Erythrokeratodermia variabilis: Immunohistochemical and Ultrastructural Studies of the Epidermis

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Immunohistochemical and ultrastructural study of the epidermis was performed in a 53-year-old female with erythrokeratodermia variabilis (EKV) before and after treatment with the aromatic retinoid Etretinate (RO 10-9359). Aberrant expression of cytokeratin PKK2 (Labsystems, Helsinki, Finland) in lesional EKV stratum corneum was observed; this feature disappeared after Etretinate therapy. A normal distribution of DR-positive dendritic Langerhans' cells was seen in diseased, control and post-treatment skin specimens. The striking finding of this study was thus the shift to a basal cell-type keratin reactivity in stratum corneum in lesional skin, perhaps a reflection of cytoskeleton features related to cell adhesion. Increased adhesion between the cells in stratum corneum might account for the retention type of hyperkeratosis characteristic of EKV. *Key words: Cytokeratin; Retinoid therapy; Langerhans' cells.* (Received July 23, 1986.)

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Erythrokeratodermia variabilis (EKV), first described in 1925 by Mendes da Costa (1), is a rare autosomal dominant inherited disorder of keratinization. It is manifested by discrete, often symmetrical, patches of skin erythema and the presence of hyperkeratotic, yellow-brown, scaly plaques in the erythematous areas. Previously considered to be a possible variant of congenital ichthyosiform erythroderma (2), it has since been established as a retention-type hyperkeratosis with distinctive histopathological (3) and genetic features (4).

Few ultrastructural and histochemical studies of EKV exist in the literature (3, 5), only one (5) includes the use of immunohistochemistry. The two studies report contradictory findings relating to Langerhans' cells (LC) in lesional skin.

In the present study we examined the immunohistochemical expression of cytokeratin and HLA-DR in EKV lesional epidermis and correlated the findings to those seen in the skin following oral retinoid therapy.

CASE REPORT

A 53-year-old female was seen in June 1985 because of a persistent skin eruption which had been histologically diagnosed 14 years earlier as EKV. The condition consisted of large erythematous areas on the trunk and upper parts of the limbs, some of which were notably hyperkeratotic, brown-pigmented and scaly (Fig. 1). The rash had been present from the age of 3 months, apart from a short period in 1977. Menstruation, pregnancy or menopause did not influence the clinical appearance of the rash, but exposure to wind and sea-bathing were aggravating factors. The family history revealed histologically confirmed EKV in the patient's mother and a similar rash in an aunt of the patient's mother. The patient's three daughters had no evidence of EKV.

The patient underwent mastectomy for mammary carcinoma in 1981; subsequent follow-ups did not show any signs of recurrence. Apart from a short period of post-operative chemotherapy, there was

no history of drug use and her general health was excellent. Previous treatment of the rash was limited to the use of moisture creams and oil-baths.

The following clinical variables were normal or negative: complete blood count, blood urea, serum creatinine, cholesterol, liver enzymes, ANA, RA latex-test, complement C3, C3d and C4. The patient was HLA type A1, B7, B17 and CW6.

Two pretreatment lesional biopsies were taken from the extensor surfaces of both thighs, providing tissue for light microscopy, immunohistochemistry and electron microscopy. Control material was obtained from clinically normal skin. Thereafter the patient was treated with Etretinate 75 mg daily, resulting in clinical normalization of all skin lesions after 6 weeks. She continued with a maintenance dose of 50 mg Etretinate daily. Two post-treatment biopsies of clinically normal skin (after 4.5 months of treatment) were taken from the previous lesional sites.

TISSUE EXAMINATION

Parallell sections of lesional, control, and post-treatment skin specimens biopsies were prepared as follows: 1) Fixed in 4% formaldehyde, paraffin-embedded, sectioned at 6 μm and stained routinely with haematoxylin and eosin; 2) Frozen and stored at –70°C before being freeze-dried in an Edwards Vacuum Tissue Dryer (model ETD 4, from Edwards High Vacuum, Crawley, England) according to the method described by Stein et al. (6) for immunohistochemistry (the freeze-drying was continued for 24 h at a fixed temperature of -60°C, and thereafter the tissue specimens were heated gradually to 56°C and paraffin embedded while still in a vacuum); and 3) Fixed with 2% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.4) and postfixed in osmium according to routine procedures for electron microscopy.

Immunohistochemical procedure

Serial sections were cut at 6 μm and dewaxed in cold xylene immediately before immunohistochemical staining. A three-step immunofluorescence avidin-biotin method was used, often performed as paired staining by combining polyclonal and monoclonal primary reagents as described previously (7). The sections were first incubated with the primary antibody reagents for 20 h at room temperature. After a wash with phosphate-buffered (pH 7.5) isotonic saline (PBS), the sections were further incubated for 3 h with biotinylated horse anti-mouse IgG and finally for 30 min with fluorescein-conjugated avidin (for paired staining mixed with rhodamine-conjugated swine anti-rabbit IgG). The sections were mounted in buffered (pH 8) polyvinyl alcohol medium before being examined in a Leitz Orthoplan microscope equipped with a Ploem-type vertical illuminator (with an Osram HBO 200 W lamp for rhodamine excitation, and an XBO 150 W lamp for fluorescein excitation).

Antibody reagents

A polyclonal reagent to epidermal keratin was prepared as described elsewhere (8). In addition commercial murine monoclonal antibodies were used to demonstrate cytokeratin PKK1 and PKK2 (Labsystems, Helsinki, Finland) and HLA-DR (Becton-Dickinson, California, USA or Dakopatts, Copenhagen, Denmark). PKK1 antibodies have been shown to react with 56-, 48-, 45- and 41-kilodalton polypeptides of cytokeratins, PKK2 reacted with the 43- and 40-kilodalton polypeptides (9). All reagents were diluted in PBS containing bovine serum albumin at 125 g/l, with the following working dilutions: rabbit anti-epidermal keratin 1:400; anti-PKK1 and PKK2 1:100; anti-HLA-DR (both reagents) 1:20.

RESULTS

The pretreatment histological findings (Fig. 2*a*) were characteristic (10). Clinically there was a complete clearing of the rash by Etretinate treatment. Post-treatment biopsy revealed a slight epidermal thickening with mild acanthosis and hyperkeratosis. Small perivascular lymphoid infiltrates appeared in the dermis. The hyperkeratosis was markedly less pronounced in post-treatment skin (Fig. 2*b*).

Aberrant keratin staining for PKK2 in the stratum corneum was noted in pre-treatment lesional skin (Fig. 3*a*). This was absent in control skin and in subsequent post-treatment biopsies (Fig. 3*b*). Repeated tests with PKK2 antibody on a second-site lesional biopsy confirmed the finding. The basal cell layer in both lesional, control and post-treatment skin

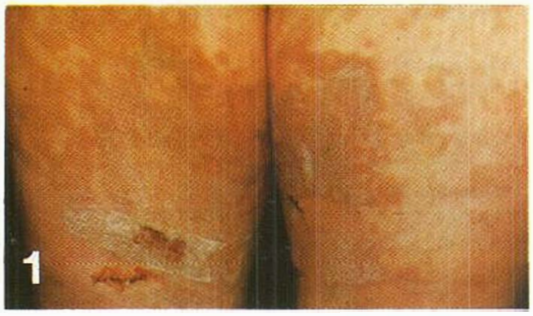
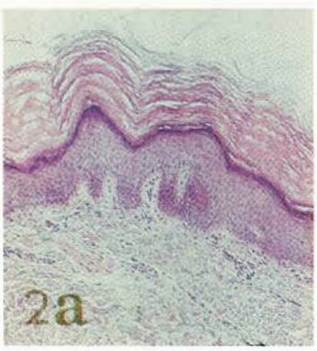


Fig. 1. Pre-treatment EKV of the thighs with biopsy sites.



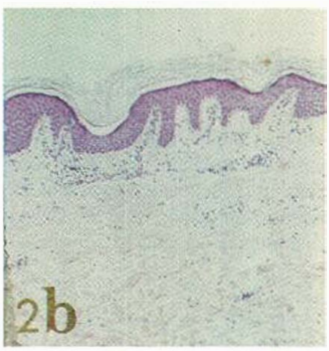
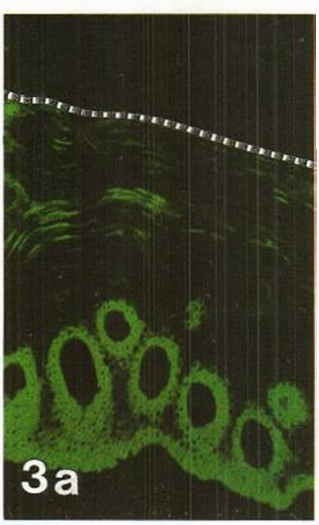


Fig. 2. Histology of lesional (*a*) and post-treatment (*b*) skin. H&E-stained sections (magnification ×44). Lesional skin (2 *a*) reveals marked hyperkeratosis, moderate acanthosis and small dermal perivascular infiltrates, in contrast to the post-treatment biopsy specimen (2 *b*).



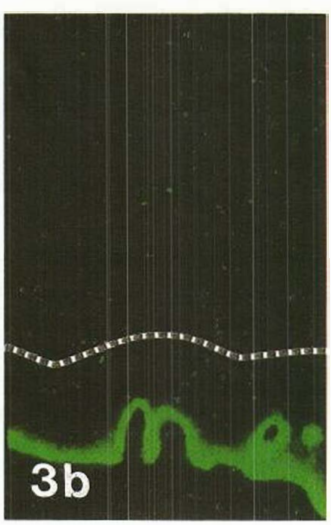


Fig. 3. Cytokeratin PKK2-reactivity in (*a*) lesional and (*b*) post-treatment skin, with abnormal band-line reactivity in stratum corneum in lesional skin. Note post-treatment normalization (magnification ×44). Top of epidermis is indicated by broken line.

was positively stained for the other cytokeratin, PKK1. The epidermal findings were otherwise unremarkable.

HLA-DR-positive dendritic cells were readily seen in lesional (Fig. 4) and control epidermis and dermis, both prior to and following Etretinate therapy. Our impression was that the number of these cells was increased in post-treatment biopsies, although no attempt was made to quantify the cells, because of methodical difficulties (5).

Electron microscopy confirmed the presence of normal-appearing LC with characteristic LC-granules in the epidermis of both lesional and normal skin, prior to Etretinate

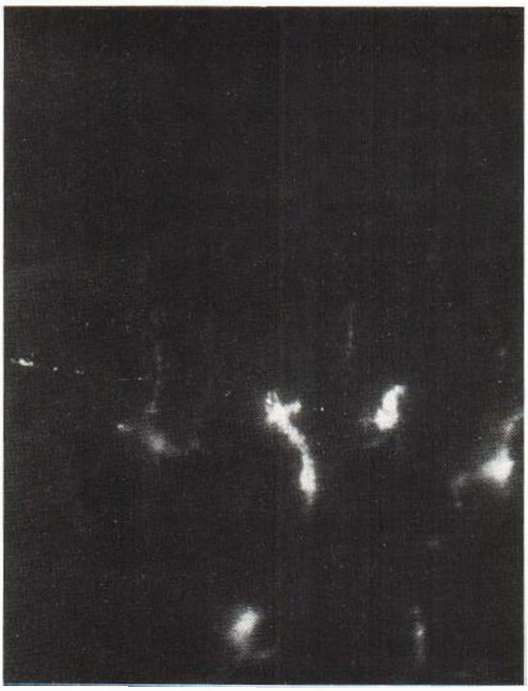


Fig. 4. HLA-DR-positive dendritic cells in lesional epidermis (magnification ×175).

therapy. Nerve fibrils were not seen in the epidermis, nor was there any notable structural difference between lesional and control stratum corneum.

DISCUSSION

In keeping with a complete clinical clearing of the rash, the hyperkeratosis and acanthosis diminished markedly histologically after the treatment with Etretinate. This is somewhat in contrast to the findings of Marks & Finlay, who reported that the pre- and post-treatment biopsies "did not differ remarkably" (11).

We confirmed the presence of normal-appearing LC in both lesional and normal EKV epidermis as reported by van der Schroeff et al. (5). Conversely Vandersteen & Muller (3) were unable to demonstrate such cells in EKV epidermis. Our results are further in contrast to those in the latter study, in that we found no ultrastructural evidence of epidermal nerve fibrils.

Antibody reagent PKK1 has previously been reported not to react with keratin in the epidermal basal cell-layer (9). In our study, the basal cell-layer constantly was positively stained with PKK1. Tissue fixation, especially cross-binding fixatives like formalin, may alter the antigenicity of cytokeratin polypeptides (9). The fact that our specimens for immunhistochemical examination were freeze-dried without fixation might explain the consistent discrepancy between our findings and those of Holthofer et al. (9).

On the basis of our observations with antibody to PKK2, the stratum corneum cells appeared phenotypically different in EKV lesions, despite the fact that no ultrastructural abnormality was seen. The stratum corneum cells possessed intermediate cytokeratin filament, usually found only in epidermal basal cells and follicular epithelial cells (12). Etretinate altered the reactivity pattern for this cytokeratin, in accord with the well-known epidermal normalization taking place after such therapy. It would then appear that the

stratum corneum is a site of pathology in EKV, and that this change is more likely biochemical, rather than ultrastructural or immunological. Modulation and rearrangement of the cytoskeleton structure in epidermis occurs during normal keratinization (13). A biochemical change in the altered cells of the stratum corneum could explain the change in intermediate filament protein configuration and thus the aberrant presentation of keratin epitopes. A shift of intermediate filament configuration to that of the basal layer cells might account for closer adhesion of stratum corneum cells in EKV lesional epidermis, resulting in a retention type of hyperkeratosis. This would agree with the findings of Schellander & Fritsch, who found normal proliferation rate in EKV epidermis by labelling with tritiated thymidine (14). Immunoelectron microscopy might help to further define this disorder of keratinization.

ACKNOWLEDGEMENTS

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