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Cornified envelopes in congenital disorders of keratinization

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SUMMARY

A morphological and biochemical analysis was made of cornified envelopes isolated from patients with different congenital disorders. Nomarski contrast microscopy of the envelopes showed that their morphology was not greatly altered in several types of keratoderma and parapsoriasis, but it was grossly modified in ichthyotic disorders. The various types of ichthyoses, keratoderma palmoplantare, KID syndrome and parapsoriasis showed, after cyanogen–bromide cleavage, peptide patterns similar to those obtained from healthy subjects. In contrast, envelopes from patients with Darier's disease, congenital pachyonychia and erythrokeratoderma variabilis showed markedly different peptide patterns.

The cornified envelope (CE) is an insoluble protein structure synthesized during terminal differentiation of epidermal keratinocytes.[1] It is formed at the level of the upper granular layer by plasma membrane-associated transglutaminase.[2,3] This calcium-dependent enzyme catalyses the covalent cross-linking of precursor proteins via $\gamma\lambda$-glutamyl-ε-lysine isopeptide bonds. In the epidermis two morphologically distinct types of CEs have recently been identified:[4] a polygonal rigid type (CE_r) and an irregularly shaped type with a fragile appearance (CE_f) which, in healthy skin, is only present in the lower part of the stratum corneum. These two types possibly represent two successive steps of CE maturation. The stratum corneum of normal subjects contains mainly CE_r, whereas the scales in psoriasis contain a much higher proportion of CE_f. Keratinocytes in submerged cultures are only able to form CE_f.[4]

Several CE precursor proteins have been identified in cultured keratinocytes.[5–7] These proteins are either cytosoluble, e.g. involucrin,[5] or plasma-membrane bound.[6] Involucrin has been proposed as the major precursor of cornified envelopes of cultured normal keratinocytes.[5] However, transformed keratinocytes (line SV-K14), which contain a very low level of involucrin, are nevertheless able to form CEs after being treated with a calcium ionophore, probably by using a different set of precursor proteins.[8] Moreover cyanogen–bromide peptide

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mapping of purified envelopes revealed differences in the composition of the CEs from cultured normal keratinocytes, SV-40-transformed keratinocytes,[8] normal epidermis and psoriatic scales.[4] These results indicate that the molecular composition of the CE may not be strictly constant and may vary according to the availability of substrate proteins when the cross-linking enzyme transglutaminase becomes activated. We have analysed the CEs from patients with various congenital hyperkeratotic lesions.

METHODS

Patients

Keratosis follicularis (Darier's disease). Five patients, aged 16–62, with typical lesions on trunk and legs.

Keratoderma palmoplantare. Three patients, aged 20–52, with lesions only on soles and palms.

KID (keratitis, ichthyosis, deafness) syndrome. A girl, aged 10, with keratoderma palmoplantare and red ichthyotic lesions especially on the face, ears and extremities.

Congenital pachyonychia. A boy, aged 15, with marked changes of nails and hair and severe keratoderma of the soles and palms.

Erythrokeratoderma variabilis. A woman, aged 21, with widespread areas of erythema and ichthyosis that she has had since birth.

Parapsoriasis. Two patients, aged 61 and 81, with circumscribed, oval, slightly scaly and persistant erythematous lesions.

Ichthyosis vulgaris. Three patients, aged 30–37, with dry skin and marked ichthyotic lesions on the extremities.

Ichthyosis, brittle hair syndrome. Two brothers, aged 16–18, with dry ichthyotic skin and brittle hair since birth. The stature was normal and an amino acid analysis had not yet been performed to exclude a diagnosis of trichothiodystrophy.

These diagnoses were confirmed both clinically and histologically.

Collection of the samples

The patients had no treatment for at least 2 months before sampling apart from moisture creams. The corneocytes were collected from the stratum corneum by curettage and the material obtained placed in an aqueous solution of 2% sodium dodecyl sulphate (SDS), 0·1% dithioerythritol and 0·01% sodium azide and stored at 4°C.

Cornified envelope purification

Purification was performed as previously described.[4] The samples were boiled with vigorous stirring for 10 min. The CEs were recovered with 5 min centrifugation at 3500 *g* and resuspended in the above described mixture. Boiling and centrifugation was repeated four times and the final pellet was suspended in an equivalent volume of Laemmli sample buffer[9] and

transferred into a dialysis bag (Spectra/por 6/132542; M_r cut off: 50,000; Spectrum Medical Industries, Los Angeles, CA, U.S.A.). The dialysis bag was immersed into an electrophoretic tank containing Laemmli running buffer and subjected to electrophoresis at 20–50 V for 24 h. The envelopes were recovered by centrifugation and the electrodialysis procedure was repeated three more times.

Cyanogen bromide (CNBr) cleavage of CEs

The protein content of the envelopes was determined by the Lowry procedure[10] after digestion with proteinase K.

The envelopes were suspended in 70% (v/v) formic acid, and 20 mg of CNBr was added per mg protein. Under these conditions, selective cleavage of peptide bonds occurs at the level of methionine residues. After 24 h of agitation at room temperature the mixture was diluted 1:5 with distilled water. After drying under a vacuum the residues were suspended in Laemmli sample buffer.

Electrophoresis

Electrophoresis of the peptides was performed according to the procedure of Laemmli.[9] Fifteen per cent polyacrylamide gels were used and 10 μg of protein were loaded per lane. The peptides were revealed by silver staining.[11]

RESULTS

Morphology of the CEs

The morphological aspects of the CEs obtained from patients affected with different dermatoses are presented in Figure 1. As a reference, CEs from normal human epidermis are shown in Figure 1a. As described[8] they mainly consisted of CE_r with only a minor proportion of CE_f. Due to the limited number of patients examined, it is not possible to determine precisely the proportion of type CE_r and CE_f in each dermatosis. As a consequence, the different diseases are described in qualitative rather than in quantitative terms.

The morphology of CEs obtained from patients with Darier's disease is shown in Figure 1b; being mainly composed of CE_r. However, their size and shape are more heterogeneous than those from normal skin.

Palmoplantar keratoderma lesions (Fig. 1c) contained mainly CE_r. The envelopes were larger than those from normal epidermis. CEs prepared from a patient with KID syndrome (Fig. 1d) and from a patient with congenital pachyonychia (not shown) were very similar to those of normal skin apart from a relative increase in CE_f. CEs from a patient with erythrokeratoderma variabilis (Fig. 1e) are similar to those from normal skin.

In parapsoriatic epidermis (Fig. 1f) the type CE_r is predominant. A characteristic feature of this disease was the presence of a nuclear-like inclusion in some envelopes which made it easy to distinguish it from psoriasis vulgaris (Fig. 1h). Even though parakeratosis is also a characteristic feature of psoriasis, there were no nuclei present in the CE preparation (Fig. 1h), indicating differences in the cross-linking reaction in both diseases.

The CEs from ichthyosis vulgaris (Fig. 1g) contained an increased number of type CE_f and those from one ichthyotic patient were reduced in size. This was also observed in the samples prepared from the two patients with ichthyosis, brittle hair syndrome.

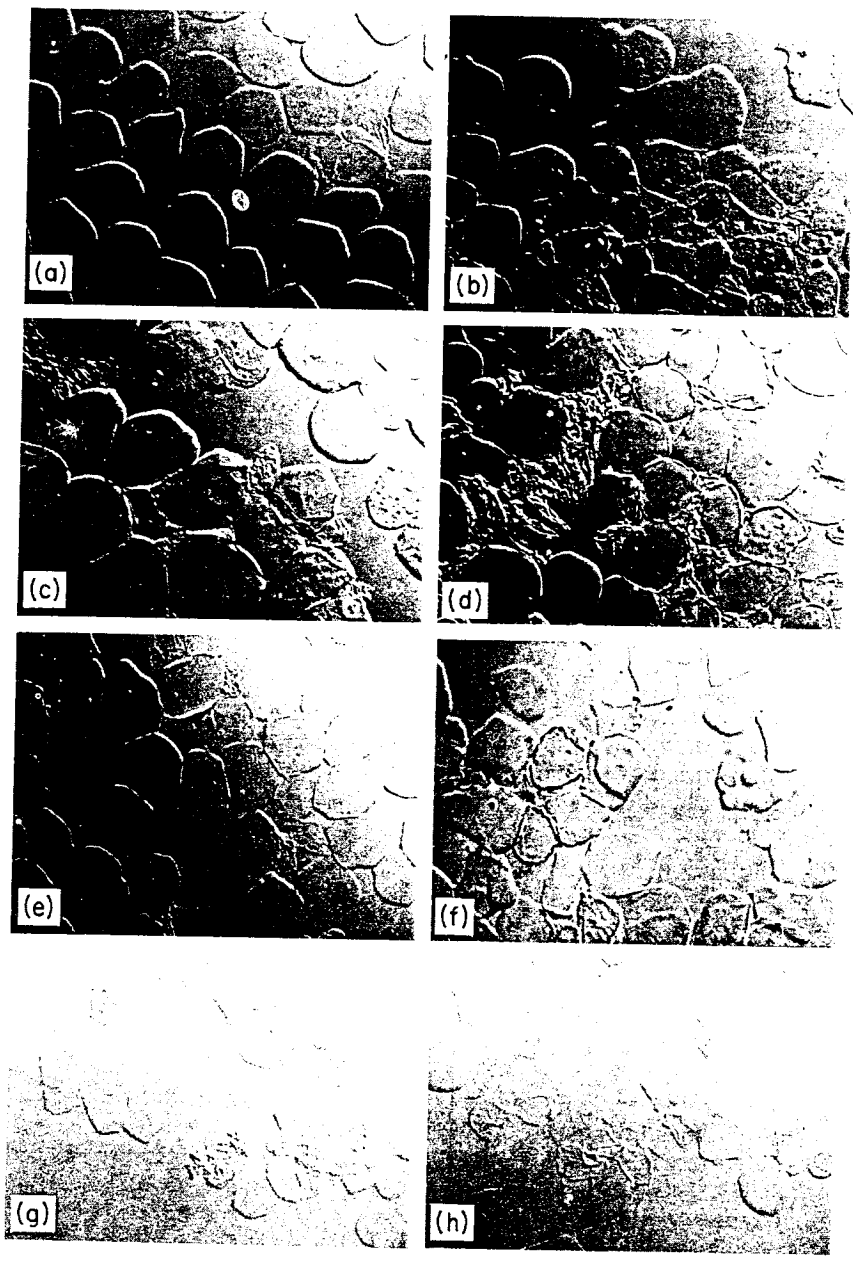

FIGURE 1. Nomarski contrast micrographs (× 120) of the cornified envelopes obtained from normal epidermis (a) and from the lesions of patients with Darier's disease (b), keratoderma palmoplantare (c), KID syndrome (d), erythrokeratoderma variabilis (e), parapsoriasis (f), ichthyosis vulgaris (g) and psoriasis vulgaris (h).

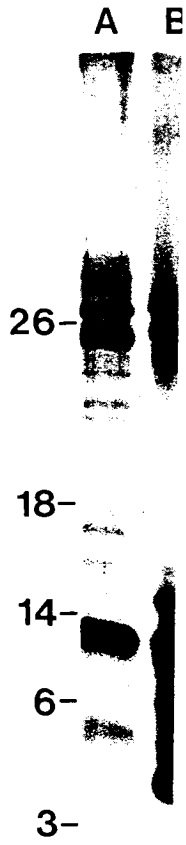

FIGURE 2. Cyanogen-bromide peptide
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(C), erythrokeratoderma variabilis (D),
position of molecular weight markers (i

CNBr peptide mapping

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The CNBr peptide maps of CEs o
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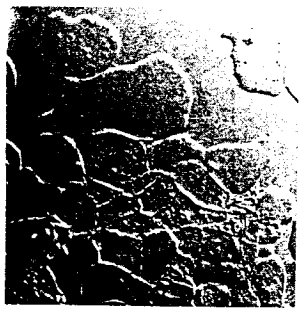

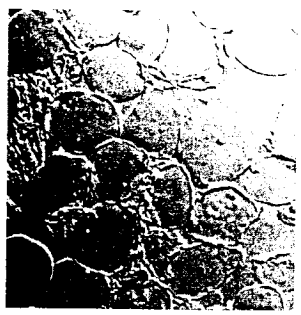

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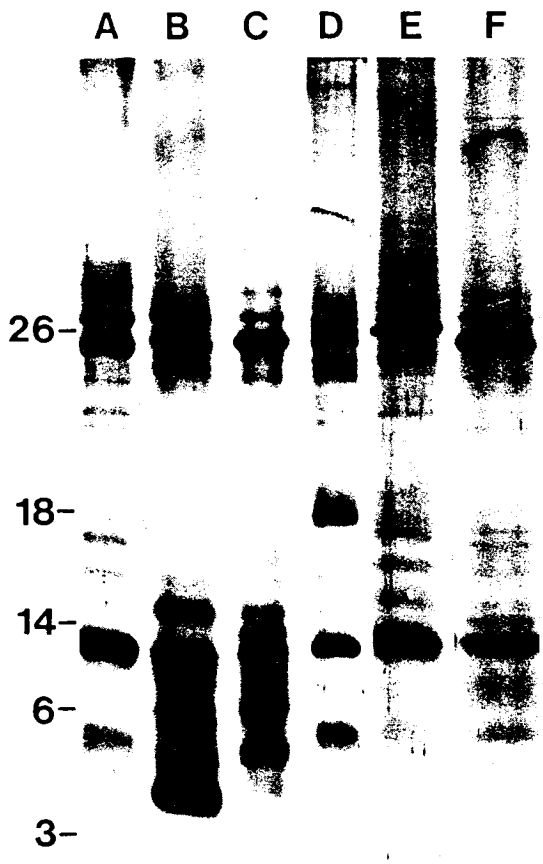

FIGURE 2. Cyanogen-bromide peptide mapping of the cornified envelopes obtained from normal epidermis (A) and from the lesional skin of patients with Darier's disease (B), congenital pachyonychia (C), erythrokeratoderma variabilis (D), ichthyosis vulgaris (E) and keratoderma palmoplantare (F). The position of molecular weight markers (in kDa) is indicated.

CNBr peptide mapping

The CNBr peptide maps of the CEs obtained from different origins are presented in Figure 2. The peptide pattern of CEs purified from the lesional skin of patients with Darier's disease differed markedly from those of normal epidermis, in particular at the level of peptides of molecular weight comprised between 18 and 6 kDa (map B to be compared with map A). A similar difference was also observed in congenital pachyonychia (map C).

The CNBr peptide maps of CEs obtained from a patient with erythrokeratoderma variabilis (map D) exhibited a characteristic peptide of molecular weight 18 kDa not present in the peptide pattern of CEs from normal skin.

The peptide maps of CEs obtained from the patients with ichthyosis vulgaris (map E), palmoplantar keratoderma (map F) and of the other diseases studied (not shown) were not significantly different from those of healthy subjects.

DISCUSSION

According to the morphology of the CEs, congenital disorders of keratinization can be divide into two classes. One comprises keratoderma palmoplantare, KID syndrome, congeni pachyonychia, erythrokeratoderma variabilis and parapsoriasis in which the morphology of th CEs is very close to that of normal skin. In the other class, that consisted of Darier's disea ichthyosis vulgaris and the ichthyosis brittle hair syndrome, the morphology of the CEs grossly altered. In the ichthyotic-like dermatoses the morphology of the CEs resemble close that of psoriatic epidermis (Fig. 1g to be compared with Fig. 1h) and contain an increas number of CE_f. The reduced size of CEs observed in some ichthyotic patients is apparent correlated with a reduced size of corneocytes.[12] In Darier's disease, the morphology of the C is heterogeneous, which could indicate that in this disorder the keratinocyte morphology already altered at the level of the upper granular layer, in which the CE is formed.

The CNBr peptide mapping of CEs revealed that the peptide composition of CEs fro patients affected with Darier's disease, congenital pachyonychia and erythrokeratoderm variabilis are different from those of normal skin. The pattern of CEs from patients wi Darier's disease and congenital pachyonychia showed an accumulation of low molecular weig peptides similar but not identical to that observed in CEs from psoriatic patients.[8] The C peptide pattern from a patient with erythrokeratoderma is strikingly different from that normal and psoriatic individuals. The peptide patterns obtained with CEs from the oth diseases resemble those of normal skin.

The morphology and peptide composition of the CEs are not strictly correlated. Ichthyos vulgaris, for example, shows abnormal CE morphology with a normal peptide pattern, where congenital pachyonychia presents a normal CE morphology but an abnormal peptide patter This shows that the morphology of CEs does not depend exclusively on the pepti composition, but may also be affected by other parameters such as the attachment of the recent described ester-linked hydroxyacyl sphingosine.[13]

Biochemical studies of congenital disorders of keratinization have been limited so far to th analysis of keratin proteins. Bowden *et al.*[14] found normal keratin expression in ichthyosis b an alteration of the normal keratin pattern in Darier's disease. Expression of normal kerat subunits in ichthyosis was confirmed by the immunoblotting technique with AE1 and AE monoclonal antibodies.[15] These results on keratin expression in diseases cannot be strict correlated with our findings on the CE peptide composition. However, not only the kerati subunits but also the CE peptide patterns of ichthyotic patients are identical to those of norm subjects.

The results show that different dermatoses can be distinguished by analysing either th morphological shape or the peptide pattern of the CEs, but further work is needed to evalua the use of CE analysis in the diagnosis and classification of dermatological disorders.

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