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Expression of Loricrin in Skin Disorders

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Loricrin, the major component of the cornified envelope, is normally expressed in the granular layer of epidermis during the last steps of keratinocyte differentiation. Using an anti-loricrin antiserum (A8–73), an increased expression of this envelope precursor was found in some disorders of hyperorthokeratosis (ichthyosiform erythroderma; lichen ruber), but not in others (keratodermia ichthyosis vulgaris). In disorders accompanied by parakeratosis, a sign of incomplete differentiation (psoriasis, prurigo nodularis) loricrin was not detected, whereas the tissue expressed filaggrin. Treatment of normal skin with retinoic acid, increasing epidermal thickness in some subjects, led to an increased expression of loricrin. Loricrin might be a useful indicator of the extent of terminal epidermal differentiation in skin disorders. ***Key words: Psoriasis; Ichthyosis.***

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Human loricrin, a 26kD cationic protein, rich in glycin and serin residues, is expressed in the stratum granulosum of epidermis and becomes the major component of the cornified envelope in the stratum corneum (1, 2). In epidermis reconstructed *in vitro*, the expression of loricrin is abolished by retinoic acid (3), an agent which inhibits terminal epidermal differentiation and causes parakeratosis (4). Similar effects of retinoic acid on loricrin have been found in classical cultures of human keratinocytes (5, 6). The present study was performed using anti-loricrin antiserum (A8–73) prepared by immunizing rabbits with a carboxy-terminal peptide deduced from a unique coding sequence of A8 cDNA (5). In the present study, the A8–73 antiserum has been used to study the localization of loricrine in skin biopsies from patients with various skin disorders.

MATERIAL AND METHODS

Punch biopsy specimens (3 mm diameter) were obtained from 8 healthy volunteers and from 64 patients presenting various skin disorders listed in Table I and mentioned in the results. Unless otherwise stated, the lesions were untreated previous to biopsy. The specimens were quick-frozen in isopentane at −70°C and stored at the same temperature until sectioning in a cryostat. Acetone-fixed sections, 6 μm thick, were first investigated for binding of A8–73 antiserum diluted at 1/20000 for 30 min, followed by the addition of biotinylated goat anti-rabbit IgG (1/200 in PBS) for 30 min. The expression of filaggrin was investigated in some sections using an anti-filaggrin monoclonal antibody (bti Stoughton, Ma), diluted at 1/400 and the peroxidase-antiperoxidase technique (7). Monoclonal antibodies to anti-Leu 6 were used as positive controls. Omission of the primary antibodies served as negative controls.

RESULTS

In skin from healthy subjects and uninvolved skin from psoriatic patients, loricrin was expressed in the granular layer (Fig. 1a). Biopsies from patients with the disorders listed in Table I also showed a normal expression of loricrin, whereas the expression was more pronounced in 3 patients with lichen planus. A clearly increased expression of loricrin in the granular layer was evident in 2 patients with non-bullous ichthyosiform erythroderma (Fig. 1b). In 3 patients with Darier's disease, there was also in addition a diffuse expression of loricrin towards the spinal layer (Fig. 1c).

Loricrin was not expressed in the lesions of non-treated plaque psoriasis (7 patients), whereas filaggrin was present (Figs. 1e-f). Loricrin was also absent in lesions of prurigo nodularis accompanied by parakeratosis, (3 patients), in the cancer cells in 1 out of 3 patients with superficial basalioma, and in one patient with Hailey-Hailey's disease.

In 4 subjects previously treated with a topical 0.05% retinoic acid cream (Aberela®), the expression of loricrin was somewhat diffuse, and especially when the thickness of epidermis had increased during the treatment (Fig. 1d). In 2 patients treated for 2 weeks with the oral retinoids etretinate and acitretin, respectively, loricrin was present, as in healthy sub-

Table I. *Skin lesions with expression of loricrin in the granular layer as in normal skin*

Figures indicate number of patients investigated.

Actinic keratosis	(2)
Alopecia areata	(2)
Atopic dermatitis	(2)
Contact dermatitis	(2)
Hailey-Hailey disease	(1)
Ichthyosis vulgaris	(2)
Ichthyosis syndrome (KID)	(1)
Keratoderma palmo-plantare	(2)
Keratosis follicularis (Darier)	(4)
Lichen planus	(3)*
Lupus erythematosus discoid	(2)**
Mycosis fungoides	(2)
Pigmented nevi	(4)
Pityriasis rosea	(1)
Porphyria cutanea tarda	(1)
Purpura	(1)
Seborrheic keratosis	(2)
Subcorneal pustulosis	(1)
Telogen effluvium	(1)
Tinea versicolor	(1)
Trichoepithelioma	(1)
Urticaria chronica	(1)
UV-erythema (18^h)	(1)
Vitiligo	(1)

*More marked than the others, **Diffuse.

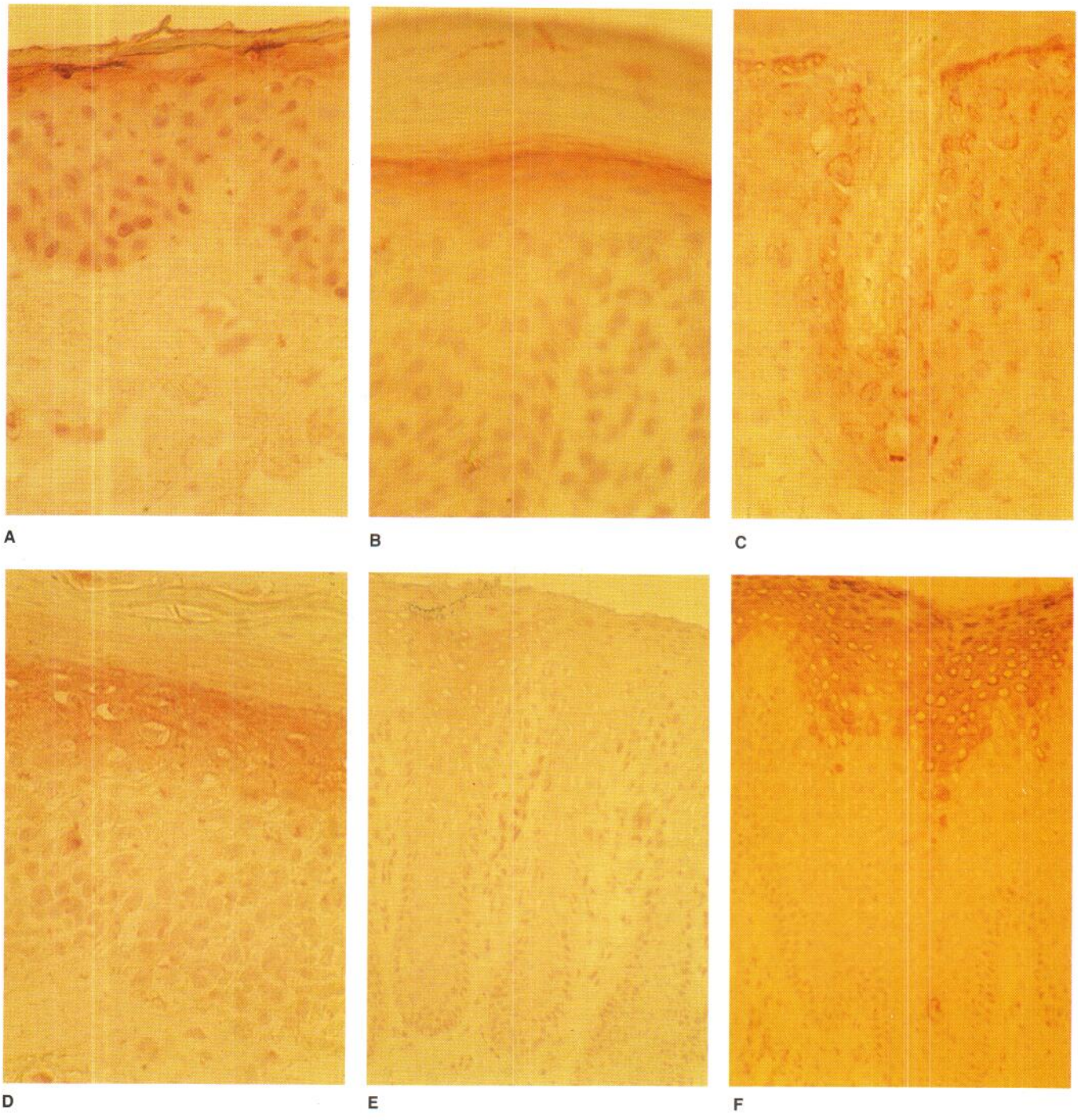

Fig. 1. Expression of loricrin (L) and filaggrin (F) in the epidermis. *a.* Normal skin (L); *b.* Ichthyosiform erythroderma (L); *c.* Darier's disease (L); *d.* Retinoic acid treated (L); *e.* Psoriasis (L); *f.* Psoriasis (F).

jects. In a squamous cell carcinoma, a few single cells in the tumour showed loricrin expression.

DISCUSSION

In general, expression of loricrin was most marked in diseases characterized by an hyper-orthokeratotic stratum granulosum, such as ichthyosiform erythroderma and lichen planus, although in ichthyosis vulgaris, KID syndrome and keratodermia no increased expression of loricrin was detected. On the contrary, in skin lesions exhibiting parakeratosis, a sign of inhibited differentiation such as psoriasis and prurigo nodularis, loricrin was not detected. These findings correlate well with *in vitro* studies on reconstructed epidermis, where retinoic acid caused parakeratosis and abolished the reactivity for loricrin (3). However, the effect of retinoic acid is different *in vivo*, since in patients treated with oral retinoids (acitretin and etretinate) we found no alteration of the expression of loricrin.

Topical treatment with retinoic acid cream increased the thickness of epidermis, without inducing parakeratosis and, in some subjects, an increased expression of loricrin was even observed (Fig. 1d). Since retinoids do not induce parakeratosis *in vivo* (8), this explains their different effects on loricrin expression *in vivo* and *in vitro*. Our findings are in agreement with recent observations of a down-regulation of loricrin in various disorders exhibiting parakeratosis (9). The same group of investigators also found a normal loricrin expression in ichthyosis vulgaris, whereas lamellar ichthyosis showed high expression of loricrin (9).

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