

Association between Expression of Stratum Corneum Chymotryptic Enzyme and Pathological Keratinization in Human Oral Mucosa

BJÖRN SONDELL^{1,2}, PER DYBERG¹, GÖRAN KB ANNEROTH³, PÄR-OLOV ÖSTMAN³ and TORBJÖRN EGELRUD¹

Departments of ¹Dermatology, ²Anatomy and ³Oral Pathology, Umeå University, Umeå, Sweden

Stratum corneum chymotryptic enzyme (SCCE) may function in the degradation of intercellular cohesive structures in the stratum corneum preceding desquamation. Previous results have suggested that SCCE may be specifically expressed in squamous epithelia undergoing terminal differentiation and keratinization. The aim of the present work was to further elucidate the association between SCCE expression and terminal differentiation in squamous epithelia. Using immunohistochemical methods, we have examined the expression of SCCE in two diseases of human oral mucosa, which produce a pathological keratinization of the epithelium at sites which are normally non-keratinized. Affinity-purified polyclonal rabbit antibodies raised against recombinant SCCE and monoclonal antibodies against the differentiation-specific keratins nos. 10 and 13 were used on formaldehyde-fixed and paraffin-embedded biopsies. Whereas there was essentially no expression of SCCE in normal, non-keratinized buccal mucosa, there was a strong expression in suprabasal cells in orthokeratotic and parakeratotic areas of the lesions of oral lichen planus (an inflammatory disease) and benign oral keratosis (a non-inflammatory disease). There was a close association between the expression of SCCE and keratin no. 10, i.e. a keratin which is specifically expressed in cornifying squamous epithelia. The results suggest that SCCE expression may be a true marker of terminal differentiation in squamous epithelia and give further evidence for a role of SCCE in the formation and/or turnover of the stratum corneum. **Key words:** SCCE; serine proteinase; human epidermis; immunohistochemistry.

(Accepted October 25, 1995.)

Acta Derm Venereol (Stockh) 1996; 76: 177–181.

B. Sondell, Department of Dermatology, Umeå University, S-901 85, Umeå, Sweden.

Stratum corneum chymotryptic enzyme (SCCE) is generally present in an active form in the outermost layer of human skin, the stratum corneum (1–3). Available data suggest that SCCE may play a role in the degradation of intercellular cohesive structures in the stratum corneum preceding desquamation (4–6). This function of SCCE, however, has still to be proven, and the physiologic function of SCCE is not fully understood.

SCCE has been cloned and expressed in recombinant form in mammalian cells. It is produced as an inactive pro-form, which obtains proteolytic activity after treatment with trypsin (7). The mechanisms of activation *in vivo* remain to be elucidated. On the mRNA level SCCE has been found to be expressed in high amounts in the skin, but not in a wide variety of other human tissues (7). In a recent immunohistochemical study with monoclonal antibodies raised against

native SCCE, we found specific staining in the epidermis, in the keratinized inner root sheet of the hair follicle, and in those parts of the normal human oral epithelium forming a stratum corneum during terminal differentiation. No staining was found in non-keratinized parts of the oral mucosa. These results suggested a close association between SCCE expression and terminal differentiation of squamous epithelia (8). By means of immunoelectron microscopy SCCE has been detected in the stratum corneum extracellular space in association with intact desmosomes and desmosomes undergoing degradation, compatible with a role of SCCE in desquamation (9). Enzymatic studies also support an extracellular location of SCCE in human plantar stratum corneum (10). It is also possible, however, that SCCE may have a function in the formation and turnover of the stratum corneum in a broader sense, e.g. in the extensive degradation of intracellular structures associated with cornification (8).

In order to further elucidate the association between SCCE expression and keratinization of squamous epithelia we have examined biopsies of human oral mucosa affected by diseases which involve a pathological keratinization at sites which are normally non-keratinized. We used SCCE-specific polyclonal antibodies raised against recombinant SCCE for immunohistochemistry on formaldehyde-fixed, paraffin-embedded biopsies. We have analyzed one set of biopsies from a non-inflammatory disorder (benign oral keratosis) and one set from lesions caused by an inflammatory disease (oral lichen planus). In addition to SCCE expression, the expression of differentiation-specific keratins was analyzed. The results give further evidence that SCCE expression is closely associated with the activation of genes involved in terminal differentiation of squamous epithelia and give further support to the idea that SCCE plays an important role in the formation and/or turnover of the stratum corneum.

MATERIALS AND METHODS

Biopsies

Biopsies from buccal lesions, which had been fixed in formaldehyde and embedded in paraffin according to routine protocols, were obtained from the Department of Oral Pathology, Umeå University. They had previously been classified by an experienced pathologist with diagnostic criteria according to recommendations put forward by the World Health Organisation (11). A total of 18 biopsies from 18 individuals, 9 with the diagnosis of benign oral keratosis and 9 with the diagnosis of oral lichen planus of the reticular type, were analyzed. Control samples ($n=5$ from 4 individuals) were taken under local anaesthesia as 3-mm punch biopsies from the buccal mucosa of healthy volunteers. The control biopsies were fixed for at least 18 h in 4% phosphate-buffered formaldehyde before being dehydrated and embedded according to the routine protocol. Punch biopsies of normal

non-palmar-plantar skin were taken under local anaesthesia from healthy volunteers and fixed and embedded as above.

Antibodies

Polyclonal rabbit anti-SCCE, provided by Symbicom AB, Umeå, Sweden, was obtained by means of intramuscular immunization of rabbits with purified recombinant pro-SCCE (7). Specific antibodies were obtained by means of immunoaffinity chromatography on a column with recombinant pro-SCCE coupled to CNBr-sepharose according to the procedure recommended by the manufacturer (Pharmacia Biotech, Uppsala, Sweden). On immunoblots the purified antibodies bound to recombinant pro-SCCE as well as to recombinant SCCE activated by trypsin treatment (7) and native human SCCE (3). When used on immunoblots of crude extracts of human epidermis the antibodies specifically detected components with electrophoretic behaviour identical to SCCE. Control rabbit IgG was prepared from sera from non-immunized rabbits by means of affinity chromatography on Protein A-sepharose (Pharmacia). Bound immunoglobulins were eluted with 0.1 M acetic acid and immediately dialysed against phosphate-buffered saline (PBS). Mouse monoclonal antibodies to human keratins no. 10 (moab K10) and no. 10/13 (moab K10/13) were purchased from Dakopatts, Älvsjö, Sweden. According to the manufacturer, moab K10/13 recognizes only keratin no. 13 on sections of formaldehyde-fixed and paraffin-embedded tissue. The detection system used (Super Sensitive Multilink/horseradish peroxidase with liquid diaminobenzidine) was obtained from BioGenex, San Ramon, CA.

Immunohistochemical procedure

Serial 5- μ m sections were collected on Superfrost Plus slides (Menzel Gläser, Braunschweig, Germany) and dried overnight at 37°C. Deparaffinization, rehydration, and quenching of endogenous peroxidase activity with hydrogen peroxide according to routine protocols were followed by antigen retrieval. This was carried out by means of incubating the sections for 25 min at room temperature without heating in 0.01 M sodium citrate, pH 6.0, which had immediately before been pre-heated to boiling in a microwave oven. After rinses 2 \times 5 min in distilled water and 5 min in PBS, the sections were incubated for 15 min at room temperature in 0.04 mg/ml pepsin (Sigma, St. Louis MO, catalogue no. P 7000) in 0.2 M HCl. After a rinse in PBS the sections were incubated with primary antibodies for 1 h at 37°C, followed by another rinse. For the detection of bound primary antibodies the protocol recommended by BioGenex was followed. Briefly, the sections were incubated at 37°C with biotinylated goat anti-immunoglobulins and horseradish peroxidase-labelled streptavidin, 5 min each, followed by the chromogenic substrate for 5 min at room temperature. Antibodies were diluted in PBS with 1% BSA and rinses were carried out in PBS. After counter staining with Mayer's hematoxylin the sections were dehydrated and mounted. The SCCE-specific antibodies and the control immunoglobulins were used at 1 μ g/ml, the monoclonal antibodies at dilution 1/100. For each biopsy sections were also stained for routine histology with hematoxylin and eosin.

RESULTS

Distribution of SCCE-specific staining in normal human skin

Fig. 1 shows the staining pattern in formaldehyde-fixed paraffin-embedded normal non-palmar-plantar epidermis with the polyclonal SCCE-specific antibodies. In the viable cell layers the staining was confined to the granular layer. The stratum corneum was homogeneously stained. Most likely due to differences in tissue processing, these results differ somewhat from previous results with acetone-fixed tissue and SCCE-specific monoclonal antibodies. In these experiments the zone of stained viable cells was slightly broader, with staining also

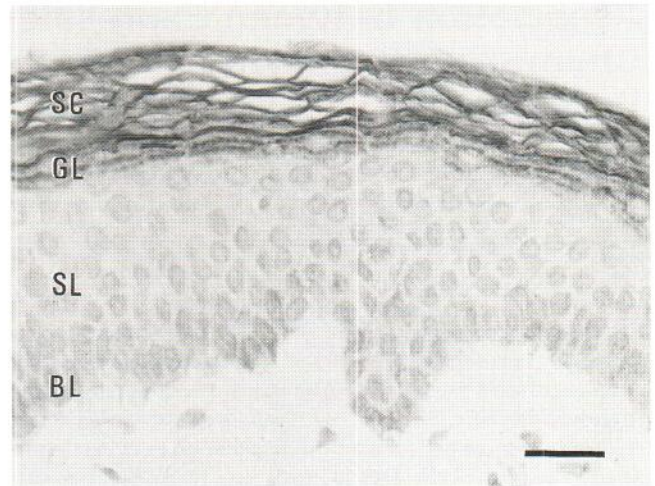


Fig. 1. Immunohistochemical staining with rabbit anti-SCCE of formaldehyde-fixed and paraffin-embedded normal non-palmar-plantar human epidermis. SCCE-specific staining was found in the granular layer and in the stratum corneum. SC = stratum corneum, GL = granular layer, SL = spinous layer, BL = basal layer. Bar = 50 μ m.

in the uppermost spinous cells, and the staining of the stratum corneum irregular and spotty (8).

Normal buccal mucosa

All control biopsies had a normal appearance on hematoxylin-eosin-stained sections with no stratum corneum, no granular cell layer, and a thickness of the epithelium around 0.7 mm. There were no inflammatory cells in the submucosa (Fig. 2). With SCCE-specific antibodies there was a weak staining in the lower part of the epithelium, which did not differ significantly from background staining (Fig. 2F). There was no staining with moab K10 (Fig. 2I), whereas moab K10/13 stained all suprabasal cells (Fig. 2L).

Oral lichen planus

In all examined lesions, areas with an orthokeratotic (fully keratinized) and sometimes also a parakeratotic (partially keratinized) stratum corneum were found. In orthokeratotic areas there was a layer of granular cells below the stratum corneum. In the underlying submucosa there was a dense inflammatory infiltrate consisting of lymphocytes (Fig. 2A). The lesional epithelium was generally thinner than normal mucosa. The staining pattern for each antibody was consistent in all biopsies.

Fig. 2, left vertical row, shows the results of routine as well as immunohistochemical stainings of a lichen planus lesion in which there was an area with apparently normal buccal mucosa surrounded by pathologically changed tissue. As shown in Fig. 2D, there was a strong staining with SCCE-specific antibodies of suprabasal cells in those parts of the biopsy where there was a stratum corneum, whereas there was no staining with these antibodies significantly above background level of the apparently normal part of the biopsy. A similar staining pattern was obtained in this biopsy with moab K10, with strong staining of suprabasal cells in keratinized areas, and essentially no staining of the non-keratinized part of the epithelium (Fig. 2G). Moab K10/13 stained suprabasal cells

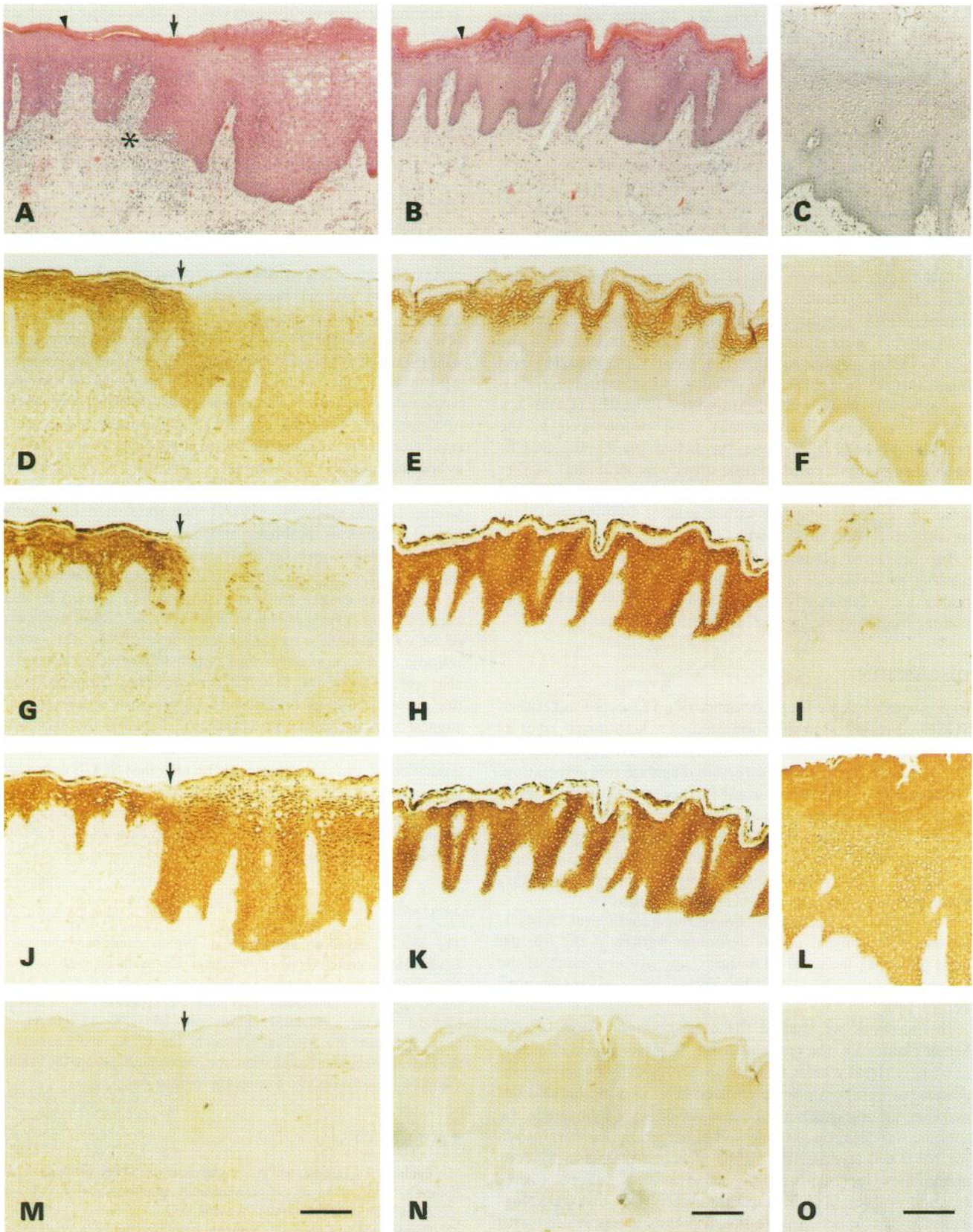


Fig. 2. Hematoxylin/eosin staining (A–C), and immunohistochemical staining with rabbit anti-SCCE (D–F), moab K10 (G–I), moab K10/13 (J–L) and normal rabbit IgG (M–O) of biopsies of human buccal mucosa with oral lichen planus (*left vertical row*), benign oral keratosis (*middle vertical row*) and normal mucosa (*right vertical row*). In each vertical row serial sections of the same biopsy are shown. *Arrows* in the left vertical row (lichen planus) show the border between affected and normal mucosa. *Arrowheads* in (A) and (B) denote the stratum corneum. In lichen planus and benign oral keratosis, but not in the normal mucosa, suprabasal cells were stained by anti-SCCE and moab K10. *Bars* = 200 μ m.

throughout the epithelium (Fig. 2J). In areas with parakeratosis the SCCE-specific staining was weaker in the cell layers just beneath the stratum corneum than in areas with orthokeratosis (results not shown).

Benign oral keratosis

All biopsies showed consistent staining patterns. The epithelium was generally thinner than normal mucosa and was dominated in most biopsies by areas with an orthokeratotic stratum corneum and a well developed granular layer. In some biopsies there were also areas with parakeratosis. Inflammatory cells were sparse or absent in the submucosa (Fig. 2B). The results for a representative biopsy with an orthokeratotic stratum corneum are shown in Fig. 2, middle vertical row. Suprabasal cells were heavily stained with the SCCE-specific antibodies (Fig. 2E), moab K10 (Fig. 2H), and moab K10/13 (Fig. 2K). The staining of the stratum corneum was generally weak with all antibodies (Fig. 2E, H and K). The SCCE-specific staining was most pronounced in the granular layer (Fig. 2E). As for lichen planus the SCCE-specific staining in parakeratotic areas was somewhat weaker in the cells just beneath the stratum corneum than in orthokeratotic areas. This coincided with a weaker staining also with moab K10 (results not shown).

In oral lichen planus as well as benign oral keratosis the number of viable cell layers stained with SCCE-specific antibodies was significantly higher than in normal non-palmar-plantar human epidermis (cf. Figs. 1 and 2D and E).

DISCUSSION

In previous work we found evidence of an association between SCCE-expression and the formation of a keratinized layer as a result of terminal differentiation of squamous epithelia (8). The aim of this work was to further elucidate this association. We considered this an important further step towards a better understanding of the function of this recently discovered human serine proteinase. We chose to study two disease conditions where the pathological changes involve the transition from a non-keratinized to a keratinized squamous epithelium: oral lichen planus and benign oral keratosis.

The squamous epithelium lining the human oral cavity is normally keratinized in the vermilion border of the lip, the hard palate, at the gingival margins, and at the dorsum of the tongue. At all other sites the epithelium is normally non-keratinized.

In this work we studied the most common form of oral lichen planus, i.e. the reticular form. It is recognised clinically as whitish streaky areas and histopathologically by an orthokeratotic or parakeratotic keratinized layer in addition to other markers, among which are a dense lymphocyte infiltrate in the submucosal connective tissue and liquefaction degeneration in the basal cell layer. Other forms of lichen planus in mucous membranes include atrophic, erosive and bullous forms. Isolated oral lesions which cannot clinically or histopathologically be discriminated from lichen planus in association with skin disease also occur. Among the possible etiological factors of these changes are irritative and contact allergic responses to dental materials (12, 13 for ref.).

The entity designation "benign oral keratosis" is used for changes in the oral epithelium which are clinically recognised

as homogeneously whitish areas which are usually well demarcated from the normal mucosa. Histologically there are changes which often remind of a normally differentiated epidermis, with well-developed granular and cornified cell layers. There are usually no or only sparse inflammatory cells in the submucosal connective tissue. The prefix "benign" denotes the absence of atypical epithelial cells indicative of a pre-malignant state. Chronic irritation is considered to be one among other etiologic factors. Since benign oral keratoses cannot be clinically differentiated from pre-malignant or malignant changes, they have to be removed and examined histopathologically (14, 15 for ref.).

As in our previous work (8), we found a very low expression of SCCE in normal buccal mucosa. In the pathologically changed buccal mucosa in lesions of oral lichen planus and benign oral keratosis, on the other hand, we found strong suprabasal SCCE-specific staining, but only at sites where a stratum corneum had been formed. The presence of a stratum corneum in the lesions was also associated with the expression of keratin 10. Whereas keratin 13 is normally expressed by suprabasal keratinocytes of the oral epithelium, keratin 10 is a specific marker of terminal differentiation in squamous epithelia and a major constituent of suprabasal layers in non-palmar-plantar epidermis (16). It thus appears that the pathological changes in the buccal mucosa in oral lichen planus as well as benign oral keratosis involve the activation of a differentiation program similar to that present in the epidermis. In an *in vitro* system with reconstituted human skin in which keratinocytes form a cornified layer (17), we found expression of SCCE in high suprabasal keratinocytes. In this system retinoic acid at concentrations which inhibited keratinization also seemed to inhibit SCCE expression (18). Taken together, the present findings and results of previous studies (8, 18) suggest that the apparent association between the expression of SCCE and the formation of a cornified layer is not merely coincidental, but rather support the idea that SCCE expression is a true marker of terminal differentiation in squamous epithelia. The present findings also give further support for an important function of SCCE in the formation and/or turnover of the stratum corneum.

ACKNOWLEDGEMENTS

The skilful technical assistance of Britta Lindgren and Mona Lindström is gratefully acknowledged. The authors wish to thank Mats Strömqvist and Annica Carlstein, Symbicom AB, for providing the SCCE-specific antibodies. This work was supported by the Swedish Medical Research Council (grant no. B95-12X-11206-01A), the Swedish Society for Medical Research, the JC Kempe Foundation, the Edvard Welander Foundation, the Finsen Foundation, the Swedish Psoriasis Association and Symbicom Sweden AB.

REFERENCES

1. Egelrud T, Lundström A. A chymotrypsin-like proteinase that may be involved in desquamation in plantar stratum corneum. *Arch Dermatol Res* 1991; 283: 108-112.
2. Lundström A, Egelrud T. Stratum corneum chymotryptic enzyme: a proteinase which may be generally present in the stratum corneum and with a possible involvement in desquamation. *Acta Derm Venereol (Stockh)* 1991; 71: 471-474.
3. Egelrud T. Purification and preliminary characterization of stratum corneum chymotryptic enzyme: a proteinase which may

- be involved in desquamation. *J Invest Dermatol* 1993; 101: 200–204.
4. Lundström A, Egelrud T. Cell shedding from human plantar skin in vitro: evidence of its dependence on endogenous proteolysis. *J Invest Dermatol* 1988; 91: 340–343.
 5. Lundström A, Egelrud T. Evidence that cell shedding from plantar skin in vitro involves endogenous proteolysis of the desmosomal protein desmoglein I. *J Invest Dermatol* 1989; 94: 216–220.
 6. Lundström A, Egelrud T. Cell shedding from plantar skin in vitro: evidence that two different types of protein structures are degraded by a chymotrypsin-like enzyme. *Arch Dermatol Res* 1990; 282: 234–237.
 7. Hansson L, Strömquist M, Bäckman A, Wallbrandt P, Carlstein A, Egelrud T. Cloning, expression and characterization of stratum corneum chymotryptic enzyme. *J Biol Chem* 1994; 269: 19420–19426.
 8. Sondell B, Thornell L-E, Stigbrand T, Egelrud T. Immunolocalization of stratum corneum chymotryptic enzyme in human skin and oral epithelium with monoclonal antibodies: evidence of a proteinase which may be specifically expressed in keratinizing squamous epithelia. *J Histochem Cytochem* 1994; 42: 459–465.
 9. Sondell B, Thornell L-E, Egelrud T. Evidence that stratum corneum chymotryptic enzyme is transported to the stratum corneum extracellular space via lamellar bodies. *J Invest Dermatol* 1995; 104: 42–46.
 10. Egelrud T. Stratum corneum chymotryptic enzyme: evidence of its location in the stratum corneum intercellular space. *Eur J Dermatol* 1992; 2: 50–55.
 11. Kramer IRH, Lucas RB, Pinborg JJ, Subin LH. Definition of leukoplakia and related lesions: an aid to studies on oral precancer. *Oral Surg* 1978; 46: 518–536.
 12. Black MM. Lichen planus and lichenoid disorders. In: Champion RH, Burton JL, Ebling FJG, eds. *Textbook of dermatology*. 5th edn. Oxford: Blackwell Scientific Publications, 1992: 1675–1698.
 13. Scully C, El-Kom M. Lichen planus: review and update on pathogenesis. *J Oral Pathol* 1985; 14: 431–458.
 14. Scully C. The oral cavity. In: Champion RH, Burton JL, Ebling FJG, eds. *Textbook of dermatology*. 5th edn. Oxford: Blackwell Scientific Publications, 1992: 2689–2759.
 15. Shklar G. Oral leukoplakia. *N Engl J Med* 1986; 315: 1544–1545.
 16. Moll R, Franke WW, Schiller DL. The catalog of human cytokeratins; patterns and expression in normal epithelia. *Cell* 1982; 31: 11–14.
 17. Régnier M, Darmon M. Human epidermis reconstructed in vitro: a model to study keratinocyte differentiation and its modulation by retinoic acid. *In Vitro Cell Dev Biol* 1989; 25: 1000–1008.
 18. Egelrud T, Régnier M, Sondell B, Shroot B, Schmidt R. Expression of stratum corneum chymotryptic enzyme in reconstructed human epidermis and its suppression by retinoic acid. *Acta Derm Venereol (Stockh)* 1993; 73: 181–184.