

Expression of Stratum Corneum Chymotryptic Enzyme in Reconstructed Human Epidermis and Its Suppression by Retinoic Acid

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The aim of the present study was to investigate the presence of stratum corneum chymotryptic enzyme (SCCE) in human epidermis reconstructed *in vitro* on dead de-epidermized dermis and the effect of retinoic acid (RA) on its expression. SCCE is a recently discovered serine proteinase which has been purified from human stratum corneum, and evidence has been presented that it may play a role in stratum corneum turnover, especially in desquamation. The SCCE-specific monoclonal antibody TE-9B showed positive immunofluorescence staining of high suprabasal keratinocytes, mainly in the stratum granulosum, in normal non-palmo-plantar human epidermis as well as in reconstructed epidermis in the absence of RA. This staining was also seen in reconstructed epidermis cultured in the presence of 10^{-8} M RA, a concentration at which the reconstructed epidermis still formed an orthokeratotic stratum corneum. At 10^{-7} M RA, however, not only the formation of the stratum corneum but also SCCE expression was suppressed. These results support the hypothesis that SCCE expression is related to and may be part of the epidermis-specific differentiation program, where the enzyme may be involved in the homeostasis of the stratum corneum, possibly by degrading the intercorneocyte cohesive structures.

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Stratum corneum chymotryptic enzyme (SCCE) is a recently discovered serine proteinase which has been purified from human stratum corneum (1–3). SCCE may play a role in the terminal epidermal differentiation program. Based on comparative studies of (i) the inhibitor profiles of SCCE, (ii) cell shedding in an *in vitro* desquamation model and (iii) the accompanying degradation of the desmosomal protein desmoglein I (4–6), it has been suggested that the physiological function of SCCE may be the degradation of intercellular cohesive structures in the stratum corneum during desquamation. This is supported by findings that SCCE may have an extracellular localization in the stratum corneum (7), and that the enzyme is active at a pH found in the stratum corneum under physiological conditions (1, 2).

In the present work we present further evidence that SCCE expression may be related to and part of the epidermal differentiation program, by studying its expression in reconstructed epidermis, cultured in the absence and presence of different concentrations of retinoic acid (RA).

MATERIALS AND METHODS

Reconstruction of an epidermis on de-epidermized dermis

Dermal substrates were prepared from split-thickness human cadaver skin as previously described (8). Briefly, strips of skin were cut into 2 cm² pieces, placed dermal side down into calcium-magnesium free phosphate-buffered saline (PBS). After 10 days at 37°C, the epidermis was peeled off with forceps. Dermal cells were killed by successive freezings and thawings. This de-epidermized dermis (DED) was stored at –70°C until use.

Epidermal cell suspensions were obtained after separation of epidermis from dermis by trypsinization of split-thickness adult human skin. Keratinocytes were dissociated by gently shaking the epidermis in a trypsin-EDTA solution. After inhibition of trypsin with fetal calf serum, the suspension was spun down for 10 min at 200 × g. Cells were resuspended in a tissue culture medium containing 10% (v/v) fetal calf serum, consisting of Dulbecco's modified Eagle's medium (DMEM) supplemented with hydrocortisone (0.4 mg per ml), epidermal growth factor (10 ng per ml) and cholera toxin (0.1 mg per ml). Epidermal cells were subcultured once by plating 4,000 cells per cm² on plastic dishes containing 3T3 cells, mitotically blocked by irradiation. Then, epidermis was reconstructed according to the technique already described (8). Pieces of DED were placed, basement membrane side up, on the bottom of a dish. Stainless steel rings were put on the dermis to delineate surfaces of 1 cm². Viable cells (5×10^5) were seeded into each ring. Medium was added to cover the dermis. One day later, the dermal substrate bearing the keratinocytes was lifted on a grid to expose the culture to the air-liquid interface. The medium was replaced by a mixture of DMEM supplemented with 10% delipidized serum (9). This medium was changed every second day.

Treatment with RA

One day after seeding the keratinocytes on the DED, medium containing delipidized fetal calf serum and eventually RA (10^{-8} and 10^{-7} M) was added to the cultures. Control cultures were supplemented with the solvent alone, i.e. 0.1% dimethyl sulphoxide. Ten days later, the cultures were prepared for histological and immunohistological studies.

Histology and immunohistology

Samples of normal non-palmo-plantar human skin and reconstructed epidermis were fixed in 10% formaldehyde; 0.5 µm sections were stained with hematoxylin-eosin. For indirect immunofluorescence, samples were embedded in OCT compound (Tissue-Tek, Miles, Naperville, Ill.) and frozen in liquid nitrogen.

The production and characterization of the SCCE-specific monoclonal antibody (Mab) TE-9B will be described in detail elsewhere. In brief, SCCE was extracted from dissociated plantar corneocytes with 1 M KCl, pH 8 (1, 4) and further purified by affinity chromatography on covalently linked soybean trypsin inhibitor (3). The purified enzyme was used to immunize mice from which antibody-producing hybridomas were produced and propagated in mouse ascites fluid. Mab TE-9B is of isotype IgG1-kappa and reacts specifically with native SCCE on immunoblots and in immunoprecipitation experiments. Five µm cryostat sections were fixed in acetone and briefly rinsed in PBS. Working dilutions of the first antibody (50 µg per ml)

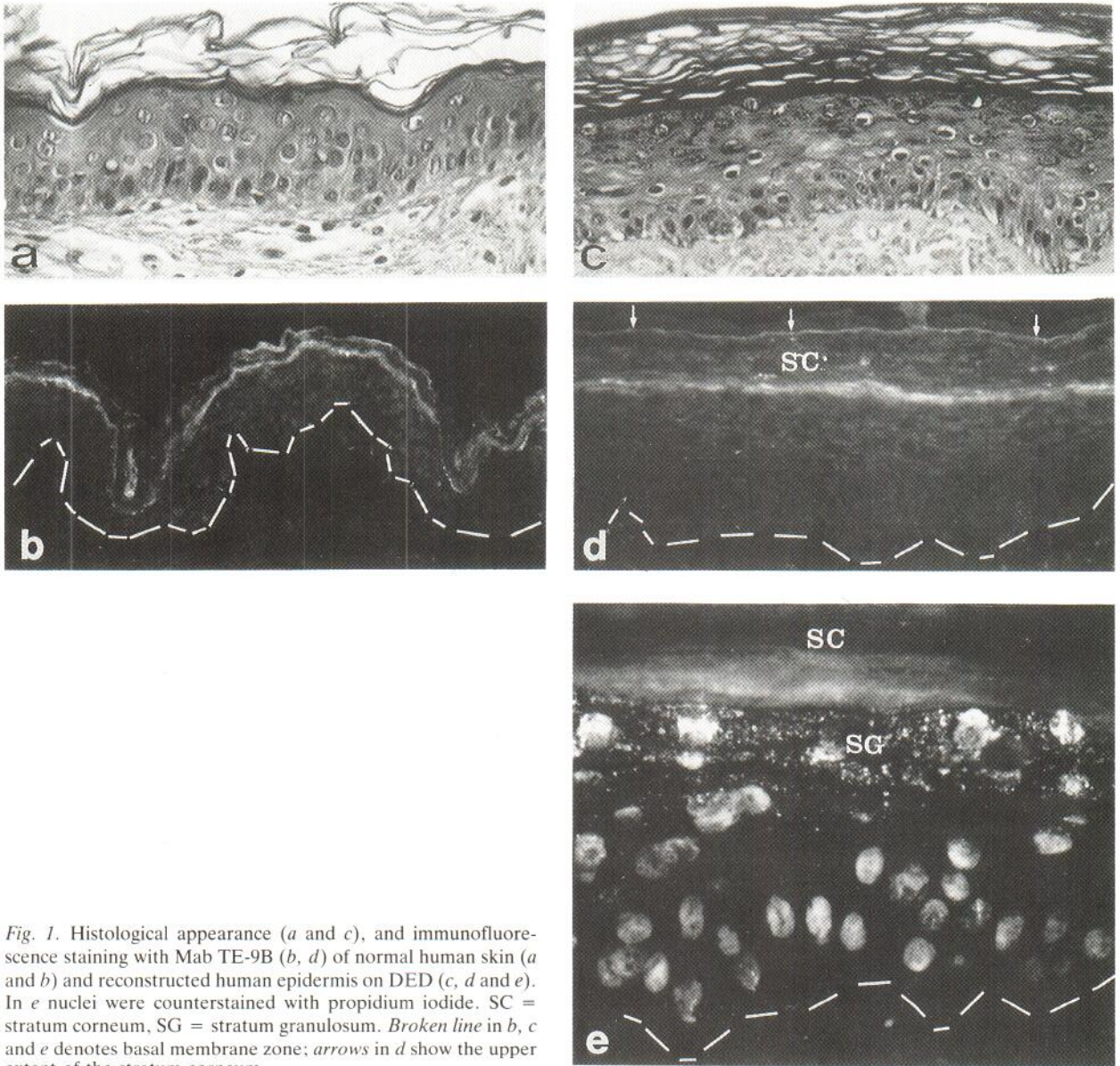


Fig. 1. Histological appearance (a and c), and immunofluorescence staining with Mab TE-9B (b, d) of normal human skin (a and b) and reconstructed human epidermis on DED (c, d and e). In e nuclei were counterstained with propidium iodide. SC = stratum corneum, SG = stratum granulosum. Broken line in b, c and e denotes basal membrane zone; arrows in d show the upper extent of the stratum corneum.

and fluorescein-conjugated goat-anti mouse immunoglobulins (Becton Dickinson, Mechelen, Belgium) 1:200 were prepared in 0.1 M Tris-HCl, pH 7, and reacted with the sections for 30 min at room temperature. Rinses were carried out in 0.1 M Tris-HCl, pH 7, except for one 5-min rinse after the second antibody, which was carried out with PBS. In some experiments, nuclei were counterstained with propidium iodide, 5 µg per ml in PBS. Sections reacted with conjugate alone were used as controls. Slides were examined with a Zeiss microscope equipped with epifluorescence.

RESULTS

Mab TE-9B stained high suprabasal cells, mainly in the stratum granulosum, in normal non-palmo-plantar skin (Fig. 1a-b). Cells in the lower parts of the epidermis were not stained. The reconstructed human epidermis on DED (Fig. 1c) exhibits a well stratified epithelium with granular and cornified layers. As in normal skin Mab TE-9B stained high suprabasal keratinocytes with the highest intensity in the granular layer

(Fig. 1d). A higher magnification with counterstained nuclei (Fig. 1e) shows that expression of SCCE starts at the mid-spinous layers in a granular cytoplasmic form.

When RA was present in the culture medium at 10^{-8} M, the reconstructed epidermis still stratified and formed a stratum corneum (Fig. 2a). As in the absence of RA, Mab TE-9B showed positive staining of cells at the transition between living and cornified layers (Fig. 2b). However, in the presence of 10^{-7} M RA the formation of a stratum corneum is suppressed, and the staining with Mab TE-9B revealed the absence of the typical SCCE expression pattern (Fig. 2c). Instead there was a rather unspecific, weak, spotty, and irregular staining of single cells throughout the epithelial sheet.

DISCUSSION

Previous studies on cultured keratinocytes (for review see 10),

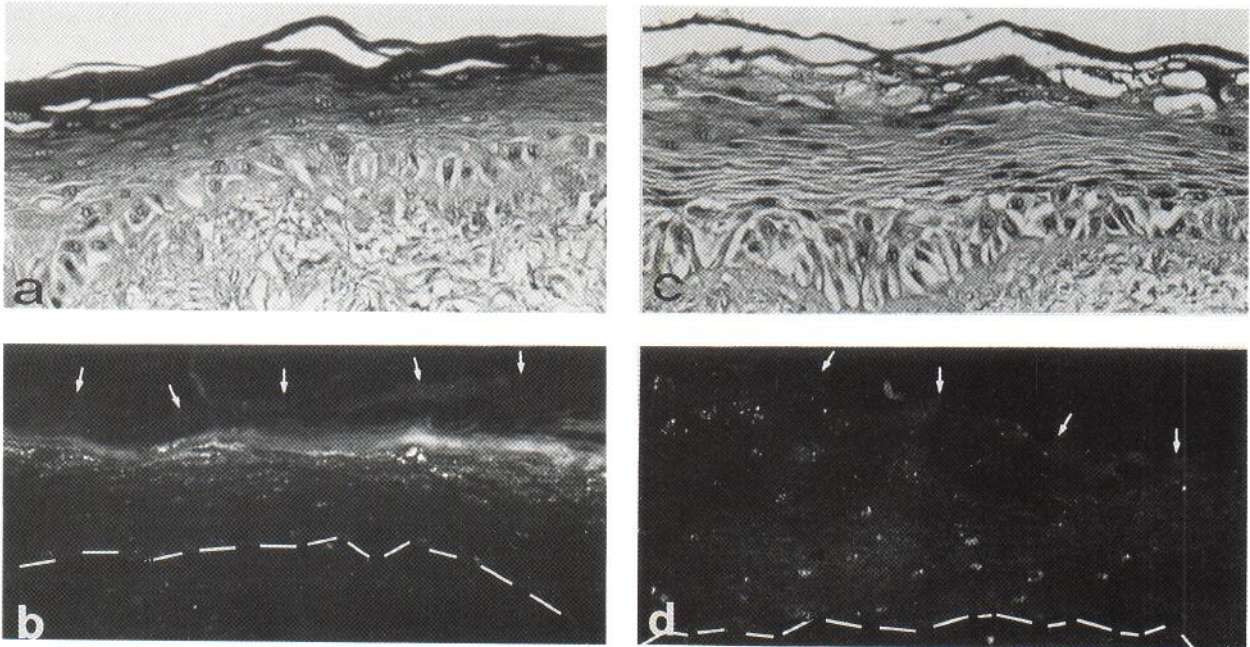


Fig. 2. Histological appearance (a and c) and immunofluorescence staining with Mab TE-9B (b and c) of reconstructed human epidermis on DED treated with 10^{-8} M (a and b) and 10^{-7} M RA (c and d). Broken line denotes basal membrane zone in b and d; arrows in b and d show the upper extent of the epidermal reconstructs.

and three-dimensional reconstructs of human epidermis on DED (8), or on fibroblast-contracted collagen lattices (11), have shown that RA in pharmacological concentrations (10^{-7} M and higher) prevents the expression of several epidermal differentiation markers, including cytokeratins K1/K10 and profilaggrin/filaggrin, and the formation of granular and cornified layers. Epidermis reconstructed in the presence of these RA-concentrations has been found to express tissue and differentiation-stage specific markers in a way resembling non-cornified oral epithelium (12). RA administered topically in vivo to rhino mice has been shown to cause the induction of hyperproliferation-related cytokeratins, a decreased expression of filaggrin, and a reduced proteolysis of stratum corneum keratins (13).

We found that in reconstructed human epidermis on DED the expression pattern of SCCE is similar to that found in normal non-palmo-plantar epidermis. It was interesting to observe that RA, at a concentration that prevented the formation of a stratum corneum, also abolished the typical expression pattern of SCCE. These results are in good agreement with recent findings that in oral epithelium SCCE is expressed only at sites with an orthokeratotic stratum corneum*.

The fact that RA, known to induce in vitro keratinocyte proliferation and to inhibit differentiation, suppresses the expression of SCCE, provides further evidence that this enzyme may be a part of the epidermal specific differentiation program. It remains to be elucidated whether its function is restricted to the degradation of inter-corneocyte cohesive structures during desquamation, or if it also participates in other

proteolytic processes during epidermal differentiation (14–18).

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