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Epidermal Overexpression of Stratum Corneum Chymotryptic Enzyme in Mice: A Model for Chronic Itchy Dermatitis

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Identification of tissue-specific mechanisms involved in the pathophysiology of inflammatory skin diseases could offer new possibilities to develop effective therapies with fewer systemic effects. The serine protease stratum corneum chymotryptic enzyme is preferentially expressed in cornifying epithelia. We have previously reported on increased expression of the stratum corneum chymotryptic enzyme in psoriasis. Here is reported an increased epidermal expression of stratum corneum chymotryptic enzyme also found in chronic lesions of atopic dermatitis. Transgenic mice expressing human stratum corneum chymotryptic enzyme in suprabasal epidermal keratinocytes were found to develop pathologic skin changes with increased epidermal thickness, hyperkeratosis, dermal inflammation, and severe pruritus. The results suggest that stratum corneum chymotryptic enzyme may be involved in the pathogenesis of inflammatory skin diseases, and that stratum corneum chymotryptic enzyme and related enzymes should be evaluated as potential targets for new therapies. ***Key words: itch/kallikrein/serine protease/skin/transgenic mice. J Invest Dermatol 118:444–449, 2002***

Common inflammatory skin diseases may result in severe handicap by causing reduced function, stigmatization, and almost unbearable sensory symptoms. For many of these diseases available treatments are still unsatisfactory. In conditions such as psoriasis and atopic dermatitis there is good evidence in favor of a central pathophysiologic role for the immune system (for reviews see Nickoloff, 1999; Leung, 1999). It seems likely that the development of the various disease-specific skin lesions and symptoms is the result of interactions at the cellular and molecular level between the immune system and skin-derived structures and molecules. Skin-specific cells produce a wide variety of adhesion molecules, cytokines, and growth factors by which they can communicate with the immune system. This type of molecules are, however, more or less generally present in cells and tissues throughout the body. Accordingly, the most effective treatments in, for example, psoriasis, which interfere with this type of general mechanism, do not have skin-specific effects, and have limited usefulness due to their systemic mode of action and unwanted side-effects. The chances of finding more specific treatments for a given skin disease could improve if one could find skin-specific structures or molecules involved in the pathogenesis of the disease.

Many of the tissue-specific molecular mechanisms of the skin are associated with the formation and turnover of the barrier-forming outermost layer of the epidermis, the stratum corneum, consisting of cornified epithelial cells surrounded by highly organized lipids. The stratum corneum is continuously being formed in the process of epidermal differentiation. In our efforts to understand the mechanisms by which a constant thickness of the stratum corneum is maintained via a continuos desquamation of surface cells, we identified two new human serine proteases, stratum corneum chymotryptic enzyme (SCCE; also named kallikrein 7) and stratum corneum tryptic enzyme (SCTE; also named kallikrein 5) (Hansson *et al*, 1994; Brattsand and Egelrud, 1999; Diamandis *et al*, 2000a; Ekholm *et al*, 2000). Both enzymes belong to the kallikrein group of serine proteases, the genes of which are localized to a short stretch of chromosome 19q13.3–q13.4 (Diamandis *et al*, 2000b). The expression of SCCE and SCTE seems to be restricted to squamous epithelia undergoing cornification and in which there is a need for desquamation (Ekholm *et al*, 2000). Results in this study are reported to elucidate the possible involvement of one of these proteases, SCCE, in skin pathology. We characterized the human and murine *scce* genes, and produced transgenic mice over-expressing human *scce* mRNA under a viral promoter. The only phenotypic changes observed were found in the skin, which showed several histologic changes similar to those seen in

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Abbreviations: SCCE, stratum corneum chymotryptic enzyme; SCTE, stratum corneum tryptic enzyme.

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inflammatory skin diseases in humans. In addition, the transgenic mice showed signs of severe itch. We also found evidence of overexpression of SCCE in chronic lesions of atopic dermatitis in humans, as we have recently shown for psoriasis (Ekholm and Egelrud, 1999). Taken together, our results give support for the idea that SCCE and related enzymes may be involved in the pathophysiology of inflammatory skin diseases, and thus are potential targets for organ-specific treatments.

MATERIALS AND METHODS

Human and murine *scce* genes The human *scce (hscce)* gene was isolated from a human leukocyte genomic library (Clontech, Palo Alto, CA) by using cDNA probes derived from the human *scce* cDNA (Hansson *et al*, 1994). Overlapping clones were isolated and the entire structural gene was sequenced and analyzed. (GenBank accession no. AF332583; also see GenBank AF166330and Yousef *et al*, 2000)

To isolate the murine *scce* (*mscce*) gene cDNA probes derived from the murine *scce* cDNA (Bäckman *et al*, 1999) were used to screen and isolate clones from a 129SVJ Lambda Fix II genomic library (Stratagene, La Jolla, CA). The entire gene sequence was determined and constructed as above (GenBank accession no. AF339930).

Transgenic mice The *scce* genomic DNA was modified by insertion of *Hin*dIII linkers 20 bp upstream of the start codon in exon 2, and 4.8 kb downstream of the stop codon, respectively. The resulting *Hin*dIII *scce* fragment was then ligated to a 325 bp *Bam*HI/*Hin*dIII fragment containing the *SV40e* enhancer and promoter elements and cloned into pBluescript SK$^+$ (Stratagene), resulting in pAM119. For gene transfer, the plasmid pAM119 was digested with *Bam*HI and *Cla*I and the *SV40e/scce* fragment of about 10.7 kb was isolated and purified by electroelution before microinjection into one-cell stage mouse fertilized oocytes. Transgenic mice were generated in C57BL/6JxCBA-f2 embryos by standard microinjection procedures (Hogan *et al*, 1986). Three transgenic lines Tg103$^{SV40e\text{–}hscce}$, Tg107$^{SV40e\text{–}hscce}$ and Tg1010$^{SV40e\text{–}hscce}$) were established by breeding heterozygous mice with C57BL/6JxCBA or with C57BL/6J. Littermates were used as controls. To identify transgenic animals, DNA was extracted from tail biopsies of 3 wk old mice and analyzed by southern blot analyses or polymerase chain reaction (PCR) with probes and primers designed to discriminate between human and murine genomic *scce* DNA. Tissue specimens for RNA or protein analyses were collected at different ages and immediately frozen in liquid nitrogen. Specimens were also processed for histologic analyses. The animal experiments had been approved by the Regional Ethical Committee for Animal Experiments.

Skin biopsies These were taken from human volunteers and patients after informed consent and with the approval of the Human Research Ethics Committee, Umeå University.

RNA isolation, cDNA synthesis, and real-time quantitative PCR Total RNA were prepared from 50 to 300 mg of tissue using RNA STAT-60 (Tel-Test "B", Friendswood, TX) according to the manufacturer. From each RNA preparation 50 μg was DNase treated (Ausubel *et al*, 1992) using RQ1 DNase (Promega, Madison, WI), and about 1.6 μg total RNA from each tissue was used for cDNA synthesis. From each tissue RNA samples from three animals with the same genetic background were pooled. Superscript Pre-amplification System for First Strand cDNA Synthesis (Life Technologies, Gaithersburg, MD) was used according to the manufacturer, using Oligo d(T)$_{12\text{–}18}$ primer. The synthesized cDNA was diluted 100 × in water. Real-time quantification was performed in triplicate for each cDNA with primers and probes derived from exons 4 and 5 of the *hscce* gene and designed to discriminate between *hscce* and *mscce* genes: As forward primer (5′-GCG-AACCCCCTGGAACAA-3′: position 427–444 of the human cDNA sequence in exon 4); and reverse primer (5′-ACATCCACGCAC-ATGAGGTCA-3′; position 490–510 of the human cDNA sequence in exon 5) were used. The real-time amplification probe (5′-CCTGTACTGTCTCCGGCTGGGGCACTACC-3′; position 445–473 of the human cDNA sequence in exon 4), was labeled with the reporter fluorescent dye FAM in the 5′-end and the quencher fluorescent dye TAMRA in the 3′-end. The amplification of PCR products and real-time detection were performed in ABI Prism 7700 Sequence Detection System (PE Applied Biosystems, Foster City CA). Amplification of a part of murine acidic ribosomal phosphoprotein P0 (GenBank accession no. X15267) was used as endogenous control for the real-time quantification studies. The relative quantification was calculated according to the formula: relative amount of mRNA = $2^{-\Delta C_T}$, where ΔC_T is the difference in threshold values between the target and the endogenous control (User Bulletin 2, PE Applied Biosystems).

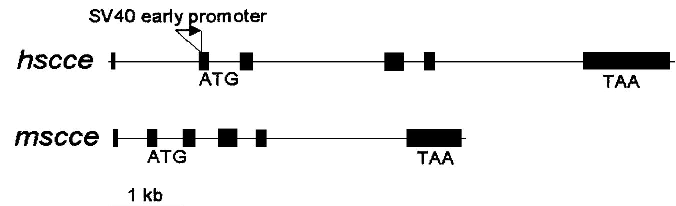

Figure 1. Organization of the human and murine structural genes and the recombinant *sv40e/hscce* gene. The *scce* genes from humans and mouse both contain six exons, here indicated as black boxes, and have the translational start located in exon 2, and the stop codon in exon 6. The most apparent difference between the structural genes from the two species is that the introns are longer in the human *scce* gene. To construct the recombinant gene, the *sv40e* transcriptional regulatory element was inserted 20 nt upstream of the start codon of the human *scce* gene, indicated by the *arrow*.

Protein and histologic analyses Polyclonal antibodies to recombinant human SCCE were prepared and affinity purified as described (Sondell *et al*, 1996). These antibodies are reactive towards human SCCE and pro-SCCE, as well as murine SCCE.

Tissue extracts for the enzyme-linked immunosorbent assay were prepared by homogenization of 200–400 mg frozen tissue in 1 ml dH_2O containing a mixture of protease inhibitors (Complete Protease Inhibitor Cocktail Tablets, Boehringer Mannheim, Germany), followed by centrifuging at 20,000 × *g* for 30 min at 4°C. Protein concentrations were determined by reaction with bicinchoninic acid, with bovine serum albumin as standard. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis, zymography, and immunoblotting were carried out as described (Ekholm *et al*, 2000). Approximately 0.1 g of mouse skin was homogenized in 10 ml of 1 M acetic acid and extracted overnight at 4°C. After clearing by centrifugation, extracts were aliquoted, lyophilized, and resolubilized in electrophoresis sample buffer. For histology and immunohistochemistry (Sondell *et al*, 1996; Ekholm and Egelrud, 1998) samples were either formaldehyde fixed and paraffin embedded according to routine protocols, or snap frozen after fixation for 2 h in formaldehyde.

Quantitative measurements of SCCE in tissue extracts were carried out with a sandwich enzyme-linked immunosorbent assay with unconjugated and alkaline phosphatase conjugated SCCE-specific antibodies and recombinant human pro-SCCE as standard.

Data were analyzed with a two-tailed, unpaired Student's t test.

RESULTS

The human and murine *scce* genes The amino acid sequences (as deduced from cDNA) of human and murine SCCE show about 80% similarity (Hansson *et al*, 1994; Bäckman *et al*, 1999). The genomic organization of the human and murine *scce* structural genes are schematically shown in **Fig 1**. Overall, the organization of the exon–intron structure of the two genes is similar, but due to shorter introns the murine gene is smaller. In the human gene the translation initiation site is found 60 nt downstream the 5′-end of exon 2, and a potential TATA-box approximately 35 bp upstream of exon 1. The murine initiation codon is positioned within the second exon, 39 nt downstream of the intron–exon junction.

Phenotypic skin changes in *SV40e–hscce* transgenic mice In order to study the effects of modified *scce* expression we constructed mice transgenic for the human *scce* gene under control of the *SV40e* enhancer and promoter element. Mice transgenic for *SV40e–hscce* integrated at a single site were obtained from three founders in the C57BL/6JxCBA genetic background. At the age of 4–5 wk macroscopic skin changes were apparent in mice of the Tg1010 line (Tg1010$^{SV40e\text{–}hscce}$), whereas no obvious phenotype was seen in mice of the two other lines. The skin changes were noted as a loss of hair from a narrow zone around the eyes in mice 4–5 wk of age. In older mice there was an apparent thinning of body hair in general, and a luster-less appearance of the coat. We could see no

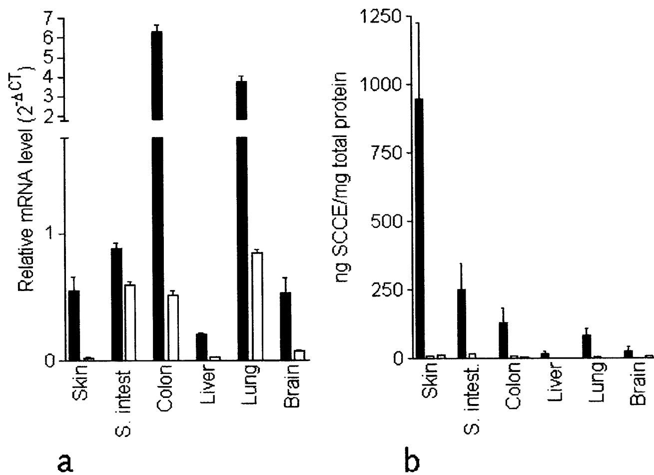

Figure 2. *scce* mRNA and SCCE protein in transgenic animals and controls. (*a*)Real-time quantitative PCR analyses of recombinant human *scce* mRNA in various tissue preparations from the transgenic lines Tg1010$^{SV40e\text{–}hscce}$ (*black bars*) and Tg107$^{SV40e\text{–}hscce}$ (*empty bars*). Analyses in triplicate were carried out on RNA samples comprising pooled material from three animals from each line. The murine acidic ribosomal phosphoprotein P0 was used as internal standard. Mean and SD. (*b*)Enzyme-linked immunosorbent assay analyses of SCCE-protein in various tissues from the transgenic lines Tg1010$^{SV40e\text{–}hscce}$ (*black bars*) and Tg107$^{SV40e\text{–}hscce}$ (*empty bars*), and nontransgenic siblings (*gray bars*). Analyses in triplicate were carried out on pooled extracts from three animals from each line and controls. Mean and SD.

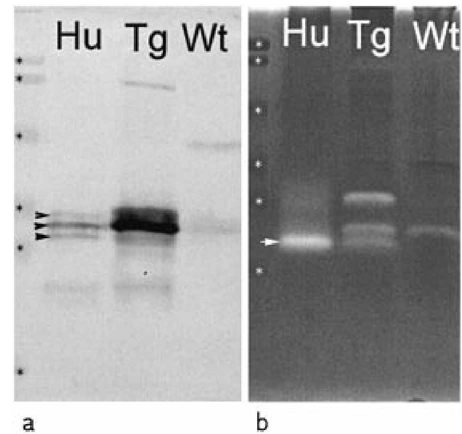

Figure 3. Pro-SCCE and active SCCE in skin from Tg1010$^{SV40e\text{–}hscce}$. *Hu* = extract of human plantar stratum corneum, prepared as described (Ekholm *et al*, 2000); *Tg* = extract of skin from Tg1010$^{SV40e\text{–}hscce}$; *Wt* = extract of skin from wild-type littermate. (*a*)Immunoblot with SCCE-specific antibodies, reduced samples. *Arrowheads* denote, from top to bottom, glycosylated pro-SCCE, mixture of unglycosylated pro-SCCE and glycosylated SCCE, and unglycosylated SCCE (Hansson *et al*, 1994). Amount of sample applied corresponding to 0.1 mg and 4.5 mg of skin for Tg and Wt, respectively. (*b*)Zymography in 12.5% acrylamide gel with 1% casein; nonreduced samples. Amount of sample applied corresponding to 0.4 mg and 4.5 mg of skin for Tg and Wt, respectively. *Arrow* denotes SCCE. To the far left (marked by asterisks) molecular weight markers; from top 106, 81, 47.5, 35.3, 28.2, and 20.8 kDa, respectively.

gross changes in hair shaft morphology. On the back the skin surface was covered with fine scales. From the age of 5–6 wk the animals showed signs of itch with scratching, the frequency of which increased with time. We therefore performed further studies on Tg1010$^{SV40e\text{–}hscce}$ in which nontransgenic littermates and transgenic mice from one of the two other lines, Tg107 (Tg107$^{SV40e\text{–}hscce}$), served as controls.

Diagnostic necropsies were carried out with microscopic examination of brain, cerebellum, intestines (duodenum/jejunum, ileum, colon, rectum), skin, heart, liver, lung, salivary gland, spleen, thymus, and thyroid. In littermate controls (3 wk, n = 5; 5 wk, n = 5) and Tg107$^{SV40e\text{–}hscce}$ (5 wk, n = 4) no significant macro- or microscopic abnormalities were observed. In Tg1010$^{SV40e\text{–}hscce}$ abnormalities were found in the skin of all animals examined, but in no other organs or tissues. In mice 3 wk of age (n = 4) skin changes included a mild to moderate epidermal hyperplasia and hyperkeratosis, and a mild cellular inflammatory reaction with mixed leukocytes in the upper dermis. In animals 5 wk of age (n = 4) the skin abnormalities were of the same type but more pronounced with a marked acanthosis-like hyperplasia and hyperkeratosis of the epidermis. In addition, the number of mast cells in the dermis was increased in some of the animals. Leukocyte invasion of the epidermis was occasionally found and then manifested as small groups of granulocytes within the thickened cornified layer, which at these sites was parakeratotic.

The level of expression of *hscce* in *SV40e–hscce* transgenic mice correlate with phenotypic skin changes Analyses by real-time quantitative PCR of *hscce* mRNA (**Fig 2*a***) in a number of tissues from transgenic mice consequently showed higher values for Tg1010$^{SV40e\text{–}hscce}$ as compared with Tg107$^{SV40e\text{–}hscce}$. The highest relative levels were found in the intestines and lungs. The most pronounced difference in *hscce* expression between the two transgenic lines was seen in skin, in which the relative level of *hscce* mRNA was about 25 times higher in Tg1010$^{SV40e\text{–}hscce}$. Enzyme-linked immunosorbent assay (**Fig 2*b***) showed amounts of SCCE protein close to or below the detection limit in tissues from Tg107$^{SV40e\text{–}hscce}$ and normal controls. In Tg1010$^{SV40e\text{–}hscce}$ SCCE protein was readily detected in several tissues, including the skin, intestines, and lung, the relative level (ng per mg total protein) being highest in the skin. It thus seemed plausible to us that the skin changes seen in Tg1010$^{SV40e\text{–}hscce}$ but not in Tg107$^{SV40e\text{–}hscce}$ was related to a higher expression of *hscce* mRNA and SCCE protein in Tg1010$^{SV40e\text{–}hscce}$.

Immunoblotting with SCCE-specific antibodies showed small amounts of a component with a molecular mass similar to human SCCE in the skin of control mice, whereas much higher amounts of the corresponding component were found in the skin of Tg1010$^{SV40e\text{–}hscce}$ (**Fig 3*a***). A proteolytic enzyme corresponding to human SCCE could be detected in extracts of skin from Tg1010$^{SV40e\text{–}hscce}$, but not in controls by zymography (**Fig 3*b***; the amounts of active murine SCCE are too low to be detected under the experimental conditions used). These results suggest that a fraction of the human pro-SCCE produced in the skin of Tg1010$^{SV40e\text{–}hscce}$ is converted to proteolytically active enzyme. This was supported also by the immunoblotting experiments (**Fig 3*a***), where small amounts of a component corresponding to active human SCCE was found. In addition to SCCE, skin extracts of Tg1010$^{SV40e\text{–}hscce}$ contained increased amounts of a proteolytic enzyme with an apparent molecular mass of about 28 kDa, the nature of which is presently not known.

Extended phenotypic analyses: overexpression of *hscce* causes itchy dermatitis Three male Tg1010$^{SV40e\text{–}hscce}$ were mated with wild-type C57BL/6J females, resulting in six litters with a total of 40 mice. Of these 19 (eight Tg1010$^{SV40e\text{–}hscce}$) were killed at the age of 7–8 wk and 21 (11 Tg1010$^{SV40e\text{–}hscce}$) were followed to the age of 13–14 wk. In the latter group scratch movements with the legs were quantified.

Of the 11 transgenic mice followed for 13–14 wk, eight animals (73%) showed signs of itch (at least one period of scratching with hind or forepaws per minute) at the age of 10–11 wk. The frequency of scratching varied. Whereas some animals showed weak or moderate signs of itch, other animals spent most of their time scratching (**Fig 4**). At the age of 13–14 wk there was a 7–10% reduction in weight in transgenic mice as compared with wild-type

litter mates (mean for males 27.0 g *vs* 30.0 g; p = 0.022; mean for females 21.7 g *vs* 23.5 g; p = 0.033).

After killing the animals, tissues (dorsal skin, large and small intestines, and lung) were prepared for microscopic analyses. Examination of routinely processed skin samples was carried out blindly (the examiner was not informed about genotype or scratching behavior). In all cases but one, typical skin changes allowed transgenic animals to be correctly identified. Epidermal thickness was 55 μm (SD = 21 μm; n = 19) for transgenic animals, and 15 μm (SD = 2.6 μm; n = 21; p < 0.001) for controls. There was no statistically significant difference in epidermal thickness between younger (7–8 wk) and older (13–14 wk) transgenic animals. Other histologic findings in the skin of transgenic animals (**Fig 5*a***, controls **Fig 5*b***) were similar to those found at diagnostic necropsy. In addition to the marked acanthosis and hyperkeratosis there was an infiltrate of inflammatory cells that was mainly confined to the dermal part but occasionally seen also in the epidermis. We also noted increased epithelial thickness of adnexal structures (hair follicle walls, and sebaceous glands and ducts). No differences could be detected by routine histology between transgenic animals and normal littermates in other organs examined (results not shown).

We next analyzed the pattern of SCCE expression by immunohistology with SCCE-specific antibodies. In skin from transgenic animals there was strong labeling of keratinocytes in the suprabasal parts of interfollicular epidermis, including the thickened cornified layer. In hair follicles and sebaceous ducts only luminal parts, including the cornified lining of follicles and ducts, were stained (**Fig 5*c***). This was in marked contrast to basal cells of interfollicular epidermis and the major peripheral parts of hair

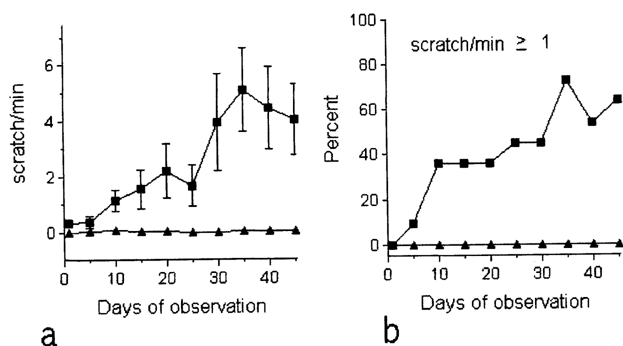

Figure 4. Scratching behavior of *scce*-transgenic mice Tg1010$^{SV40e-hscce}$. Twenty-one mice (11 transgenics, five females; 10 wild-type litter mates, two females) were observed every fifth day for 45 d, starting when the mice were 5–6 wk of age. At each observation point mice were transferred to individual cages, and episodes of scratching with hind or front paws were counted during three 5 min periods with 2.5 min lapsing from the transfer to the cage to the first counting, and between counting periods. The results for the three observation periods were pooled and the number of episodes of scratching per minute calculated. In *a* the number of episodes of scratching (mean and SEM for all animals in each group) is shown, in *b* the percentage of animals with at least one episode of scratching per minute is given. *Filled squares*: Tg1010$^{SV40e-hscce}$, *filled triangles:* wild-type litter mates.

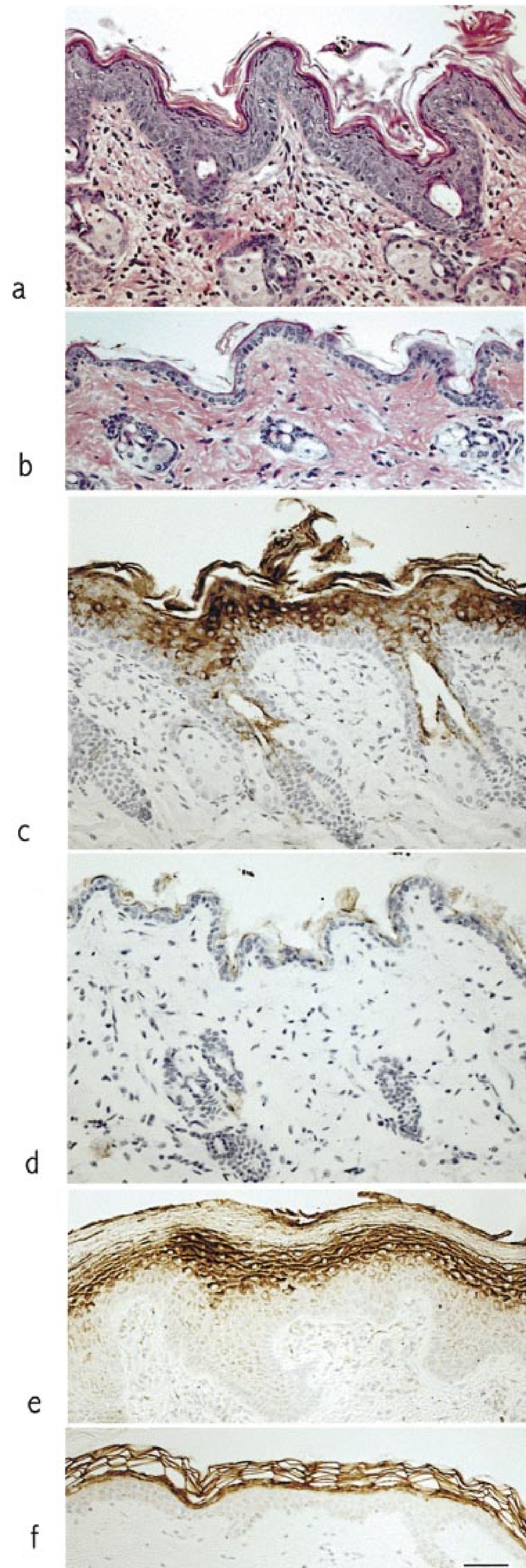

Figure 5. Histology and SCCE immunohistology of skin from *scce* Tg1010$^{SV40e-hscce}$ and control; comparison with normal human skin and chronic lesion of atopic dermatitis. Formaldehyde-fixed and paraffin-embedded samples. (*a*, *b*) Stained with hematoxylin and eosin. (*c*–*f*) Immunoperoxidase staining with SCCE-specific antibodies, contra-staining with hematoxylin. (*a*, *c*) Tg1010$^{SV40e-hscce}$, 5 wk of age. (*b*, *d*) Nontransgenic littermate. (*e*) Atopic dermatitis. (*f*) Normal human skin. *Scale bar*: 50 μm.

follicles and sebaceous ducts and glands, where no or very weak labeling by the antibodies was seen. The unexpectedly low expression of SCCE in basal cells may suggest that regulatory elements in the *hscce* gene overrides the *SV40e* promoter and direct expression to more differentiated cells. In controls there was a relatively weak labeling of high suprabasal cells, the stratum corneum, and luminal parts of hair follicles (**Fig 5*d***). This pattern was similar to that previously described for normal human epidermis (Ekholm and Egelrud, 1998). In the intestines of transgenic animals SCCE-specific labeling was seen in irregularly distributed epithelial cells. Stained cells were more numerous at the tips of villi in the small intestine and in the luminal parts of colonic epithelium. In the lungs of transgenic animals apical parts of bronchiolar epithelial cells were weakly labeled. At higher antibody concentrations there appeared to be diffuse labeling also of the alveolar epithelium. No SCCE-specific staining was seen in the intestines and lungs of control animals (results for intestines and lung not shown).

Similarities between skin changes in Tg1010$^{SV40e\text{–}hscce}$ and chronic lesions of atopic dermatitis in humans In previous preliminary studies in humans with various skin diseases we had noted that in chronic lesions characterized by acanthosis, hyperkeratosis, and itch, the number of suprabasal epidermal cell layers expressing SCCE was often increased. We had seen similar changes in psoriasis (Ekholm and Egelrud, 1999). From these results it was not possible to tell whether the changes in SCCE expression played a significant pathophysiologic role, or if they were merely secondary to other changes in the epidermis. As the results presented herein suggested that increased epidermal expression of SCCE could by itself cause, in addition to severe itch, skin changes, which in several aspects mimic chronic lesions seen in, e.g., atopic dermatitis, we extended this study to include a number of patients with this disease. Biopsies were taken from chronic eczematous lesions on the flexural sides of lower arms of five adults with atopic dermatitis and processed for microscopy as above. Biopsies from corresponding sites were obtained from volunteers. In routine stained sections (not shown) the lesions showed, as expected, marked acanthosis, hyperkeratosis, and a sparse dermal infiltrate consisting mainly of lymphocytes. Immunohistology with SCCE-specific antibodies showed a drastic increase in the number of labeled suprabasal cell layers as compared with controls (**Fig 5*e*, *f***). As regards the acanthosis, hyperkeratosis, and pattern of SCCE-specific staining the differences seen between lesional and normal skin were strikingly similar to those seen between the skin of Tg1010$^{SV40e\text{–}hscce}$ and control mice.

DISCUSSION

In a number of previous studies we collected evidence that SCCE may take part in stratum corneum turnover and desquamation. Although *in vitro* experiments as well as the expression pattern and tissue localization of SCCE suggested that SCCE may indeed take part in desquamation, and that its function may be epidermis-specific (Egelrud, 2000), this remained to be confirmed by more direct evidence. Further studies on the function of SCCE was necessitated also by the fact that we had found expression of SCCE at the RNA level also in a number of other tissues in addition to skin, although at much lower levels (Hansson *et al*, 1994; Bäckman *et al*, 1999). By construction of a mouse transgenic for the human *scce* gene linked to the *SV40e* promoter, we speculated that SCCE would be produced in a number of organs, and that there would be phenotypic changes that would be informative as regards the physiologic function as well as effects of overexpression of SCCE. The results presented in this report strongly suggest that the major site of function of SCCE may be the skin and, in particular, the epidermis. Another important finding was that SCCE may be involved in skin pathophysiology.

We have thus shown that, whereas there is an elevated expression of SCCE in lesions of human skin diseases such as psoriasis (Ekholm and Egelrud, 1999) and atopic dermatitis, overexpression of SCCE in the epidermis of transgenic mice causes skin changes that have similarities to inflammatory skin diseases in humans.

In *SV40*–h*scce* transgenic mice with phenotypic skin changes aberrant expression of SCCE, RNA as well as protein, was found also in other organs, especially the small and large intestine, and lungs. The fact that no pathologic changes were seen in these organs may be explained either by a resistance or unresponsiveness to effects mediated by SCCE, or by a lack of SCCE-activating enzymes in unaffected organs. SCCE (human as well as murine) is produced as an inactive precursor, which is converted to active protease by tryptic cleavage at a conserved site (Hansson *et al*, 1994; Bäckman *et al*, 1999). The enzyme responsible for SCCE activation in the epidermis has not yet been identified. Our finding that skin of transgenic animals contained increased amounts of active SCCE suggests that elevated levels of pro-SCCE in the epidermis also leads to increased proteolytic activity. On the other hand, these results also suggest that conversion of pro-SCCE to active SCCE is under strict control, as only a minor fraction of the total SCCE protein in the skin was present as active enzyme.

The mechanisms by which SCCE can cause a thickened epidermis with hyperkeratosis, a dermal inflammatory infiltrate, and itch remain to be elucidated. According to the current view the SCCE precursor is synthesized in high suprabasal epidermal keratinocytes and stored in lipid-rich lamellar bodies. In the process in which a terminally differentiated keratinocyte is transformed from a viable cell to a corneocyte, i.e., a building block of the cornified surface layer of the epidermis – the stratum corneum – the contents of the lamellar bodies, including the SCCE precursor, are secreted to the extracellular space, where conversion of pro-SCCE to active protease is taking place (Sondell *et al*, 1995). One possibility is that SCCE, which has been activated as postulated, diffuses through the epidermis to the superficial parts of the dermis, and via its proteolytic activity induces epidermal thickening, dermal inflammation, and activation of itch-mediating nerve endings. Alternatively an increased proteolytic activity in the transition zone between viable epidermal layers and the stratum corneum may lead to the release of mediators, which diffuse to other parts of the skin where they give rise to the observed changes. A possible candidate may be interleukin 1β. We have recently shown that this pro-inflammatory cytokine is activated by an alternative mechanism in plantar and psoriatic epidermis, possibly involving SCCE (Nylander-Lundqvist and Egelrud, 1997a, b; Brattsand and Egelrud, 1998). The proliferative response of the epidermis could be a result either of direct effects of released mediators on keratinocytes, or an effect that is secondary to the dermal inflammation. A third possibility is that the altered skin phenotype reflects adaptive responses to a deterioration of the barrier function of the stratum corneum caused by increased proteolytic degradation of structures responsible for intercellular cell cohesion in the cornified layer. Experiments aimed at elucidation of these questions are under way.

Mechanisms of itch, and the molecular basis of differences between inflammatory skin conditions as regards degree and quality of associated pruritus, are poorly understood. The only fully established inflammatory itch mediator is histamine, which, however, is not likely to be responsible for the pruritus in diseases other than various forms of urticaria (Greaves and Wall, 1996). It has long been known that proteolytic enzymes, when injected intradermally in humans, can cause itch (Arthur and Shelley, 1955). The mechanisms involved may vary between enzymes used. Whereas pancreatic trypsin and mast cell chymase appeared to cause itch via release of histamine from mast cells, the itch caused by papain and plasma kallikrein was likely not to involve histamine (Hägermark *et al*, 1972; Hägermark, 1974). We have seen no changes in scratching behavior in SCCE-transgenic mice treated with the antihistaminic drug loratidine at 1.7 or 3.6 μg per g (T. Egelrud and A. Ny, unpublished results), suggesting that histamine is not a major cause of pruritus in these animals. The fact that signs of itch were not seen before the age of 5 wk, whereas

overexpression of SCCE was found also in younger animals, may suggest that the pruritus was not a direct effect of SCCE, but was secondary to the epidermal and dermal changes. In this context it should be noted that conditions in experimental settings where enzymes are injected intradermally may differ significantly from the situation in our SCCE-transgenic animals, in which production of active protease may take place in high suprabasal parts of the epidermis.

Recently a direct association between a defect in epidermal barrier function and aberrant proteolysis in an inherited human condition with severe skin disease has been described. Strong evidence has been presented that the disease-causing mutations in Netherton's syndrome are localized to the SPINK5 gene coding for the multidomain serine protease inhibitor LEKTI (Chavanas *et al*, 2000; Sprecher *et al*, 2001). Furthermore, associations between polymorphisms in SPINK5 and atopic dermatitis have been reported (Walley *et al*, 2001). These results, together with our present results, suggest that increased activity of serine proteases present in the skin may indeed play a significant part in skin pathophysiology. Epidermal serine proteases such as SCCE should therefore be explored as potential targets for new therapeutic principles in inflammatory skin diseases.

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REFERENCES

Arthur RP, Shelley WB: The role of proteolytic enzymes in the production of pruritus in man. *J Invest Dermatol* 25:341–346, 1955

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K, (eds): *Current Protocols in Molecular Biology*. New York: Greene & Wiley, 1992

Bäckman A, Stranden P, Brattsand M, Hansson L, Egelrud T: Molecular cloning and tissue expression of the murine analog to human stratum corneum chymotryptic enzyme. *J Invest Dermatol* 113:152–155, 1999

Brattsand M, Egelrud T: Purification and characterization of interleukin 1 beta from human plantar stratum corneum. Evidence of interleukin 1 beta processing in vivo not involving interleukin 1 beta convertase. *Cytokine* 10:506–513, 1998

Brattsand M, Egelrud T: Purification, molecular cloning, and expression of a human stratum corneum trypsin-like serine protease with possible function in desquamation. *J Biol Chem* 274:30033–30040, 1999

Chavanas S, Bodemer C, Rochat A, *et al*: Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. *Nat Genet* 25:141–142, 2000

Diamandis EP, Yousef GM, Clements J, *et al*: New nomenclature for the human tissue kallikrein gene family. *Clin Chem* 11:1855–1858, 2000a

Diamandis EP, Yousef GM, Liu-Ying L, Magklara A, Obiezu CV: The new human kallikrein gene family—implications in carcinogenesis. *Trends Endocrinol Metab* 11:54–60, 2000b

Egelrud T: Desquamation in the stratum corneum. *Acta Derm Venereol (Suppl.) (Stockh)* 208:44–45, 2000

Ekholm E, Egelrud T: The expression of stratum corneum chymotryptic enzyme in human anagen hair follicles: further evidence for its involvement in desquamation-like processes. *Br J Dermatol* 139:585–590, 1998

Ekholm E, Egelrud T: Stratum corneum chymotryptic enzyme in psoriasis. *Arch Dermatol Res* 291:195–200, 1999

Ekholm IE, Brattsand M, Egelrud T: Stratum corneum tryptic enzyme in normal epidermis: a missing link in the desquamation process? *J Invest Dermatol* 114:56–63, 2000

Greaves MW, Wall PD: Pathophysiology of itching. *Lancet* 348:938–940, 1996

Hägermark Ö: Studies on experimental itch induced by kallikrein and bradykinin. *Acta Derm Venereol (Stockh)* 54:397–400, 1974

Hägermark Ö, Rajka G, Bergqvist U: Experimental itch in human skin elicited by rat mast cell chymase. *Acta Derm Venereol (Stockh)* 52:125–128, 1972

Hansson L, Strömqvist M, Bäckman A, Wallbrandt P, Carlstein A, Egelrud T: Cloning, expression, and characterization of stratum corneum chymotryptic enzyme. A skin-specific human serine proteinase. *J Biol Chem* 269:19420–19426, 1994

Hogan B, Constanini F, Lazy E: *Manipulating the Mouse Embryo: a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Laboratory Press, 1986

Leung DYM: Pathogenesis of atopic dermatitis. *J Allergy Clin Immunol* 104:S99–S108, 1999

Nickoloff BJ: The immunologic and genetic basis of psoriasis. *Arch Dermatol* 135:1104–1110, 1999

Nylander-Lundqvist E, Egelrud T: Biologically active, alternatively processed interleukin-1 beta in psoriatic scales. *Eur J Immunol* 27:2165–2171, 1997a

Nylander-Lundqvist E, Egelrud T: Formation of active IL-1 beta from pro-IL-1 beta catalyzed by stratum corneum chymotryptic enzyme in vitro. *Acta Derm Venereol (Stockh)* 77:203–206, 1997b

Sondell B, Thornell LE, Egelrud T: Evidence that stratum corneum chymotryptic enzyme is transported to the stratum corneum extracellular space via lamellar bodies. *J Invest Dermatol* 104:819–823, 1995

Sondell B, Dyberg P, Anneroth GK, Ostman PO, Egelrud T: Association between expression of stratum corneum chymotryptic enzyme and pathological keratinization in human oral mucosa. *Acta Derm Venereol (Stockh)* 76:177–181, 1996

Sprecher E, Chavanas S, DiGiovanna JJ, *et al*: The spectrum of pathogenic mutations in SPINK5 in 19 families with Netherton syndrome: implications for mutation detection and first case of prenatal diagnosis. *J Invest Dermatol* 117:179–187, 2001

Walley AJ, Chavanas S, Moffatt MF, *et al*: Gene polymorphism in Netherton and common atopic disease. *Nat Genet* 29:175–178, 2001

Yousef GM, Scorilas A, Magklara A, Soosaipillai A, Diamandis EP: The KLK7 (PRSS6) gene, encoding for the stratum corneum chymotryptic enzyme is a new member of the human kallikrein gene family—genomic characterization, mapping, tissue expression and hormonal regulation. *Gene* 254:119–128, 2000