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Filaggrin Immunoreactive Composite Keratohyalin Granules Specific to Acrosyringia and Related Tumours

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The luminal cell layer of acrosyringia contains heterogeneous globular keratohyalin granules, some of which contain basophilic and eosinophilic components. Using immunoelectron microscopy we found that the majority of the granules, which are basophilic, are strongly reactive to an anti-filaggrin antibody, while the minority, which are eosinophilic, are not. Similar heterogeneous keratohyalin granules were observed in syringomas and in an eccrine syringofibroadenoma. Since such granules are not normally observed in other human cutaneous epithelia, it is suggested that these composite keratohyalin granules with partial filaggrin immunoreactivity might serve as a useful marker for acrosyringial differentiation in both normal and pathological material. ***Key words: Eccrine sweat duct; Immunoelectron microscopy; Syringoma; Eccrine syringofibroadenoma.***

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Filaggrin is a protein that is expressed in differentiated epidermal keratinocytes (1, 2). It is synthesized as the inactive phosphorylated precursor, profilaggrin, which is stored in irregularly shaped electron-dense keratohyalin granules (3, 4) that were recently named F-granules because of their filaggrin immunoreactivity (3). The morphology of keratohyalin granules varies among epithelial tissues from different species, anatomical sites, and developmental stages (5–10). The variations include size, shape, electron density and cytochemical properties.

It has recently been reported that the luminal cell layers of acrosyringia have two types of globular keratohyalin granules which vary in electron density (9). The less electron-dense forms occur in small numbers, whereas the more electron-dense forms are more numerous. Both types of granules may combine to form a composite granule. Because the biochemical composition of these keratohyalin granules is unknown, we studied filaggrin immunoreactivity within these granules using immunoelectron microscopy. We also examined some eccrine sweat gland tumours to see whether these characteristic granules could serve as an acrosyringial differentiation marker in pathological tissues.

MATERIAL AND METHODS

Studies of normal human skin and acrosyringia

Samples of normal human thigh and sole skin from adult men and women were obtained under local anesthesia. For light microscopic observation, paraffin-embedded sections of formalin-fixed material were stained with hematoxylin and eosin. For transmission electron microscopy, the tissues were sequentially fixed with 5% glutaraldehyde in 0.08 M cacodylate buffer and with 1% osmium tetroxide in distilled water. After en bloc staining with uranyl acetate, the tissues were dehydrated with ethanol and embedded in Araldite (Oken, Tokyo, Japan). Ultrathin sections were stained with uranyl acetate and lead citrate.

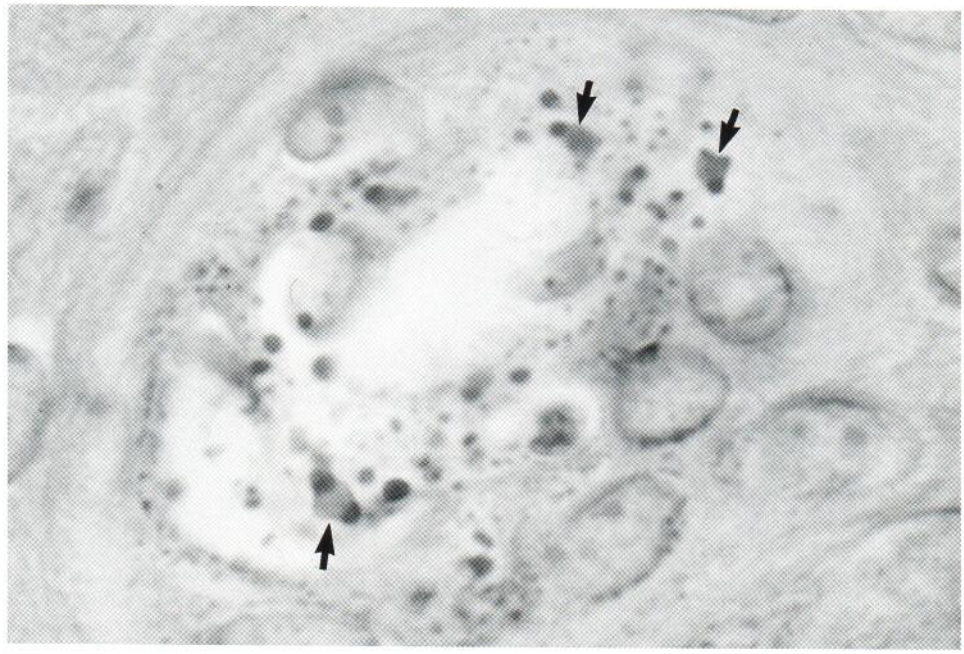

Fig. 1. Light micrograph showing heterogeneous globular keratohyalin granules in an acrosyringium. Note three unique composite granules with a larger eosinophilic part and smaller basophilic parts (*arrows*). Hematoxylin-eosin staining. ×2,000.

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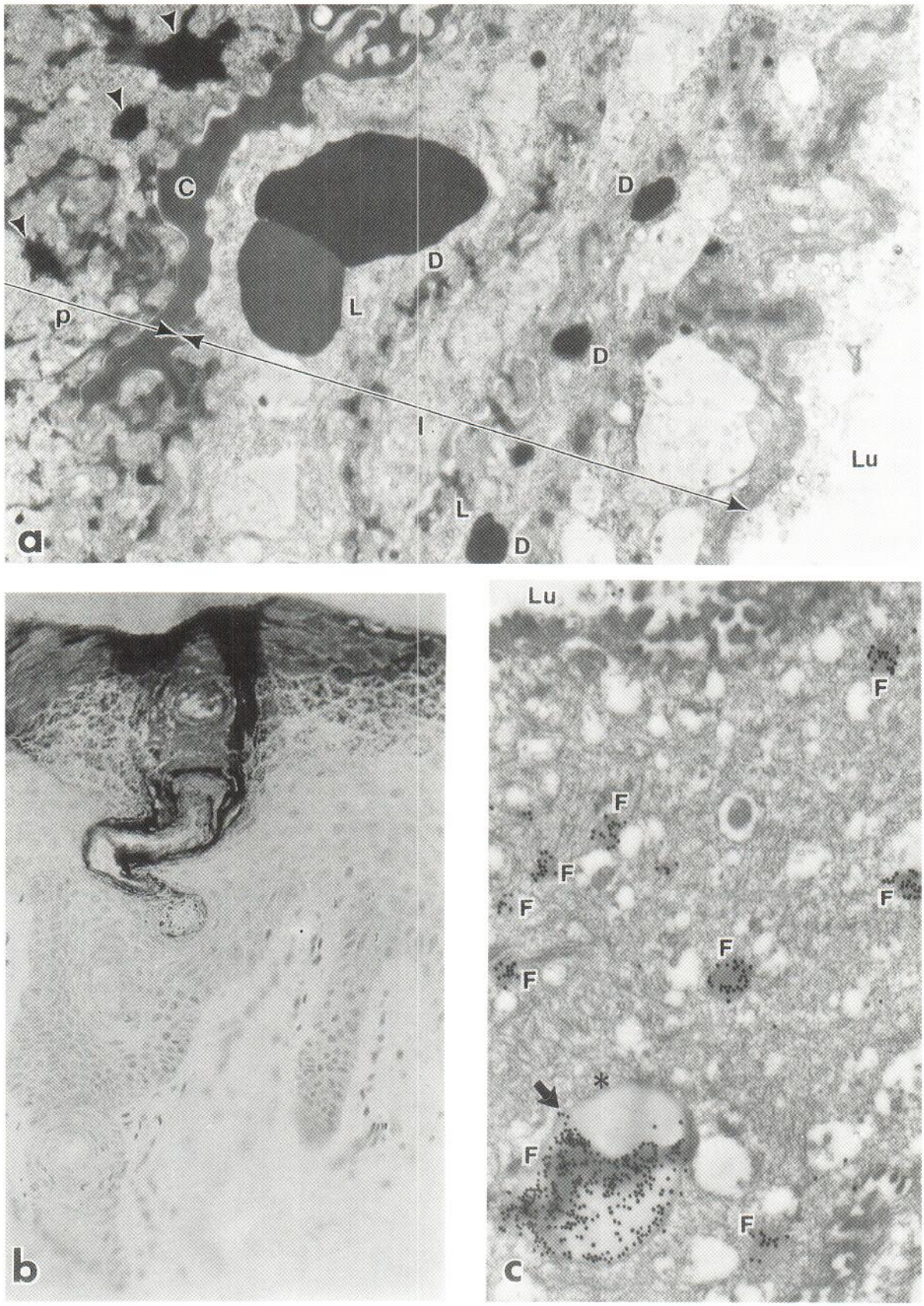

Fig. 2a. Electron micrograph of part of an acrosyringium at the mid-epidermis level. Luminal cells (1) contain smoothly outlined intracytoplasmic granules, the majority having a high electron density (*D*). There are also composite granules with a variable electron density (dense (*D*) and less dense (*L*)). By contrast, the peripheral cells (*p*) have irregularly shaped granules with high electron density (*arrowheads*). Note a cornified cell (*C*) at interface between luminal and peripheral layers. *Lu* = lumen. ×14,400. (*b*) Light micrograph of an LR White resin embedded semi-thin section that has been stained by immunogold-silver stain to demonstrate filaggrin immunoreactivity, counter-stained with toluidine blue. Immunoreactivity is seen in the upper portions of acrosyringia as well as in granular and horny layers of epidermis. ×256. (*c*) Electron micrograph of an LR White section showing immunogold labelling of filaggrin in a luminal acrosyringial cell. There are many filaggrin-stained smoothly outlined granules (*F*). One granule (*arrow*) is a composite granule with filaggrin positive (*F*) and negative (*) components. 30 nm gold label. ×16,900.

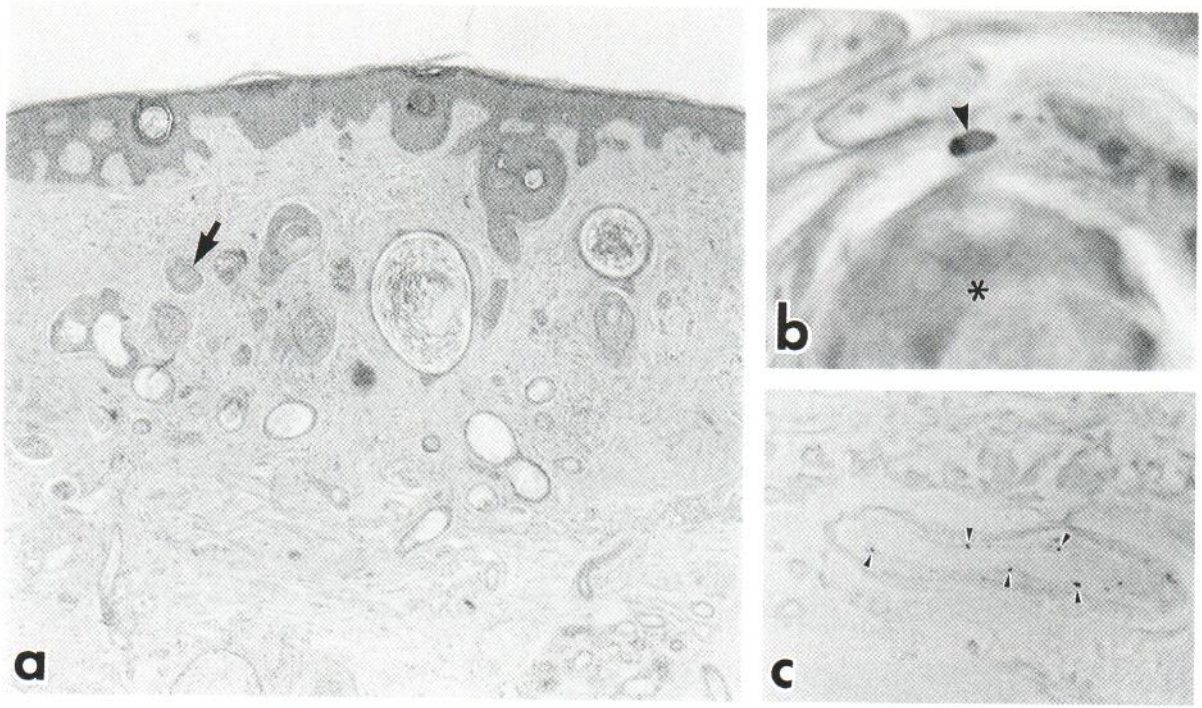

Fig. 3. Light micrographs of syringomas. (*a*) Many small ducts are embedded in a fibrous dermal stroma. Hematoxylin-eosin stain. ×55. (*b*) A high-power view of a duct shown with an arrow in *a*. In the duct wall a composite keratohyalin granule with eosinophilic and basophilic parts is seen (*arrowhead*). The duct contains amorphous eosinophilic material (*). ×1,660. (*c*) Filaggrin immunohistochemistry on syringoma. The duct wall cells contain small filaggrin immunoreactive granules (*arrowheads*). ×330.

For immunocytochemistry, the tissues were fixed with 1% glutaraldehyde and 0.2% picric acid in phosphate-buffered saline and embedded in LR White resin (The London Resin, Hampshire, UK) according to the method described elsewhere (3). As a primary antibody, anti-filaggrin monoclonal antibody (Biomedical Technologies Inc., Stoughton, MA) was used. For light microscopic observation, 1 μm thick sections were immunolabelled and stained by an immunogold silver enhancement technique, using 1 nm colloidal gold conjugated goat anti-mouse antibody (BioCell, Cardiff, UK) and a silver enhancement kit (Amersham International, Amersham, UK) as described before (11). The sections were briefly stained with toluidine blue. For electron microscopic observation, ultrathin sections mounted on nickel grids were incubated with the primary antibody and labelled with secondary antibody conjugated with 30 nm sized colloidal gold (Amersham International, Amersham, UK). The sections were contrasted only with uranyl acetate. For control studies, conditioned medium from a mouse myeloma cell line SP2/0-Ag14 was used in place of the primary antibody.

Studies of some skin tumours

Formalin-fixed, paraffin-embedded and hematoxylin-eosin-stained specimens from 37 cases of syringoma (age from 9 to 85, male 8, female 29), an eccrine syringofibroadenoma (58-year-old male), 17 eccrine poromas, 20 seborrheic keratoses and 10 trichoepitheliomas were examined. Transmission electron microscopy was performed on material from the case of eccrine syringofibroadenoma. Light microscopic filaggrin immunohistochemistry was also performed on deparaffinized and trypsin-treated sections from 2 cases of syringomas and the case of eccrine syringofibroadenoma, using an immunogold silver staining method as described above.

RESULTS

Light microscopy

Acrosyringial luminal cells contained globular keratohyalin granules in their superficial portions (Fig. 1). The majority of the granules were small and basophilic, whereas the minority were large and eosinophilic. The latter granules were partially surrounded by the former, forming composite granules (for example, see the granules indicated with arrows in Fig. 1).

Electron microscopy

It was clear that the acrosyringium was composed of two distinct cell layers, an inner or luminal cell layer and an outer or peripheral cell layer (Fig. 2*a*). The peripheral cell layer contained irregularly shaped keratohyalin granules associated with keratin filaments. The cells also formed lamellar granules, whose contents could be seen in the intercellular spaces beneath the cornified cells. Cornification in this layer was characterized by the formation of cornified cell envelopes and compaction of keratin filaments.

In contrast, the luminal layer had smoothly outlined keratohyalin granules which had little association with keratin filaments. Granules with different electron density were present, the less dense granules forming the minority. Composite granules containing both low and high density components were also seen (Fig. 2*a*). In the more superficial part of the duct, the luminal cells lost their nuclei and keratohyalin granules, leaving keratin filaments and remnants of cellular organelles. The cytoplasm was often vacuolated. The microvilli on the luminal surface were still recognizable and the lumen was open. Neither

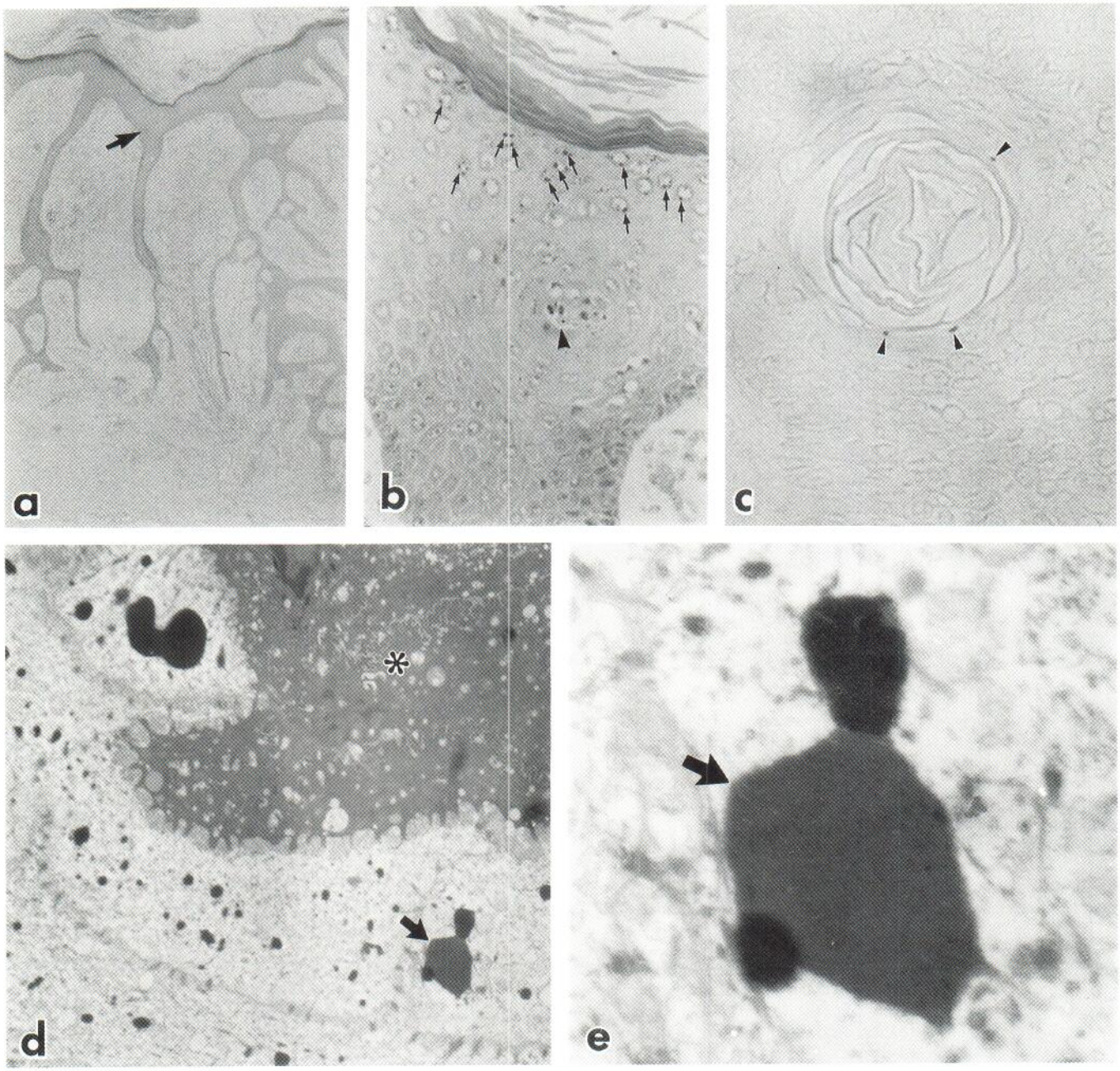

Fig. 4. An eccrine syringofibroadenoma. (*a*) Branching epithelial cords extend from the epidermis into the fibrous mid-dermis. Higher magnification of the portion marked with an arrow is shown in b. Hematoxylin-eosin stain. ×26. (*b*) Globular keratohyalin granules are seen beneath the cornified tumour surface (*arrows*) and around a ductal structure (*arrowhead*). ×260. (*c*) Filaggrin immunohistochemistry. The tumour cells surrounding a ductal structure contain filaggrin-immunoreactive keratohyalin granules (*arrowheads*). ×690. (*d*) An electron micrograph showing numerous globular keratohyalin granules on the tumour cells. Note one composite granule (*arrow*). Adjacent tumour cells have keratinized (*). ×5,800. *e*. Higher magnification of a composite granule shown in d. ×29,000.

lamellar granules nor cornified cell envelopes were formed during the differentiation process of luminal acrosyringial cells.

Light microscopic immunohistochemistry

Filaggrin immunoreactivity was seen in granular and cornified layers of the epidermis and in the upper portions of acrosyringia (Fig. 2*b*).

Immunoelectron microscopy

Filaggrin immunoreactivity was found on the irregularly shaped keratohyalin granules of epidermal granular cells from thigh and sole skin. In sole skin, however, there were some keratohyalin granules which were not immunoreactive to filaggrin. The keratohyalin granules in peripheral acrosyringial cells were filaggrin immunoreactive (not shown). In luminal acrosyringial cells, the majority of granules, which probably corresponded to those of higher electron densities in conventional transmission electron microscopy, were stained with the filaggrin antibody, but the other type was not (Fig. 2*c*). In the more differentiated inner cells, which had lost their keratohyalin granules, filaggrin

labelling was seen throughout the cytoplasm (not shown), in a distribution similar to that occurring in cornified cells of epidermis or peripheral acrosyringial layers.

Skin tumours with or without acrosyringial differentiation

Heterogeneous, sometimes composite globular keratohyalin granules were observed in 17 out of 37 syringomas (Fig. 3) and in a case of eccrine syringofibroadenoma (Fig. 4) but not in the eccrine poromas examined in the present study. Eight out of the 20 seborrheic keratoses and all of the trichoepitheliomas showed globular keratohyalin granules, but no apparent heterogeneity or composite granules were observed. In the syringomas and the eccrine syringofibroadenoma positive filaggrin immunoreactivity was observed in the cells forming ductal structures (Figs. 3*c*, 4*c*).

DISCUSSION

Variations of keratohyalin granules

In hematoxylin-eosin-stained sections, epidermal keratohyalin granules are seen as basophilic intracellular granules. Ultrastructurally, the granules have irregular outlines and a homogeneous high electron density. They are closely associated with keratin filaments at their periphery and appear to be deposited on the keratin filaments. Profilaggrin, the phosphorylated precursor of filaggrin, has been identified as a constituent of keratohyalin granules (2).

In the luminal cells of acrosyringia, however, keratohyalin granules are generally globular and lack close association with keratin filaments. Two types of keratohyalin granules with different electron densities have been recognized by electron microscopy in these cells (9). Although this distinction has not been well documented at light microscopic level, the different staining properties of these two types of granules are clearly shown in the present study (Fig. 1). The majority of granules are small and basophilic, whereas the minority are larger and eosinophilic. These different granules form unique composite granules. Using immunoelectron microscopy, we have demonstrated that these two types of granules are also distinct in terms of filaggrin immunoreactivity. The basophilic or more electron dense granules can be stained with a filaggrin antibody, while the eosinophilic or less electron-dense granules cannot.

Variations in morphology of keratohyalin granules have been observed in epithelial tissue from different species and anatomical sites and from different developmental stages (5–10). For example, in rodent tongue epithelia and epidermis two types of granules have been observed (3, 5–8). One is phosphorus-rich and filaggrin-immunoreactive (F-granules) and the other is cystein-rich and loricrin-immunoreactive (L-granules). Loricrin is a recently described cornified cell envelope precursor protein (12) and L-granules seem to participate in cornified cell envelope formation. The filaggrin-negative granules described in this study, in the human luminal acrosyringial cells, are morphologically different from the rodent L-granules, which are small electron-dense granules. Furthermore, the absence of an appreciable cornified cell envelope in the luminal acrosyringial cells suggests functional differences between the rodent L-granules and human filaggrin-negative luminal acrosyringial granules. Heterogeneity in keratohyalin granules was also described in palmo-plantar skin. The granules consist of two components; a larger, electron-dense, irregularly shaped and filaggrin-immunoreactive portion and a smaller, less electron-dense, round and filaggrin-negative component (13). These findings were confirmed by us in the present study (not shown). Although the latter filaggrin-negative granules seem to be different morphologically from the acrosyringial filaggrin-negative granules, further studies are required to clarify their differences or similarities biochemically.

Composite granules with different immunohistochemical properties have also been observed in human tongue papillae and in some specimens of pathological epidermis (14, 15). These granules are composed of filaggrin and trichohyalin-immunoreactive parts. Our preliminary immunoelectron microscopy studies revealed that the acrosyringial granules are not trichohyalin-immunoreactive (Ishida-Yamamoto, Iizuka and Manabe, unpublished observation).

Composite keratohyalin granules as a marker for acrosyringial differentiation

We examined some skin tumours to see whether the "acrosyringial composite granules" are preserved in neoplasms with acrosyringial differentiation. Although the number of tumours examined in the present study is rather small, we could demonstrate that certain sweat duct-related tumours, syringomas (16) and eccrine syringofibroadenomas (17–19), do possess the unique composite keratohyalin granules. Therefore the presence of such granules might be a useful marker for acrosyringial differentiation. Indeed there is a report showing similar composite keratohyalin granules in palmo-plantar keratoderma, where the authors suggest that acrosyringia might play a significant part in the pathogenesis of that condition (20).

In conclusion, we have presented evidence for a highly characteristic and possibly unique composite keratohyalin granule in the human acrosyringium. The function of this granule is not known but is probably associated with a specialized form of epithelial differentiation. The granules can be identified in eccrine tumours and may therefore serve as a marker for acrosyringial differentiation.

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