association with MTX-LPDs. EMU is a clinical subtype of B-cell LPDs, which was first proposed by Dojcinov et al. (4) and presents with indolent mucocutaneous ulcers located around the lips and within the oral cavity of immunosuppressed patients. However, it is noteworthy in our case that the skin ulcers were impressively deep and large, unlike ulcers in the previous cases of MTX-associated mucocutaneous ulcers, which may provide a clue for diagnosis of B-cell neoplasms. The infiltration of CD30$^+$ EBV$^+$ large B-cells is a pathognomonic hallmark of EMU (4). Since inflamed ulcers develop gradually and may even partially regress, this condition may be initially misdiagnosed as inflammatory and infectious disorders until a skin biopsy is performed (5).

EBV-associated mucosal lesions in immunosuppressed individuals have previously been reported as LPDs or B-cell lymphomas. Although EMU shares some features with other B-cell lymphomas with RS-like cell infiltrations including classical Hodgkin's lymphoma, T-cell-rich B-cell lymphoma and lymphomatoid granulomatosis, there are distinctive clinical and pathological differences. The majority of RS cells in classical Hodgkin's lymphoma are CD20$^-$ and CD15$^+$ and CD30$^+$, while the neoplastic cells in T-cell-rich B-cell lymphoma are CD20$^+$ and CD14$^-$ or CD30$^-$ (6). Lymphomatoid granulomatosis characteristically shows angiocentric infiltration of lymphocytes (7). Resolution of EMU has been reported in more than 30% of reported cases after restoration of immunosuppression. In the case of MTX-LPDs, especially, tumours were observed to regress dramatically (8–11), a feature also seen in our case. Although several cases of MTX-associated skin ulcers due to the drug toxic effect have been reported (12–14), some of these cases might include EMU associated with MTX treatment.

Since the skin seems easily to be affected by this disease, special attention should be given to skin lesions in immunosuppressed patients (5, 15).

The authors declare no conflicts of interest.

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A Case of Lymphomatoid Papulosis with Extensive Limb Disease Followed by Extracutaneous Involvement and Acquired Ichthyosis

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Primary cutaneous CD30$^+$ T-cell lymphoproliferative disorders include: (*i*) lymphomatoid papulosis (LyP), (*ii*) primary cutaneous anaplastic large cell lymphoma (pcALCL), and (*iii*) borderline lesions (1). LyP represents the benign end of this spectrum of disorders; however, some cases are associated with progressive cutaneous lesions and/or extracutaneous involvement (2). Although pcALCL usually has a favourable prognosis, extensive limb disease (ELD) is believed to indicate a poorer prognosis (3). We report here the case of a man who presented with ELD, extracutaneous involvement, and acquired ichthyosis during the typical course of LyP.

CASE REPORT

A 41-year-old man presented with self-healing, but recurrent, erythematous papules on the face, trunk, and extremities, which were first noted when he was 28 years old (Fig. 1A). Histological examination revealed a typical picture of LyP, with a dermal wedge-shaped cell infiltrate (Fig. 2A), which comprised anaplastic large cells in an inflammatory background. The tumour cells had an anaplastic morphology with irregular-shaped nuclei, prominent nucleoli, and abundant cytoplasm (Fig. 2B). Immunohistochemically, they were diffusely positive for CD30 (Fig. 2C), partially positive for CD3 and CD4, but negative for CD8, CD15, CD20, CD25, anaplastic lymphoma kinase, and epithelial membrane antigen. Also, he had exhibited ichthyosiform eruptions on the extremities and abdomen since he was 40 years old (Fig. 1A). His family members had no history of similar skin symptom. The histology showed orthokeratotic hyperkeratosis with a decreased granular cell layer. All together, we diagnosed the patient as LyP associated with acquired ichthyosis. He had been treated with a topical steroid agent and phototherapy (PUVA), but did not achieve complete remission of the papules. The ichthyosiform eruptions improved slightly with 20% urea ointment. At the age of 42 years, left inguinal lymphadenopathy abruptly developed. Histologically, the aggregation of anaplastic large cells with the same phenotype as those of papules in the cortex was observed. Radiation therapy (total radiation dose: 46 Gy/cm^2) was performed, resulting in temporal remission. At the age of 45 years, an ulcerative, erythematous tumour abruptly developed on proximal and flexor sites of the left thigh, followed by recurrent left inguinal lymphadenopathy. The tumour was excised, but multiple tumours soon developed on the operation scar (Fig. 1B). Histologically, the tumour mass was composed mainly of anaplastic large tumour cells (Fig. 2D) involving the whole dermis. The tumour cells showed a phenotype identical to those of the papules and lymph nodes. Southern-blotting analysis of T-cell receptor (TCR) Cβ1 and Jγ genes in the tumour showed no clonal TCR gene rearrangement, as with the papules and lymph node. Imaging tests detected no further systemic involvement. We re-started local radiation therapy, and the lymph node was sensitive to irradiation (total radiation dose: 28 Gy/cm^2). The tumours were also sensitive to radiation; however, new tumours continuously developed in surrounding, non-irradiated areas over approximately a 3-month period. Finally, the tumours expanded to the left popliteal fossa, and thus we decided to increase the irradiated areas (total radiation dose: proximal site: 64 Gy/cm^2, distal site: 32 Gy/cm^2). We commenced treatment with low-dose (15 mg/week) methotrexate (MTX). The tumours responded well to MTX and stopped developing approximately 2 weeks after MTX introduction. Recently, we have tapered MTX (15 mg/2 weeks), with a few papules remaining; however, no new skin tumours and systemic lesions have been found.

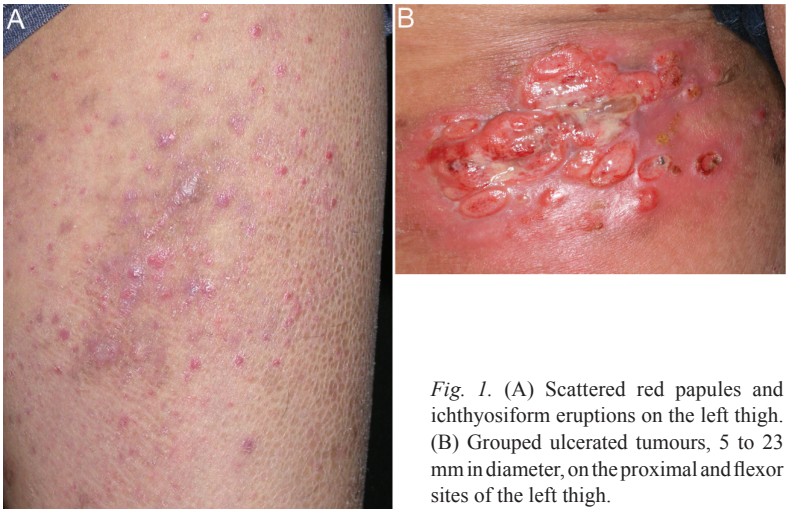

Fig. 1. (A) Scattered red papules and ichthyosiform eruptions on the left thigh. (B) Grouped ulcerated tumours, 5 to 23 mm in diameter, on the proximal and flexor sites of the left thigh.

DISCUSSION

LyP was referred to as a benign, skin-limited, chronic disease in its original description (4). However, Bekkenk et al. (2) have indicated that approximately 20% of patients with LyP are associated with lymphomas, and 10% of those are associated with extracutaneous involvement. Most patients with pcALCL present with solitary or localized tumours, classified as T1 or T2 according to the ISCL-EORTC TNM classification system for primary cutaneous lymphomas other than mycosis fungoides and Sézary syndrome (5). The prognosis is usually favourable, with a 10-year disease-specific survival of approximately 90% (6). However, Liu et al. (7) indicated that a subset of patients with pcALCL with ELD might follow a more aggressive clinical course. Patients with ELD were noted to present with extensive tumour involvement of a single limb, classified as T2b or T2c, or

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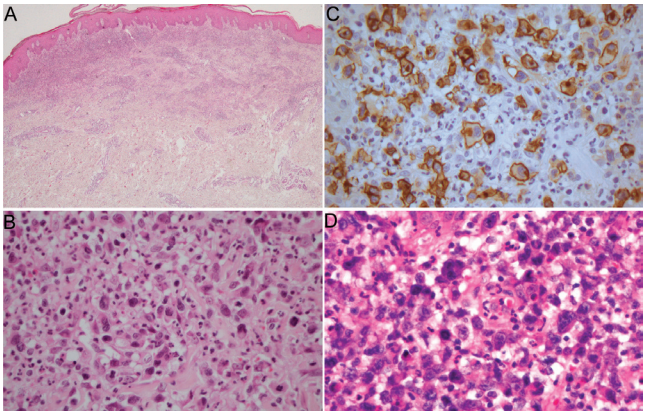

Fig. 2. (A) A papule shows wedge-shaped cell infiltrate in the dermis (haematoxylin-eosin staining × 100). (B) The cell infiltrate is composed of anaplastic large cells in an inflammatory background. (C) Immunohistochemical staining of a papule. Large cells are positive for CD30. (D) Anaplastic large cells proliferate in the tumour. (haematoxylin-eosin staining × 400).

a single limb with contiguous body regions, classified as T3b. Woo et al. (3) have reported a study of 48 patients with pcALCL, including 4 with ELD. This study also included 8 patients with pcALCL with extracutaneous involvement. Univariate analysis demonstrated that ELD and extracutaneous involvement were significant prognostic factors for the 5-year disease-specific survival. The clinical course indicated that our case was a poor one in which LyP progressed to pcALCL, classified as T2c. Although no monoclonal rearrangement of TCR genes has been identified, they should be regarded as ELD because of the aggressive clinical course. In the study by Woo et al. (3), 4 patients with ELD were characterized by the lack of a durable response to treatment. All but one with ELD progressed regionally within 3 months of completing chemotherapy or local radiation. They also failed to salvage treatments, including various chemotherapy regimens. Our case was similar to their cases, in that ELD progressed regionally over an approximately 3-month period in spite of local radiation, but was different, in that low-dose MTX therapy was effective and no new skin tumours and systemic lesions have been found for approximately 3.5 years.

Acquired ichthyosis is a rare and significant manifestation, as it is generally associated with underlying lymphoproliferative disorders, such as Hodgkin's disease (8). Yokote et al. (9) demonstrated a case of LyP preceded by ichthyosis. Their case was similar to ours, in that LyP was followed by inguinal lymphadenopathy. Morizane et al. (10) reported that, of 10 patients with pcALCL, 3 (30%) were associated with ichthyosis. All 3 cases of PC-ALCL with ichthyosis were in the advanced stages and showed internal lymph node involvement. In our case, inguinal lymphadenopathy initially occurred 2 years after the onset of ichthyosis, followed by ELD and the recurrence of lymphadenopathy. Overall, we hypothesize that LyP or pcALCL patients with acquired ichthyosis have a poorer prognosis than those without ichthyosis. Thus, more careful follow-up may be required when identifying ichthyosis during the course of LyP or pcALCL.

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Serum Levels of CC Chemokine Receptor 4 and CXC Chemokine Receptor 3 Ligands in CD8$^+$ Sézary Syndrome

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Sézary syndrome is an erythrodermic cutaneous T-cell lymphoma with haematological evidence of leukemic involvement, which is typically characterized by a monoclonal proliferation of atypical CD4$^+$CD45RO$^+$ lymphocytes (Sézary cells). We report here a case of CD8$^+$ Sézary syndrome that was treated successfully with methotrexate. Sézary cells were CD3$^+$, CD4$^-$, CD8$^+$, CD45RO$^+$. They also expressed CC chemokine receptor 4 and 10, but not CXC chemokine receptor 3. The number of Sézary cells and serum levels of soluble IL-2 receptor were positively correlated with serum levels of CC chemokine ligand 17, 22 and 27, whereas they were negatively correlated with serum levels of CXC chemokine ligand 10.

CASE REPORT

A 55-year-old man was referred in February 2007 for the investigation of erythroderma and lymphadenopathy. He had been treated with topical corticosteroids with no notable improvement. Clinical examination revealed generalized infiltrated erythema, palmoplantar hyperkeratosis, and bilateral axillary and inguinal lymphadenopathy (Fig 1A). Histological examination of the skin showed acanthosis and dermal perivascular infiltrate of atypical lymphocytes with little epidermotropism (Fig 1B). Immunohistochemically, these atypical lymphocytes were CD3$^+$, CD4$^-$, CD8$^+$, CD45RO$^+$, CCR4$^+$, CCR10$^+$, and CD56$^-$ (Fig 1C–E). Laboratory tests showed a leukocyte count of 19,260/mm^3 with 53.5% atypical lymphocytes (10,300/mm^3). Lactate dehydrogenase was 599 IU/ml (normal 119–229 IU/ml), and soluble IL-2 receptor (sIL-2R) was 1,632.4 U/ml (206–713 U/ml). Blood flow cytometry showed that 90.6% of lymphocytes were CD8$^+$ (CD4/CD8 ratio: 0.06), and 78.3% of CD8$^+$ cells expressed CC chemokine receptor 4 (CCR4), but not CXC chemokine receptor 3 (CXCR3). Serum levels of CC chemokine ligand 17 (CCL17), CCL22, CCL27, and CXC chemokine ligand 10 (CXCL10) were 25,400 pg/ml, 7,810 pg/ml, 873 pg/ml, 304 pg/ml, respectively. Infiltration of atypical lymphocytes was also found in the inguinal lymph nodes and bone marrow by histological analyses. Molecular analysis of clonality using a Vγ–Jγ PCR revealed the presence of an identical predominant clonal rearrangement in the blood and in a skin biopsy sample. Collectively, the patient was diagnosed with CD8$^+$ Sézary syndrome.

Since narrowband ultraviolet B (NB-UVB) and psoralen plus ultraviolet A (PUVA) therapies were not

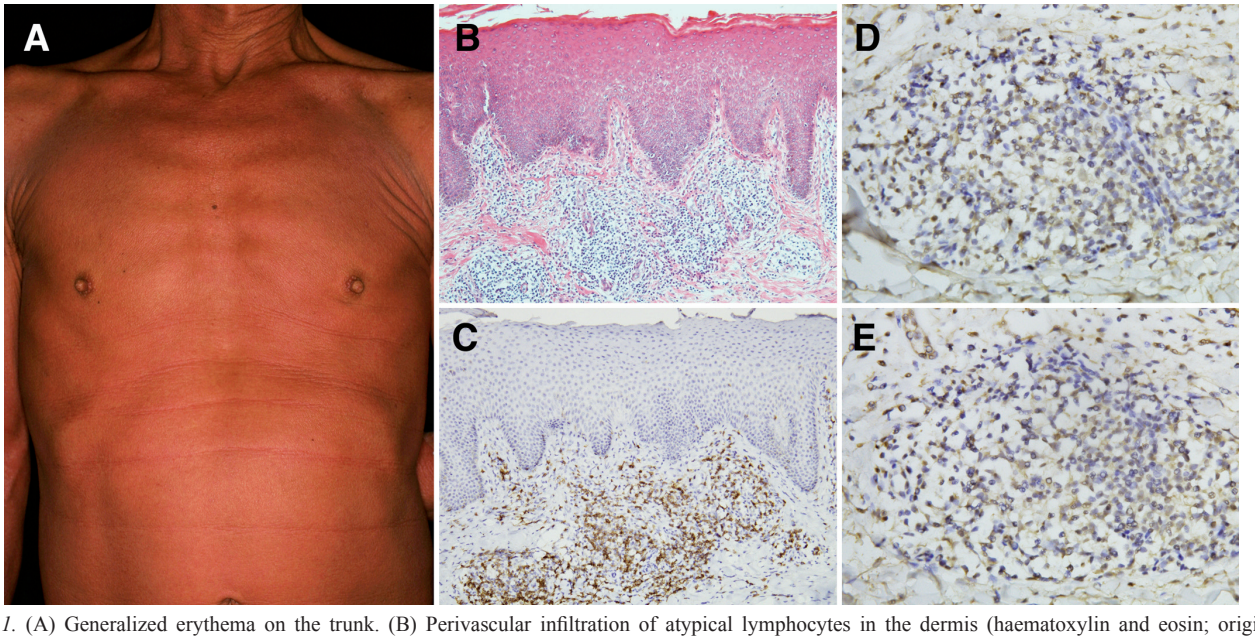

Fig. 1. (A) Generalized erythema on the trunk. (B) Perivascular infiltration of atypical lymphocytes in the dermis (haematoxylin and eosin; original magnification × 100). Immunohistological staining for (C) CD8 (original magnification × 100), (D) CCR4 (original magnification × 400), and (E) CCR10 (original magnification × 400).

effective, we began treatment with methotrexate (5 mg/day, twice a week) in September 2007, and the patient's symptoms gradually improved with a decrease in the number of Sézary cells in the blood to 200/mm^3 in 14 months. In keeping with the reduction of the number of Sézary cells and serum levels of sIL-2R, serum levels of CCL17, CCL22, and CCL27 were decreased, whereas those of CXCL10 were increased (Fig. 2). Correlation coefficients between serum levels of chemokines, Sézary cell number in the blood, and serum levels of sIL-2R were 0.93 (CCL17 and Sézary cells; $p < 0.001$), 0.97 (CCL22 and Sézary cells; $p < 0.001$), 0.83 (CCL27 and Sézary cells; $p = 0.007$), –0.85 (CXCL10 and Sézary cells; $p = 0.004$), 0.94 (CCL17 and sIL-2R; $p < 0.001$), 0.78 (CCL22 and sIL-2R; $p = 0.013$), 0.69 (CCL27 and sIL-2R; $p = 0.051$), and –0.64 (CXCL10 and sIL-2R; $p = 0.065$).

DISCUSSION

Sézary cells usually show a mature helper T-cell phenotype (CD3$^+$, CD4$^+$ and CD45RO$^+$). Only a few cases of CD8$^+$ Sézary syndrome have been reported, and no report was available concerning chemokine release in the blood and the expression pattern of chemokine receptors in this particular disease (1, 2). We showed for the first time that CD8$^+$ Sézary cells express CCR4 and CCR10, but not CXCR3, as previously shown in typical CD4$^+$ Sézary cells (3–7), and that the number of Sézary cells and serum levels of sIL-2R were positively correlated with serum levels of CCR4 ligands (CCL17 and CCL22) and CCR10 ligand (CCL27), whereas they were negatively correlated with serum levels of CXCR3 ligand (CXCL10) in CD8$^+$ Sézary syndrome. Atypical T cells in cutaneous T-cell lymphomas are known to express several chemokine receptors, such as CCR4, CCR10, and CXCR3. Interactions between CCR4, CCR10 and their respective ligands have been linked to the recruitment of atypical T cells to the skin (6–8), whereas CXCR3 may play a role in their further infiltration into the epidermis, and CXCR3 is frequently expressed in cutaneous T-cell lymphomas, which show prominent epidermotropism, such as early stages of mycosis fungoides and pagetoid reticulosis (3, 4, 6). Serum levels of CCL17, CCL22, and CCL27 in typical CD4$^+$ Sézary syndrome are known to be higher than those of normal subjects (4, 9), and a decrease in serum CCL17 and CCL27 in a case of Sézary syndrome was reported after successful treatment with NB-UVB (10). On the other hand, no report is available concerning serum levels of CXCL10 in Sézary syndrome. Although further studies are needed, we conclude that serum levels of those chemokines could be markers of disease activity in CD8$^+$ Sézary syndrome.

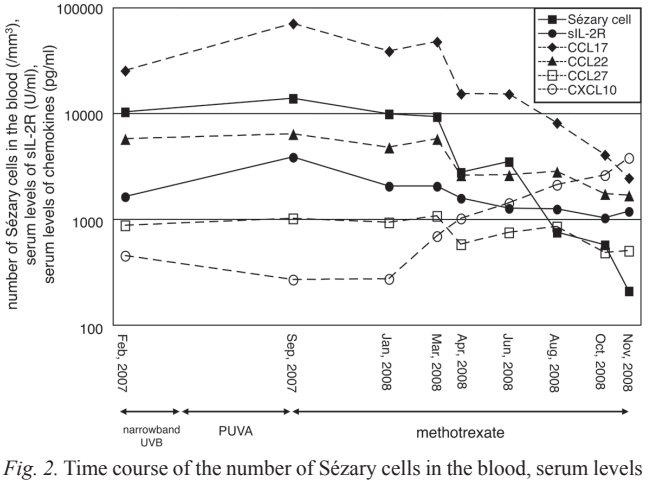

Fig. 2. Time course of the number of Sézary cells in the blood, serum levels of soluble IL-2 receptor (sIL-2R) and serum levels of CCL17, CCL22, CCL27 and CXCL10. PUVA: psoralen plus ultraviolet A; UVB: ultraviolet B.

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Serum IL-31 Levels are Increased in Patients with Cutaneous T-cell Lymphoma

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Interleukin-31 (IL-31) is expressed by activated Th2 cells, signalling through a receptor complex composed of IL-31RA and oncostatin M receptor β (OSMRβ) (1). Atopic dermatitis (AD) is an inflammatory skin disease with intense pruritus. The mechanisms underlying dermatitis and pruritus are not fully understood, but there is emerging evidence that IL-31 may play an important role. Transgenic mice over-expressing IL-31 developed dermatitis mimicking AD with severe pruritus (1). In an AD-like murine model (NC/Nga mice), high IL-31 mRNA expression is associated with scratching behaviours (2). Previous studies have reported that plasma or serum IL-31 levels were elevated in AD patients (3), and positively correlated with disease severity (4).

Mycosis fungoides (MF) and Sézary syndrome (SS) are the most common types of cutaneous T-cell lymphoma (CTCL). MF is a malignant proliferation of neoplastic T cells that preferentially traffic to the skin. SS is characterized by erythroderma, lymphadenopathy and leukaemic involvement. Laboratory findings in CTCL are similar to AD, such as eosinophilia, high serum levels of immunoglobulin E and Th2-associated chemokines (5, 6). Thus, we decided to investigate IL-31 involvement in CTCL.

MATERIALS AND METHODS

Forty patients with AD (mean ± standard deviation (SD) age: 33.9± 11.1 years), 38 patients with CTCL (34 MF cases and 4 SS cases; 58.8 ± 13.9 years), and 23 healthy control subjects (39.4 ± 15.2 years) were enrolled in this study. Patients with CTCL were subgrouped into patch and plaque ($n = 22$), tumour ($n = 11$), and erythroderma ($n = 5$) according to their types of skin lesions defined by International Society of Cutaneous Lymphoma (ISCL) and the European Organization of Research and Treatment of Cancer (EORTC) (7). They were also subgrouped into stage I (21 cases), stage II ($n = 7$), stage III ($n = 2$) and stage IV ($n = 8$) according to the staging system proposed by the ISCL/EORTC (7). The healthy controls had no history of allergy, AD, CTCL or other immune diseases. Serum samples were stored at –20ºC until use. The medical ethics committee of the University of Tokyo approved all described studies and the study was conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained to use sera. IL-31 was quantified by an IL-31 (Human) enzyme-linked immunosorbent assay kit (Avnova, Taipei City, Taiwan). The measured values from individual patients were plotted using dots. Statistical analysis between multiple groups was performed using the one-way analysis of variance (ANOVA) (Kruskal-Wallis test) followed by Dunn's Multiple Comparison test. Correlation coefficients were determined by using the Spearman's rank correlation test. p-values < 0.05 were considered statistically significant.

RESULTS

As previously reported (4), serum IL-31 levels were significantly higher in patients with AD (2.13 ± 0.26 pg/ml) than those of healthy controls (0.94 ± 0.28 pg/ml; $p < 0.01$; Fig. 1a). In addition, serum IL-31 levels were significantly higher in patients with CTCL (3.19 ± 0.74 pg/ml) than those of healthy controls ($p < 0.05$; Fig. 1a). We subsequently examined serum IL-31 levels in CTCL patients with different types of skin lesions. Serum IL-31 levels in patients with patch and plaque, tumour and erythroderma were 1.05 ± 0.30 pg/ml, 6.25 ± 2.11 pg/ml and 5.00 ± 1.46 pg/ml, respectively (Fig. 1b). Serum IL-31 levels in patients with tumour were extremely high, which were significantly higher than those with healthy controls ($p < 0.01$) and patients with patch and plaque ($p < 0.05$). Serum IL-31 levels in patients with erythroderma were also significantly higher than those in healthy controls ($p < 0.01$). Serum IL-31 levels in patients with stage I, stage II, stage III and stage IV were 0.95 ± 29 pg/ml, 3.97 ± 1.43 pg/ml, 2.07 ± 0.00 pg/ml and 7.98 ± 2.56 pg/ml, respectively (Fig. 1c). Serum IL-31 levels in patients with stage IV were significantly higher than those with healthy controls and patients with stage I ($p < 0.05$). Serum IL-31 levels in patients with stage II were significantly higher than those with healthy controls ($p < 0.05$). We also found that serum IL-31 levels correlated significantly with serum sIL-2R and LDH levels ($r = 0.43$, $p < 0.05$ and $r = 0.34$, $p < 0.05$, respectively; Fig. 1d, e), which have been reported to reflect disease activity of CTCL (8, 9). Thus, serum IL-31 levels correlate with disease activity of CTCL.

DISCUSSION

IL-31 as well as IL-31RA and OSMRβ, receptors for IL-31, is expressed in varieties of tissues other than skin, such as thymus, testis, and trachea (1), suggesting that this cytokine may have potential multiple, pleiotropic physiological functions. Moreover, IL-31 stimulates secretion of proinflammatory cytokines, chemokines, and matrix metalloproteinases from human colonic subepithelial myofibroblasts (10). In this study, patients with CTCL exhibited elevated levels of serum IL-31 compared with healthy controls. A variety of cytokines and chemokines are reported to be involved in the development of CTCL (11), some of which may be regulated by IL-31.

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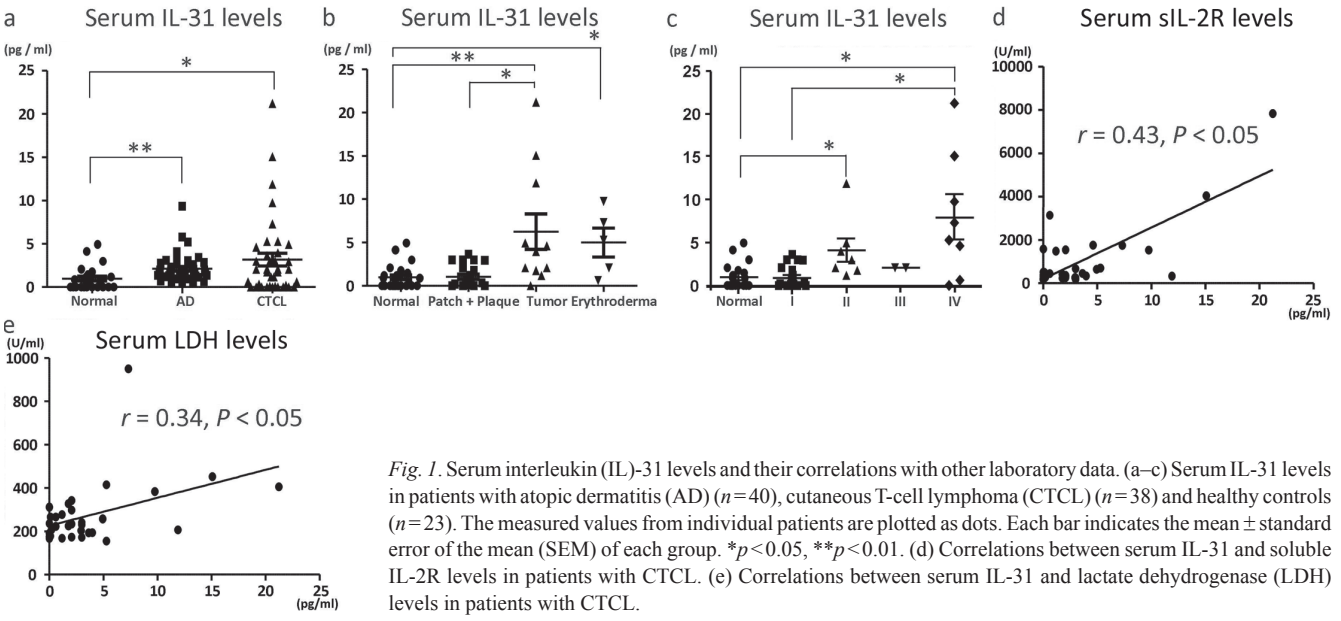

Fig. 1. Serum interleukin (IL)-31 levels and their correlations with other laboratory data. (a–c) Serum IL-31 levels in patients with atopic dermatitis (AD) ($n=40$), cutaneous T-cell lymphoma (CTCL) ($n=38$) and healthy controls ($n=23$). The measured values from individual patients are plotted as dots. Each bar indicates the mean ± standard error of the mean (SEM) of each group. *$p<0.05$, **$p<0.01$. (d) Correlations between serum IL-31 and soluble IL-2R levels in patients with CTCL. (e) Correlations between serum IL-31 and lactate dehydrogenase (LDH) levels in patients with CTCL.

Serum IL-31 levels reflected the severity of CTCL and had correlations with serum sIL-2R and LDH levels. Serological immunomarkers might be useful for disease monitoring during treatment (9). IL-31, as well as other immunomarkers, may have prognostic value for predicting the clinical course. In addition, a 2005 National Cutaneous Lymphoma Foundation survey revealed that 340 (53.9%) of 640 MF patients are affected by pruritus (12). IL-31 may play a role in causing pruritus in CTCL patients, as has been reported in AD patients (1–4).

In conclusion, serum IL-31 levels are significantly elevated and associated with disease severity in CTCL. IL-31 may play an important role in inflammatory conditions and pruritus in CTCL.

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Localized AL Amyloidosis in a Patient with Diffuse Large B-cell Lymphoma of the Breast

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Amyloidosis refers to a variety of conditions in which a protein polysaccharide complex, amyloid protein, is accumulated locally or systemically in tissues or organs. Amyloid in the skin may be derived directly from keratinocytes, or secondarily from immunoglobulin light chain fragments (AL type), fragments of the acute-phase reactant serum amyloid A (AA type), etc. Nodular amyloidosis (NA) is a rare type of localized AL amyloidosis, with most reported cases being of the lambda light chains (1) and is often histopathologically indistinguishable from systemic amyloidosis. Its association with lymphoma has been observed infrequently.

CASE REPORT

An 88-year-old woman initially noticed a 5×3 cm mass in her right breast in 1990. She was diagnosed with non-Hodgkin's lymphoma (NHL) (diffuse large immunoblastic B-cell type, stage IE) after the excision of the mass in a tertiary referral centre. Bone marrow biopsy revealed no evidence of lymphoma involvement. She achieved complete remission after chemotherapy. Six years after the diagnosis of lymphoma she developed multiple enlarging nodules on her legs. On examination, there were coalescing, waxy, skin-coloured, nodules with some foci of haemorrhage, central ulceration and crusts on the legs (Fig. 1). Skin biopsy revealed homogenous, eosinophilic, amorphous nodular deposit of amyloid in the dermis, with involvement of the blood vessel walls (Fig. 2A). Lymphoplasmacytic cells aggregation with erythrocytes extravasation was also observed. Congo red stain revealed green birefringence under polarized light (Fig. 2B). Transmission electron microscopy showed some rigid, straight, 6–10 nm-sized, non-branching filaments. No restrictive cardiomyopathy or hepatosplenomegaly was found by whole-body computer tomography and echocardiogram. Random biopsy of rectal mucosa and abdominal skin did not demonstrate amyloid deposits. Technetium-99m-pyrophosphate (Tc-99m PYP) scintigraphy showed multifocal areas of increased uptake of Tc-99m PYP in the subcutaneous tissues of the legs and right thigh. Laboratory investigations showed a positive antinuclear antibody with a titre of ≥1:1280 in a speckle pattern and an anti-centromere antibody with a titre of ≥1:1280. Urine immunofixation electrophoresis (IFE), bone marrow biopsy, anti-double-stranded DNA, anti-Ro (SSA) and anti-La (SSB) autoantibodies were all negative. IFE was initially negative, but detected an IgG λ monoclonal gammopathy 7 years after the skin biopsy. Based on the patient's clinical, pathological, and laboratory data, she was diagnosed with NA associated with diffuse large B-cell lymphoma of the breast. She subsequently received intralesional triamcinolone acetonide (5 mg/ml), Super Lizer™ infrared light and topical psoralen plus ultraviolet-A (PUVA) therapy, but NA progressed. Only partial response to topical PUVA was observed. The nodular lesions became ulcerated and haemorrhagic and were complicated with several episodes of cellulitis in the following years. Besides, her lymphoma relapsed in 2004.

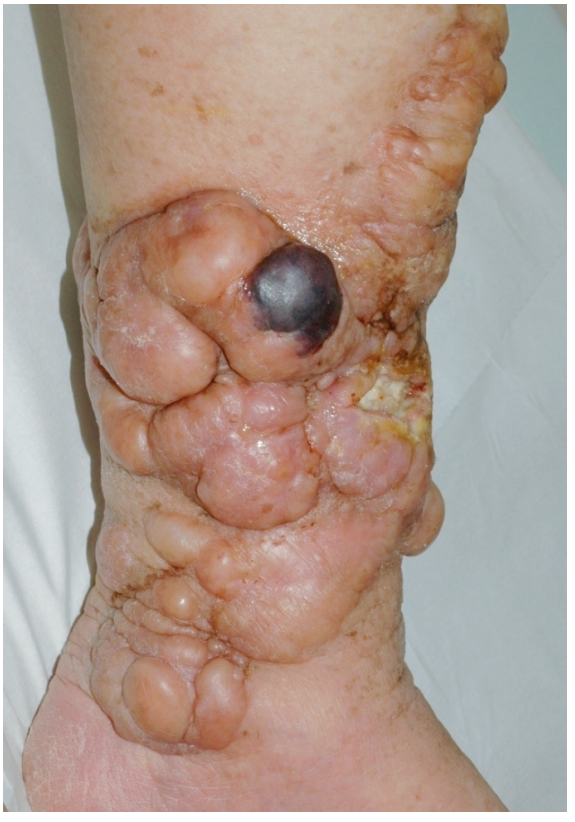

Fig. 1. Multiple waxy, skin-coloured, verrucous nodules with some foci of haemorrhage and central ulceration involving the entire lower legs.

DISCUSSION

NA is a localized cutaneous AL amyloid deposit with a tendency to affect acral sites. It may be primary or associated with autoimmune and lymphoproliferative disorders. The exact mechanism of NA remains unclear. Gene rearrangement studies have confirmed clonality of the amyloid-producing plasma cells in the skin, but not in the bone marrow, and suggested that NA might arise from local plasma cell dyscrasia or plasmacytoma (1). Several disorders, including systemic sclerosis, primary biliary cirrhosis, systemic lupus erythematosus, Sjogren's syndrome, and rheumatoid arthritis, have been associated with NA (2).

NHL of the breast, a rare malignancy, accounts for approximately 2.2% of extranodal NHL and 0.2% of breast malignancy (3). Diffuse large B-cell lymphoma was the predominant histological type (3). Monoclonal gammopathy had been found in 2.4% of adult patients with NHL (4). Amyloid deposition associated with

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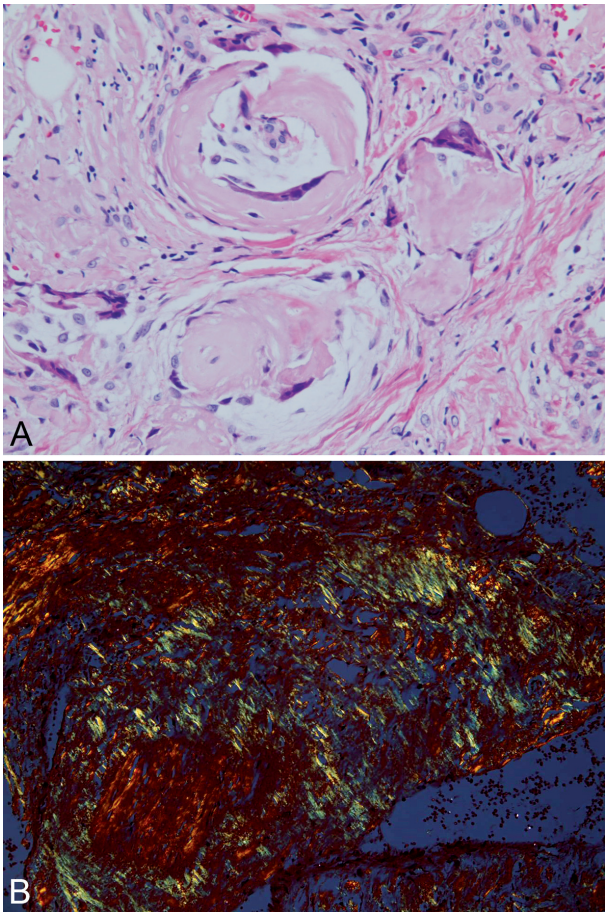

Fig. 2. (A) Homogenous, eosinophilic, amorphous nodular deposition of amyloid in the dermis with involvement of the blood vessel walls (haematoxylin and eosin stain; original magnification ×400). (B) Green birefringence on Congo red stains viewed under polarized light.

NHL, which can be systemic (5) or localized (6), had been reported. In most patients, amyloidosis is usually AL-type, and AA-type amyloidosis (7) in patients with NHL is extremely rare. Local amyloid deposits are usually localized in areas adjacent to the lymphoma (7) and had been found in nasal sinuses, lung, urinary tract, tongue, gastrointestinal tract, breast, brain, and soft tissues, despite the presence of circulating light chains (5). NA associated with NHL is rare and only one case of a lymphoplasmacytoid lymphoma with NA of salivary gland extending to the skin had been reported (6). However, lymphoma with non-co-localized cutaneous amyloid deposition had not been reported.

In 1970, Brownstein & Helwig (8) reported a 50% progression rate of primary cutaneous NA into systemic amyloidosis in a case series of 10 patients. However, recent studies by Woollons & Black. (9) and Moon et al. (10) showed that systemic amyloidosis progression rate was 7% in two independent studies, of 15 patients, respectively.

Clinical features of NA are variable and need to be differentiated with colloid milium, sarcoidosis, pseudolymphoma, deep fungal infection, nodular pretibial myxoedema, elephantiasis nostras verrucosa, neoplasms and other deposit diseases. Treatment of NA is often difficult, and our case also showed that various treatments, such as intralesional steroid, PUVA and Super Lizer™, were disappointing.

Although immunohistochemical staining was negative for lambda and kappa light chains on amyloid deposits in our patient, clinical appearance of waxy nodules combined with amyloid deposits on skin biopsy favoured the diagnosis of nodular amyloidosis (localized AL amyloidosis). Because no other possible aetiology possibly leading to amyloidogenesis, such as pruritic dermatoses, could be identified, and due to the development of NA in our lymphoma patient with monoclonal gammopathy, we reasonably thought that there was an association between NA and lymphoma. However, our case report provided limited proof for this connection.

In summary, complete evaluation at initial diagnosis with subsequent follow-up for systemic amyloidosis, autoimmune disease and lymphoproliferative disorders is indicated in patients with NA.

The authors declare no conflict of interest.

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Cutaneous Cryptococcosis Mimicking Basal Cell Carcinoma in a Patient with Sézary Syndrome

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Cryptococcosis is an opportunistic yeast infection that is the most common systemic fungal infection in immunocompromised patients. Skin involvement is a feature in 10–20% of cases of disseminated cryptococcal infection (1). We report here a case of a 63-year-old woman with Sézary syndrome (T4, N3, M0, B1) with an ulcerated preauricular tumour that developed during photopheresis with a combination of methotrexate and steroid treatment. We highlight the importance of differential diagnosis of cryptococcosis in the case of any atypical or non-healing lesions observed in an immunosuppressed patient.

CASE REPORT

A 63-year-old Caucasian woman who had breast cancer and underwent mastectomy in 1989 followed by chemotherapy was diagnosed in 1998 with parapsoriasis through clinical signs and histological examination in the County Hospital. In 2001 she presented with generalized pruritus and erythroderma at our clinic. She responded poorly to oral psoralen and ultraviolet A (PUVA) therapy. In 2002 clinical signs (weakness, general condition, intermittent fever) were suspicious for Sézary syndrome or Cutaneous T-cell lymphoma. Sézary syndrome was confirmed in 2003 by peripheral blood examination (28% $CD4^+$, $CD7^-$ cells), and electron-microscopy of the lymph nodes from two biopsies. At that time skin biopsies showed mild acanthosis, parakeratosis and some perivascular mononuclear cell infiltrate in the papillary dermis, whereas bone marrow biopsies were normal. After ineffective treatment with local steroid and bath PUVA she received systemic steroid plus azathioprine treatment (1 mg/kg) with moderate effect. The $CD4^+$ cell percentage increased to 84%, consisting of 69% $CD4^+/CD7^-$ cells. Due to the systemic appearance of the disease (Ann Arbor stage IV) in April 2004 one cycle of cyclophosphamide + vincristine + prednisolone (CVP) therapy was given in the Department of Internal Medicine, with poor response. She was treated with oral steroid with moderate effect. At this point another lymph node biopsy was performed that confirmed Sézary syndrome, and further treatment included total body electron irradiation in 2005 at the Oncology Center in Budapest. From February to September 2007 photopheresis in combination with methotrexate and steroid treatment had no effect on her symptoms and the skin infiltration increased, therefore methylprednisone, 8–16 mg daily, was administered constantly to control the symptoms (Fig. 1A). In May 2007, she developed a small hyperaemic nodule in the preauricular region, suspicious for basal cell carcinoma. We planned to perform a biopsy from the lesion, but the patient did not agree to the procedure at that time because of its localization. By August, at the next check-up, the lesion had a size of 2×3 cm with minor ulceration, and increased continuously in size to 5×7 cm by October (Fig. 1B). Differential diagnosis was performed for basal cell carcinoma, atypical mycobacteriosis, deep fungal infection, pyoderma, exulcerated lymphoma, spinocellular carcinoma, pyoderma gangrenosum, leishmaniasis, insect bite, burn injury, trauma, and artefact. Biopsy specimens revealed necrotic epidermis, ulceration, a granulomatous infiltrate in the dermis, and numerous round organisms with capsules measuring 2 ± 10 µm in diameter present both extracellularly and within the vacuolated spaces of macrophages (Fig. 1C). The organisms were also stained with Giemsa and showed periodic acid-Schiff (PAS)-positive reaction (Fig. 1D and E). Serology for Cryptococcus antigen was positive in the blood. Chest X-ray and computed tomography (CT) revealed Cryptococcus infection of the lung, cerebrospinal fluid was negative for the presence of Cryptococcus and there were no signs or symptoms of meningeal involvement.

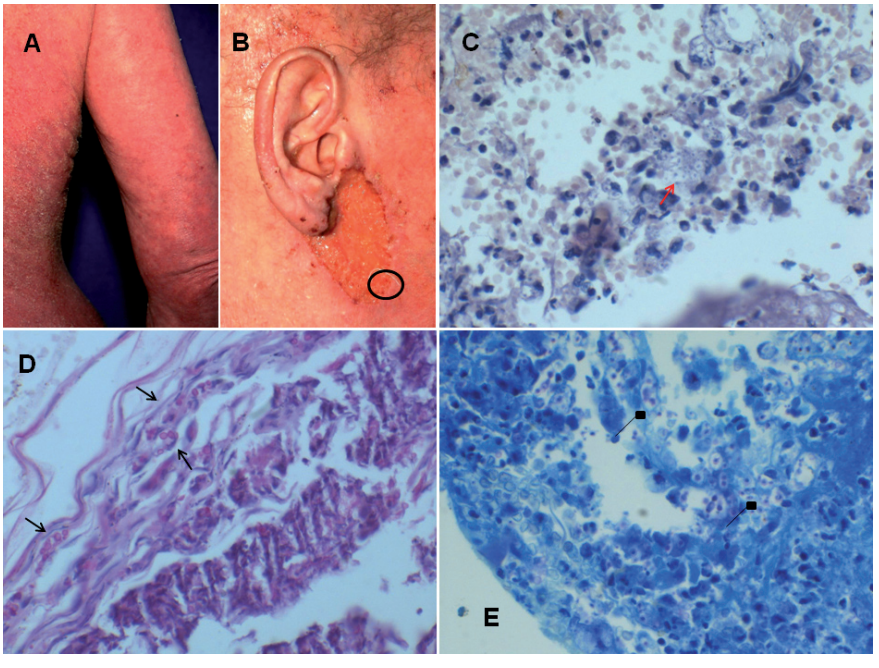

Fig. 1. (A) Generalized exfoliative erythroderma of the patient with advanced Sézary syndrome before extending photopheresis treatment with a combination of methotrexate and steroid. (B) Ulcer affecting the pre-auricular region mimicking basal cell carcinoma that developed during immunosuppressive treatment. Note the biopsy site (*circle*). (C) Histological examination of the skin biopsy from the ulcer showed innumerable thick capsulated round organisms measuring 2 ± 10 µm both extracellularly and within the vacuolated spaces of macrophages (*red arrows*) (haematoxylin and eosin; original magnification ×40). (D) Cryptococcal organisms stain red with periodic acid-Schiff (PAS) (*black arrows*) (original magnification × 100) and (E) blue with Giemsa stain (*diamond arrows*) (original magnification ×40).

In October 2007 the patient started receiving intravenous amphotericin B and fluconazole. Despite all our efforts Cryptococcus antigenaemia was still detected. The patient died 6 weeks later from acute cardiac and renal failure. Autopsy revealed cutaneous cryptococcal infection in the skin of the submammal region of the thorax and in the lungs.

DISCUSSION

Cryptococcal species are yeast-like fungi. Based on their virulence they are classified as pathogenic or non-pathogenic. Classically, *C. neoformans* is the only pathogenic cryptococcal species present in high concentrations in pigeon faeces, but also in soil, fruit, and other sources in nature, such as eucalypt reservoirs. It includes four distinguishable serotypes: A (*C. neoformans var. grubii*) has a worldwide distribution; D (*C. neoformans var. neoformans*) found mostly in Europe; and B and C (*C. neoformans var. gattii*), which are limited to tropical and subtropical areas (2, 3).

Cryptococcus infection is acquired by inhalation and resides in the lung, mostly in the immunocompromised host, as was the case in our patient, due to Sezary syndrome and continuous steroid immunosuppressive treatment with photopheresis extended with a combination of methotrexate. Infection can also involve patients with intact immune systems who are predominantly infected with *C. neoformans var. gattii* (4). The infection can resolve or disseminate, mostly spreading to the meninges, but bones, viscera and the skin can also be involved. In the diagnoses of Cryptococcus infection, culture from smears of body fluids, secretions, exudates, or other specimens is definitive, but X-ray and serological examination are also of importance.

Involvement of the skin can result in a great variety of lesions, most commonly affecting the face and neck, from papules, nodules, acneiform lesions, granulomas, herpetiform vesicles, abscesses, and ulcers, resembling other cutaneous disease, such as molluscum contagiosum, vasculitis, Kaposi's sarcoma, varicella, basal cell carcinoma, cellulitis, cutaneous ulceration, atypical mycobacteriosis and whitlow (5–8). The two forms of histological manifestation are gelatinous and granulomatous reactions. While the granulomatous type results in pronounced tissue reaction, with histiocytes, giant cells, lymphocytes, and fibroblasts, with a low number of yeasts, that vary in size from 2 to 20 μm, in the gelatinous type, masses of organisms occur, with the accumulation of cryptococcal capsular polysaccharide causing mucoid degeneration of the invaded tissue, with only minimal signs of inflammation (6).

Treatment of the systemic infection includes appropriate antifungal drugs, in which the mainstay is intravenous amphotericin B in combination with flucytosine, which is often followed by fluconazole for many months or, depending on the patient's immune status, even life-long. If fluconazole is not available or is contraindicated, acceptable alternatives include itraconazole, or extended-spectrum azoles (voriconazole and posaconazole). Occasionally, localized cryptococcal infections of the lung not responding to medical therapy may require surgical resection for cure (9). In the case of cutaneous Cryptococcus infection successful treatment with oral fluconazole alone has also been reported, and incision, with local irrigation and debridement, topical application of anti-inflammatory agents and antifungal agents was also recommended (8, 10).

Our case illustrates the importance of serological tests with histological examination, and pathogen-culturing from any atypical or non-healing lesions observed in immunocompromised patients for an accurate diagnosis of opportunistic infection. Importantly, a primary infection of the lung and disseminated infection involving the central nervous system may be present with dermatological rather than pulmonary or neurological manifestations (11).

The authors declare no conflicts of interest.

The authors pay tribute to Dr Agnes Begany, the outstanding dermatologist and person, who stays with us in our thoughts and words.

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Mycosis Fungoides in the Setting of T-cell Large Granular Lymphocyte Proliferative Disorder

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Large granular lymphocytes (LGLs) are medium-to-large cells, of either T- or natural killer (NK)-cell lineage, characterized by eccentric nuclei, condensed chromatin, and abundant pale cytoplasm containing coarse azurophilic granules (1, 2). Proliferative conditions of T- and NK-LGLs represent a complex spectrum of different clinico-pathological entities, ranging from benign reactive lymphocytosis to overt malignant leukaemia. Although patients with mycosis fungoides (MF) are known to be at increased risk of additional haematological neoplasms (occurring either before or following the appearance of MF lesions) (3, 4), no association with LGL proliferative disorders has been described. We report here a patient who developed MF in the setting of T-LGL proliferative disorder, and discuss the possible pathogenetic implications of this previously unreported association.

CASE REPORT

In April 2010, a 62-year-old man presented to our department with a widespread erythemato-squamous dermatosis of 5 years' duration. He reported persistent pruritus, with partial improvement of cutaneous lesions following sun-exposure. Ten years previously he had been diagnosed at a different institution with indolent T-LGL leukaemia; treatment with cyclosporine (CyA) (5 mg/kg/day) had been administered since February 2000, virtually without interruption.

Physical examination revealed a sub-erythrodermic status, characterized by the presence of several erythematous, mildly scaling macules and patches, at times exhibiting a finely wrinkled appearance, widely scattered over the trunk and proximal limbs (Fig. 1A); diffuse xerosis and skin changes secondary to scratching were also evident. The physical examination was otherwise unremarkable. Histopathological evaluation of two 4-mm punch biopsy specimens taken from representative patches on both arms revealed features consistent with early-stage MF: mild epidermal hyperplasia, disproportionate epidermotropism of atypical lymphocytes in areas with only scant spongiosis, and an expanded, slightly fibrotic superficial dermis harbouring a patchy-lichenoid mononuclear cell infiltrate. Several solitary lymphocytes exhibiting pleomorphic and hyperchromatic nuclei were also observed aligned along the dermo-epidermal junction (Fig. 1B). Immunohistochemical stains revealed that intraepidermal lymphocytes, as well as 65–75% of dermal lymphocytes, were $CD3^+$, $CD4^+$, $CD8^-$, $CD20^-$, $CD30^-$, $CD56^-$ cells. Polymerase chain reaction (PCR) analysis of gamma T-cell receptor (TCR) gene rearrangement in the skin failed to detect any clonal population of T cells; a result still compatible with patch-stage MF (3).

In-depth staging studies, including total body computed tomography (CT) scan, human T-cell lymphotropic virus-1/2 serology (assessed by enzyme-linked immunosorbent assay (ELISA)), peripheral blood smear and flow cytometry (FC) analysis, and FC assay for Vbeta TCR repertoire, failed to produce any abnormal result. Bone marrow (BM) biopsy, however, revealed an abnormal, interstitial infiltrate of $CD3^+$ $CD8^+$ mononuclear cells, replicating the picture observed in previous BM studies. Accordingly, a diagnosis of patch-stage MF (Stage IB; TII, N0, M0), together with subclinical T-LGL proliferative disorder, was made. Oral CyA was interrupted; the patient was started on treatment with narrowband ultraviolet B (UVB) along with close haematological monitoring. At the time of last follow-up (January 2010) the patient's skin lesions and reported itching had significantly improved, while his haematological status was stable.

DISCUSSION

With the exception of muco-cutaneous pyogenic infections secondary to chronic neutropaenia, reports of dermatological manifestations associated with chronic LGL proliferative disorders have been scarce and fragmentary, with significant overlap between diseases of T- and NK-cell origin. The overwhelming majority of such cutaneous features can be classified schematically within three major clinico-pathological categories: (*i*) cutaneous small- or medium-sized vessel vasculitis, usually presenting as palpable purpura, necrotic pustules, urticaria vasculitis, or polyarteritis nodosa-like ulcers (5, 6); (*ii*) vasculopathy without histological evidence of true vasculitis, manifesting as livedoid vasculopathy and/or eruptive telangiectatic lesions (5, 7, 8); (*iii*) persistent ulcerations with histological demonstration of intravascular LGL, often localized to the lower limbs

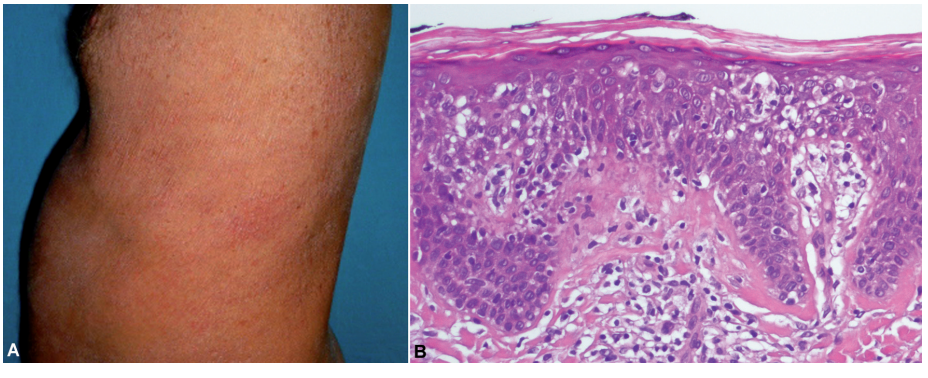

Fig. 1. (A) Multiple erythematous scaling macules and patches on the trunk in a pattern reminiscent of parapsoriasis. (B) Biopsy specimen showing sparse lymphoid infiltrate in the papillary dermis and intraepidermal aggregates of lymphocytes without significant spongiosis (haematoxylin and eosin × 200).

(7, 9). Interestingly, Mallo et al. (10) reported a case of indolent T-LGL leukaemia with persistent generalized pruritus as the only alleged manifestation. There are striking similarities between this case and the one we report; indeed, the clinico-pathological picture described by Mallo et al. would perfectly fit with the so-called "invisible dermatosis" presentation of MF, which is an uncommon, but well-known, scenario (3).

Patients with either T-LGL proliferative disorders or MF appear to be subjected to heightened risk of developing discordant lymphomas (1, 4, 11), defined as second, histologically distinguishable lymphoid neoplasms involving different anatomical sites (12). In this regard, Assaf et al. (13) described a case of indolent T-cell-prolymphocytic leukemia (T-PLL) with subsequent development of MF, lymphomatoid papulosis, and primary cutaneous $CD30^+$ anaplastic large cell lymphoma; molecular studies revealed identical monoclonal TCR genes rearrangements as well as cytogenetic abnormalities in T-PLL and cutaneous T-cell lymphoma (CTCL) cells, indicating that leukaemic and cutaneous malignant T lymphocytes derived from the same clone. According to the authors, T-PLL and CTCL might have arisen concomitantly from the same progenitor cell, or, more likely, T-PLL might have evolved linearly to generate the three cutaneous disorders. We could not employ a similar investigative approach, as cytogenetic aberrations are not a common feature of either T-LGL disorders or MF, and no monoclonal TCR gene rearrangements could be detected in either peripheral blood or skin. Nonetheless, malignant $CD8^+$ T-LGL are thought to be antigen-activated T cells arising, at least in most cases, out of oligoclonal proliferations of a physiological subset of post-thymic $CD8^+$ cytotoxic T cells; this view has also been supported by analysis of V-beta receptor transcripts. Malignant transformation could stem from insensitivity to apoptosis, acquired through constitutive, activating phosphorylation of Stat3 (14). Interestingly, similar Jak3/Stat3 signalling aberrations may play a key role in the pathogenesis of MF (15). Speculative, is it, in our case, a common stem cell/lymphoid precursor, having undergone one or more somatic events predisposing to cytokine-independent activation of the Jak3/Stat3 pathway, generated, through divergent differentiation, two distinct aberrant populations of T lymphocytes (i.e., $CD8^+$ LGL and $CD4^+$ cells, respectively).

Secondly, loss of immune competence resulting from long-standing CyA treatment may have favoured development and/or progression of MF in our case (4, 11): cases have been reported where treatment with low-dose CyA (< 5 mg/kg/day) following a misdiagnosis of inflammatory dermatoses led to clinical worsening and aggressive transformation of MF (16, 17). Of note, in our patient the effect of continuous CyA administration may have been compounded by the relative immunosuppression which is known to be inherent in patients with lymphoproliferative conditions, including T-LGL disorders.

Lastly, although no proven risk factor seems to be shared by T-LGL disorders and MF (1, 3, 11), an independent transformation of distinct lineages of lymphocytes secondary to common exposure to environmental carcinogens or oncogenic viruses cannot be ruled out.

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