Meibomian gland studies: histologic and ultrastructural investigations

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Heightened interest in meibomian gland dysfunction has prompted us to evaluate the normal morphological and ultrastructural characteristics of the meibomian gland. Histologic analysis of human, primate, steer, and rabbit glands revealed evidence of keratinized epithelium extending throughout the meibomian gland duct. Characteristic ultrastructural features of keratinized epithelium identified in primate and rabbit glands included tonofilaments, keratohyaline granules, lamellar bodies, and keratinized squamous cells. Comparison of the meibomian gland duct to the pilosebaceous canal and the sebaceous duct brought out certain dissimilarities such as (1) the lack of a well-developed stratum granulosum and (2) the absence of lipid inclusions within transitional cells from duct to acini. We postulate that abnormalities of the keratinizing process may be responsible for meibomian gland dysfunction states.

Key words: meibomian gland, sebaceous gland, chalazia, keratinization, electron microscopy

Meibomian gland function is receiving increased attention as a potential cause of the widespread clinical problems of lid margin disease. At present, new therapies such as tetracycline for underlying meibomian gland dysfunction have been proposed based on an assumed similarity between disorders of the lid margin and meibomian gland to that of the skin and sebaceous gland. If such an assumption is true, study of the meibomian ductal epithelium may be important because of the involvement of the sebaceous duct and pilosebaceous canal in sebaceous gland disease. Although the normal histology, histochemistry, and ultrastructure of the meibomian gland has been studied, the literature has dealt principally with the structure and function of the meibomian gland acinar cells. Furthermore, the few reports dealing with the meibomian gland ductal epithelium are fragmentary and conflicting. It has been stated that keratinized epidermis extends into the ductal orifice, but details concerning the nature of the ductal epithelium have not been reported. Furthermore, the association of the meibomian gland orifice to the mucocutaneous junction is unclear. Does the keratinized epidermis end at the orifice, or does keratinization extend more posteriorly?

Studies in our laboratories have been made toward defining normal meibomian gland morphology, ultrastructure, and lipid composition. This report is a study of the morphology and ultrastructure of meibomian glands, with special emphasis on the ductal components. Histologic and electron microscopic evidence of keratinization occurring within the ductal epithelium is included.
Fig. 1. A, Light micrograph of rabbit lid demonstrating meibomian gland embedded within the tarsal plate. Note central duct (D) extends throughout length of gland and connects to single acini by means of short ductules (arrow). Arrow head, Meibomian gland orifice; C, conjunctiva. Inset Distal portion of duct. (H&E; ×25.) B, Light micrograph of steer meibomian gland. Note presence of a main duct (D), although not communicating with meibomian gland orifice in this section. (H&E; ×30.)
Fig. 2. Meibomian gland ductal orifice from the human lid stained for keratin. Note the dark staining of stratum corneum extending into and beyond the orifice and ending abruptly at the mucocutaneous junction posterior to the orifice (arrow head) (Keratin stain; X200.)

Materials and methods

We obtained superior and inferior lid specimens from primates (Macaca speciosa and Macaca mulatta), steer, rabbit, and human exenteration specimens. Light microscopic samples were fixed in 10% phosphate-buffered formalin (pH 7.4) and submitted for histologic processing, including stains for neutral lipid (oil red O) and keratin and prekeratin by the method of Ayoub-Shklar (cited in ref. 12).

Rabbit and primate tissue for transmission electron microscopy were obtained fresh from whole lid samples and dissected into 2 by 6 mm tissue blocks while in cold (4° C) fixative. Each block contained approximately two glands. Tissues were fixed in one of the following solutions: (1) 3% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.4; (2) 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M sodium cacodylate buffer, pH 7.4; or (3) 1% osmium tetroxide in 0.1M sodium cacodylate buffer, pH 7.4. After overnight fixation, samples were washed in cold 0.1M sodium cacodylate buffer and then postfixed for 1 hr in 1% osmium tetroxide, dehydrated in graded ethanols ending with propylene oxide, and embedded in Epon-Araldite or Spurr ERL 4206. Sections (1 μm) were cut on a MT2 Sorvall microtome and stained with toluidine blue to locate the gland orifice. Thin sections were stained with 4% uranyl acetate in 50% methanol and in lead citrate. We viewed the sections on 0.25% Formvar-coated 400 μm slotted grids in a Zeiss EM 10-A electron microscope.

Results

Light microscopy. The meibomian glands of human, primate, and rabbit are simple branched acinar glands embedded within the tarsal plate. Each gland contained a single
long central duct whose orifice was located at the lid margin (Fig. 1, A). The length of duct corresponded approximately to the length of the tarsal plate, with the width appearing to remain constant throughout its length (Fig. 1, A, inset). Along the length of the duct, acini were connected by means of short ductules. In contrast, the meibomian glands of the steer was characterized by a single short main duct approximately 0.5 to 1.0 mm in length (Fig. 1, B). Acini were not encountered along the length of the main duct, as in...
the human; instead, the terminal end of the duct branched into multiple ductules connecting to saccular glands.

In all specimens studied, the mucocutaneous junction was located just posterior to the meibomian gland orifice (Fig. 2, arrow). Well-developed stratum granulosum and stratum corneum were located not only at the anterior and posterior borders of the orifice but also extended into the distal portion of the duct. However, keratohyaline granules, as visualized by both hematoxylin and eosin (H&E) and keratin stains, were only occasionally encountered past the first set of acini communicating with the central duct.

The acinar cells in all specimens were identified at various stages of holocrine differentiation. Neutral lipid was demonstrable in these acinar cells and in the material contained within the duct. A periodic acid–Schiff (PAS)–positive material sensitive to diastase digestion, indicating glycogen, was identified within the epithelial cells of the distal portion of the duct. A PAS-positive line was observed adjacent to the basal cells of the duct and acini. This material was not sensitive to diastase digestion, suggesting presence of a basement membrane.

**Electron microscopy.** We achieved optimal fixation and sectioning with the use of 2.5% glutaraldehyde and 2% paraformaldehyde fixative with embedding in Spurr ERL 4206. Other fixatives resulted in poor definition of lamellar bodies.

**Ductal epithelium.** Ductal epithelium consisted of a basal cell layer, intermediate cell layer, and a horny cell layer of the (A, B, and C in Fig. 3). Comparison of the characteristic features identified by electron microscopy varied little between distal and proximal portions of the duct. No differences were noted between the rabbit and primate glands.

In all samples, the basal cell layer consisted of a single layer of cuboidal to column-shaped cells adjacent to the basal lamina. Basal cells were characterized by a high nucleus/cytoplasm ratio, occasional nucleoli, and an electron-dense cytoplasm containing numerous tonofilaments, desmosomes, and hemidesmosomes. Numerous ribosomal particles were also present, along with a few rough endoplasmic reticula (RERs). The basal lamina was made up of a fine fibrillar material, 200 Å wide, separated from the basal cells by a 200 Å space. The membrane adjacent to the basal lamina also contained numerous pinocytotic vesicles, 600 Å in diameter. Interlocking villus processes were observed between adjacent cells (Fig. 3, inset). Occasional large (0.8 μm) intercellular extensions were observed, composed of finely granular electron-lucent matrix containing cross-sections of fine filaments or tubules. We saw no zonula occludentes.

Overlaying the basal cells was one or two layers of nucleated polygon-shaped intermediate cells. As compared to basal cells, intermediate cell cytoplasmic matrix was distinctly less electron-dense. RER appeared to be slightly increased, whereas the number of tonofilaments was decreased. However, these cells appeared to have a smaller nucleus/cytoplasm ratio, which may account for these apparent differences in organelle numbers. Nucleoli were rarely present. Transitional forms between basal cells and intermediate cells were also identified above the basal layer.

Contained within the apical cytoplasm of the intermediate cells were considerable numbers of electron-dense granules of approximately 0.1 μm in diameter (Fig. 4, A). These granules were membrane-bound and contained a material arranged in a lamellar pattern (Fig. 4, A, insets). Such granules have previously been well described for keratinized epidermis; however, there have been no reports concerning the finding of such lamellar granules within other epithelial tissue.

A lamellar material similar to that contained within the lamellar bodies was also present in the extracellular space between intermediate cells and horny cells, as well as within infoldings of the plasma membrane of the intermediate cell (Fig. 4, B, open arrows). Such findings suggest that the lamellar material contained within the lamellar bodies was deposited in the extracellular space by fusion of the lamellar body membrane to the
Fig. 4. For legend see facing page.
intermediate cells apical plasma membrane. This interpretation would be consistent with the observations of Zelickson\textsuperscript{14} concerning the deposition of lamellar body material of the stratum granulosum cells of keratinized epidermis into the extracellular space between the stratum granulosum and the stratum corneum.

Flattened anucleated horny cells containing a homogeneous, finely granular, cytoplasmic matrix and occasional areas of coarsely granular material lined the luminal surface. Large electron dense inclusions having the appearance of keratohyaline granules were also discernible. Other intracellular organelles were not observed. No clear layer of fragmentation or shedding of horny cells into the lumen of the duct was evident. A lamellar material was present in the intercellular space between horny cells, which was arranged into broad sheets extending along the cell surface (Fig. 4, B, arrow). This material has a similar appearance to the material contained within the lamellar bodies and to the material deposited at the junction between the intermediate cells and horny cells. These findings could indicate that the material contained within the lamellar bodies of the intermediate cells was deposited in the extracellular space and that this material remained associated with the apical plasma membrane during cellular differentiation to fully keratinized squamous cells. Such a hypothesis would be in agreement with the observations of Elias et al.\textsuperscript{15} who have morphologically identified intercellular lamellar material between stratum corneum cells of the epidermis by freeze-fracture and further linked this material by histochemical lipid analysis to the lamellar bodies of the stratum granulosum.

The transition zone between ductal epithelium and acinar cells (meibocytes\textsuperscript{16}) was characterized by an abrupt change from keratinizing cells to meibocytes (Fig. 5). No transitional elements were discernible. Keratinizing cells containing lamellar bodies were observed surrounded by differentiating meibocytes. However, no keratinizing cells were identified that contained lipid vesicles, nor were any meibocytes identified that contained lamellar bodies or keratohyaline granules.

Connective tissue adjacent to the gland contained dense collagen, fibroblasts, and capillaries. Numerous nonmyelinated nerve axons associated with Schwann cell sheaths were encountered most notably in rabbit tissue. Junctions between nerve cell axons and epithelial or acinar cells could not be identified.

**Acini.** Three types of acinar cells were discernible in the order of increasing holocrine maturation: (1) basal cells, (2) differentiating cells, and (3) degenerating cells. A finely granular, electron-dense cytoplasmic matrix containing numerous ribosomes, mitochondria, and tonofilaments characterized the basal cells. Desmosomes were present along with hemidesmosomes that attached the basal portions of these cells to the basal lamina. Differentiating cells overlaid basal cells and occasionally were located with the basal layer. A well-developed smooth endoplasmic reticulum (SER) with numerous meibum vesicles\textsuperscript{16} of increasing size, depending on the degree of maturation, characterized these cells. Occasionally, SER was found to be arranged around the vesicles in a concentric pattern (Fig. 6, A). Membrane-bound electron-dense inclusions characteristic of lysosomes were also identified within differentiating cells. Numerous ribosomes,
Fig. 5. Transition zone between ductal epithelium and meibocytes. Note absence of lipid vesicles in keratinized epithelial cell adjacent to meibomian acinar cells. L, Lumen; A, acinar cell; MV, meibomian vesicle; open arrow, basal lamina; arrow, lamellar bodies.
Fig. 6. A, Electron micrograph of a differentiating meibocyte. Note concentric pattern of smooth endoplasmic reticulum surrounding meibomian vesicle (arrow). MV, Meibomian vesicle; N, nucleus; M, mitochondria. B, Electron micrograph of well-differentiated meibocyte. Note shrunken and electron-dense appearance of mitochondria (arrow). MV, Meibomian vesicle; open arrow, lysosome.
mitochondria, and occasional RER made up the balance of the intracellular organelles. In well-differentiated cells, mitochondria were shrunken in size and more electron-dense compared to those in cells that were less well differentiated (Fig. 6, B). Degenerating cells were observed in the center of the acinus adjacent to the lumen. These cells were characterized by large meibum vesicles surrounded by a finely granular, electron-dense cytoplasmic matrix containing a few intracellular organelles. Organelles that were present, including nuclei, appeared to be condensed and electron-dense. Within the lumen of the ductule, fragments of these cells were observed to be composed of condensed matrix.

Discussion

Clinical therapy for meibomian gland dysfunction is being prescribed, based on the assumed similarity of the meibomian gland to the sebaceous gland. Although there are similarities, there are also differences, both in the morphology as pointed out here and in the chemical nature of the lipid each gland produces. Our results regarding the keratinization of the meibomian gland duct also point to some interesting similarities and dissimilarities. The question arises as to how these findings are related to the pathology of this gland.

Knutson, in studying the development of comedo formation in acne vulgaris, showed that keratinization of the sebaceous gland duct leading into the pilosebaceous canal was important in the pathology of this gland. The normal pilosebaceous canal has been characterized as a well-developed keratinized epithelium with a stratum granulosum that contains ultrastructural elements such as tonofilaments, keratohyaline granules, and lamellar bodies and a stratum corneum. The sebaceous duct also contains ultrastructural elements of keratinization, although a distinct stratum granulosum and stratum corneum are lacking. Significantly, lipid inclusions have been found within the keratinized cells of the normal sebaceous gland duct. Increased occurrence of these inclusions have been seen in early stages of acneogenesis. Such findings indicate a defect in keratinization, which has been postulated to result in abnormal desquamation leading to plugging of the pilosebaceous canal.

The meibomian gland does not have a structure comparable to the pilosebaceous canal. A well-developed stratum granulosum and stratum corneum are absent except at the very distal portion of the meibomian gland duct. Furthermore, the multiple acini of the meibomian gland open into a common central duct whose orifice opens onto the lid margin. This is distinct from the single acini of the sebaceous gland which open onto the hair shaft of the pilosebaceous canal. Nevertheless, our ultrastructural analysis indicates that the meibomian gland duct does contain elements of keratinization such as occur in the sebaceous duct, e.g., tonofilaments, keratohyaline granules, and lamellar bodies. These morphologic features, in particular the finding of lamellar bodies and intercellular lamellar substance between horny cells, which have previously only been described in association with well-developed keratinized epithelium, clearly align this tissue with keratinizing epidermis and the pilosebaceous unit.

Lipid inclusions characteristic of transitional sebaceous duct epithelium were not identified in the meibomian ductal epithelium nor within the abrupt transitional area between the ductal and acinar cells (meibocytes) from the primate or rabbit. The importance of these inclusions in acne and sebaceous gland disease has been mentioned above. However, the literature is unclear as to whether these inclusions are a normal finding or represent an underlying subclinical sebaceous disease. In the latter case, the absence of lipid inclusions in normal glands from primate and rabbit would be expected. Weingeist states that lipid inclusions do occur within the meibomian gland ductal epithelium of human specimens. However, no further details were given in this fragmentary report. It is not known, for example, whether this represents a normal or abnormal finding in the human. If abnormal, such a finding
would support the theory that abnormalities of keratinization that affect the sebaceous gland would similarly result in meibomian gland disease.

The similarity in keratinization between the meibomian gland and sebaceous gland is therefore an important finding as regards possible pathogenetic mechanisms for meibomian gland dysfunction. However, differences in glandular structural organization, as pointed out earlier, suggest different types of pathologic presentations. For example, abnormalities in keratinization along the central duct would lead to blockage of the more proximal portion of the duct or only single acini, leading to microchalazia.

The fact that abnormalities in keratinization indeed affect meibomian gland function has recently been demonstrated by the report of polychlorinated biphenyl (PCB) intoxication, which resulted in replacement of meibomian glands with keratin cysts. This response to PCB suggests that other agents which result in hyperkeratosis may similarly affect these glands. The finding of a great number of environmental hydrocarbons present in the excreta from human meibomian glands, as reported by Nicolaides et al., suggests that the potential for such disease is highly relevant.

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REFERENCES