Special Issue

The International Workshop on Meibomian Gland Dysfunction: Report of the Subcommittee on Anatomy, Physiology, and Pathophysiology of the Meibomian Gland

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The tarsal glands of Meibom (glandulae tarsales) are large sebaceous glands located in the eyelids and, unlike those of the skin, are unassociated with hairs. According to Duke-Elder and Wyler,[1] they were first mentioned by Galenus in 200 AD and later, in 1666, they were described in more detail by the German physician and anatomist Heinrich Meibom, after whom they are named.

Lipids produced by the meibomian glands are the main component of the superficial lipid layer of the tear film that protects it against evaporation of the aqueous phase and is believed also to stabilize the tear film by lowering surface tension.[2] Hence, meibomian lipids are essential for the maintenance of ocular surface health and integrity.

Although they share certain principal characteristics with ordinary sebaceous glands, they have several distinct differences in anatomy, location, secretory regulation, composition of their secretory product, and function.

Functional disorders of the meibomian glands, referred to today as meibomian gland dysfunction (MGD),[3] are increasingly recognized as a discrete disease entity.[4–8] In patients with dry eye disease, alterations in the lipid phase that point to MGD are reportedly more frequent than isolated alterations in the aqueous phase. In a study by Heiligenhaus et al.,[9] a lipid deficiency occurred in 76.7% of dry eye patients compared with only 11.1% of those with isolated alterations of the aqueous phase. This result is in line with the observations by Shimazaki et al.[10] of a prevalence of MGD in the absolute majority of eyes with ocular discomfort defined as dry eye symptoms. These observations noted that 64.6% of all such eyes and 74.5% of those excluding a deficiency of aqueous tear secretion were found to have obstructive MGD, or a loss of glandular tissue, or both.[10] Horwath-Winter et al.[11] reported MGD in 78% of dry eye patients or, if only non-Sjögren patients are considered, in 87% compared with 13% with isolated aqueous tear deficiency. It may thus be accepted that MGD is important, conceivably underestimated, and possibly the most frequent cause of dry eye disease due to increased evaporation of the aqueous tears.[5,9–12]

After some excellent reviews of MGD[4,7,8,13,14] in the past, many new findings have been reported in recent years, and other questions remain to be identified and resolved. A sound understanding of meibomian gland structure and function and its role in the functional anatomy of the ocular surface[15] is needed, to understand the contribution of the meibomian glands to dysfunction and disease. Herein, we seek to provide a comprehensive review of physiological and pathophysiological aspects of the meibomian glands.

Heinrich Meibom

Heinrich Meibom the younger (1638–1700; Fig. 1)[16] came from a scholarly family. He was the son of the physician Johann Heinrich Meibom and the grandson of the German historian and poet Heinrich Meibom the elder (1555–1625),[17] who was professor of history and poetry at the University of Helmstedt in Germany. Heinrich Meibom the younger was born on June 29, 1638, in Lübeck, Germany, and later traveled around in Europe and received a cosmopolitan education. In a short article[18] that commemorated the 300th anniversary of his birth in 1938, the *British Medical Journal* characterized him as follows: "Like so many of his contemporaries, he was indeed a child of Apollo, god of culture, poetry, rhetoric, and healing. While still a medical student (he became MD at Angers in 1663) he was appointed to, and in 1664 took up, the professorship of medicine in the University of Helmstadt. Fourteen years later he accepted the additional chairs of history and of poetry. He further showed his versatility by straying into the pleasant fields of archaeology, philology, and philosophy, and all his life he was an insatiate traveller." Apparently a man of many talents, in 1666, shortly after receiving the chair of medicine, Heinrich Meibom published the first detailed description, including a drawing, of the oil glands inside the tarsus of the eyelid, that later were named the meibomian glands. His description appeared as a book with the title *De Vasis Palpebrarum Novis Epistola*.[19] This early drawing showed basic characteristics of the glands, such as multiple single gland streaks along the extension of the tarsus with openings onto the lid margin, similar to but not as detailed as another much later and more well-known drawing (Fig. 2).

Anatomy, Embryology, and Physiology

Anatomy of the Meibomian Glands

Arrangement. A single meibomian gland is composed of clusters of secretory acini that are arranged circularly around a

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FIGURE 1. Heinrich Meibom, the younger (1638–1700). In 1666, he published the first detailed description of the tarsal glands in the eyelid, which later became known as the meibomian glands. Reprinted with permission of the Herzog August Bibliothek, Wolfenbüttel, Germany, Signatur B 100.

long central duct and connected to it by short ductules. This arrangement has been compared with a chain of onions.[20] One end of the central duct is blind, and the other end opens close to the posterior lid border, just anterior to the mucocutaneous junction, at the lid margin, where the oily secretion is delivered onto the tear meniscus.[21]

These separate glands are arranged in parallel in a single row throughout the length of the tarsal plates in the upper and lower lids,[1,22,23] and they presumably act in a coordinated fashion that is influenced by hormonal and neural regulation and by the mechanical forces of muscle contraction during the eye blink.[24]

The extent of the meibomian glands roughly corresponds to the dimensions of the tarsal plates in the upper and lower eyelids and hence differs between them (Fig. 2). In the upper lid the tarsal plate has the shape of a half circle that extends upward centrally for approximately 1 cm and narrows on the temporal and nasal sides, whereas the tarsal plate in the lower lids is smaller and forms a strip of rather equal length (~0.5 cm) from the nasal to the temporal side.[24]

Dimensions and Number of Glands. The reported dimensions of the meibomian glands differ to a certain extent in different studies. The number of separate glands in the upper lid is given in one study[20] as 25 and in another[1] as 40, with a median number of approximately 31.[25] The number of glands in the lower lid is given in the former study[20] as 20 and in the latter[1] as 30, with a median of approximately 26 glands.[25] The length of the individual glands is reported as approximately 5.5 mm in the middle of the upper lid and approximately 2 mm in the lower lid, and hence their calculated total volume is also higher: approximately double in the upper lid (26 μL) versus the lower lid (13 μL).[25] The meibomian glands in the lower lids tend to be wider than those in the upper lids. The number of secretory acini along a single meibomian gland is reported[20] to be approximately 10 to 15 and is also higher in the upper than in the lower lid.

The secretory capacity of the meibomian glands in the upper lids should therefore be roughly double of that in the lower lids, but most investigations focus on the lower lid because of its greater accessibility. The differential secretory capacity in the upper versus the lower lid has not been investigated.

Embryologic Development of the Meibomian Gland

The embryologic growth of the meibomian glands occurs from the third to the seventh month of gestation, during the sealed-lid phase of eyelid development.[26–28] During this time, the loose connective tissue of the mesoderm in the lid folds differentiates into the tarsal plate and muscles (orbicularis and Riolan's muscle), the blood vessels, and the loose connective tissue underlying the outer lid skin and the conjunctiva. The development of the meibomian glands from the anlage (the initial clustering of embryonic cells that serves as a foundation from which the organ develops) of the meibomian glands shows considerable similarities to that of the hair follicles, the hair anlage. Both of them grow from the ectodermal sheet, which seals the fused lid folds down into the mesoderm, although the meibomian anlage is larger, grows deeper, and takes longer for complete development as investigated in detail by Ehler's group.[28]

Similar to the hair anlage of the eyelashes, which develops associated glands (holocrine sebaceous glands of Zeis and modified sweat glands of Moll), the epithelial cord of the meibomian anlage develops lateral outgrowths that later differentiate into the connecting ductules and secretory holocrine sebaceous acini. Inside the epithelial cylinder of the meibomian anlage, similar to the hair anlage, the production of lipids leads to the formation of a central canal that later develops into the central duct. The production of lipids is followed by the occurrence of keratohyalin granules in the luminal epithelial

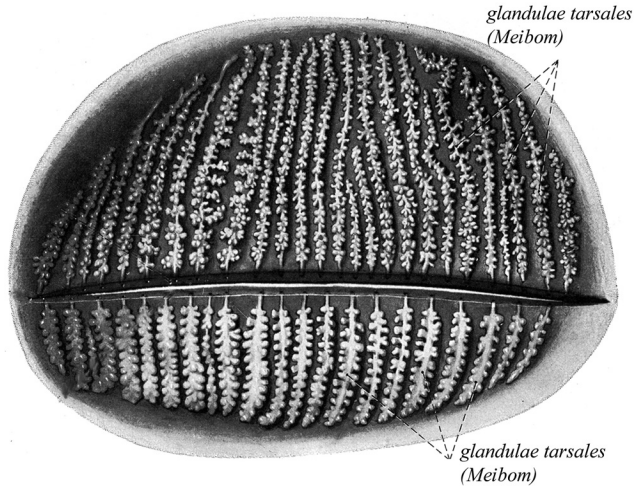

FIGURE 2. Topography of the meibomian glands within the tarsal plates of the upper and lower eyelids. The extension of a single meibomian gland follows the shape of the tarsal plate, which is different in both lids. The drawing depicts a posterior view with the anterior part of the lid removed, and the tarsal connective tissue made translucent so that the glands are exposed. The proximal ends of the glands extend toward the proximal margin of the tarsal plates and the secretum (meibum) is delivered at the distal end of the tarsus via a short excretory duct through the orifice onto the lid margin. Reproduced from Sobotta Đ. *Atlas der Anatomie des Menschen.* Ferner H, Straubesand J, eds. Ed. 18, Vol. 1, p. 215, Urban & Schwarzenberg 1982, with the kind permission of Elsevier.

cells, and therefore the lipid synthesis and keratinization events were once assumed to be somehow related.[28] Lipid production by the more mature meibomian anlage and by the ciliary glands of Zeis has also been found to be related to the formation of a canal between the two sealed lid folds, which leads to the separation of the then fully differentiated upper and lower lids in the seventh month of gestation.[28] In the mouse, it is thought that an increasing amount of keratinization, rather than lipid secretion, contributes to the separation of the upper and lower eyelids.[29]

Hence, the central meibomian duct can be compared to the hair follicles of the eyelashes in embryology and also shows distinct similarities in structure and epithelial differentiation, including the keratinization status, in the adult. The meibomian gland can hence be regarded as a "hair follicle without a hair shaft."[30] This observation may offer the conclusion that hyperkeratinization is a typical disease of the meibomian gland.

Histologic Appearance of the Meibomian Gland

The meibomian glands are composed of secretory acini that are connected via smaller ductules to the larger, long, straight central duct (Fig. 3) that extends throughout the length of the tarsal plate and opens onto the free lid margin close to the posterior lid border. The whole internal ductal system is lined by a stratified squamous epithelium with signs of incipient keratinization. Full keratinization (cornification), as indicated by the presence of luminal keratin lamellae, is physiologically only present in the terminal part of the central duct that is lined by an ingrowth of the cornified epidermis from the surface of the free lid margin.[1,23,24,31,32]

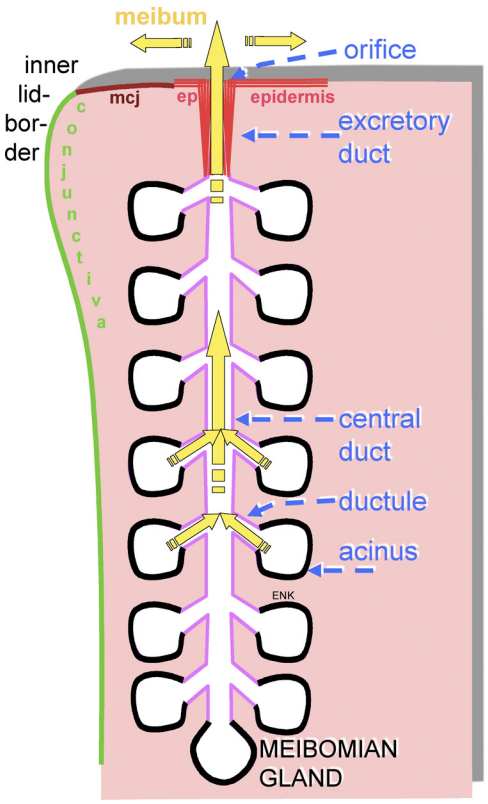

FIGURE 3. Morphology of a single meibomian gland. A single meibomian gland (located within the tarsal plate near the conjunctiva) is composed of multiple holocrine secretory acini that are arranged circularly around a long central duct to which they are connected via short, lateral, connecting ductules. The terminal part of the central duct is lined by an ingrowth of the epidermis (ep) that covers the free lid margin and hence forms a short excretory duct that opens as an orifice at the posterior part of the lid margin just anterior to the mucocutaneous junction (mcj) near the inner lid border. The oily secretum (meibum) is synthesized within the secretory acini and transported (*yellow arrows*) in a distal direction toward the orifice. Knop N, Knop E. [Meibomian glands. Part I: anatomy, embryology and histology of the Meibomian glands] Meibom-Drüsen Teil I: Anatomie, Embryologie und Histologie der Meibom-Drüsen. *Ophthalmologe*. 2009;106:872–883, with the kind permission of Springer Science and Business Media.

Acinus. As a special type of sebaceous gland, the secretory acini of the meibomian glands follow a holocrine secretion mode that is reflected by their structure (Fig. 4A). The numerous secretory acini have an elongated or spherical shape of approximately 150 to 200 μm diameter. They are completely filled with secretory cells, termed meibocytes.[33] The basal cells are smaller and darker. The meibocytes, located more toward the center of the acinus, show a progressive accumulation of lipids in the cytoplasm and hence appear increasingly foamy and pale in conventional histology of paraffin-embedded sections because of the extraction of the lipids during processing. During their maturation, the most central cells undergo shrinkage, compaction, and disintegration of the nucleus (pyknosis). Eventually, disintegration of the cell membrane occurs at the transition from the acinus to the ductule. Hence, the whole cell contents form the oily secretory product termed meibum.[33] A gradient in maturation with more undifferentiated, immature cells in the basal layer is also supported by transmission electron microscopy in mouse[34] and human[35] meibomian glands. The basal acinar cells contain a medium dense nucleus that is rich in heterochromatin and has a prominent nucleolus. The basal acinar cells have sparse cytoplasm that contains a large number of keratin filament bundles together with numerous mitochondria and many free ribosomes, as is characteristic for synthesis of internal cell proteins, whereas the rough endoplasmic reticulum and Golgi apparatus, for export of secretory products, are scarce. The basal layer of meibocytes in the periphery of the acinus serves as a proliferating progenitor cell population that constantly gives rise to new meibocytes. In the rat, it has been shown that it takes approximately 4 days to generate new meibocytes.[36]

Connecting Ductule. Typically one, or sometimes more, acini are connected to a ductule that is approximately 150 μm long and has a luminal diameter of approximately 30 to 50 μm. The ductules are lined by a stratified (four layers) squamous epithelium. At the junction from the acinus to the ductule, a sharp transition from the peripheral layer of basal meibocytes to the ductal epithelium has been reported in electron microscopy of the monkey and rabbit.[32] This conclusion was reached from the observation that the epithelial cells of the ductule did not contain lipid droplets, as found in neighboring meibocytes, but instead contained keratohyalin granules. Histology in the human meibomian gland does not necessarily confirm a sharp transition based on the shape and arrangement of the epithelial cells in this region, because the spherical brighter epithelial cells of the basal meibocytes are seen to transform gradually into the cells of the multilayered ductal epithelium that have a slightly denser cytoplasm and a more elongated shape (Fig. 4B). Keratohyalin granules are also observed in ductal epithelial cells of the normal human meibomian gland (Knop E, et al. *IOVS* 2009;50:ARVO E-Abstract 4833).

Central Duct. The connecting ductules enter the long central duct, typically in an oblique direction that leads to the formation of a sharp tissue spur that is mainly composed of epithelial cells and contains a narrow internal core of connective tissue at the entrance into the central duct (Fig. 4C).

The central duct is also lined by a four-layered, stratified squamous epithelium but has a wider lumen approximately 100 to 150 μm in diameter. The central duct extends through-

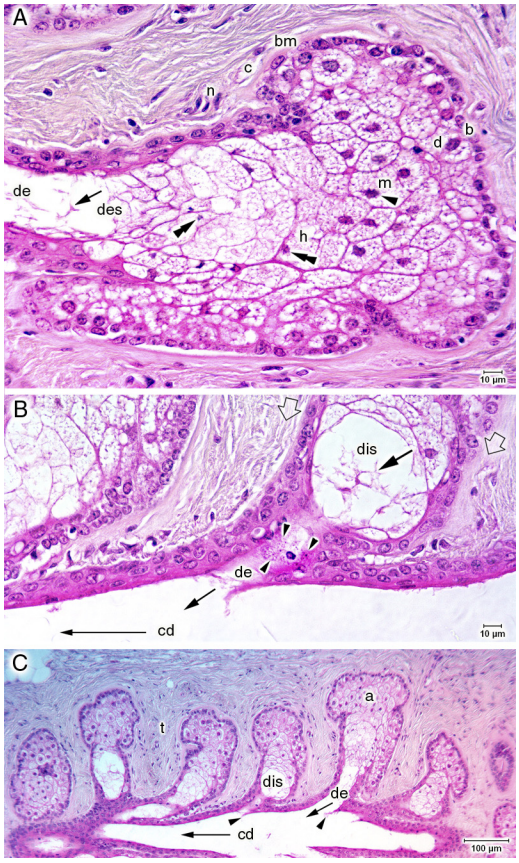

FIGURE 4. Structure of the acini and ductal system of a normal meibomian gland. (**A**) The holocrine acini of the meibomian gland are filled with the secretory cells (meibocytes) and surrounded by a basement membrane (bm). In the periphery of the acinus, a capillary (c) and a small nerve fiber (n) are seen. From the basal cells (b) at the peripheral margin, differentiating meibocytes (d) start with the production and accumulation of lipids within lipid droplets that occur as vacuoles in routine histology, because the lipids are dissolved in the histologic preparation. Toward the center of the acinus, there is an increase in the number and size of their internal lipid droplets as the cells differentiate into mature meibocytes (m). These remain vital, as indicated by their intact nucleus (*arrowhead*). In the very large hypermature meibocytes (h), the nucleus becomes pyknotic (*double arrowheads*) [compare with Fig. 7]. The cytoplasmic membrane of these cells disintegrates, and the components of the whole cell form the secretory product, termed meibum, in the disintegration zone (des) close to the connecting ductule (de). Remnants (*arrow*) of the meibocytes are still found inside the ductule and sometimes in the central duct. (**B**) In the area of the disintegration zone, located at the transition of the acinus to the ductule, the basal cell layer is replaced (*open arrows*) by the multilayered squamous epithelium of the ductule, which is about four cell layers thick. If the ductal epithelium is observed in an oblique plane of section, it is seen to contain keratohyalin granules (*arrowheads*) in the luminal cell layer that represent an incipient stage of keratinization. (**C**) Numerous acini of spherical to elongated shape are radially arranged around the central duct (cd) of a gland, seen here in a longitudinal section. Ductules enter (**B**, **C**, *arrows*) the central duct, typically in an oblique direction, which results in the formation of a sharp tissue spur (**C**, *arrowheads*) toward the central duct. The direction of flow of the meibum inside the gland is indicated by a *large arrow* in (**B**) and (**C**). Light microscopic images of paraffin-embedded sections stained with hematoxylin and eosin (H&E); size markers are shown in the images. Reprinted from Knop N, Knop E. [Meibomian glands. Part I: anatomy, embryology and histology of the Meibomian glands] Meibom-Drüsen Teil I: Anatomie, Embryologie und Histologie der Meibom-Drüsen. *Ophthalmologe*. 2009;106:872–883 with the kind permission of Springer Science and Business Media.

out the total length of the gland, which corresponds roughly to the extension of the tarsus.[1,20,25,37]

Around the terminal part of the central duct and among the terminal acini close to the free lid margin, there are various amounts of striated fibers of Riolan's muscle (Fig. 5, also noted in Fig. 18A) that are split from the orbicularis muscle by the down-growth of the hair anlage of the cilia, deep into the tarsal fold during embryologic development. These muscle fibers appear to encircle the terminal part of the meibomian gland.[20,38] The terminal part of the central duct preceding the excretory duct is often slightly dilated, thus forming a kind of ampulla, conceivably due to its physiological content of secreted meibum.

Excretory Duct. The cornified epithelium of the free lid margin (epidermis) extends into the terminal part of the mei-

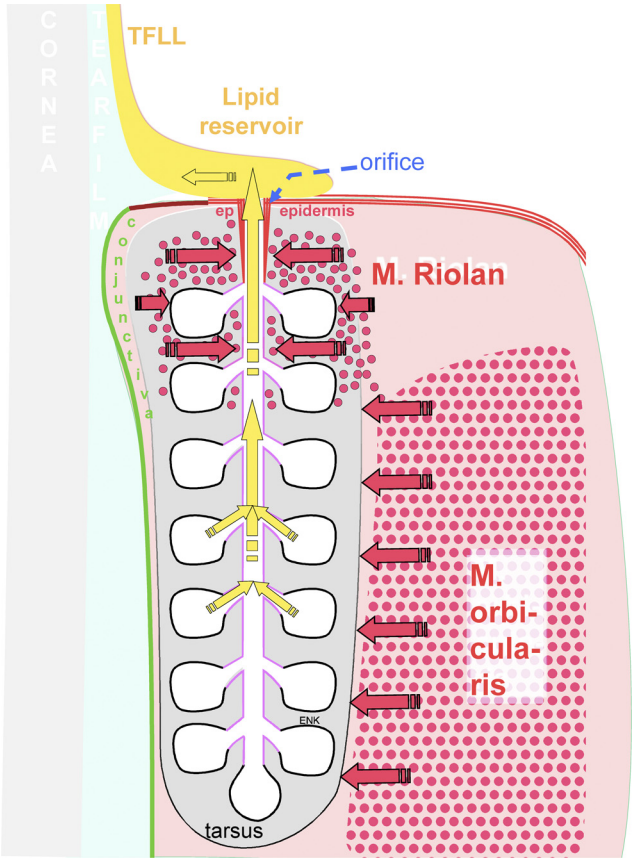

FIGURE 5. Driving forces for the delivery of meibomian oil onto the lid margin and tear film. A schematic drawing of a single meibomian gland inside the connective tissue of the tarsus at the posterior lid margin. The driving forces that result in the eventual delivery of meibomian oil (meibum) onto the lid margin and tear film are (1) the continuous secretion of meibum by the secretory acini, which generates a secretory pressure that pushes the meibum (*yellow arrows*) into the ductal system and further toward the orifice and (2) the mechanical muscular action by muscle fibers (*red dots*) of the pretarsal orbicularis muscle (M. orbicularis), located on the outside of the tarsus, and of the marginal muscle of Riolan (M. Riolan), which encircles the terminal part of the meibomian gland. During a blink, these muscles may exert a compression (*red arrows*) of the meibomian gland that drives the oil out of the orifice into the marginal lipid reservoir, where it eventually constitutes the tear film lipid layer (TFLL), as observed clinically [compare with Figs. 3, 7, and 18]. Reprinted from Knop E, Knop N, Schirra F. [Meibomian glands. Part II: physiology, characteristics, distribution and function of meibomian oil] Meibom-Drüsen Teil II: Physiologie, Eigenschaften, Verteilung und Funktion des Meibom-Öls. *Ophthalmologe*. 2009;106:884–892, with the kind permission of Springer Science and Business Media.

bomian gland[1,22,23,32] for about 0.5 mm. It has a keratinizing layer that contains numerous dense keratohyalin granules (granular layer) and a superficial layer of fully keratinized (cornified) keratin lamellae. At about 0.5 mm internal to the orifice, the epithelium gradually transforms into the ordinary ductal epithelium of the meibomian gland by losing the keratin lamellae and the granular layer and by reducing the stratification from about six to eight layers to four layers. Since the epithelium in this terminal part has a different structure compared with the rest of the central duct, it appears justified to term it as an excretory duct (Knop E, et al. *IOVS* 2009;50:ARVO E-Abstract 4833).

Physiology of the Meibomian Glands

Secretion Mode. Basal meibocytes move during their maturation, which includes the production and accumulation of lipids, from the basal compartment of the acinus toward its center[36] and eventually toward the entrance of the ductule (Fig. 4, also indicated in Fig. 3). The biochemical characteristics of the secretion process and its products are considered in the section on lipid synthesis.

During this process, the meibocytes go through several stages that can be differentiated morphologically (basal, differentiating, mature, and hypermature), depending on several structural characteristics as described by Gorgas.[34] Cell organelles that are necessary for lipid production inside the cells increase in number and size during this process—in particular, the smooth-surfaced endoplasmic reticulum (sER) and peroxisomes.[34] The lipid droplets are encircled by multilamellar membrane structures that are assumed to originate from the membrane of the sER.[34] Similar multilamellar structures are also integrated into the lipid droplets and hence contribute to the meibum.[35] All the components of the whole cell including lipids, proteins, and nucleic acids contribute to the oily secretory product, which is also called meibum.[33]

The ductal system may contribute to the final secretory product that is released onto the posterior lid margin in an active or passive way, since (1) nerve fibers are observed not only around the acini but also around the ductal system,[39] and (2) the originally secreted meibomian lipids are conceivably, at least in part, modified by hydrolyzing enzymes from commensal bacteria inside the ductal system that lead to a breakdown of triglycerides into free fatty acids and small portions of mono- and diglycerides and other modifications[40] in patients with blepharitis.[41] and even in the normal condition. Commensal bacterial species have been cultured in most expressed meibum samples[42] from blepharitis patients, but similarly also from normal subjects.

Mechanisms of Secretion and Delivery. Because of the length of the meibomian glands, there is frequently a long distance between the cell biological process of secretion of the meibum in the secretory acini and its actual delivery onto the lid margin where it exerts its functions. Therefore, it appears advisable to follow the nomenclature suggested by Bron and Tiffany[43] and to separate secretion of the meibum from its delivery.

The constant production of new meibocytes in the secretory acini and their disintegration into the final secretory product generates a continuous secretory force that drives the meibomian oils within the ductal system of the gland toward the orifice at the free lid margin (Fig. 3). The hypothesis of a continuous production is not only supported by the observed generation time of new meibocytes[36] but also by the finding that in the morning after sleep, during which the lids are closed, an increased amount of lipid that has apparently accumulated within the ductal system is then delivered in increased amounts onto the lid margin.[44,45] The constant secretion of meibum further represents a basis for the generation of increased pressure within an obstructed gland.

The same observation further supports the conclusion that the mechanical action of the lid muscles contributes to delivery (Fig. 5), as suggested by Linton et al.[46] During the movement of the eyelids during a blink, the orbicularis muscle, located on the external side of the tarsal plate, generates a compression of the tarsal plate and the enclosed tarsal glands of Meibom. It was concluded that this "would promote the flow of secretion by a milking action."[46] It has been suggested that the embedding of the glands inside the tarsal tissue provides for a homogeneous effect on compression of the individual glands along the lid margin.[37] The contraction of Riolan's muscle conceivably exerts compression of the terminal part of the ductal system and acini and contributes to the delivery of the oily meibum onto the surface of the lid margin. This notion may be supported by the incidental observation that meibum is released onto the lid margin in the form of jets of liquid.[47] It has been assumed that the constrictive forces of Riolan's muscle may also close the terminal part of the meibomian gland and hence prevent the outflow (i.e., act in a somewhat antagonistic way on the flow of meibum compared to the pretarsal orbicularis muscle).[46] It has been further speculated that, in the act of a blink, the pretarsal orbicularis performs a "milking action" while Riolan's muscle is relaxed and, conversely, between blinks, while the orbicularis is relaxed, Riolan's muscle contracts to prevent the outflow of meibum.[46] Contraction of Riolan's muscle may also aid in limiting unwanted outflow of meibum (e.g., at night), although then the orbicularis muscle is relaxed, according to Linton et al.,[46] and does not perform a propulsive action on the meibum. Although these speculations may offer attractive explanations for the delivery of meibum, there is no evidence of such an antagonistic action of the marginal muscle of Riolan compared with the pretarsal orbicularis muscle. There is also no evidence of a potential influence of the smooth fibers of the superior and inferior tarsal muscles or of an influence of age-related or pathologic changes in the composition and shape of the tarsal plate and lids that could influence meibomian gland function and meibum delivery. These questions may need further investigation.

After an absence of blinking and muscular action overnight, the accumulated meibum from within the ductal system is delivered in increased amounts. This action has been observed by meibometry[44] in the morning during the first hour or so after awaking and has been clinically observed after a prolonged time of concentrated work associated with reduced blinking frequency.[45,46] Consequently, muscular action during repeated enforced blinks significantly increases the lipid layer thickness[48] and is also an appropriate therapeutic approach to overcoming a certain minor degree of obstruction in patients with incipient obstructive meibomian gland disease.[3]

Lid Topography and Meibomian Gland Function. The differential contribution of the meibomian glands in the upper versus lower eyelids has been insufficiently investigated to date. Because of the calculated higher volume of the meibomian glands in the upper lids,[25] it can be assumed that they also have a higher secretory capacity and contribute more to the lipid pool at the lid margin and subsequently to the tear film lipid layer. However, because of the better accessibility of the margin of the lower lids, most investigations of the morphology and secretory capacity of the meibomian glands have focused on the lower lids.

This focus applies in particular to meibometry, introduced by Bron and Tiffany[43] and Chew and colleagues,[44,45] which is able to measure effective amounts of lipid on the lid margin.[44] Research has shown the amount of meibomian lipids on the lid margin, and the rate of delivery is dependent on age, sex, and diurnal conditions.[45,49] In these investigations it has been as-

sumed that mixing of the meibum of the upper and lower lids during blinking means that the lower reservoir is likely to be similar to the value obtained from the upper lid. The amount of lipid in the marginal reservoir is lower in children younger than 14 years compared with adults, higher in adult males than in adult females, equal in both sexes after the age of 50 years, and stable or even slightly increased up to the eighth decade of age.[45,50] The rising amount of lipids in the marginal reservoir with age is in some contrast to the described decrease of active meibomian glands with age[50] and can probably be explained by a decreased removal of lipids from the lipid layer and reservoir.[43,50,51]

The secretory activity of glands, as analyzed by Norn[52] by the staining of delivered lipid at the meibomian orifice, found active delivery in only 45% of gland openings at one time point and a decrease of active glands by 50% from the age of 20 years to the age of 80 years.[52] The morphologic equivalent of such a decrease in function may be represented by an age-dependent disappearance of gland tissue (gland dropout) as observed more recently by meibography.[53] The secretory activity of glands was analyzed in more detail by Korb and Blackie[51,54,55] by their ability to deliver a liquid secretory product on diagnostic expression involving application of mild external pressure in the physiological range of 1.25 g/mm^2, performed to reveal delivery without overcoming a potential obstruction of the orifice. These studies supported that not all glands deliver oil at the same time. In addition, it was observed for the first time, that the number of active glands in lower lids depends on their location along the lid margin and is highest in the nasal third,[51] lower in the middle of the lid, and lower still in the temporal third meibomian glands. It was also observed that there is a correlation between the number of actively delivering meibomian glands in the lower eyelid and dry eye symptoms.[51] The time necessary for full expression of a gland, at the specified mild pressure until delivery stopped was approximately 12 seconds, on average, and the recovery time until new oil could again be expressed from the same gland was approximately 2 hours.[54] When individual glands were repeatedly expressed, with intervals of 3 hours between expressions over a daytime period of 9 hours (i.e., four times), it was observed that a single gland is capable of secreting oil on demand over the course of a working day.[55] This continuous activity of the meibomian glands also showed a similar dependence on the position along the lid margin, as observed before.

Investigations on the secretory activity and capacity of the meibomian glands in the upper lid are desirable, to better analyze the physiological functions of the glands and their alterations in different types of disease.

Innervation. In contrast to the sebaceous glands of the skin elsewhere in the body that are mainly regulated via hormones and other factors, the meibomian glands also have a distinct neural innervation.

The meibomian glands of the human have a dense meshwork of unmyelinated nerve fibers (nerve plexus) around the acini that are described by electron microscopy[32] and also by histochemistry.[56] The network consists of nerve fibers that have terminal buttons containing small vesicles filled by granules that contain neurotransmitters. The nerve endings are located closely around the acini but remain outside the basement membrane and constitute so-called synapses en passant that lack a direct postsynaptic structure in the target cell, as is characteristic of the autonomic nervous system. Such nerve fibers are also observed around the ductal system,[56] which may indicate that the ductal epithelium contributes to the composition of the finally delivered meibum. Many nerve fibers occur around and within the wall of the small vessels[39] that build a dense meshwork around the acini.[20]

The nerve fibers of the human meibomian gland are mainly positive for acetylcholinesterase and are hence supposed to represent a part of the cholinergic parasympathetic nervous system.[56] In addition they contain the neuropeptides calcitonin gene-related peptide (CGRP) and substance P,[57] which are markers for the sensory nervous system but also occur in the parasympathetic system, and, in addition, the parasympathetic vasoactive intestinal polypeptide.[58] These results substantiate a prevailing parasympathetic innervation of the meibomian gland.

In summary, the innervation of the meibomian glands is maintained via a dense meshwork of nerve fibers that contain different neurotransmitters and originate from different sources. They include, besides the mainly parasympathetic nerves from the pterygopalatine ganglion, sympathetic nerves from the superior cervical ganglion and sensory fibers from the trigeminal ganglion. In the rat it has been shown, that the parasympathetic fibers via the pterygopalatine ganglion originate from the superior salivatory nucleus[59] that is also responsible for the innervation of the lacrimal gland. This innervation pattern offers the possibility of a common regulation[24] of the ocular surface glands that contribute the different components of the tear film (meibomian glands for the lipids and lacrimal gland and accessory glands for the aqueous phase) to achieve an optimal composition of the tear film. The goblet cells that produce the secreted mucins which represent the main component of the mucous phase of the preocular tear film appear to be regulated in the same way.[60] Whether and how the meibomian glands are actually integrated into the neural feedback loop,[61,62] similar to the lacrimal gland, is yet to be learned.

Less information is available at present on the release of the transmitters observed in the nerve fibers, on respective receptors on the target tissue, and on the mode of action that is transmitted by their interaction.

Keratinization

Process of Epithelial Keratinization. Meibomian glands share principal features of the embryologic developmental course and of the structural organization with the hair follicles of the eyelashes. These features include a general commitment of the epithelium to keratinization. Keratohyalin granules represent incipient stages of keratinization and contain proteins such as filaggrin that are later released into the cytoplasm and serve the function of interconnecting the intermediate keratin filaments leading to the formation of a densely packed meshwork. The keratin meshwork increasingly occupies the cytoplasm of the keratinizing epidermal cells, termed keratinocytes.[63] The cross-linking of keratin bundles goes along with an enforcement of the cell membrane that is transformed into the cornified envelope.[64] After degeneration and loss of their nuclei, these cells form the superficial keratin lamellae that indicate full keratinization (cornification)[63] and serve the purpose of protecting against physical and chemical stress factors.

Incipient Keratinization

Electron microscopy has shown that the ductal epithelium of normal meibomian glands contains keratohyalin granules in the apical cell layer of the rabbit and monkey.[32] Although no obvious keratinization occurs in the normal human meibomian gland, recent histologic investigations (Knop E, et al. *IOVS* 2009;50:ARVO E-Abstract 4833) could verify that the whole epithelium of the central duct and ductules of the human meibomian gland also contains keratohyalin granules and hence preserves a commitment to keratinization. Thus, the meibomian gland can in principle be regarded as a "hair follicle without a hair shaft."[30]

Meibomian Gland Stem Cells and Cell Dynamics

As a special type of large sebaceous gland, the meibomian glands, unassociated with hairs, share certain principle rules of biology and cell dynamics with the more conventional hair-associated sebaceous glands of the skin. However, compared with the latter, the knowledge of basic information on stem cells and cell dynamics is very limited for the meibomian gland in general and in particular for the human. The same applies to another sebaceous gland without association to hairs, the preputial gland. Therefore, it is at present frequently necessary to consider more general phenomena of sebaceous gland biology in the skin, the validity of which is awaiting experimental proof for the meibomian gland.

Meibocyte Generation and Migration

As a sebaceous gland, the meibomian gland produces its secretum (meibum) by the holocrine secretion mechanism. This means that the contents of the whole glandular cells form the meibum, as shown in Figure 4A. After a process of maturation including lipid synthesis and accumulation, centripetal cell movement, and eventual cell degeneration and membrane disintegration, the lipids and other cell components are shed into the lumen of the ductal system. This holocrine secretion process hence results in the structural consequence that the whole secretory acinus is filled by secretory cells and in the dynamic consequence that secretory cells are continuously lost and replaced. This process is in distinct contrast to the merocrine secretion mode of the aqueous lacrimal gland, where only the secretory products are released from intact secretory cells.[65] The continuous loss of acinar cells requires a consequent continuous production of new cells and therefore a continuous cell turnover and differentiation within the acinus. Even though it has long been assumed that the regeneration of the meibocytes arises from the peripheral layer of basal cells located on the basement membrane,[66] in a way that is equivalent to the regeneration of other epithelial tissues from their basal layer,[67] this was only proven in 2001 by Olami et al.[36] A gradient in maturation, with more undifferentiated immature cells in the basal layer, has been observed by transmission electron microscopy in the mouse[34] and the human[35] meibomian gland and has also been indicated by histology.

Olami et al.[36] labeled dividing cells in the mouse meibomian gland with the radioactive nucleotide [3H]-thymidine, which is integrated as a marker into the nuclei during mitosis. They were able to show, after observation at different time points, that the labeled dividing cells were initially only found in the basal cell layer. At later time points, the number of labeled cells gradually increased, indicating basal cell mitosis and multiplication. Later, labeled cells were observed in locations closer to the center of the acinus, thus verifying the assumed centripetal movement of meibocytes. It has been calculated that in the mouse meibomian gland, the meibocytes have a generation time of 4.1 days between each division. Newly formed cells move from the basal layer at a velocity of 0.62 μm per day toward the center of the acinus and need approximately 9 days from their formation in the basal layer for their movement and eventual shedding in the center. These results[36] showed for the first time the location of meibocyte progenitor cells in the basal cell layer, together with the constant and synchronous centripetal movement of their progeny. This movement explains the constant secretion of meibum, as observed by meibometry,[44] and provides the basis for a previously assumed constant secretory force[43] that, together with the muscular action[46] of the orbicularis muscle on the outside of the tarsal plate and of Riolan's muscle around the terminal parts of the meibomian glands, leads to the delivery of the oil onto the posterior lid margin. It can be assumed that basic similar characteristics also apply to the human meibomian gland, but, since the human acinus is larger in diameter and is filled by more cells, the exact numerical values may be slightly different.

Meibomian Gland Stem Cells

Olami et al. concluded from their observations that the stem cells of the meibomian glands lie at the circumference of each acinus. There have been almost no investigations of stem cells in the meibomian gland to date, with the exception of Olami's work and one abstract (Lavker RM, et al. *IOVS* 2003;*44*:ARVO E-Abstract 3781). The latter group was concerned with meibomian gland stem cells in general and also reported that, after labeling with bromodeoxyuridine (BrdU) or [3H]-thymidine, most of the rapidly cycling cells were seen in the "basal sebocytes," which refers to the basal acinar meibocytes. However, these cells are not regarded as real stem cells which are defined as a slow-cycling cell population,[68] similar to those in the corneal limbus[69–71] and skin,[72] analogous to those defined in the hematopoietic system.[73] Rather they are regarded as progenitor cells (transient amplifying [TA] cells). TA cells are daughters of the stem cells, with more rapid division but a limited further number of divisions and a restricted differentiation program,[68] that eventually give rise to terminally differentiated cells. There were only a few if any slow-cycling stem cells observed in the acini (Lavker RM, et al. *IOVS* 2003;*44*: ARVO E-Abstract 3781), but most of the fast-cycling cells of the meibomian glands were located there.

The presence of fast-cycling TA cells in the basal acinar epithelium appears sufficient to explain the continuous generation of meibocytes, if it is additionally assumed that these basal cells are continuously replenished by the migration of young TA cells from a stem cell source outside the acinus. Similarly, the corneal epithelium is apparently maintained by the migration and division of TA cells that originate from the stem cell source at the limbus and can also remain intact after a prolonged time of limbal stem cell insufficiency.[69–71] The same applies to the skin epithelium, which is also regenerated by several generations of basal TA cells that originate from the stem cells located in the hair follicles[68] or in interfollicular epidermal rete pegs.[74]

Slow-cycling, and hence label retaining, putative stem cells have been found concentrated in the ductal epithelium of the meibomian gland (Lavker RM, et al. *IOVS* 2003;*44*:ARVO E-Abstract 3781). In addition, many of the cells "in the uppermost portion of the meibomian gland ductal epithelium" have show label dilution indicative of rapid dividing TA cells (Lavker RM, et al. *IOVS* 2003;*44*:ARVO E-Abstract 3781). Such highly proliferative cells also occurred farther out in the zone of the mucocutaneous junction on the inner lid margin.

Similarities to Hair Follicles. This situation in the meibomian glands shows immediate similarity with the arrangement of stem cells and TA cells in the hair follicle— consistently downward with the lower hair root epithelium and upward with the skin epithelium. Cotsarelis et al.[75] observed that, in the hair–skin unit, the slow-cycling putative stem cells are almost exclusively localized in a specific zone (the hair bulge) at about the middle of the hair follicles where the permanent upper part of the hair follicle ends and the arrector pili muscle inserts into the follicle. Later investigations by Taylor et al.[68] confirmed the initial hypothesis that TA cells originate from slow-cycling cells in the hair-bulge zone and populate two different tissue compartments. From the differential location and cell-cycling characteristics it was concluded that they form (1) the lower hair follicle

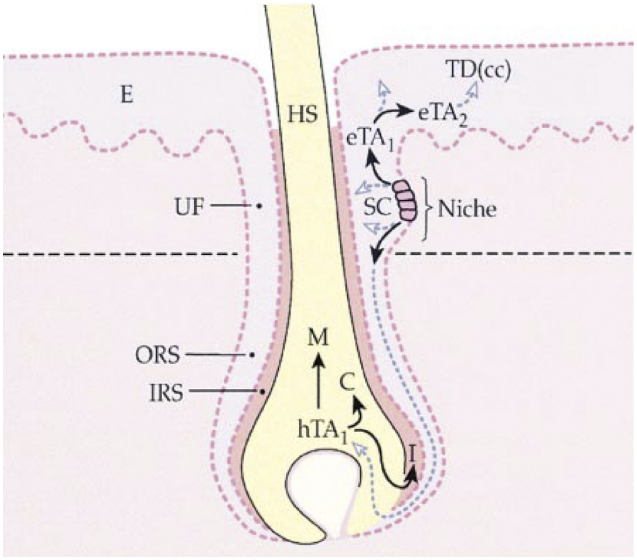

FIGURE 6. Stem cells in the hair follicle as a potential model for the meibomian gland. Stem cells (SCs) are located in the bulge area (Niche) of the outer root sheet (ORS) of the hair follicle. Two different populations of transient amplifying (TA) cells arise from this stem cell source and migrate (*solid arrows*) into two directions: hair-forming TA cells (hTA) migrate downward, whereas epidermis-forming TA (eTA) cells migrate upward. Both of them gradually differentiate and mature via different intermediate stages (TA1, TA2. . . to TAn) into the terminally differentiated cornified cells (TDcc) of the epidermis and hair, respectively. Inner root sheet (IRS) and (I); upper follicle (UF); hair medulla (M); hair shaft (HS); cortex (C); skin epidermis (E). In the bulge region and epidermis further differentiated cells generally move upward (*dashed arrows*) to the lumen. Schematic drawing of a section through the skin and a hair follicle. Reprinted from Taylor G, Lehrer MS, Jensen PJ, Sun TT, Lavker RM, Involvement of follicular stem cells in forming not only the follicle but also the epidermis, *Cell*, 102, pp. 451–461, ©2000 with permission from Cell Press.

and also the hair shaft and (2) the epithelium of the upper hair follicle and the epidermis.

It was therefore concluded that the hair-bulge stem cells are bipotent, follow two distinct differentiation pathways, and are able to give rise to two different populations of TA cells that form either the hair or the epidermis (Fig. 6). The reported results of Lavker RM, et al. (*IOVS* 2003;44:E-Abstract 3781) in the meibomian gland share certain similarities with the situation observed earlier in the hair follicle.[68,75] In addition to a slow-cycling stem cell zone in the epithelial lining of a canal (either of the hair follicle or of the meibomian duct) two regions of more rapidly cycling, differentiating TA cells occur. TA cells are located downstream (in either the lower hair follicle or in the acinus of the meibomian gland) and upstream (in either the upper hair follicle and lid margin epidermis on the outside or in the uppermost part of the meibomian gland duct and in the MCJ on the outside). It is therefore tempting to speculate that the renewal of the epithelial tissue by stem and TA cells follows a course in the meibomian gland similar to that in the hair follicle. A basic similarity between the hair follicles of the cilia and the meibomian glands, that would explain why hyperkeratinization is a typical pathology in MGD, is further validated by their similar structure and their joint embryologic development,[26–28] as reviewed in Knop et al.[24]

Cell Dynamics and Functional Compartments

The meibomian glands have conceivably at least three structural and functional domains (Fig. 7) that require a unique differentiation status maintained by differential regulation of intracellular signaling pathways. These are constituted by (1) the holocrine secretory compartment of the acini filled with the secretory meibocytes that produce the meibomian oil, (2) the tubular four-layered squamous ductal system that drains the oil, and (3) the uppermost (distal) part of the long central duct, the excretory duct, that delivers the oil onto the posterior lid margin and represents an extension of the multilayered cornified skin epithelium (epidermis).

At present it is unclear how the boundaries of such structural and functional compartments are established and maintained. It can be assumed that different receptors are expressed on the surface of cells in the excretory duct versus the ductal system versus the secretory meibocytes. Furthermore, a differential expression of proteins in the cytoplasm that are related to the differential maturation and function of these tissues can be assumed. Little detailed information on these assumptions is available today, even though microarray analyses by Sullivan, Schirra, and colleagues[76–81] have generated substantial data on the gene expression profiles of the meibomian glands in health and disease.

Cell Dynamics in Sebaceous Glands

The development of sebaceous glands, some of which are the meibomian glands, is governed by a hierarchy of factors: (1) A lineage commitment of undifferentiated stem cells into sebaceous gland stem cells is followed by (2) their proliferation and organization into a gland structure, which is completed by (3) the terminal differentiation of secretory cells inside the acinus and their functional lipid production.[82,83] Each of these steps is governed by regulatory factors, the specificity and interaction of which is insufficiently resolved to date.

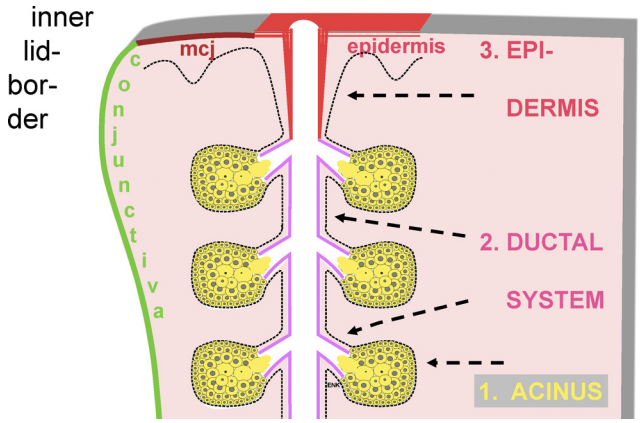

FIGURE 7. Structural and functional domains of the meibomian glands. The human meibomian glands are composed of at least three different structural and functional compartments. These are (1) the holocrine acinus with its basal cycling and luminal differentiating, lipid-producing meibocytes (*yellow*); (2) the four-layered stratified squamous epithelium of the ductal system (connecting ductules and long central duct), which has physiological incipient (*pink*) keratinization; and (3) the epidermis of the excretory duct, which represents an ingrowth of the stratified squamous, fully cornified (*red*) epidermis of the skin from the free lid margin. It can be assumed from studies of stem cells of the epidermis and of hair-associated sebaceous glands that each of these compartments is provided with lineage-committed progenitor cells. The basement membrane that separates the epithelial tissues from the underlying connective tissue is indicated by a *dotted line*. Schematic drawing of a section through a meibomian gland and the posterior lid margin, mcj, mucocutaneous junction [compare with Fig. 3]. Modified from Knop N, Knop E. [Meibomian glands, Part I: anatomy, embryology and histology of the meibomian glands]. Meibom-Drüsen, Teil I: Anatomie, Embryologie und Histologie der Meibom-Drüsen. *Ophthalmologe*. 2009;106:872–883 with the kind permission of Springer Science and Business Media.

Location and Lineage Commitment of the Sebaceous Gland Stem Cells. Even though there is a relatively large body of literature on sebaceous glands, the formation of the sebaceous gland tissue from stem cells is still insufficiently understood. From Taylor et al.,[68] it can be assumed that the subpopulation of hair-bulge stem cells that migrate upward to populate the epidermis also represent the stem cells for the hair-associated sebaceous glands that are located on the way from the bulge toward the epidermal surface. This notion is supported by studies in which it was found that isolated and transplanted hair-bulge stem cells can form all skin cell lineages and constitute the respective tissues (i.e., hair follicles, sebaceous glands, and epidermis).[84,85] More recent results indicate that epidermal stem cells from regions between hair follicles (interfollicular epidermis) are also bipotent, similar to the hair-bulge stem cells,[68] and form two lineages.[86] One of them differentiates into epidermal cells that express keratinization markers, and the other differentiates into sebocytes under the influence of the transcription factor c-myc. Another study[74] indicated multiple classes of stem cells are present in cutaneous epithelium, independent of the hair-bulge cells, and can contribute to the development of epidermal structures including hairs and sebaceous glands. This group also showed, by retroviral lineage tracing in the mouse, that in approximately one third of the labeled hair units, only the sebaceous gland was selectively labeled, which indicates the presence of a pool of long-lived, slow-cycling cells that conceivably represents a pool of lineage-specific glandular stem cells (Fig. 8). These presumed gland progenitors are located at the transition zone from the acinus to the hair follicle,[74] and, in the same position, presumed mouse sebaceous gland–specific progenitors were later characterized by a transcription factor.[82] This finding is in some contrast to the location of slow-cycling progenitors in the ductal epithelium of the mouse meibomian gland (Lavker RM, et al. *IOVS* 2003;44:E-Abstract 3781).[87] Differences in the location of holocrine acinar stem cells may be related to the fact that the mouse hair–associated skin sebaceous glands apparently do not have a distinct connecting ductule, in contrast to the human hair sebaceous glands and the meibomian glands.

At present, it is thought that there are at least three independent stem cell populations in the skin[88]: the multipotent hair-bulge stem cells, for the cyclic reformation of the hair, and lineage-committed stem cells for the sebaceous glands and the interfollicular epidermis. In addition, there are isthmus-resident cells in the upper hair follicle close to the sebaceous gland. Under pathologic conditions such as wounding, the bulge stem cells become activated and can in fact replenish the sebaceous glands[82] and the epidermis between the hair follicles.[88]

Development of Sebaceous Glands. The transcription factor c-myc governs the expression of a large number of genes, including some that are essential for skin development. In skin, c-myc represents a kind of switch that determines the development of stem cells into keratinizing epidermal cells versus sebaceous gland cells. Increased expression of c-myc favors their differentiation into sebocytes.[83] The differentiation of sebocytes can also be induced by c-myc activation in stem cells of the interfollicular epidermis between hair follicles.[86]

The further differentiation and proliferation of stem cells committed to sebaceous gland development is governed by the transcription factor B lymphocyte-induced maturation protein 1 (BLIMP1), originally discovered as a factor that inhibits the further proliferation but promotes the differentiation of B-lymphocytes into antibody-secreting plasma cells. Experiments in the mouse showed that a loss of the Blimp1-positive gland progenitor cells at the transition from the acinus to the hair follicle sebaceous gland (Fig. 8) resulted in increased cell activity in the hair-bulge stem cell compartment and led to reformation of gland tissue, although in a hyperplasic state.[74] From these observations it was concluded that the BLIMP1-positive cells represented the unipotent, lineage-committed progenitor cells that control the development and homeostasis of the sebaceous gland. A later study in the mouse and human, however, did not show that BLIMP1 distinguishes stem cells undergoing differentiation into sebaceous gland versus hair follicle versus interfollicular epidermal cells.[86] Recently, it was observed that the BLIMP1 protein does in fact occur in all appendages of the skin including hair follicles, nail organs, sebaceous glands, and the epidermal granular layer, at least in the human.[89] In addition, BLIMP1 protein was mainly found in the most mature cells; therefore, the authors concluded that BLIMP1 has a major role in terminal differentiation, including a central function in skin barrier homeostasis, as indicated by its presence in the epidermal granular layer.[89] However, the function of BLIMP1 may be more complex, since in BLIMP1 knockouts, an increased cell division activity has been observed in the multipotent stem cells of the hair bulge, conceivably serving to replace the dysfunctional sebaceous gland cells. This occurrence results in a disturbance of sebaceous gland homeostasis with formation of enlarged, hyperplasic glands and an

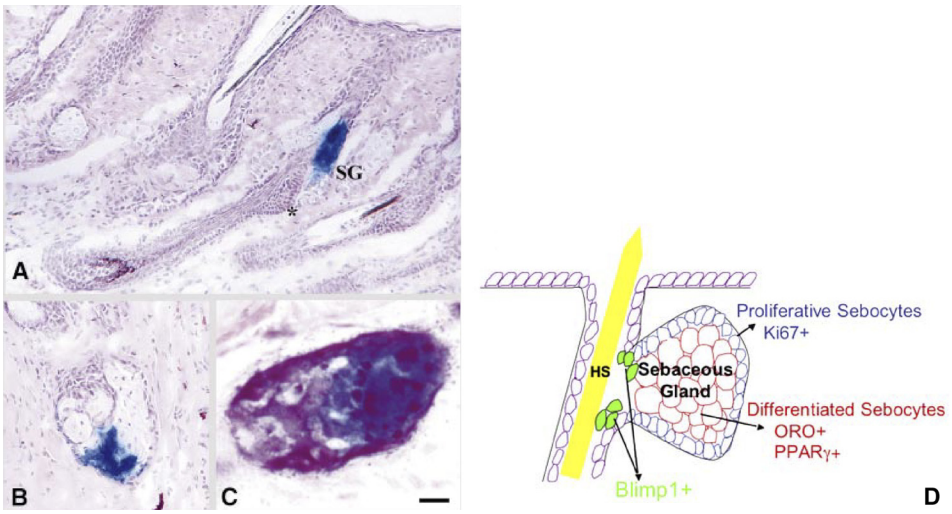

FIGURE 8. Location of sebaceous gland progenitors in the mouse skin. The expression of sebaceous gland–committed progenitor cells was found to be restricted to the transition zone between the acinus and the hair follicle in skin sebaceous glands. Such progenitor cells, labeled by retroviral transfer (**A–C**), are seen in a hair met in longitudinal section (**A**) as well as in cross-sections (**B**, **C**). Another marker (BLIMP1) that is assumed to characterize lineage-committed sebaceous gland progenitors indicates respective cells at the same position in a schematic drawing (**D**). (**A–C**) Reprinted by permission from Macmillan Publishers Ltd: *EMBO J*, Ghazizadeh S, Taichman LB. Multiple classes of stem cells in cutaneous epithelium: a lineage analysis of adult mouse skin. 2001;76:1215–22, © 2001. (**D**) Reprinted from Cell, 126, Horsley V, O'Carroll D, Tooze R et al., Blimp1 defines a progenitor population that governs cellular input to the sebaceous gland, 597–609, © 2006 with, permission from Cell Press.

oily fur.[82] Other regulatory factors also contribute to sebocyte differentiation and proliferation via the Hedgehog, Wnt, Notch, and other signaling pathways.[90] Hedgehog[91] and Indian hedgehog[92] signaling stimulates sebocyte proliferation, whereas their inhibition results in suppression of gland development.

Terminal Maturation of Sebocytes. The maturation of sebocytes within the sebaceous gland involves signaling by peroxisome proliferator activated receptor-γ (PPAR-γ), a member of the PPAR subfamily of nuclear hormone receptors. It is a ligand-activated transcription factor that plays an important role in the control of gene expression in a large number of tissues with lipid-producing cell types, even including keratinocytes,[93] and is activated by fatty acids.[94] PPARs regulate multiple lipid metabolic genes via PPAR response elements located in cell organelles, such as peroxisomes, microsomes, and mitochondria, which are involved in lipid metabolism.[95] PPAR-γ is necessary for the differentiation of adipose tissue in vivo and in vitro.[96] Signaling to PPAR-γ, (e.g., by long–chain fatty acids such as linoleic acid)[97] initiates lipogenesis and the accumulation of large intracellular lipid droplets.[98] PPAR-γ is also involved in the terminal differentiation of sebocytes in the non–hair-associated rat preputial gland.[97] The growth and development of sebaceous glands is dependent on androgens, but they are not sufficient for the full maturation of the sebocytes, as dissected in cell culture experiments.[99] Androgens appear to influence early steps of sebocyte differentiation, probably including the upregulation of PPAR-γ, whereas full differentiation and maturation of sebocytes, including the formation of the typical large lipid droplets, requires the action of PPAR-γ.[97] Therefore, the action of androgens is related to but distinct from that of PPAR-γ. Still, in cell culture, the effects of dihydrotestosterone and the specific PPAR-γ ligand thiazolidinedione BRL-49653 were additive in increasing the lipidogenesis of rat preputial sebocytes,[97] which points to the importance of sex hormones—in particular, androgens—in the regulation of sebaceous gland function.

Cell Differentiation and Dynamics in the Meibomian Gland

Molecules that are involved in the differentiation and function of sebaceous glands are also present in the meibomian glands, as observed by Nien et al.[87] BLIMP1 was described as a differentiation marker for the sebaceous lineage stem cells,[82] but was observed later in the epidermis and basically in all skin appendages[89] and was also found in the meibomian gland ductal system[87] and in the luminal layers of the eye lid epidermis. The sebocyte differentiation marker PPAR-γ was also observed in mouse meibocytes. It showed an age dependence and changed from a cytoplasmic staining of basal cells of young animals, to cytoplasmic staining in most meibocytes of young adults, to a nuclear staining of all meibocytes in old animals.[87] This rearrangement was paralleled by a decrease in acinar size and in lipid production, as verified by oil red O staining. Furthermore, in young and young adult animals, many of the basal acinar cells were recognized as proliferative by Ki67 staining, whereas the proliferation rate decreased in older animals. The noted absence of BLIMP1 from basal acinar cells may be explained by the recent finding that it does not represent a lineage marker for sebaceous stem cells, as originally assumed[82] and does not discriminate between stem cells of different lineages in the mouse,[86] but in fact occurs everywhere in the human epidermis.[89]

The terminal differentiation of meibocytes shares similarities with that of the secretory cells (sebocytes) of sebaceous glands, as can be assumed from their structure and function for lipid production. Intracellular lipids, after their synthesis via a PPAR-γ-dependent pathway, are maintained inside specialized compartments (lipid droplets) that contain the associated molecule adipose differentiation-related protein (ADRP, or adipophilin) in their periphery.[100] ADRP is an intrinsic lipid storage protein found in lipid droplets of different cell types and in all cells that produce lipids to any degree, from muscle cells to adipocytes. ADRP stimulates the uptake of long-chain fatty acids and its own expression is also upregulated[101] by the presence of these fatty acids that represent a prominent component in the meibomian oil (Green-Church KB, et al. *IOVS* 2009;50:ARVO E-Abstract 533).[102–105] ADRP has been described in rat meibomian gland tissue by Northern and Western blot analysis and by immunohistochemistry.[106] The latter showed ADRP localization at the margins of the lipid droplets with a generally higher level of expression in the more mature meibocytes located toward the center of the acinus in situ and in more mature cells in the cell culture of isolated meibocytes. ADPR was hence suggested to be a differentiation marker for mature meibocytes.

Relation of Stem Cells to Meibomian Gland Disease

Defects in stem cell formation and their migration may contribute to MGD and disease. This assumption is mainly based on observations in cutaneous hair-associated sebaceous glands, but also on findings in the meibomian glands itself.

In the skin, these defects are relevant (e.g., in the onset of acne[107] and sebaceous cancer).[108] Related diseases also occur in the meibomian gland in the form of hyperkeratinizing seborrheic conditions[7,109,110] and cancers of the meibomian gland tissue.[111]

Alterations of sebaceous glands can occur on all levels of development, from commitment to stem cell lineage, to gland tissue formation, to terminal sebocyte maturation inside the acini, as discussed by Horsley et al.[82] Overexpression of the basal transcription factor c-myc, which acts as a kind of switch between the developmental directions into epidermis versus sebaceous gland formation, favors sebaceous gland hyperplasia,[112] which conceivably results in primary seborrhea. BLIMP1, a more downstream transcription factor that represses c-myc and inhibits further proliferation but stimulates the differentiation of progenitor cells,[113] is essential for lineage determination of sebaceous gland stem cells.[114,115] Disturbance of sebaceous gland homeostasis due to loss of BLIMP1-positive, gland-committed progenitors can result in excessive repair processes with formation of enlarged, hyperplastic glands and seborrhea.[82] The terminal maturation of sebocytes inside the acinus is maintained by PPAR-γ, which is a key factor for all lipid-producing cells.[98] Cells with deficiency of PPAR, such as knockout cells, can only poorly contribute to the formation of secretory acini.[96] In line with this observation in sebaceous glands may be the finding that the expression pattern of PPAR-γ gradually redistributes from a cytoplasmic location in young and young adult mice to a nuclear location in old mice. This change in location is paralleled by development of acinar atrophy with a decrease in the size of the acini in general and of the lipid droplets within individual meibocytes in particular.[87] A lack of PPAR-γ could therefore contribute to potential age-related atrophic processes of the meibomian gland. Agonists and antagonists of PPAR-γ can modulate the function of sebaceous glands and may also provide potential therapeutic approaches.[87]

In wounding conditions, stem cell differentiation tends to develop a higher degree of plasticity,[74] and the lineage commitment can switch, leading to a replacement of altered or lost progenitor cells.[82,88] This process may also apply to events observed in MGD that may exert stress on the cells, such as that due to stasis with downstream mechanical pressure stress

in obstruction or due to increased bacterial growth and downstream release of bacterial lipases, toxic mediators, or inflammatory mediators. Stem cell–based repair mechanisms, however, do not always result in intact tissue reconstruction but can lead to alterations in structure and function. This alteration can result in hyperplastic acini with increased lipid production[82] that could contribute to the pathogenesis of MGD, as similarly described in the pathogenesis of acne,[107] but it may also be involved in the development of meibomian gland cancer.[111]

Lipid Synthesis in Meibomian Glands

Overview

There are few studies on lipid synthesis or uptake in the meibomian gland. A primary reason for the scant number of studies is that information about which lipids are synthesized has been lacking. Meibomian lipid characterization has reached a point where it may now be possible to identify those lipid synthetic pathways that lead to very long fatty acids. Science remains challenged, however, in that not all animal models would produce the same lipid mixtures as humans, and because the energy needs and hormonal stimulation needed for lipid production are not fully understood. Enormous amounts of energy are required to add two carbons to a growing lipid chain (1ATP, 2NADPH; 16C palmitic acid requires 7ATP plus 14NADPH). For this to occur, the meibocytes need both a sufficient supply of oxygen and a reliable carbon source. Although the basal cells in the acini have access to oxygen from capillaries, as the cells mature, they continually plump up with lipids, and they distance themselves from the capillaries (Fig. 4). Both glucose (the typical carbon source) and oxygen do not diffuse well through lipids—hence, the conundrum as to how these resources are supplied to the maturing acinar cells. In mature meibocytes according to Jester et al.[32] "the mitochondria are shrunken and electron-dense." In contrast, Gorgas and Völkl[34] have not reported any degeneration of mitochondria in mature meibocytes but only the presence of osmophilic, dense inclusion bodies that occur in all stages of meibocyte differentiation. Despite this, the cells fill with lipids; therefore, the resources for producing the lipids must be accessible to the central acinar cells. It remains unclear whether this process occurs via transport or diffusion.

There are also questions about hormonal regulation of the lipid production process, because insulin and glucagon are typically involved with fat and sugar metabolism. They are water soluble and would have little access to the maturing acinar cells. By contrast, steroid hormones (particularly androgens) are known to influence the acinar cells, and their lipid solubility would give them access to the maturing acinar cells. In addition to the synthesis of meibomian lipids, the ultrastructure of meibocytes indicates that there are special features of stacked membrane arrays and peroxisomes, which means that these cells must also have the machinery to synthesize polar lipids, such as phospholipids and cholesterol, for synthesis of their internal membranes. There remains the possibility, however, that some of these polar lipids and others, such as (*O*-acyl)-ω-hydroxy fatty acids, are specifically synthesized for secretion. Before the puzzle of how the synthesis of these lipids is controlled versus those specifically destined for secretion can be systematically considered, a consensus must be reached on the composition of normal meibomian secretions. It is also highly likely that some of the enzymes associated with the lipid synthesis are membrane bound, which makes them difficult to study. Immunohistochemical studies may resolve this dilemma, because they will help determine the compartmentalization of the enzymes—an important factor, in that enzyme location is an indication of the end destination of the lipids. Unfortunately, no easily accessible primer text about the biochemical pathways has been published to date.

The most abundant components of the meibomian lipids are wax and sterol esters, consisting of fatty acids and fatty alcohols, long-chain (>20C) fatty acids and alcohols, and sterols, particularly cholesterol. The most abundant fatty acid is oleic acid, which has 18C and is monounsaturated (18:1(*cis*Δ9), which means it has 18C with the ninth bond being a *cis* double bond. Wax esters are formed by condensation of fatty alcohols with fatty acids, and sterol esters are formed by condensation of sterols with fatty acids.[103,116] Theoretically, these lipid components could either be synthesized de novo in the acinar cells or taken up from the bloodstream or both. The evidence for de novo synthesis is supported because the synthetic enzymes for the components and the transesterases to form the final products have been detected either directly or indirectly (mRNA) in the acinar cells.[77,81,117] There is no direct evidence to date that the lipids are taken up from the bloodstream (this does not refer to steroid hormones, which are lipids, being taken up by the acinar cells as part of their hormonal action) and this is an area that warrants further investigations. Such uptake could show variations in lipid composition with change in diet, and the plasma cholesterol levels warrant further investigation relative to the cholesterol[118] or cholesterol esters in tear fluid or meibomian secretions. Recently, patients with blepharitis and taking flaxseed oil (omega-3) showed no difference in omega-3 fatty acids, omega-6 fatty acids, total monounsaturated, poly, or monounsaturated fatty acids, despite levels of omega-3 fatty acids being higher in the blood.[119] And if diet is important, it would not explain how the meibomian lipids of koalas, which have a very restricted and specific lipid dietary intake (sole diet of eucalyptus leaves), have a lipid profile similar to other mammals (Butovich IA, Millar TJ. *IOVS* 2009;50:ARVO E-Abstract 2545). However, a correlation has been shown between polar lipid profiles and variations in diet in women with Sjögren's syndrome; that is, those with a single polar lipid peak after HPLC analysis had about double the intake of omega-3 fatty acids compared with those who had multiple polar lipid peaks.[120] Therefore, it is most likely that all the lipids secreted by the meibomian glands are synthesized by the gland and we must understand fatty acid and cholesterol synthesis, the key enzymes involved in these pathways, and in which cellular compartments they are located.

A Skeletal Overview of Lipid Synthesis

Fatty acid synthesis catalyzed by fatty acid synthase occurs in the cytoplasm, but the carbons come from the mitochondria (Fig. 9). Therefore, mitochondria are necessary, not only for generating the large amounts of energy needed for lipid synthesis, but also for the carbons of lipids. This fact is enigmatic in the context of meibomian glands, where the more mature acinar cells continue to plump up with lipids, but at the same time their mitochondrial number decrease, and they are further displaced from their oxygen source.

Fatty Acid Synthesis

The carbon chain of the fatty acids is built two carbons at a time after repeated enzymatic cycles. As part of this process, the acetyl-CoA has to be activated to a higher energy level. This activation is induced by adding carbon dioxide to it to form malonyl-CoA (Fig. 10). With each cycle of fatty acid synthesis, two new carbons from malonyl-CoA are added to the chain, and the third carbon is released as CO_2 (Fig. 11). With each cycle, through several different reactions catalyzed by different domains of the enzyme fatty acid synthase, two reductions

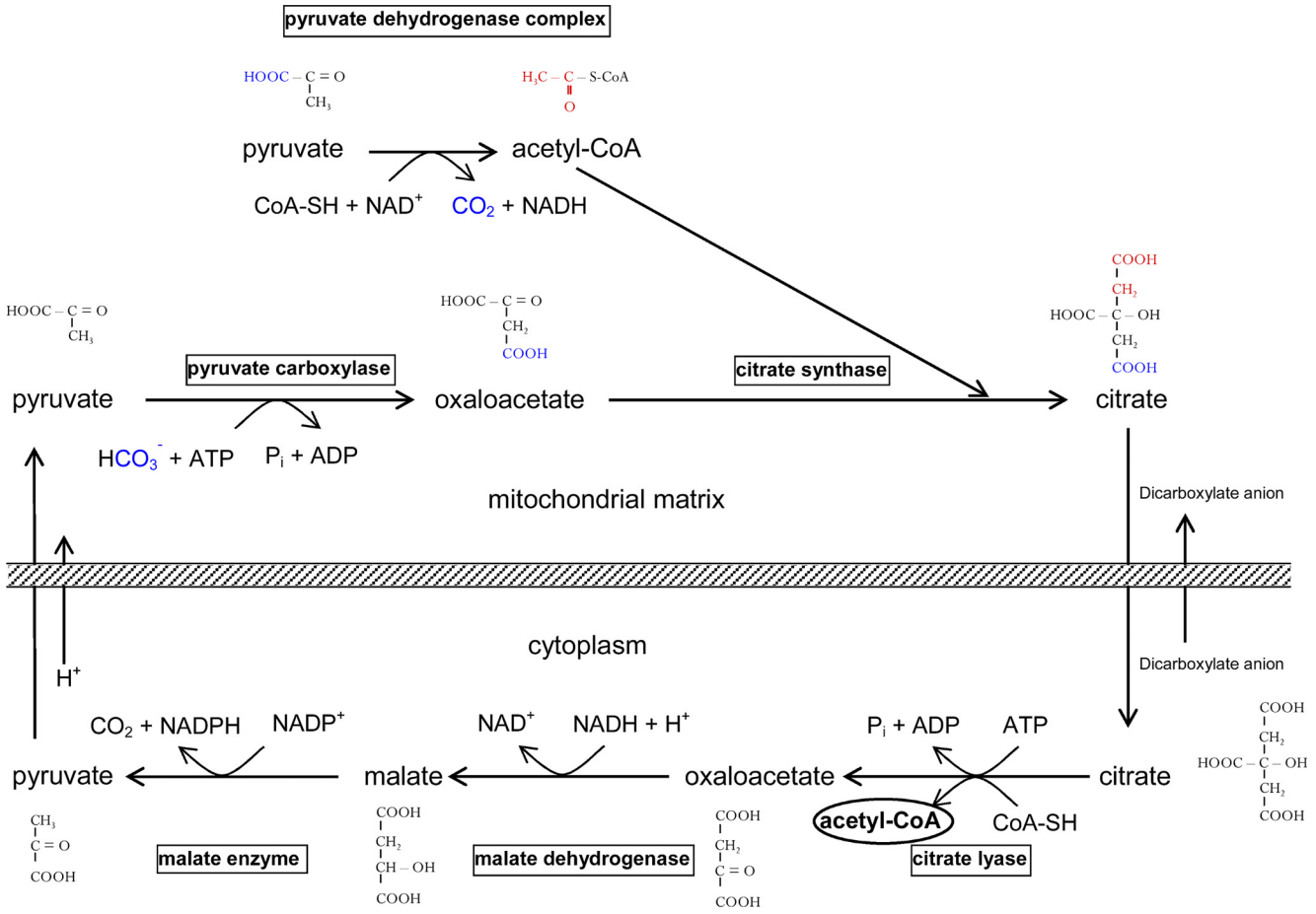

FIGURE 9. Transfer of carbons for lipid synthesis from the mitochondria to the cytoplasm. When the tricarboxylic acid (TCA) cycle in the mitochondria is blocked due to an excess of the high-energy molecule NADH, there is a buildup of mitochondrial acetyl-CoA that indicates to the cell that it has a surfeit of energy and therefore does not need to oxidize carbons to obtain more energy. Instead, it is more desirable to store the carbons as fats until the energy is needed. The acetyl group (2C) of acetyl-CoA is passed to oxaloacetate (4C) to form citrate (6C), and citrate is transferred across the mitochondrial membrane into the cytoplasm. It is then lysed (citrate lyase) and coupled to cytoplasmic CoA to form cytoplasmic acetyl-CoA, which is used for fatty acid synthesis, and oxaloacetate, which is cycled back (indirectly) to the mitochondrial matrix. Figure courtesy of Tom Millar.

occur, each of which converts one NADPH to NADP. Each of these steps is about the same as consuming three ATPs. The result is an even-numbered fatty acid, typically 16C long (palmitic acid), which is cleaved from the enzyme by a thiolase. Many of the fatty acids in the meibomian gland are much longer than 16C, and the further elongation requires different enzymes. If an odd-numbered straight-chain fatty acid is to be synthesized, propionyl-CoA (4C) is initially used as the carbon source instead of malonyl-CoA, and when CO_2 is displaced, three carbons are added to the chain, resulting in an odd-numbered fatty acid.

Straight-Chain Fatty Acids. For chain elongation, the palmitoyl component of palmitoyl ACP is transferred to coenzyme A (CoA-SH) to form palmitoyl-CoA. Then, elongation (C18–C28) occurs in the endoplasmic reticulum,[121] and some of these are converted by additional enzymes into fatty alcohols. The elongation process occurs in the same way as described above, except that in this case, CoA is the carrier. Two new carbons are loaded from malonyl-CoA onto palmitoyl-CoA, followed by reduction, dehydration, and reduction to form the new saturated chain extended by 2C. In forming the most prominent fatty acid in meibomian gland secretions, oleic acid, the palmitic acid (16C), has to be elongated and then desaturated. Fatty acyl-CoA desaturase catalyzes the introduction of a double bond into the acyl chain (bond 9 of stearic acid), to form oleic acid. This oxidation occurs on the inner face of the endoplasmic reticulum and involves coupled electron transfers through cytochrome-*b5*, FADH2, and NADPH, which is converted to $NADP^+$. This enzyme in mammals readily desaturates the bond between C9 and C10 but does not desaturate the bonds between C10 and the terminal methyl group. Fatty acids with desaturation between C10 and the terminal such as lino-

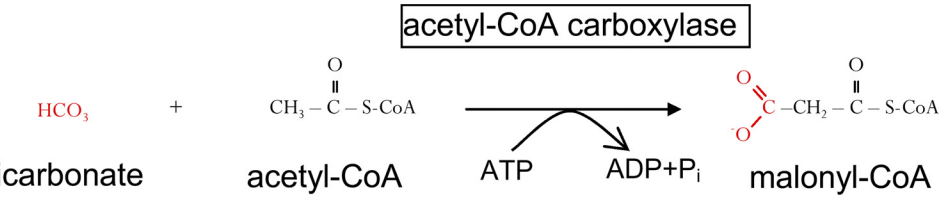

FIGURE 10. Formation of malonyl-CoA. Figure courtesy of Tom Millar.

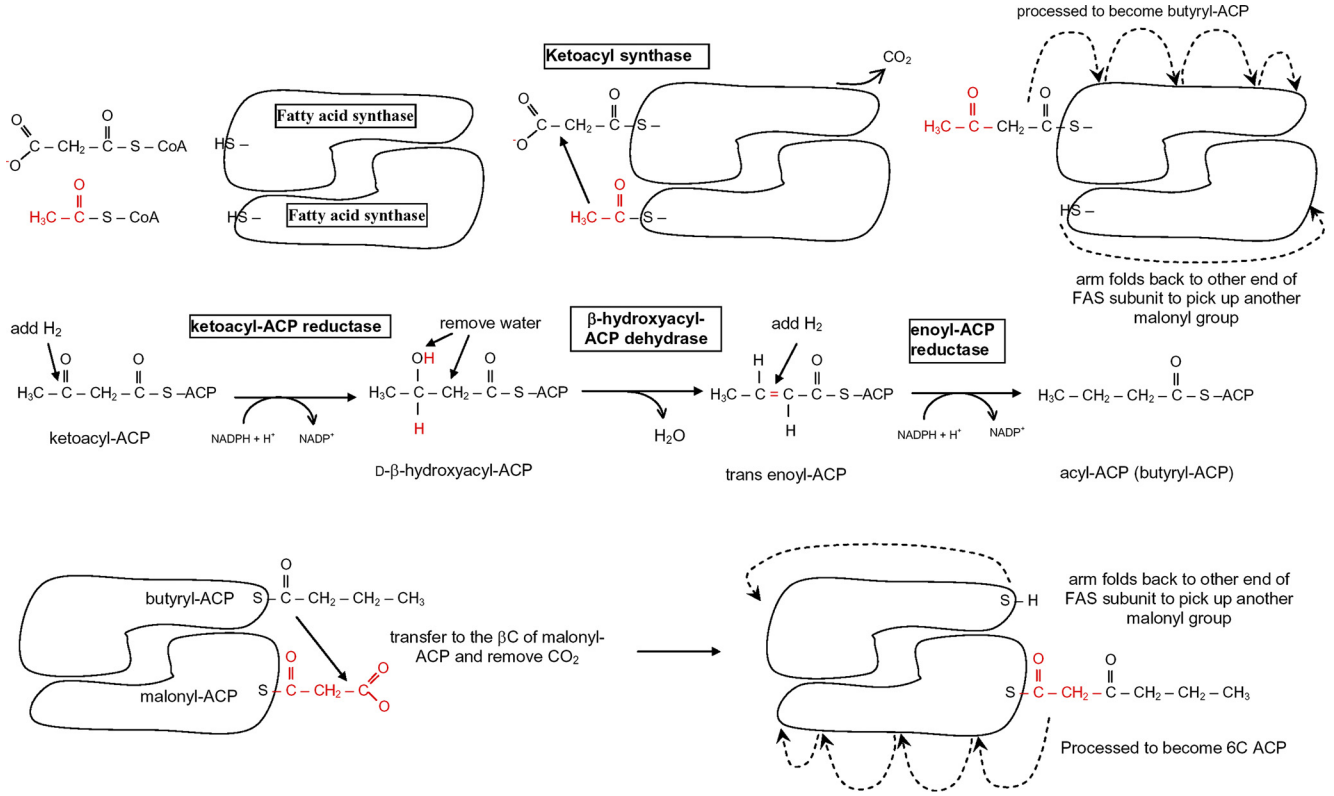

FIGURE 11. Various activities of fatty acid synthase. The functional enzyme is a dimer with multiple functions in different regions. On each cycle, the growing chain is transferred to a malonyl-loaded acyl carrier protein domain of the protein, and in so doing displaces CO_2, which increases the chain length by 2C. Figure courtesy of Tom Millar.

leic acid (18:2; all-*cis*$\Delta^{9,12}$) and linolenic acid (18:3; all-*cis*$\Delta^{9,12,15}$) are normally obtained from the diet. These fatty acids have been detected in human meibomian gland secretions, albeit in small amounts.[122] One possibility is that there are additional desaturase enzymatic activities in the meibomian gland that are able to desaturate ω-3 or ω-6 bonds in C18 fatty acids. One example of such enzymatic activity is the product of the gene *fat-1*, which has been found in animals.[123] These desaturases are membrane bound and probably require other membrane-linked cofactors, such as cytochrome-*b5* for their activity. The presence of large numbers of ordered peroxisomes in more mature cells in the meibomian gland acini[34] may be the location of these enzymes, but this still has to be demonstrated. That these enzymes are membrane bound means that they are very difficult to purify, and therefore searching for their gene expression is more likely to reveal their presence. Another important highlight of plant membrane-bound desaturases is that they use glycerolipids as their substrates and not acyl-CoA. The presence of this substrate is also likely to be the case for similar desaturases (e.g., FAT-1), if they exist, in meibomian glands. This notion implies that the carbon chain source would be from the membrane lipids of the endoplasmic reticulum.

Branched-Chain Fatty Acids. Some branched-chain fatty acids have been detected as components of meibomian lipids.[116,124,125] It appears from studies of rabbit meibomian glands, with the use of radioactive labeled precursors, that the branched carbon chain is supplied from branched side-chain amino acids. In rabbits, isoleucine has been used predominantly in vivo, whereas, in vitro, valine has also been used. In neither case has leucine been used as a precursor.[126] For these, the incorporation must have been at the initial loading of the acyl carrier domain of fatty acid synthase, because the branched fatty acids were either iso- or anteiso-branched (at the omega end of the carbon chain). Since the new chain grows from the carboxyl end, if incorporation were not at initiation, there would be multiple branching along the length of the chain. Similarly, if it were incorporated after the initial synthesis of palmitic acid, the extension phase for longer chain fatty acids, they would also occur randomly and possibly as multiple branched methyl groups near the carboxyl end, but this is not the case. Whether the preference order for the amino acid precursors for branched-chain fatty acids is the same in other animals as it is in rabbits still has to be established.

Fatty Alcohols. Fatty alcohols are synthesized from their corresponding fatty acids. The fatty acid component of acyl CoA is reduced to form the corresponding fatty alcohol (acyl-CoA reductase).[127,128] Whether the cofactor in the meibomian gland is NADPH or NADH is not known, but two high-energy compounds are consumed in the reaction. In the meibomian gland, there appears to be a preference for long-chain fatty acids, because long-chain fatty alcohols seem to be the predominant species found to be associated with cholesterol and wax esters. This association may come about because of an enzymatic preference for longer chain fatty acids or because the shorter chain fatty acids are preferentially used for the acid component of wax esters or (*O*-acyl)-ω-hydroxy fatty acids.[122] The location of the fatty alcohols could be in the peroxisomes,[34] whereas in other tissues they are involved in making ether bonds (condensation of two alcohols), but in this case it would be likely that they form ester bonds with fatty acids to form the wax esters.

Cholesterol Synthesis. An overview of cholesterol synthesis is that all 27C compounds come from acetic acid, in the form of acetyl-CoA. As above, the acetyl component of acetyl-

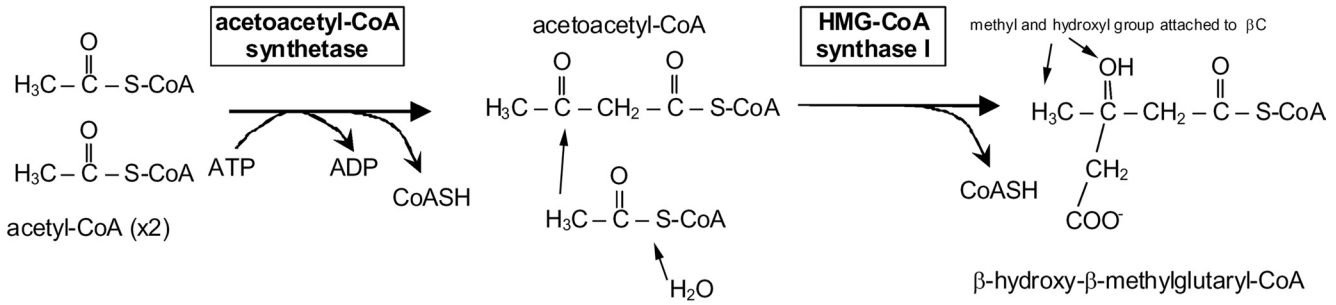

FIGURE 12. β-Hydroxy-β-methyl glutaryl-CoA (HMG-Co A). HMG-CoA synthase 1 (4.1.3.5) is located in the cytoplasm, unlike HMG-CoA synthase 2 (4.1.3.4), which is located in the mitochondria. Figure courtesy of Tom Millar.

CoA is transferred from the mitochondria to the cytoplasm via citrate (Fig. 9). It is then converted to HMG-CoA (Fig. 12), which is converted to mevalonate (Fig. 13). This reaction is catalyzed by HMG reductase, which is activated by insulin and inhibited by glucagon. Therefore, it would be of interest to examine the composition of meibomian lipids from patients with uncontrolled diabetes, as in theory, this critical enzyme would have decreased activity, and hence less cholesterol would be synthesized. Mevalonate is converted to a 5C compound, isopentenyl pyrophosphate (Figs. 13, 14). Three of these are joined to form a 15C compound—farnesyl pyrophosphate—and two farnesyl pyrophosphates are joined to form a 30C compound, squalene (Fig. 15). Squalene is cyclized to form lanosterol, and three methyl groups are then removed to form cholesterol.

The mRNAs for the enzymes associated with this pathway have been identified in mouse meibomian gland extracts, and most of them are increased by testosterone, which is an indication that this pathway is upregulated by testosterone.[77,121] This suggestion is supported by the histochemical detection of various hydroxysteroid dehydrogenases in meibomian gland acinar cells. These enzymes were located in developing and mature, but not basal, acinar cells of the meibomian gland.[129] These enzymes are not found in all sebaceous glands, but are associated with sebaceous glands of the face and neck.[130,131] In sebaceous glands, the levels of these enzymes decrease with age, which does not seem to be the case with meibomian glands. Perra et al.[56] did not mention this, even though samples were taken from 18- to 60-year-olds. In terms of function, the enzymes are located in the endoplasmic reticulum and are able to catalyze the conversion of androgens into their potent metabolic forms, particularly dihydrotestosterone, which is associated with upregulation of gene transcription for enzymes associated with fatty acid and cholesterol synthesis, as well as other functions associated with lipid metabolism.

Other Synthetic Pathways

Triglycerols have also been reported to be components of meibomian lipids. The pathway for synthesis of triglycerols involves having one of the intermediates of glycolysis, dihydroxyacetone phosphate, converted to glycerol 3-phosphate (glycerol 3-phosphate dehydrogenase), which uses NADH as the hydrogen source. Acyl transferases sequentially catalyze the transfer of acyl groups from acyl-CoA to C_1 and then C_2 of the glycerol 3-phosphate to form phosphatidic acid. Phosphatidic acid phosphatase catalyzes the dephosphorylation of phosphatidic acid to form diacylglycerol, which is then converted to triacylglycerol by transfer of an acyl group from acyl-CoA (acyl transferase).

The suggestion and, more recently, the detection of (*O*-acyl)-ω-hydroxy fatty acids[33,121] means that ω-hydroxy fatty acids must be synthesized in the meibomian glands. The enzymatic pathway for ω-hydroxy fatty acids is not known. At this stage, only long-chain ω-hydroxy fatty acids have been detected, which tends to indicate that the hydroxylation occurs at the end of acyl chain synthesis, not at the beginning. Hydroxylation of an inert terminal methyl group would be very unusual and normally needs several intermediates. Such intermediates have yet to be detected. However, many of these compounds, which may be in low amounts, would be undetected unless specifically sought.

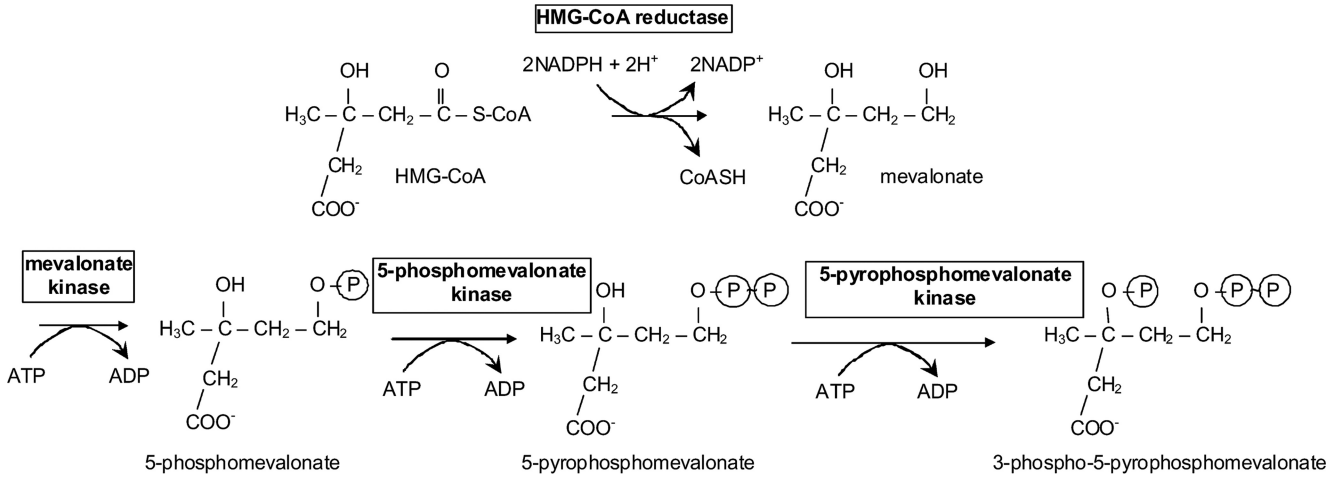

FIGURE 13. Formation of 3-phospho-5-pyrophosphomevalonate. 3-Hydroxy-3-methyl glutaryl-CoA is converted on the endoplasmic reticulum to the energetically activated 3-phospho-5-pyrophosphomevalonate. Figure courtesy of Tom Millar.

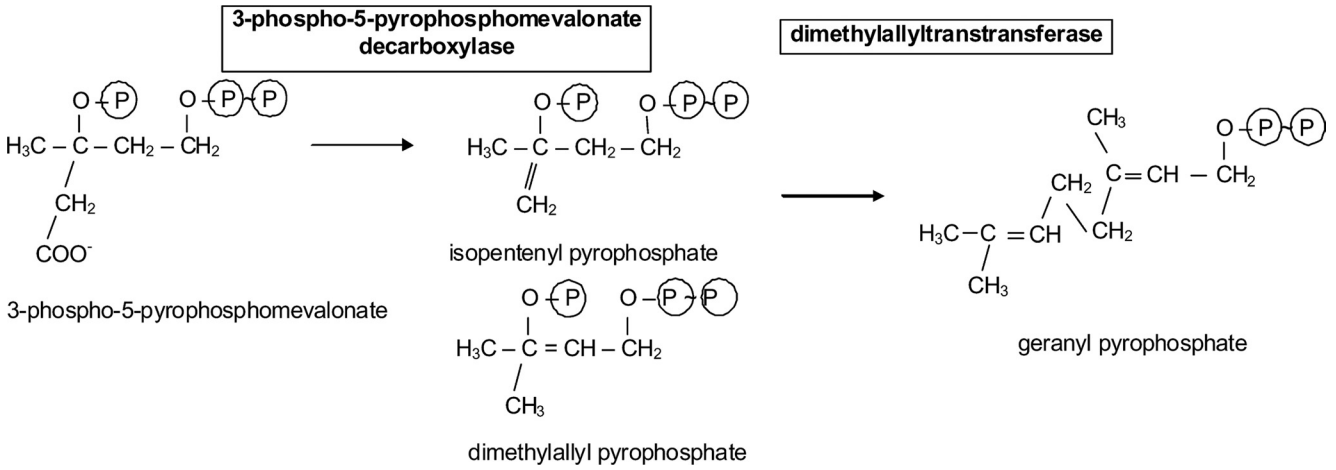

FIGURE 14. Formation of 10C geranylpyrophosphate. The 5C isopentenylpyrophosphate and its isomer are formed from 3-phospho-5-phosphomevelanate, which are then joined to form 10C geranylpyrophosphate. Figure courtesy of Tom Millar.

Cholesterol esters tend to have long-chain (>C20) fatty acids attached to them,[132] which indicates that the cholesterol transacylase(s) involved prefer long-chain acids. This preference differs from that of the wax acyl-CoA:alcohol transacylases, which do not appear to be specific for chain lengths.[125] The (*O*-acyl)-ω-hydroxy fatty acids tend to have long-chain ω hydroxyl fatty acids (C30:1, C32:1, and C34:1) acetylated through their ω-hydroxyls by a C18:1-FA,[132] which indicates that the transacylases may be very specific.

Comments on the Synthesis of Lipids in Meibomian Glands

Vascular Supply. Fat synthesis needs both energy and an excellent blood supply as the source of its oxygen. This necessity is reflected in adipose tissue development, where lipocyte density correlates positively with capillary density, and the cells tend to cluster around large blood vessels.[133] Examination of the published literature on the structure of the meibomian gland and structural studies of meibomian gland development have not paid particular attention to capillary size and density. Although this may not be particularly essential in development, a study of the capillaries could be of benefit in understanding the aging process, in which gland atrophy has been noted. Local swelling, such as occurs when there is a blockage of the meibomian gland duct, may also lead to poor blood flow and atrophy of the glands. In other fat cells, angiogenesis factors such as PGE2 (ubiquitous) and L-butyrylglycerol (specific to fat cells) are synthesized and secreted by adipocytes. To date, secretion of these factors by meibocytes has not been investigated.

The predominant source of energy in these synthetic pathways is NADPH, typically produced by a side path of glycolysis, the pentose phosphate pathway. Key enzymes associated with this pathway (glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) have been detected in the differentiating and degenerating cells of human meibomian glands, but not in the basal cells. The need for this pathway means that, to produce lipids, meibocytes require a rich supply of glucose and therefore depend heavily on insulin and a rich blood supply for glucose uptake. Insulin also stimulates lipid synthesis (many kinases that control activation of lipid synthetic enzymes are sensitive to insulin). Similarly, glucocorticoids have been long known to increase adipose tissue mass via their hypertro-

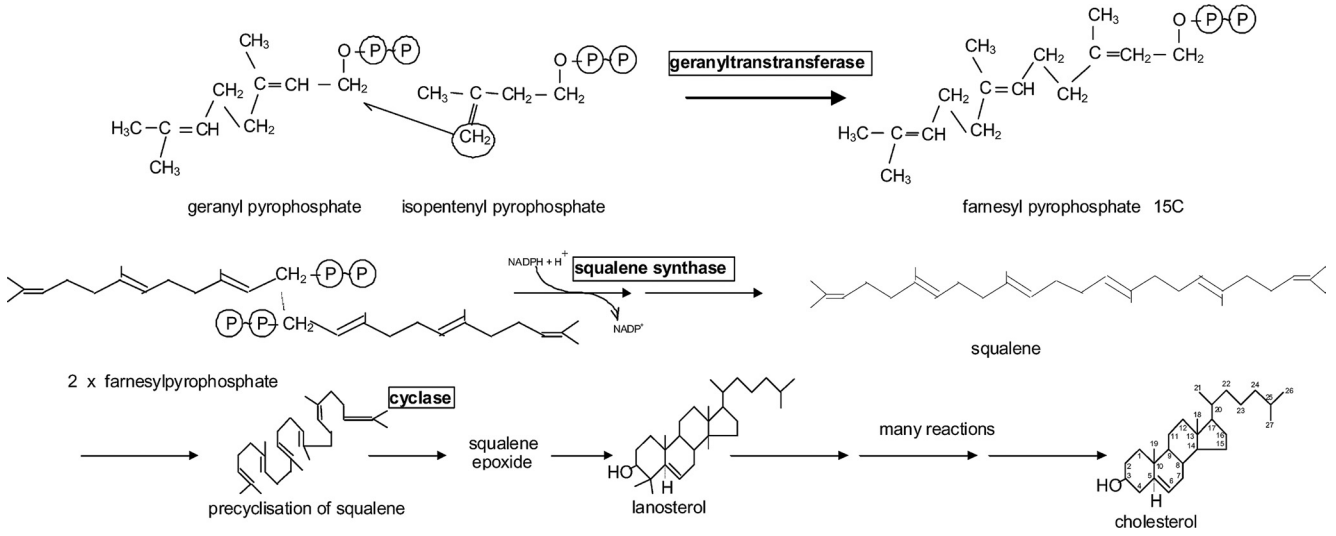

FIGURE 15. Formation of cholesterol. Formation of 15C farnesyl pyrophosphate, followed by 30C squalene, which is then converted through a variety of reactions to 27C cholesterol. Figure courtesy of Tom Millar.

phic effect. A clinical method of testing this hypothesis would be the assessment of the meibomian glands that are altered in patients who have Cushing's syndrome or are receiving cortisol treatment.

The membranes between acinar cells are particularly and unusually interdigitated[35] and could be a region of cytoplasmic exchange. On the positive side, published micrographs also show, on casual inspection, that there is a concentration of mitochondria around lipid droplets that is consistent with the molecules that come from the mitochondria being a carbon source for the new lipids.[32] Similarly, some lipid droplets are surrounded by organized concentric layers of endoplasmic reticulum. This arrangement is consistent with the enzymes associated with fatty acyl chain elongation and oxidation (formation of double bonds) occurring in these regions. It may also mean that, at this stage, different lipids are in different lipid droplets, but it probably has no impact at the end, when the cells disintegrate as part of the holocrine secretion.

Proteins and the Meibomian Gland

There is emerging evidence that specific lipoproteins[134] or other proteins (Thangavelu M, et al. *IOVS* 2010;51:ARVO E-Abstract 2373) may be associated with meibomian lipids. There has been a study on adipose differentiation-related protein, a protein that marks the differentiation of adipose tissue.[106] This protein seemed to localize adjacent to the lipid droplets and, in micrographs, appeared to be present at lower levels in the most mature cells in the follicle. The low magnification made this difficult to ascertain. Lipocalin has also been found in human meibomian gland secretions,[134] and hence it is possible that this lipocalin also sequesters lipids. It may originate from the meibomian gland, given that lipocalin mRNA has been identified in the mouse meibomian gland.[81,135]

Physical Properties of Meibomian Lipids

The transition temperature from a solid to a liquid for meibomian lipids is actually a range, 28°C to 32°C, because of the mixture of lipids. This transition has been explained by the arrangement of lipids from their transconformation (ordered and rigid) to their gauche conformation (disordered and fluid), as evidenced by infrared spectroscopy.[136] The temperature of the eyelids will therefore affect the liquidity of meibomian lipids and hence their viscosity. In liquids, viscosity (η) is a measure of resistance to flow at a particular temperature. In substances that exhibit Newtonian viscosity, if the force is doubled, the flow rate doubles, and hence the resistance to flow (viscosity) remains the same. Other substances alter their viscosity (non-Newtonian) depending on the force applied. (A good example is toothpaste, which is very viscous when sitting on a toothbrush, but flows easily out of a tube when force is applied.) This phenomenon is called shear thinning. Tiffany and Dart[137] have reported the viscosity of human meibomian lipid samples to vary between 9.7 and 19.5 Pa · s, (cf. honey, 10 Pa · s; glycerine, 1 Pa · s; olive oil, 0.1 Pa · s; and water, 1 mPa · s), depending on the force applied. Therefore, the viscosity of meibomian lipids exhibits non-Newtonian behavior. The temperature at which the viscosity was measured in these experiments was not indicated, but considering the data, it is likely that it was below the transition temperature (30°C). In a practical sense, these data represent the viscosity of the meibomian secretion sitting in the ducts. Blinking would apply shearing force that would lower the viscosity, making the lipids easier to eject from the meibomian orifices. This shearing force would be increased by having narrow openings in comparison to the diameter of the duct (as anatomic studies indicate is the case), and this effect would also reduce the viscosity. A lower viscosity would also occur because the temperature of the eyelid (35°C–37°C) would be above the transition temperature. In the same experiments, the viscosity of lipids extracted from chalazion secretions were so high that only one measurement, despite heating to 70°C, could be made, and that was 69.9 Pa · s. This material contained more phospholipids, free fatty acids, cholesterol, and triglycerides than do normal meibomian lipids.

Viscosity measurements on thin films are complex, and several different experimental parameters, such as frequency and amplitude of shear forces and surface pressure of the film, are varied to come up with appropriate measures of viscosity.[138–140] Films made from human meibomian lipids show increasing viscosity (complex viscosity) with surface pressure and attain a viscosity of 1 mPa · s at a surface pressure of approximately 25 mN/m, dropping to 0.1 mPa · s at 35°C, at a shear frequency of 6.2 rads^{-1}.[141]

The refractive index of meibomian lipids varies between 1.46 and 1.53 per the visible spectrum, with a small, relatively linear decrease over a temperature range from 25°C to 45°C. The refractive index of human skin lipids is approximately 0.013 less. This high refractive index probably has little overall refractive influence over a pure air–water interface because the lipid layer is very thin.[142]

Although there seems to be an abundance of lipids available on the eyelid margins (300 μg) compared with the amount of lipids estimated to be in the lipid layer of the tear film (9 μg),[48] we still do not know what enables them to spread from the reservoir across the ocular surface to form a film. There is indirect evidence that a bigger reservoir of lipids on the eyelid margins leads to a thicker lipid layer. The amount of lipids is higher on the eyelid margins just after waking,[45] and there is a thicker oil film on the eye surface in the morning.[143] A surfactant is needed for lipids to spread across an aqueous surface. In the absence of a surfactant, the lipids form lenses on the surface. One of the main questions about meibomian lipids centers on what enables them to spread. Holly[144] proposed that this could be achieved by an initial spread of polar lipids over the aqueous surface followed by nonpolar lipids. However, it is most likely that the lipid film is not respread on each blink, but instead collapses and expands, as indicated by the same interference patterns seen over multiple blinks; only after this is a new layer formed. What conveys these properties is still not understood and whether proteins from the aqueous layer become part of this outer layer of the tear film, as some models suggest, still has to be demonstrated. Conversely, there is also no evidence they are not part of this outer layer.

Regulation of the Meibomian Gland in Health and Disease

Sebaceous glands are present throughout the body and are classified into two major types: pilosebaceous, which are associated with hair follicles, and free (i.e., preputial and meibomian), which occur in the transitional zone between the skin and mucous membranes.[145] An extensive amount of information is known about sebaceous glands, and a review of their physiological regulation in health and disease would be challenging. More than 6000 articles have been published about these glands since 1904, and numerous factors are known to modulate the development, proliferation, differentiation, maturation, lipogenesis, and secretion of sebaceous glands throughout the body (Table 1). These factors include sex steroids, corticosteroids, hypothalamic and pituitary hormones, insulin, retinoids, thyroxine, melanocortins, neurotransmitters, growth factors, and peroxisome proliferator-activated receptor ligands (Table 1).[40,98,145–156] The control points for sebaceous gland regulation often involve effects on

TABLE 1. Control of Sebaceous Gland and/or Sebocyte Growth and Lipid Production

Factor	Growth	Lipid Production
Adrenocorticotropic hormone	↑	
Androgen	↑	↑
Basic fibroblast growth factor	↑	
Calcitonin gene-related peptide	R	R
Corticotropin-releasing hormone	↑	↑
Epidermal growth factor	↑	
Estrogen	↓	↓
Growth hormone	↑	
Hydrocortisone	↑	
Insulin	↑	
Insulin-like growth factor-I	↑	
Insulin-like growth factor-II	↑	
Keratinocyte growth factor	↑	
Neuropeptide Y	R	R
Peroxisome proliferator activated receptor ligands		↑
Pituitary extract (bovine)	↑	
Progestin		~
Retinoid	↓	↓
Substance P	↑	↑
Thyroid-stimulating hormone	↑	
Thyroxine	↓	↑
Transforming growth factor-α	↑	
Vasoactive intestinal peptide	R	R
α-Melanocyte stimulating hormone	↑	↑
β-Adrenergic agonist		Permissive
β-Endorphin	↓	↑

Growth refers to sebocyte proliferation, sebocyte differentiation, and/or glandular size. Lipid production refers to lipogenesis and/or lipid secretion. R, functional receptors have been identified, but their role has yet to be determined; ↑, increase; ↓, decrease; ~, increase, decrease, or no effect, depending, on the type of sebaceous gland.

gene expression, protein synthesis, and lipid production. There appear to be considerable differences in the control mechanisms, however, as well as in the lipid composition, of sebaceous glands between species and between different types of sebaceous glands.[145]

In contrast to sebaceous glands in general, there is relatively little information about the physiological regulation of the meibomian gland. Fewer than 850 articles about the meibomian gland have been published in the past 106 years (Table 2), and fewer than 50 of these papers, including reviews, address the topic of physiological control. The paucity of studies is astonishing, given that recent research has demonstrated the presence of more than 270 receptor mRNAs in the mouse meibomian gland alone (Table 3). Yet it is unknown whether most of these transcripts are translated and functional. Of particular interest is the lack of knowledge about the neural influence on the meibomian gland. This tissue is the only sebaceous gland in the human body that has rich sensory, sympathetic, and parasympathetic innervation,[145] including contact with nerve fibers reactive for acetylcholinesterase, substance P, vasoactive intestinal peptide, dopamine β-hydroxylase, nitric oxide synthase, tyrosine hydroxylase, somatostatin, neuropeptide Y (NPY), and CGRP.[39,56,57,59,81,157–169] Further, the meibomian gland contains mRNAs for serotonin, adrenergic, CGRP, cholinergic, dopamine, γ-aminobutyric acid, glutamate, NPY, neurotensin, and somatostatin receptors (Table 3).[81] It is entirely unclear, however, whether neurotransmitters are released in the vicinity of the meibomian gland, act on glandular receptors, or induce a physiological effect.

Almost all of our understanding of the physiological, as well as pathophysiological, regulation of the meibomian gland originates from research exploring the effects of androgens, estrogens, progestins, all-*trans* retinoic acid, and growth factors on this tissue and/or its epithelial cells. This topic is discussed in the following sections.

Androgens

Androgen Regulation of Sebaceous Glands. Androgens exert a significant impact on the meibomian gland.[81] This influence is not surprising, given that androgens control the development, differentiation, and lipid production of sebaceous glands throughout the body.[40,81,145,146,148,151,153,156,170–188] Androgens act primarily on acinar epithelial cells in sebaceous glands, and these cells contain both androgen receptor mRNA and protein (in their nuclei). Acinar cells respond to androgens by increasing the transcription of multiple genes and synthesizing proteins that augment both the elaboration and secretion of lipids. Sebaceous gland activity and secretion may be inhibited by orchiectomy or topical antiandrogen treatment.[182,189–192] Of particular interest, sebaceous gland function declines with age.[193] This aging-associated dysfunction has been correlated with both an atrophy of acinar epithelial cells and a decrease in serum androgen levels.[193] Indeed, the age-related cellular shrinkage in certain sebaceous glands has been directly correlated with a reduction in androgen levels in the surrounding skin.[193]

Androgen activity in sebaceous glands is significantly influenced by the activity of certain enzymes, particularly 5α-reductase (converts testosterone into the potent androgen 5α-dihydrotestosterone), aromatase (converts testosterone to 17β-estradiol, androstenedione to estrone), and 17β-hydroxysteroid dehydrogenase (HSD; regulates the interconversion of 17-ketosteroids with their corresponding 17β-hydroxysteroids and is necessary for the intracrine formation and/or inactivation of all active androgens and estrogens in sebaceous glands).[194–200] These enzymes are vital, given that most of the androgens and estrogens in humans are synthesized in peripheral tissues (e.g., sebaceous glands) from adrenal sex steroid precursors (i.e., dehydroepiandrosterone [DHEA] and DHEA-sulfate) and that these enzymes regulate the critical steroidogenic pathways (Fig. 16).[194–197] Of interest, the activity of these enzymes may vary according to sex, tissue location in the body, or cellular position within a pilosebaceous unit and may also be induced by microenvironmental factors (e.g., proinflammatory cytokines).[200–203]

Androgens also regulate numerous pathways of lipid metabolism. For instance, depending on the tissue, androgens control:

TABLE 2. Articles with the Phrase "Meibomian Gland" Cited in PubMed from 1903 through November 2009

Topic	Number of Articles	% of Articles
Clinical assessment and treatment	405	48.0
Cancer	141	16.7
Lipid analysis, properties and synthesis	99	11.7
Literature reviews (partial)	52	6.2
Anatomy and histochemistry	50	5.9
Physiological regulation	48	5.7
Pathology (experimental)	25	3.0
Neural innervation	17	2.0
Culture systems	4	0.5
Stem cells	3	0.4

A total of 844 articles were evaluated. The category Literature Reviews (Partial) does not include topics related to lipid analysis, neural innervation, or physiological regulation. Those reviews are placed in their specific topic areas.

TABLE 3. Receptor mRNAs Present in the Mouse Meibomian Gland[83]

5-hydroxytryptamine (serotonin) receptor 1a
5-hydroxytryptamine (serotonin) receptor 1b
5-hydroxytryptamine (serotonin) receptor 1d
5-hydroxytryptamine (serotonin) receptor 3b
5-hydroxytryptamine (serotonin) receptor 5b
5-hydroxytryptamine (serotonin) receptor 6
5-hydroxytryptamine (serotonin) receptor 7
7 transmembrane g-protein coupled receptor
Activin a receptor, type 1
Activin a receptor, type 1b
Activin a receptor, type ii-like 1
Activin receptor iia
Activin receptor iib
Adenosine a2b receptor
Adenosine receptor retinoid x receptor interacting protein
Adenylate cyclase activating polypeptide 1 receptor 1
Adrenergic receptor, α 1b
Adrenergic receptor, α 2a
Adrenergic receptor, α 2c
Adrenergic receptor, β 3
Adrenomedullin receptor
Arginine vasopressin receptor 1a
Arginine vasopressin receptor 1b
Arginine vasopressin receptor 2
Aryl hydrocarbon receptor nuclear translocator
Aryl hydrocarbon receptor nuclear translocator 2
Aryl hydrocarbon receptor nuclear translocator-like
Aryl-hydrocarbon receptor
Aryl-hydrocarbon receptor repressor
Aryl-hydrocarbon receptor-interacting protein
Asialoglycoprotein receptor 1
B-cell receptor-associated protein 29
B-cell receptor-associated protein 31
B-cell receptor-associated protein 37
Benzodiazepine receptor, peripheral
Bone morphogenetic protein receptor, type 1b
Bradykinin receptor, β
Cadherin EGF lag seven-pass g-type receptor
Cadherin EGF lag seven-pass g-type receptor 2
Calcitonin gene-related peptide-receptor component protein
Calcitonin receptor
Calcitonin receptor-like
Candidate taste receptor t2r19
Cation-independent mannose 6-phosphate/insulin-like growth factor ii receptor precursor
Cd36 antigen (collagen type i receptor, thrombospondin receptor)-like 2
Chemokine (c-c) receptor 2
Chemokine (c-c) receptor 4
Chemokine (c-c) receptor 5
Chemokine (c-x-c) receptor 2
Chemokine (c-x-c) receptor 3
Chemokine (c-x-c) receptor 4
Chemokine orphan receptor 1
Cholecystokinin a receptor
Cholinergic receptor, nicotinic, a polypeptide 1
Cholinergic receptor, nicotinic, a polypeptide 6
Cholinergic receptor, nicotinic, b polypeptide 1
Colony stimulating factor 2 receptor, a, low-affinity
Colony stimulating factor 2 receptor, b 1, low-affinity
Colony stimulating factor 2 receptor, b 2, low-affinity
Complement component 5, receptor 1
Complement receptor related protein
Corticotropin releasing hormone receptor 2
Coxsackievirus and adenovirus receptor
Cytokine receptor-like factor 3
D6 b-chemokine receptor
Dopamine receptor 4
Endothelial differentiation, lysophosphatidic acid g-protein-coupled receptor 7
Endothelial differentiation, lysophosphatidic acid g-protein-coupled receptor 4
Endothelial differentiation, sphingolipid g-protein-coupled receptor, 8
Endothelial-specific receptor tyrosine kinase
Endothelin receptor type b
Eph receptor a2
Eph receptor a4
Eph receptor a5
Eph receptor a6
Eph receptor b6
Eph-related receptor tyrosine kinase
Epidermal growth factor receptor
Epidermal growth factor receptor pathway substrate 15
Epidermal growth factor receptor pathway substrate 15, related sequence
Epidermal growth factor receptor pathway substrate 8
Estrogen receptor β
Estrogen receptor-binding fragment-associated gene 9
Estrogen related receptor, α
Fc receptor, IgE, high affinity i, γ polypeptide
Fc receptor, IgE, low affinity ii, α polypeptide
Fc receptor, IgG, low affinity iib
Fc receptor, IgG, low affinity iii
Fc receptor, IgG, α chain transporter
Formyl peptide receptor-related sequence 4
Formyl peptide receptor-like 1
G protein-coupled receptor 34
G protein-coupled receptor 35
G protein-coupled receptor 37
G protein-coupled receptor 44
G protein-coupled receptor 49
G protein-coupled receptor 66
G protein-coupled receptor 83
G protein-coupled receptor 87
G protein-coupled receptor g2a
G protein-coupled receptor kinase 5
G protein-coupled receptor kinase-interactor 2
G protein-coupled receptor, family c, group 5, member d
γ-aminobutyric acid (gaba-a) receptor, subunit rho 1
γ-aminobutyric acid receptor-associated protein
γ-aminobutyric acid (gaba-a) receptor, subunit gamma 3
Gastrin-releasing peptide receptor
Glial cell line-derived neurotrophic factor family receptor α 3
Glial cell line derived neurotrophic factor family receptor α 4
Glutamate receptor, ionotropic, kainate 2 (β2)
Glutamate receptor, ionotropic, kainate 5 (γ2)
Glutamate receptor, ionotropic, δ2
Golgi snap receptor complex member 1
G-protein coupled receptor 12
G-protein coupled receptor 25
G-protein coupled receptor 26
G-protein coupled receptor 3
G-protein-coupled receptor 50
Growth factor receptor bound protein 14
Growth factor receptor bound protein 2
Growth factor receptor bound protein 2-associated protein 1
Growth factor receptor bound protein 7
Growth hormone receptor
Histamine receptor h 2
Hyaluronan mediated motility receptor
Hybrid receptor gp250 precursor
Inositol 1,4,5-trisphosphate receptor (type 2)
Insulin receptor
Insulin receptor-related receptor
Insulin-like growth factor i receptor
Interferon (α and β) receptor
Interferon γ receptor
Interleukin 1 receptor antagonist
Interleukin 1 receptor, type i
Interleukin 1 receptor, type ii
Interleukin 1 receptor-associated kinase
Interleukin 10 receptor, β
Interleukin 11 receptor, α chain 1
Interleukin 13 receptor, α2
Interleukin 15 receptor, α chain
Interleukin 2 receptor, β chain
Interleukin 2 receptor, γ chain
Interleukin 3 receptor, α chain
Interleukin 4 receptor, α
Interleukin 7 receptor
Killer cell lectin-like receptor subfamily g, member 1
Killer cell lectin-like receptor, subfamily a, member 5
Kinase insert domain protein receptor
Laminin receptor 1
Low density lipoprotein receptor related protein 1
Low-density lipoprotein receptor-related protein 10
Macrophage scavenger receptor 1
Macrophage scavenger receptor 2
Macrophage stimulating 1 receptor
Mammary tumor virus receptor 2
Mannose receptor, c type 1
Mannose-6-phosphate receptor, cation dependent
Nerve growth factor receptor associated protein 1
Neuromedin b receptor
Neuropeptide Y receptor Y2
Neurotensin receptor
Neurotrophic tyrosine kinase, receptor, type 2
Nmda receptor-regulated gene 1
Nuclear receptor binding factor 1
Nuclear receptor binding factor 2
Nuclear receptor coactivator 4
Nuclear receptor coactivator 6
Nuclear receptor coactivator 6 interacting protein
Nuclear receptor co-repressor 1
Nuclear receptor co-repressor 2
Nuclear receptor subfamily 1, group h, member 2
Nuclear receptor subfamily 2, group c, member 1
Nuclear receptor subfamily 2, group e, member 3
Nuclear receptor subfamily 2, group f, member 6
Nuclear receptor subfamily 4, group a, member 1
Nuclear receptor subfamily 4, group a, member 2
Nuclear receptor subfamily 4, group a, member 3
Nuclear receptor subfamily 5, group a, member 2
Odorant receptor a16
Olfactory receptor 17
Olfactory receptor 37c
Olfactory receptor 4 cluster, gene 3
Olfactory receptor 64
Olfactory receptor 70
Olfactory receptor 71
Olfactory receptor 7b
Oncostatin receptor
Opioid receptor, sigma 1
Opioid receptor, δ1
Oxidized LDL receptor
Parathyroid hormone receptor
Peroxisome proliferator activated receptor α
Peroxisome proliferator activated receptor γ
Pheromone receptor v3r4
Phosphatidylserine receptor
Phospholipase a2, group ib, pancreas, receptor
Photolyase/blue-light receptor homolog 2
Platelet derived growth factor receptor, α polypeptide
Platelet derived growth factor receptor, β polypeptide
Progesterone receptor membrane component 1
Prolactin receptor
Prostacyclin receptor
Prostaglandin e receptor 4 (subtype ep4)
Prostaglandin f receptor
Protein tyrosine phosphatase, receptor type, a
Protein tyrosine phosphatase, receptor type, c polypeptide-associated protein
Protein tyrosine phosphatase, receptor type, k
Protein tyrosine phosphatase, receptor type, l
Protein tyrosine phosphatase, receptor-type, f interacting protein, binding protein 2
Protein tyrosine phosphatase, receptor-type, n
Purinergic receptor p2x, ligand-gated ion channel 4
Purinergic receptor p2x, ligand-gated ion channel, 1
Pyrimidinergic receptor p2y, g-protein coupled, 4
RAR-related orphan receptor α

(*continues*)

TABLE 3 (*continued*). Receptor mRNAs Present in the Mouse Meibomian Gland[83]

RAR-related orphan receptor γ	Thyroid hormone receptor interactor 4	Tumor necrosis factor receptor superfamily, member 11a
Receptor (calcitonin) activity modifying protein 2	Thyroid hormone receptor interactor 6	Tumor necrosis factor receptor superfamily, member 11b
Receptor-interacting serine-threonine kinase 1	Tumor necrosis factor receptor associated factor 4	Tumor necrosis factor receptor superfamily, member 17
Receptor-type protein tyrosine phosphatase	Tumor necrosis factor receptor-associated factor 1	Tumor necrosis factor receptor superfamily, member 18
Retinoic acid receptor, α	Tumor necrosis factor receptor-associated factor 3	Tumor necrosis factor receptor superfamily, member 1a
Retinoid x receptor-γ	Tumor necrosis factor receptor-associated factor 5	Tumor necrosis factor receptor superfamily, member 4
Ryanodine receptor type 2	Toll-like receptor 1	Tumor necrosis factor receptor superfamily, member 8
Scavenger receptor class b1	Toll-like receptor 2	Type 1 tumor necrosis factor receptor shedding aminopeptidase regulator
Serine/threonine kinase receptor associated protein	Toll-like receptor 3	Tyrosine kinase receptor 1
Signal recognition particle receptor, b subunit	Toll-like receptor 4 adaptor protein	Vanilloid receptor-like protein 1
Signal sequence receptor, δ	Toll-like receptor 9	Very low density lipoprotein receptor
Single ig il-1 receptor related protein	Tumor necrosis factor receptor-associated factor and umor necrosis factor receptor associated protein	Vomeronasal 1 receptor, a5
Skeletal muscle ryanodine receptor	Transferrin receptor	Vomeronasal 2, receptor, 15
Somatostatin receptor 2	Transferrin receptor 2	Vps10 domain receptor protein sores 2
Somatostatin receptor 3	Transforming growth factor, β receptor ii	Zona pellucida 3 receptor
Steroid receptor RNA activator 1	Transient receptor potential cation channel, subfamily m, member 7	
Steroid receptor-interacting snf2 domain protein	Transient receptor protein 1	
Stromal cell derived factor receptor 2	Transient receptor protein 2	
Taste receptor, type 1, member 2	Transient receptor protein 4, associated protein	
Taste receptor, type 1, member 3	Tumor necrosis factor receptor superfamily, member 10b	
T-cell receptor β, variable 13		
Thymic stromal-derived lymphopoietin, receptor		

All receptor mRNAs had an intensity level greater than 0.5 in CodeLink Bioarrays. Data originate from the National Center for Biotechnology Information's Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo) via series accession number GSE1582.

- the induction of genes involved in fatty acid and cholesterol synthesis, including fatty acid synthase, ATP-citrate lyase, malic enzyme, acetyl-CoA-carboxylase, 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) synthase, HMG-CoA reductase, glycerol 3-phosphate acyl transferase, farnesyl diphosphate synthase, and FAR-17c;
- fatty acid saturation and branching;
- the incorporation of fatty acids into neutral lipids and phospholipids;
- the content of total lipids, neutral lipids, cholesterol, phospholipids, triglycerides, and neutral glycosphingolipids;
- the secretion rate of wax esters and various other lipids; and
- the activity of lipases and the metabolism of lipoproteins.[184,204–219]

In addition, androgens stimulate the expression of sterol regulatory element-binding proteins (SREBPs), which are transcription factors that play an important role in the coordinate regulation of lipogenic enzymes.[215–218,220,221]

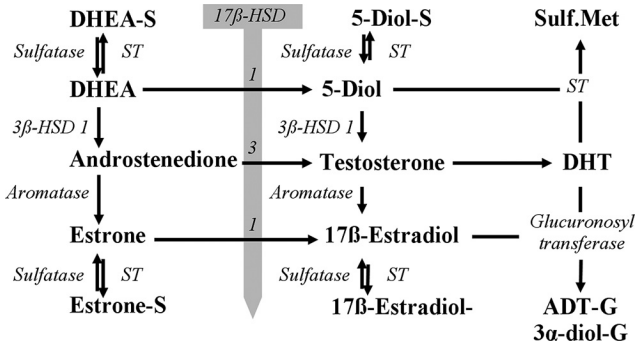

FIGURE 16. Major biosynthetic and inactivation pathways of androgens and estrogens in humans. Direction of enzymatic action is shown by *arrows*. Abbreviations include Sulfatase, steroid sulfatase; ST, sulfotransferase; Sulf Met, sulfated metabolites; HSD, hydroxysteroid dehydrogenase; DHEA, dehydroepiandrosterone; DHEA-S, DHEA sulfate; Estrone S, estrone sulfate; DHT, dihydrotestosterone; 5-diol, 5-androstene-3β,17β-diol; ADT-G, androsterone-glucuronide; 3α-diol-G, androstane-3α, 17β-diol-glucuronide. Reproduced from Schirra F, Suzuki T, Dickinson DP, Townsend DJ, Gipson IK, Sullivan DA. Identification of steroidogenic enzyme mRNAs in the human lacrimal gland, meibomian gland, cornea, and conjunctiva. *Cornea.* 2006;25:438–442 with permission from Wolters Kluwer/Lippincott Williams & Wilkins.

Androgen Regulation of the Meibomian Gland. Androgens regulate the meibomian gland and modulate gene expression and lipid production within this tissue.[77,81,117,222–225] These hormonal actions appear to be mediated, at least in part, through binding to classic nuclear receptors. Sex steroid receptors typically bind their specific hormone, and the activated hormone–receptor complex then associates with a response element in the regulatory region of target genes and controls gene transcription and eventually protein synthesis.[226–228] The meibomian glands of male and female rats, rabbits, and humans contain androgen receptor mRNA and androgen receptor protein within the acinar epithelial cell nuclei.[223,229,230] Further, androgens regulate the expression of numerous genes in mouse, rabbit, and human meibomian glands.[77,117,222,224,225,231] These genomic actions appear to be dependent on the presence of functional androgen receptors.[224,225]

The effects of androgens and estrogen on the human meibomian gland may be exerted predominantly after the local formation of sex steroids from adrenal precursors. Human meibomian glands contain all the following steroidogenic and metabolic enzyme mRNAs: steroid sulfatase, 3β-HSD-Δ^5-Δ^4-isomerase type 1,17β-HSD types 1 and 3, types 1 and 2 5α-reductase, aromatase, glucuronosyl-transferase, and sulfotransferase.[78,229] Moreover, at a minimum, 3α-HSD, 3β-HSD, and 17β-HSD are known to be translated in epithelial cells of the human meibomian gland.[129] These findings suggest that the human meibomian gland harbors the enzymatic machinery necessary for the intracrine synthesis and metabolism of sex steroids.

Androgens exert a significant influence on gene expression in the meibomian gland. Testosterone, for example, regulates the expression of more than 1580 genes in the male mouse meibomian gland.[77,81,117,222,225] Many of the upregulated genes are related to lipid metabolism (Fig. 17), lipid transport, sterol biosynthesis, fatty acid metabolism, intracellular protein transport, oxidoreductase activity, peroxisomes, mitochondria and early endosomes.[77,81,117,222,225] Moreover, some of the proteins and pathways encoded by these upregulated genes have been the focus of recent research. For example:

- ATP-citrate lyase, acetyl-CoA-synthase, acetyl-CoA-carboxylase, acetoacetyl-CoA-synthase, fatty acid synthase, HMG-CoA synthase, HMG-CoA reductase, mevalonate kinase, phospho-

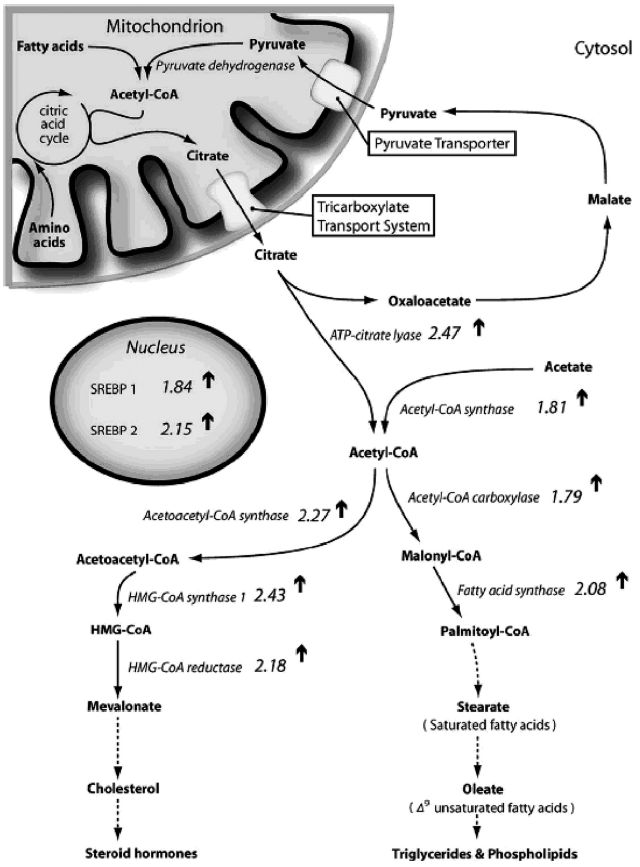

FIGURE 17. A schematic diagram of the two major lipogenic pathways,[77] which result in the synthesis of cholesterol (and steroid hormones) and fatty acids (and triglycerides and phospholipids). Both pathways typically require the generation and secretion of acetyl-CoA from mitochondria, the transcriptional control by SREBPs in the nucleus, and the action of lipogenic enzymes in the cytosol. The extent of the androgen upregulation of specific genes for SREBPs and enzymes is shown within the diagram. Reprinted from *Exp Eye Res*, 83, Schirra F, Richards SM, Liu M, Suzuki T, Yamagami H, Sullivan DA, Androgen regulation of lipogenic pathways in the mouse meibomian gland, 291–296, © 2006, with permission from Elsevier.

mevalonate kinase, mevalonate pyrophosphate decarboxylase, isopentenyl pyrophosphate isomerase, geranylgeranyl pyrophosphate synthase, squalene epoxidase, lanosterol demethylase, and Δ7-sterol reductase are all key enzymes involved in the imitation and progression of cholesterol, fatty acid, sex steroid, and/or lipid synthesis.[232,233] Fatty acid synthase is also known to be regulated by androgens in other tissues[214,218] and is expressed in meibomian gland epithelial cells (Richards SM, et al. *IOVS* 2002;43:ARVO E-Abstract 3150).

- Fatty acid transport protein 4 facilitates the cellular uptake and metabolism of long- and very-long-chain fatty acids.[234]
- The elongation of very-long-chain fatty acid-like types 1 and 3 enhance the tissue-specific synthesis of very-long-chain fatty acids and sphingolipids.[233,235] These proteins could be involved in the androgen-induced increase of long-chain fatty acids in the total lipid fraction of rabbit meibomian glands.[223]
- Monoglyceride lipase promotes hydrolysis of tri- and monoglycerides to fatty acids and glycerol.[236]
- Abca1 and Abcd3, which are members of the ATP-binding cassette family, transport various molecules across extra- and intracellular membranes. Abca1 serves as a cholesterol efflux pump in the lipid-removal pathway[237] and thereby functions as a key regulator of cholesterol distribution.[238,239] Abcd3 regulates the importation of fatty acids and/or fatty acyl-CoAs into peroxisomes.[240]
- Oxysterol-binding protein-like 1A, sterol carrier protein 2, liver, lipocalin 3, and phosphatidylcholine transfer protein are involved in the binding and/or transfer of phospholipids.[233]
- SREBP 1 promotes the fatty acid synthesis pathway, and SREBP 2 enhances the cholesterol synthesis pathway.[241–244]
- Multiple genes are involved in the peroxisome proliferator-activated receptor signaling pathway, which modulates lipogenesis, ketogenesis, lipid transport, and fatty acid transport and oxidation.[98]

Androgens also modulate a series of genes that may be very important in the endocrine regulation of the meibomian gland.[81] For instance, testosterone increases the mRNA levels of:

- 17β-HSD 7, an enzyme that regulates the interconversion of 17-ketosteroids with their corresponding 17β-hydroxysteroids.[245] This enzymatic activity is critical for the metabolism of all active androgens and estrogens in peripheral tissues[245] and may mediate the local intracrine synthesis of androgens from adrenal precursors in the meibomian gland;
- insulin-like growth factor 1, a pleiotropic protein that stimulates sebaceous cell DNA synthesis and differentiation[147];
- estrogen receptor (ER) β, a receptor that is upregulated by androgen in the prostate[246] and may inhibit the activity of ERα[247]; and
- 11β-HSD 1, an enzyme that catalyzes the conversion of cortisol to the inactive metabolite, cortisone.[233]

Of interest, testosterone downregulates genes associated with keratinization,[81,135] as well as the gene for retinaldehyde dehydrogenase 3,[81] an enzyme that promotes retinoic acid biosynthesis.[233] Androgens also stimulate the expression of genes involved in the sorting, trafficking and hydrolysis of proteins in various cellular locations, including the endosome, Golgi apparatus, endoplasmic reticulum, lysosome, proteasome, nucleus, and mitochondrion.[81]

In addition to these findings, investigators have found that androgen treatment significantly influences the expression of more than 1000 genes in the female mouse meibomian gland.[135] Many of the genes modulated by testosterone in female tissues are identical with those controlled by androgens in male meibomian glands. Some genes, however, are regulated in a sex-specific manner.[135]

Androgens also affect the lipid, and possibly protein, composition within the meibomian gland. Orchiectomy causes a marked change in the lipid pattern of rabbit meibomian glands, whereas the topical or systemic administration of 19-nortestosterone (versus placebo) for 2 weeks begins to restore the lipid profile toward that found in intact animals.[223] Further, researchers speculate that androgen signaling machinery regulates the expression of secretoglobin in human meibomian gland epithelial cells.[248] This protein may be secreted and have a lipocalin-like function in the tear film.[248]

Whether all effects of androgens on the meibomian gland are mediated through classic receptors is unclear. It is possible that the action also involves binding to glandular membrane receptors, stimulation of signal transduction cascades, and consequent alteration of gene transcription.[249,250] In addition, androgens may act indirectly, by regulating the secretion of the hypothalamic and anterior pituitary hormones that influence the meibomian gland.

Influence of Androgen Deficiency and Treatment. Given the influence of androgens on meibomian gland function, researchers have hypothesized that androgen deficiency, such as occurs during menopause (decrease in ovarian and adrenal androgen secretion),[251] aging in both sexes (decline in the total androgen pool),[196,251] autoimmune disease (e.g.,

Sjögren's syndrome, systemic lupus erythematosus, and rheumatoid arthritis),[201,252,253] complete androgen insensitivity syndrome (CAIS; women with dysfunctional androgen receptors),[254,255] and the use of antiandrogen medications (e.g., for prostatic hypertrophy or cancer)[256] all lead to MGD, altered lipid profiles in meibomian gland secretions, decreased tear film stability, and evaporative dry eye.[257–260]

When compared with control groups, it has been found that subjects taking antiandrogen therapy have significant changes in their meibomian glands, including orifice metaplasia (a condition defined as an abnormal growth and keratinization of the duct epithelium),[261] reduced quality of secretions, a striking alteration in the neutral lipid profile of secretions, and a morphologic appearance consistent with severe disease.[258,260] Many of the lipid changes are all or none, identical in both eyes, and feature characteristic shifts in fatty acid patterns.[260] In addition, patients have a significantly greater frequency of tear film (i.e., debris, abnormal menisci, and instability), conjunctival (i.e., tarsal injection, and inferior staining), corneal (staining), and lid (i.e., irregular posterior lid margins, sleeves, and collarettes) abnormalities, as well as an increased appearance of ocular surface symptoms (i.e., light sensitivity, painful eyes, and blurred vision).[258] These findings add to another that determined that leuprolide acetate administration to reduce testosterone levels is associated with ophthalmic problems and blurred vision in some patients.[262] These results may help explain the significant association between the use of medications to treat benign prostatic hyperplasia and dry eye disease.[263]

Researchers have also discovered that androgen receptor dysfunction in patients with CAIS is associated with a significant increase in dry eye signs and symptoms. This particular group of patients have a significantly higher frequency of meibomian gland orifice metaplasia and irregular posterior lid margins, as well as a decreased quality of meibomian gland secretions, when compared to normal, age-matched males and females. Patients with CAIS also have striking alterations in the neutral and polar lipid patterns of their meibomian gland secretions, relative to those of normal male and female controls.[257,259] The normal aging process is also associated with a significant reduction in the quality of meibomian gland secretions and a significant increase in the frequency of metaplasia of meibomian gland orifices.[105,264] Both the polar and neutral lipid profiles of meibomian gland secretions are significantly altered with aging.[105,264] These findings were observed when comparing 37- and 70-year-old patients, and the period between the fourth and eighth decades coincides with a dramatic decline in androgen levels in both sexes.[251]

In addition to these observations, investigators have found that patients with nonautoimmune dry eye and MGD are androgen-deficient,[265] and others have observed that the topical administration of DHEA, an androgen precursor, to humans, rabbits, and dogs stimulates the production and release of meibomian gland lipids and prolongs the tear film break-up time (TBUT).[266] Further, studies have reported that reduced serum levels of testosterone are more prevalent in women with dry eye and correlate with the subjective severity of ocular symptoms[267] and that serum testosterone concentrations correlate positively with meibomian gland secretion volume and orifice diameter in pre- and postmenopausal women, respectively (Suzuki T, et al. *IOVS* 2007;48:ARVO E-Abstract 434; Suzuki T, et al. *IOVS* 2008;49:ARVO E-Abstract 92). However, the meaning of the results in the latter studies is unclear, given that serum testosterone levels represent only a very small fraction (<0.2% in women) of the total androgen pool in humans[268] and have little or no value except as an index of ovarian activity.[251,268,269] The majority of or all androgens (75% before and 100% after menopause) in women are synthesized in peripheral tissues from adrenal sex steroid precursors (DHEA and DHEA-sulfate).[269] Perhaps the only valid and reliable estimate of the total androgen pool in humans is the serum concentration of conjugated dihydrotestosterone metabolites (androsterone glucuronide and androstane-3α 17β-diol-glucuronide)[251,270,271] that reflect the total intracrine production and metabolism of androgens in peripheral tissues throughout the body.[269]

Overall, these findings suggest that the meibomian gland is an androgen target organ, androgens promote lipogenesis and suppress keratinization within this tissue, and androgen deficiency may lead to MGD and evaporative dry eye. This apparent interrelationship of androgen deficiency with MGD and evaporative dry eye may help to explain why topical or systemic androgen administration has been reported to alleviate the signs and symptoms of dry eye in women and men.[266,272–278] Efforts directed at alleviating this endocrine imbalance (e.g., topical androgen application) may prove beneficial in the treatment of MGD and the associated evaporative dry eye, in androgen-deficient individuals. Consistent with this possibility are clinical trial results that suggest that treatment of MGD with topical testosterone would improve the quality of meibomian gland secretions and reduce ocular discomfort (Schiffman RM, et al. *IOVS* 2006;47:ARVO E-Abstract 5608).

Estrogens

Estrogen Regulation of Sebaceous Glands. Estrogens cause a significant decrease in the size, activity, and lipid production of sebaceous glands in a variety of species.[145,181,192,279–283] Indeed, estrogen was once termed the prototype agent for the suppression of sebum production,[282] and for several years, estrogen treatment was used to reduce sebaceous gland function and sebum secretion in humans.[180,181,280,281,284,285]

One mechanism proposed for this hormone action is that estrogen induces the release of lysosomal enzymes within sebocytes, leading to premature cellular destruction and decreased sebum output.[282,286] Additional suggested mechanisms are that estrogens reduce testosterone uptake, interfere with testosterone's conversion to dihydrotestosterone, and antagonize androgen action in the sebaceous gland.[279,282,283] In fact, estrogens have been described as the mainstay of treatment to decrease the effects of androgens on the sebaceous gland.[180] These antiandrogen actions of estrogens are dose-dependent, and may be overridden by treatment with physiological levels of androgens.[181,280]

Of interest, androgen treatment causes a significant decrease in the number of estradiol-binding sites,[177,286] and both hormones antagonize each other's modulation of their own receptors[283] in sebaceous glands. Further, some androgen effects are believed to be dependent on low levels of estrogen.[287]

Estrogen Regulation of the Meibomian Gland. The meibomian gland contains estrogen receptor mRNA and protein,[230,288,289] and estrogen administration to ovariectomized mice appears to result in characteristic alterations in glandular morphology.[290] Estradiol-17β also regulates the expression of almost 200 genes in mouse meibomian glands,[291] including those related to tyrosine kinases (fibroblast growth factor receptor 1) immune factors (interleukin 1 receptor, type II), extracellular matrix components (matrix metallopeptidase 3), steroidogenesis (17β-hydroxysteroid dehydrogenase 7, which converts estrone to biologically active estradiol[292]), prolactin activity (prolactin receptor), and lipid metabolism, to name a few.

Estrogen increases the expression of selected genes associated with lipid dynamics, such as phosphatidylcholine transfer protein,[291] which replenishes the plasma membrane with

phosphatidylcholines,[293] and downregulates others,[291] including carboxylesterase 3, a lipase.[294] These hormone actions suggest that 17β-estradiol promotes lipid production in the meibomian gland; however, researchers have found that most of estrogen's effects are not consistent with this conclusion. Rather, 17β-estradiol seems to have an overall negative influence on lipid generation. For example, estrogen stimulates the expression of several genes involved in lipid and/or fatty acid catabolism (the anti-lipogenic STAT5A[295]) and suppresses genes involved with lipid biosynthesis, mobilization, processing, and membrane trafficking.[291]

Given these latter antagonistic effects, as well as the impact of estrogens on sebaceous glands in general, it is logical to presume that estrogen treatment reduces lipid synthesis in the meibomian gland and promotes both MGD and evaporative dry eye. The following studies support this statement:

- An epidemiologic evaluation of 25,665 postmenopausal women found that those who receive estrogen replacement therapy had a significantly higher prevalence of severe dry eye symptoms and clinically diagnosed dry eye syndrome than did women who never received the treatment.[296]
- An assessment of 44,257 women with dry eye showed that one of the highest prevalences of comorbid conditions was the use of estrogen replacement therapy.[297]
- Estrogen treatment of women in two studies led to tear film instability, foreign body sensation, contact lens (CL) intolerance, and ocular surface dryness.[298,299]

Other genes in the meibomian gland are suppressed by 17β-estradiol, but stimulated by testosterone.[291] Some of these genes encode for secreted acidic cysteine-rich glycoprotein (regulates cell growth), vascular endothelial growth factor A (promotes cell migration), cathepsin K (degrades extracellular matrices), and matrix metalloproteinase 3 (degrades fibronectin, laminin, gelatins, and collagens). These genes could be involved in cell maturation, migration, and holocrine secretion in the meibomian gland. If so, these hormone responses would be consistent with an antisebaceous gland effect of estrogens and a prosebaceous gland effect of androgens.

Progestins

Progestin Regulation of Sebaceous Glands. It was once believed that progesterone was the trophic hormone that regulated sebaceous gland secretion in women,[181] analogous to androgens in men, because progestin treatment significantly increased sebum production.[146,181,300] However, earlier and later studies disagreed, finding that progestin administration had no effect on sebaceous gland output,[191,300] and still others reported that these hormones reduce sebaceous gland function by inhibiting local androgen metabolism and activity.[301–304] One explanation of these conflicting findings is that progestin's effect on different types of sebaceous glands seem to be significantly influenced by the dose, endocrine environment, and the subject's sex.[302,305–307]

Progestin Regulation of the Meibomian Gland. The meibomian gland contains progesterone receptor mRNA and protein[230,288] and responds to progesterone exposure with an apparent change in morphology.[290] The addition of progestin to estrogen hormone replacement therapy also causes a significant decrease in the estrogen-related symptoms of dry eye,[296] which may reflect a positive influence on meibomian gland activity. A definitive link between the action glandular progestin and ocular surface symptoms has yet to be shown.

Researchers have demonstrated recently that progesterone has a significant influence on gene expression in the mouse meibomian gland.[291] Most genes are downregulated by progesterone, including those associated with immune processes, gluconeogenesis, and energy transduction.[291] The most striking effect is the downregulation of all genes related to ribosome biogenesis, assembly, and structure,[291] suggesting that progesterone has an overall suppressive impact on protein, macromolecule, and cellular biosynthesis in the meibomian gland.

Combined administration of progesterone with estradiol-17β also has a significant effect on the expression of more than 300 genes in the mouse meibomian gland.[291] Most molecular biological responses duplicate those of estradiol or progesterone treatment alone. However, some do not, including a unique upregulation of genes involved with the localization ontology.[291] The explanation of this combined progestin/estrogen response is unclear.

Sex Steroid Involvement in Sex Differences in the Meibomian Gland

In summary, sex steroids have a significant impact on meibomian gland function and, depending on the specific steroid, may prevent or promote MGD and evaporative dry eye. In addition, the differential action of sex steroids, as with other sebaceous glands,[172,175,181,189,308] may well mediate the sex-related differences known to occur in the morphologic appearance, gene expression, neutral and polar lipid profiles, and secretory output of the meibomian gland.[44,76,105,120,259,263,264,290,309–312] Consistent with this proposal is the observation that androgens appear to mediate almost 30% of the sex-associated variations in gene expression of the mouse meibomian gland.[76] Moreover, it is possible that sex-specific aromatase activities also play a role in the sex-related differences of the meibomian gland (Liu S, et al. *IOVS* 2007;48:ARVO E-Abstract 5657).

Effect of all-*trans* Retinoic Acid on the Meibomian Gland

The compound all-*trans* retinoic acid decreases sebocyte growth, development, and lipid production and causes blepharoconjunctivitis.[313–318] It is also known to be converted in sebocytes to 13-*cis* retinoic acid (isotretinoin),[319] which leads to thickening and keratinization of meibomian gland ducts,[320,321] degeneration and necrosis of meibomian gland acinar cells,[322] periacinar fibrosis, and reduced lipid content of meibomian tissue[320,321] in animal models. Isotretinoin is the agent that has revolutionized the dermatologic treatment of severe acne over the past several decades.[323]

In humans, the administration of 13-*cis* retinoic acid results in blepharoconjunctivitis, abnormal meibomian gland secretions, meibomian gland atrophy, decreased TBUT, increased tear film osmolarity, and dry eye signs and symptoms.[324–331] In effect, the retinoic acid derivatives promote MGD and evaporative dry eye.[330,332,333]

Major mechanisms involved in the action of retinoic acid include the suppression of androgen receptor mRNA and protein[334,335] and the inhibition of retinol dehydrogenase-4, which leads to a decrease in the local production of dihydrotestosterone.[336] Understanding the influence of retinoic acid on the meibomian gland is very important, given that this compound is the key ingredient of many antiaging cosmetics for use around the eye, and as people age they become more susceptible to the development of dry eye.[263,312]

Influence of Growth Factors and Other Agents and Conditions on the Meibomian Gland

Various other components and conditions are known to influence the physiology and pathophysiology of the meibomian gland. These include:

• epidermal growth factor and bovine pituitary extract, which promote the proliferation and possibly the differentiation, of immortalized human meibomian gland epithelial cells (Liu S, et al. *IOVS* 2009;50:ARVO E-Abstract 3669). Epidermal growth factor has also been shown to stimulate the differentiation of rabbit meibomian[337] and human sebaceous[155] gland epithelial cells in vitro. Treatment of cancer patients with epidermal growth factor inhibitors is associated with the appearance of MGD (Joshi J, et al. *IOVS* 2008;49: ARVO E-Abstract 2363);

• topical epinephrine, which induces hyperkeratinization of the duct epithelium and leads to plugging and dilation of the meibomian gland[338];

• ω-3 fatty acid, the intake of which has been correlated with variations in the polar lipid profile[120] and saturated fatty acid content[119] of human meibomian gland secretions. In addition, a reduced intake of ω-3 fatty acids has been found in women with Sjögren's syndrome,[339] and these women typically have MGD[340] (Krenzer KL, et al. *IOVS* 1999;31:ARVO Abstract 2864);

• hypothalamic hypogonadism, which was found to be associated with obstructive MGD in a 35-year-old male patient.[162] The clinicians proposed that this lid condition was due to androgen deficiency[162];

• aldosterone, possibly, given that mineralocorticoid receptors are present in human sebaceous glands.[341] However, a potential aldosterone target in human meibomian gland tissue is unknown. Aldosterone has no effect on the neutral lipid or ganglioside composition of rabbit meibomian glands[342];

• multiple endocrine deficiency (Addison's disease and hypoparathyroidism), which was found to be associated with severe MGD.[343] Given this finding, it is of particular interest that parathyroid hormone receptor mRNA is present in the meibomian gland (Table 3), but whether this receptor mRNA is translated and is functional has yet to be determined.

It is also intriguing that the human meibomian gland, like other sebaceous glands,[200,344–347] secretes not only lipids, but also proteins.[134] Such proteins may significantly influence the stability of the tear film as well as the appearance of the ocular surface.[134] However, the regulation and role of this protein secretory process await clarification.

Clearly, further studies are needed to delineate the nature and extent of the endocrine, neural, nutrient, and growth factor (among others) influence on the meibomian gland, to increase our understanding of the physiological mechanisms controlling this tissue in both health and disease.

Pathophysiology and Pathology

Hyperkeratinization

Hyperkeratinization is a major reason for obstructive MGD and causes degenerative gland dilatation and atrophy without inflammation. That this is a typical pathology of the meibomian glands comes as no surprise in view of the glands' embryologic development. Hyperkeratinization could represent the removal of a developmental block of full keratinization (cornification) of the ductal epithelium that occurs because of various internal and external factors, rather than a de novo acquirement. Factors increasing epithelial keratinization and meibomian gland obstruction range from advancing age[50,87,348] and hormonal disturbances,[257,291,349] the toxic effects of medication and chemicals[350,351] and the breakdown products of meibomian lipids,[41,352,353] or influences of external factors such as epinephrine eye drops,[354] to CL wear.[355]

Obstructive MGD due to hyperkeratinization was first described by Korb and Henriquez[3,356] in patients who had only minimal or transient symptoms suggestive of ocular dryness, but became clinically symptomatic because of CL intolerance. Manual expression of their meibomian glands verified an obstruction of the orifices and revealed hyperkeratotic clusters consisting of desquamated epithelial cells and thickened meibum. After expression and removal of the plugs, the tear film normalized and CL intolerance disappeared. Histology of obstructed glands verified dilatation of the central duct by cell debris and sebaceous material.[3,356]

Later histologic examinations of meibomian glands from patients with symptomatic dry eye disease who had inspissations of duct orifices and expressible highly viscous meibum, verified signs of obstruction of the excretory duct by increased keratinization. Inside the gland, this resulted in obstruction and dilatation of the ducts as well as cystic degeneration and loss of secretory meibocytes (Figs. 18, 19) that were replaced by a squamous metaplasia of the acinar epithelium.[357] These alterations occurred without the presence of inflammatory leukocytes. It can be assumed that the observed acinar degeneration and atrophy that follows dilatation of the ductal system and results from obstruction of the gland leads to a later secondary hyposecretion due to the loss of secretory meibocytes. A similar pathology was observed in glands with cystic dilatation that were obstructed by surgical procedures or by neoplasia. Also in these cases, dilatation of the ductal system was reported, together with atrophy of the acini.[31] The dilatation of the ductal system appears more pronounced in this condition when compared with findings in obstructive MGD.[357] A large histopathologic study of the meibomian glands in 72 autopsies confirmed the morphology of cystic dilatation of ducts and acini and reported these pathologic alterations in 34.7% (25) of the cases.[358] At present, obstructive MGD (Figs. 18, 19) appears to be the disease's most common form.[3,4,10,357–362]

Animal Models of MGD

Several naturally occurring or induced animal models of MGD have been identified or developed. These models, in turn, may be very useful in exploring the pathophysiological mechanisms underling this condition. Rabbit[320,338,354,363,364] and monkey[365] models that feature hyperkeratinization have been produced by the topical application of epinephrine,[338,354,363,364] the systemic administration of isotretinoin,[320] and polychlorinated biphenyl poisoning.[365] As shown in Figure 20A, a common histopathologic finding in these rabbit and monkey models is an abnormal dilatation of the ducts, which show lumina filled with keratinized materials. In addition, the epithelium of the ductal orifices is typically hyperkeratinized and obstructed (Fig. 20B). It is currently believed that hyperkeratinization of the orifice itself, as well as the ductal epithelium, contributes significantly to the luminal plugging observed in obstructive MGD.

Mouse models of MGD and meibomian gland hyperplasia (MGH) are also available. These are either natural or have been generated by immunization, mutation, or transgenic or knockout technologies (Table 4). The resulting strains may present a variety of phenotypes, such as ductal hyperkeratinization, acinar cell loss, and progressive glandular atrophy; or no meibomian gland; or glandular enlargement (Table 4). Some of these models may well have utility for studies of evaporative dry eye and corresponding ocular surface sequelae. Consistent with this proposal are the findings that the meibomian gland atrophy in acyl-CoA:cholesterol acyltransferase-1–knockout mice is associated with corneal erosions,[366] and that the meibomian gland's absence in X-linked anhidrotic–hypohidrotic ectodermal dysplasia is often paralleled by corneal defects (e.g., neovascularization and keratinization) and ocular surface inflammation.[367] The ocular surface problems in these models, however, may not solely depend on glandular impairment.[367]

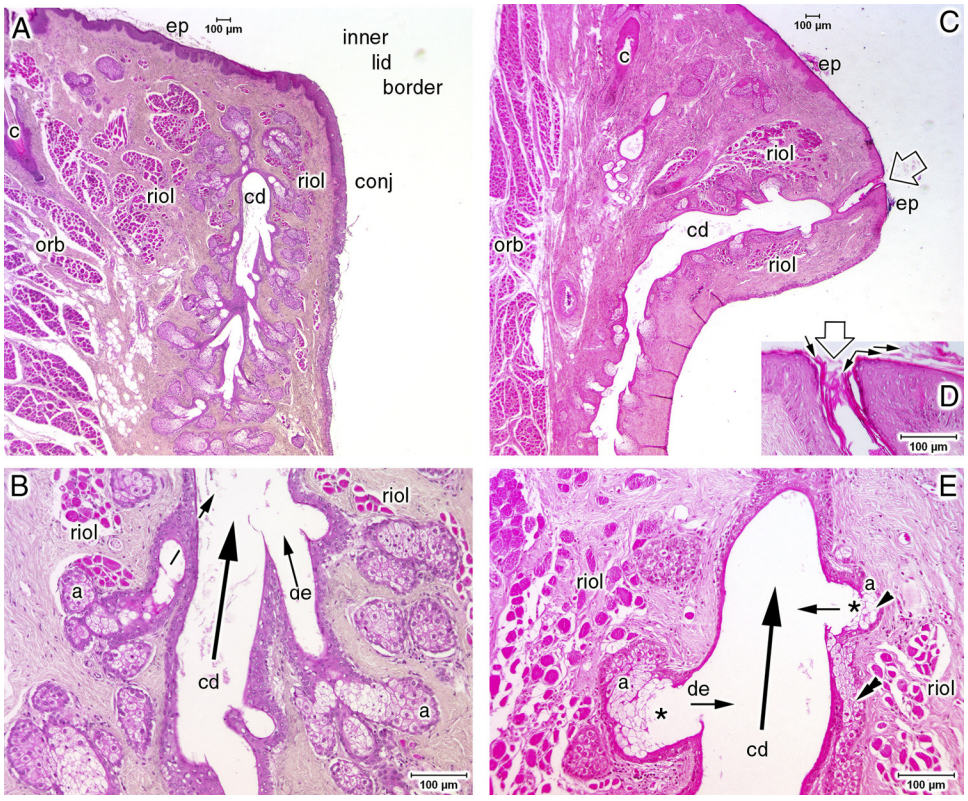

FIGURE 18. Comparison of the structure of a normal and an obstructed human meibomian gland. (**A**, **B**) A histologic section through a normal meibomian gland at the inner lid border. (**A**) The terminal part of the central duct (cd) and the terminal acini are encircled by fibers of Riolan's muscle (riol), which represents the marginal inner part of the orbicularis muscle (orb) and is split by the downgrowth of the ciliary (c) hairs [compare with Fig. 5]. The free lid margin is covered by the keratinized epidermis (ep), which transforms at the inner lid border into the conjunctival mucosa (conj). The section does not pass through the orifice of the central duct (cd). (**B**) In a magnification of (**A**), it is seen that the connecting ductules (de) from the acini (a) of a normal gland are typically narrow and enter the central duct in an oblique direction. (**C**–**E**) Section through a meibomian gland with obstructive MGD. (**C**) The orifice (*open arrow*) is in the typical position, still within the keratinized epidermis, which extends for about half a millimeter into the central duct and forms an excretory duct. Even though the obstruction is not very advanced, as judged from the moderate dilatation of the central duct (cd), there are distinct alterations of the gland structure. The cd is already partly dilated, the epithelium of the wall is thinner than in the normal gland, and the wall is partly undulated. (**D**) The orifice is obstructed by numerous keratin lamellae (*small arrows*). (**E**) The secretory acini (a) are distinctly smaller and more roundish than in a normal gland, whereas the ductules (de) are dilated and enter the central duct (cd) at about right angles (*small arrows*). An atypical lumen (*asterisk*) has formed within the acini, and the secretory meibocytes are reduced in number and form only a few remaining cell layers (*arrowhead*). In one location, the residual meibocytes of a presumably disrupted acinus appear integrated into the wall of the central duct (*double arrowhead*). Inflammatory leukocytes are not apparent. Taken together, these findings indicate atrophy of the dilated meibomian gland. Light microscopic images of paraffin-embedded sections stained with hematoxylin and eosin (H&E); size markers are shown in the images. Reprinted from Knop E, Knop N, Brewitt H et al. [Meibomian glands, Part III: meibomian gland dysfunction (MGD)—plaidoyer for a discrete disease entity and as an important cause of dry eye.] Meibom-Drüsen, Teil III: Meibomdrüsen Dysfunktionen (MGD)—Plädoyer für ein eigenständiges Krankheitsbild und wichtige Ursache für das Trockene Auge. *Ophthalmologe*. 2009;106:966–979 with the kind permission of Springer Science and Business Media.

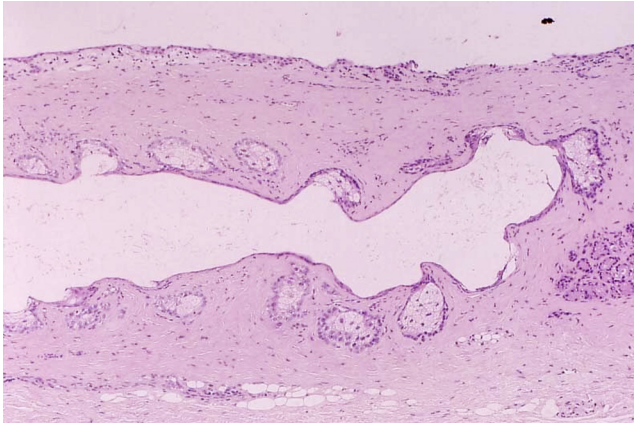

FIGURE 19. Cystic dilatation of a human meibomian gland. In cystic dilatation due to obstruction of the meibomian gland orifice, the ductal system is distinctly dilated, together with a dilation of the connecting ductules and atrophy of the acini. Figure reprinted from Obata H, Horiuchi H, Miyata K, Tsuru T, Machinami R. Histopathological study of the meibomian glands in 72 autopsy cases (in Japanese). *Nippon Ganka Gakkai Zasshi*. 1994;98:765–771 with permission from the Japanese Ophthalmological Society.

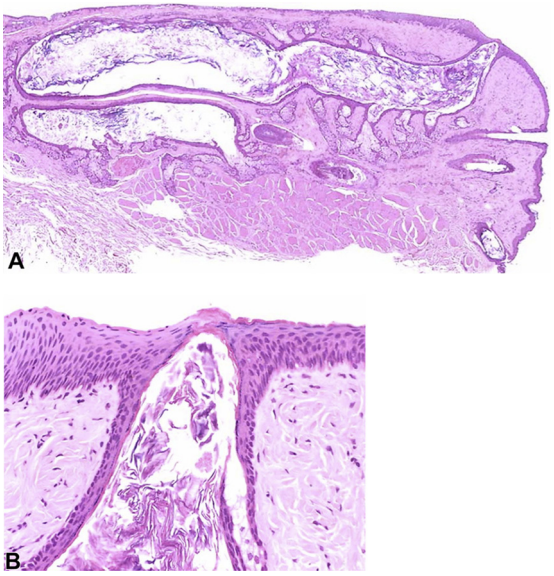

FIGURE 20. Epinephrine-induced MGD in rabbit. (**A**) The lumina of the dilated ducts are filled with keratinized material, representing keratin lamellae that are shed from the hyperkeratinized ductal wall. (**B**) The epithelium of the orifice was also hyperkeratinized and obstructed. Figure courtesy of Hiroto Obata.

TABLE 4. Possible Mouse Models of MGD, MGH, and Evaporative Dry Eye Syndrome*

Condition	Factor	Effect
Gene knockout		
	Stearoyl-coenzyme A desaturase 1 (3 types)	No meibomian gland
	Ectodysplasin-A (3 types)	No meibomian gland
	Ectodysplasin-A receptor (3 types)	No meibomian gland
	Acyl-CoA:cholesterol acyltransferase-1	Meibomian gland atrophy, corneal erosions
	Melanocortin-5 receptor	Decreased production of sebaceous lipids
	Smad4	Ectopic row of hair follicles in place of meibomian glands (distichiasis)
	Aire	T cell infiltration in meibomian glands
	Blimp1	Enlarged meibomian glands
	Tumor necrosis factor receptor-associated factor 6	Modified meibomian glands
Transgenic or gene overexpression		
	Human apolipoprotein C1	Meibomian gland atrophy
	Biglycan overexpression, under control of the keratocyte-specific keratocan promoter	Meibomian gland aplasia
	Rat erbB2 overexpression in basal layer of mouse epidermis, under control of the bovine keratin 5 promoter	Sebaceous gland enlargement
	Smad7 or parathormone-related protein overexpression	Sebaceous gland hyperplasia
	c-Myc overexpression	Enhanced sebum production
	K14-noggin	Formation of ectopic pilosebaceous units at the expense of meibomian glands
	Ectodysplasin-A	Sebaceous gland hyperplasia
	Ectodysplasin receptor	Enlarged meibomian glands
	Keratin 5-glucocorticoid receptor	No meibomian gland
Mutation		
	Rhino	Meibomian gland ductal hyperkeratinization, acinar cell loss and eventual atrophy
	Rough fur (ruf)	Sebaceous gland hypertrophy
	Downless (dl) locus	Meibomian gland defects
Experimental systemic lupus erythematosus (SLE)		
	Murine immunization with a human monoclonal anti-DNA antibody, bearing a major Id 16/6Id	Hypertrophic meibomian glands and chronic eyelid inflammation
Natural		
	Crinkled	No meibomian gland
	Bare skin	Sebaceous glands rudimentary

* See Refs. 82, 320, 338, 354, 363–384.

Additional mouse models that display significant alterations in sebaceous gland structure and function[82,320,338,354,363–384] (Table 4) may also serve as MGD or MGH models, but this possibility has not yet been evaluated.

Cytology of Meibum: Meibomian Secretion

It is believed that normal meibomian gland secretion (i.e., the meibum), is clear; however, it may have turbid, inspissated, or toothpaste-like consistency in MGD (Fig. 21A).

To examine the cytologic features of turbid meibum, impression cytology of the meibum was performed in a large study by Obata et al. (*IOVS* 2002;43:ARVO E-Abstract 60) in 50 elderly patients at least 60 years of age. The results demonstrated that keratinized materials were present in almost all cases, similar to the material first described by Korb and Henriquez[3,356] and later analyzed by Ong et al.[385] with biochemical and immunologic methods. Inflammatory cells were not detected in most cases (Fig. 21B), which may serve as a supportive indication that inflammation, at least based on the immigration of inflammatory leukocytes, does not represent a major etiologic factor in obstructive MGD (Obata H, et al. *IOVS* 2002;43:ARVO E-Abstract 60), as previously observed in histol-

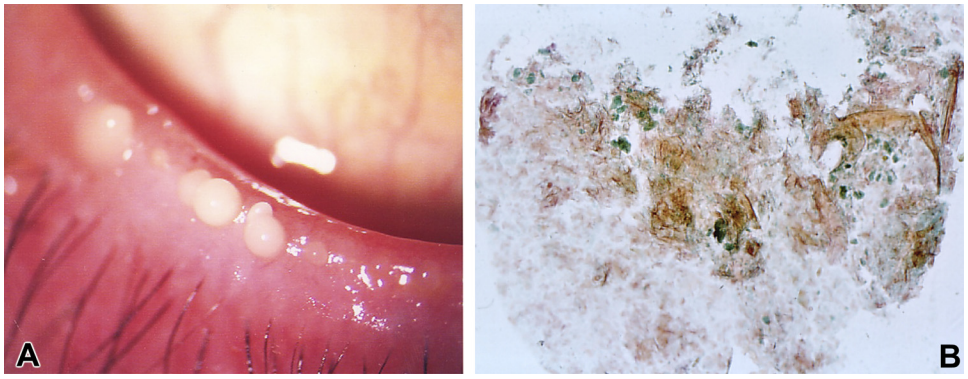

FIGURE 21. Features of pathologic meibum. (**A**) Yellowish white, turbid meibum from a 72-year-old woman. (**B**) Impression cytology of yellowish white, turbid meibum from a 71-year-old man. An orange, keratinized material is seen on the nitrocellulose membrane. Cellular components such as inflammatory cells are not seen. Papanicolaou staining. Figure courtesy of Hiroto Obata.

ogy.[31,357,358] The absence of inflammatory cells in meibum may not totally exclude a periacinar inflammatory event. However, a relative absence of overt glandular inflammation supports the notion that hyperkeratinization is one of the primary components in the pathogenesis of obstructive MGD.

Obata et al. (*IOVS* 2002;43:ARVO E-Abstract 60) divided meibum by appearance into three groups—oily, creamy, and toothpaste-like—and the color was classified into three groups: yellow, yellowish white, and white. The cytologic features were similar in each group, irrespective of the consistence or color of meibum. This finding suggests that a change in the consistency and color of meibum may reflect the change in lipid composition and not the degree of keratinization. It is assumed that color changes may be caused by lipid peroxidation.

At present, obstructive MGD is thought to be caused by hyperkeratinization of the excretory duct and orifice, dependent on several endogenous and exogenous factors as explained earlier. Secreted meibomian lipids can be altered by bacterial enzymes (e.g., lipases and esterases), which are produced by commensal bacteria.[41,353] The bacteria are located on the lid margin[42] but are also cultivated from freshly expressed meibum[353] in normal subjects and in patients with blepharitis.[42] These bacterial enzymes lead to the release of lipid breakdown products, such as free fatty acids, that cause irritation of the epithelia and stimulate more keratinization, as is thought to be the case on the lid margin[41] and has been shown for the skin.[386] Altered lipid composition may also increase the melting point, leading to a lipid of high viscosity that mixes with desquamated epithelial cells and causes obstructive MGD.

On the other hand, it is possible that changes in the lipid profile, such as those in hormonal disturbances,[223,257,259] also occur because of alterations in lipid synthesis within acinar epithelial cells of the meibomian gland. The extent to which such changes contribute to meibomian gland obstruction remains to be determined.

Acinar Atrophy

In addition to the large number of reports that show the causative influence of hyperkeratinization and gland obstruction on the clinical picture of obstructive MGD as the most frequent pathology found in the meibomian gland, there is also evidence that the gland may undergo a degenerative atrophic process with progressive destruction of the tissue inside the lid.[3,31,356,357,387] Atrophic degeneration can be explained by the increase in intraglandular pressure due to the stasis of continuously produced secretum. There is also evidence that atrophy occurs with advancing age, as in other organs of the body.[53,358,387]

Influence of Aging. A natural aging process is indicated, not only by increasing alterations of the posterior lid margin, as described by Hykin and Bron,[348] but also by a decreasing number of active glands, as evidenced by vital stains,[50] diagnostic expression,[50] and a decrease in visible gland tissue (gland dropout).[53] Norn[50] found that the number of active oil-delivering glands decreases by half between the ages of 20 and 80 years. However, the amount of lipids on the lid margin is more or less stable or even increases slightly,[44] conceivably because its excretion from the ocular surface follows a different dynamic[4] with reduced removal of lipid in elderly compared with younger individuals.[37]

The influence of aging on the mouse meibomian gland was investigated in a recent study by Nien et al.[87] who observed several indications of a reduced gland function in the sense of atrophic changes due to an alteration of factors that are essential for the maturation of sebaceous glands. Immunohistochemical results showed that with increasing age, a significant reduction in mitosis in the proliferating cells, as evidenced by Ki67 staining, occurred in the basal meibocyte layer. This reduction was paralleled by a decrease in the size of the acini and a relocation of the meibocyte maturation marker PPAR-γ from a cytoplasmic expression in young (2 months old) and young adult (6 months old) animals to a nuclear expression in old (2 years old) animals. Also, a decrease in lipid production, as verified by oil red O staining, from numerous smaller lipid droplets in young individuals to fewer and larger ones in old individuals, was observed. In addition, cells that were positive for BLIMP1 and for the bone marrow cell marker molecule CD45 were observed around the acini. It is not clear from these observations whether the increase in the number of these cells, which occurred in the old animals and was assumed to reflect increased infiltration of leukocytes, represented an event accompanying the atrophic alterations or whether it contributed to the development and progression of acinar atrophy as a causative factor.

Age-dependent alterations in humans were reported by Obata et al.,[358,387] who described acinar atrophy without distinct dilatation as one of the pathologic findings in meibomian glands, which may suggest a primary acinar atrophy that leads to a decrease in the meibomian gland secretion with aging. Atrophic acini were observed as small and irregularly shaped acini, as opposed to normal round-shaped acini (Fig. 22). Age-dependent alterations in humans also were reported by Arita et al.[53] who, using the technique of infrared meibography, showed a strong age-dependent increase in meibomian gland dropout (disappearance of the glandular tissue inside the tarsal plates) between the third and eighth decades of life. This increase was accompanied by a decrease in TBUT. At present, it is not clear whether gland dropout really indicates the physical disappearance of glandular tissue or whether it becomes invisible because it assumes the characteristics of the surrounding tarsal tissue. Later, the same group observed that CL wearers also had a high meibomian gland dropout rate.[355] Young CL wearers had a dropout rate comparable to normal individuals in their 80s. The dropout was dependent on CL wearing time in lifetime years but independent of the type of lens. The pathogenesis of gland dropout in CL wearers is not yet clear, as discussed by Arita et al.,[355] and may include obstructive events as well as chronic mechanical traumatization of the conjunctival and tarsal tissue. Inflammatory events, as observed in giant papillary conjunctivitis can also result in gland dropout.[388] It can hence be assumed that inflammatory mediators find their way from the conjunctival tissue through the tarsus toward the meibomian gland and conceivably contribute to gland dropout and also potentially to acinar atrophy, as discussed by Knop and Knop.[389]

The age-dependent atrophic changes in the mouse meibomian gland[87] were different from those typically observed in acinar atrophy due to obstructive MGD, because they did not show the well-known hyperkeratinization and dilatation of the ductal system and the acini.[3,4,31,357,358] It is not clear at present how this may relate to the supposed primary atrophy occasionally observed in humans.[358,385] Therefore, there is apparently a difference in the mechanisms of atrophy in murine age-dependent atrophy versus that based on pathologic obstruction. Both of these atrophic events, however, most likely result in a decrease in oil production by the meibomian glands, with downstream negative influences on the ocular surface and tear film.

While the possibility should be considered that there is a primary, age-dependent form of MGD that leads to a gradual decline of glandular function[358] (Figs. 22), the predominant form of MGD apparently is due to hyperkeratotic obstruction of the gland, which presents the characteristic signs of dilatation (Figs. 18, 19).

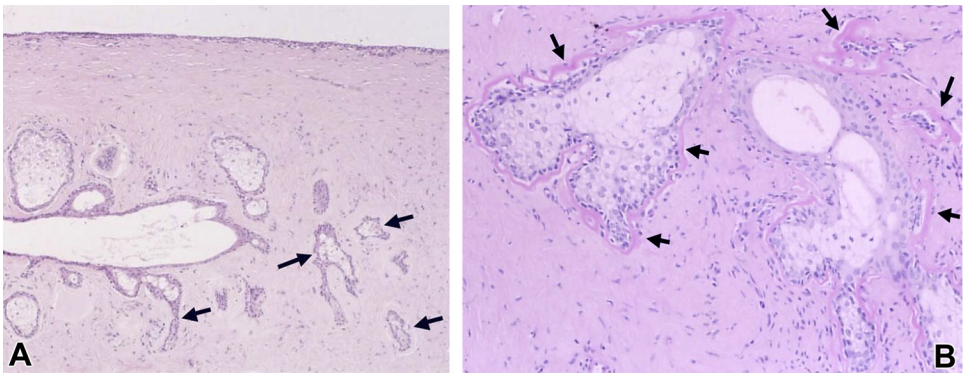

FIGURE 22. Acinar atrophy of human meibomian gland. (**A**) Acinar atrophy: Atrophic acini show a small and irregular, not rounded, shape (*arrows*); the duct appears slightly dilated. No inflammatory cell infiltration is seen. Figure reprinted from Obata H, Horiuchi H, Miyata K, Tsuru T, Machinami R. Histopathological study of the meibomian glands in 72 autopsy cases (in Japanese). *Nippon Ganka Gakkai Zasshi*. 1994;98:765–771 with permission from the Japanese Ophthalmological Society. (**B**) Basement membrane thickening of the acini: Basement membrane thickening (*arrows*) is frequently associated with atrophy of acini. Periodic acid-Schiff (PAS) staining. Figure courtesy of Hiroto Obata.

Atrophic events occur also in other ocular glands, such as the acinar atrophy in the human lacrimal gland that was also predominantly found in aged tissues.[390] Extended periods of autonomic denervation and transient denervation by intraglandular injection of botulinum toxin cause acinar atrophy in salivary glands.[391,392] Straatsma[31] speculated that obstruction of the orifices enlarges the ducts and acini, which encounter resistance from the dense connective tissue of the tarsus, and thus leads to cystic changes. Eventually, increasing intracystic pressure may inhibit cell differentiation. Turning our attention to other tissues, it is well known that obstruction by duct ligation, for instance, causes atrophy in the salivary gland,[391] and it can be assumed that this effect is based on mechanisms similar to those in the meibomian glands. Ischemia or hypoxia causes atrophy in the prostate and pancreas.[392,393] Such phenomena are well known as disuse atrophy, which is a common consequence of glandular obstruction.

Basement Membrane Thickening of Acini. Basement membrane thickening of acini is frequently associated with atrophy of acini and can be observed in hematoxylin and eosin (H&E) staining, but more clearly in periodic acid-Schiff (PAS) staining[387] (Fig. 22B). It is unclear whether the basement membrane thickening is a primary or a secondary change after acinar atrophy. In any case, basement membrane thickening may interfere with blood supply from capillaries around the acini and may hence negatively influence the homeostasis of the meibomian gland.

Influence of Blood Supply. Large blood vessels are not present in the tarsus and are inconspicuous in routine H&E-stained paraffin-embedded sections. Blood vessels in the tarsus are best observed by immunohistochemistry with a marker of vascular endothelium. This labeling shows that capillary vessels surround the acini (Fig. 23) and that they contribute to the nutrition of the meibomian gland.

Inflammation

McCulley et al.[394] stated that primary meibomitis did not appear to be a primarily infectious entity, but represents a facet of generalized sebaceous gland dysfunction. The definition and involvement of inflammation in MGD have been unclear in the past for several reasons. First, there have been several coexisting terms, such as posterior blepharitis, meibomitis, and MGD, used interchangeably and ambiguously in distinguishing definitively between the different disease entities. Second, there has been an ongoing discussion of how to define inflammation. It can be defined as an inflammatory cell infiltration in classic pathology or as the involvement of inflammatory cytokines in current molecular biology, even when no inflammatory cell infiltration is found by light microscopy. The latter would constitute a proinflammatory state, as opposed to overt inflammation, that would still be able to alter the differentiation of the tissue. Third, inflammation can be divided into two categories: infectious and noninfectious. Although it was found that the observed bacteria were commensal species that occur in normal individuals and, in a larger number, in those with blepharitis,[41,42,353] it reflects an increased growth rather than an infection.[42] Unifying definitions for these findings have been suggested.

Few reports are available that mention an inflammatory cell infiltration in the meibomian glands in MGD. In a histopathologic study of tarsal tissues obtained at autopsy,[358,387] lipogranulomatous inflammation and granulation tissue showing inflammatory cell infiltration were observed. However, it is not certain whether these changes represent pathologic features of MGD. It is important to note that, in the presently available studies, an inflammatory cell infiltration was not observed in

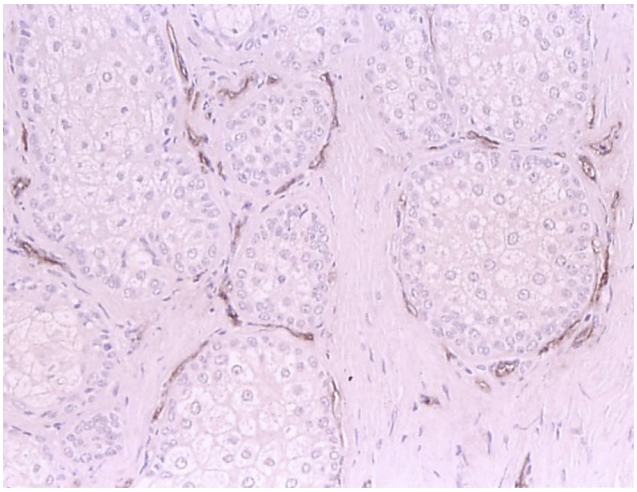

FIGURE 23. Capillary vessels in a normal human meibomian gland. Immunostaining of factor VIII, a marker of vascular endothelial cells, reveals capillary vessels surrounding the acini. Figure courtesy of Hiroto Obata.

specimens of cystic dilatation and acinar atrophy of the meibomian gland.[31,357,358,387] In contrast, in vivo confocal microscopy reported inflammation in the tarsal conjunctival epithelium and stroma in patients with blepharitis and meibomitis, suggesting that the presence of an inflammatory infiltrate enables the differentiation between MGD and meibomitis.[395] In another in vivo confocal microscopy study, a periglandular inflammatory cell infiltration was observed in the eyelids of patients with obstructive MGD, and the infiltration was cleared by treatment with topical levofloxacin, topical 0.1% fluorometholone, and oral minocycline.[396] The differentiation between individual cell types is difficult in confocal microscopy, however, and frequently it is not possible to differentiate clearly between activated stromal cells and leukocytes. As such, in vivo confocal microscopy cannot identify the presence or absence of inflammation as clearly as has been achieved by histopathology.[3,31,356,357,387]

If obstructive MGD increases the intraglandular pressure, ductal and acinar epithelia may undergo cell stress. It is speculated that this stress also triggers MAP kinase activity in the meibomian gland, with downstream release of chemokines and cytokines and ultimately the occurrence of inflammation, as observed in the epithelium of the conjunctiva and cornea.[397] Further studies are needed to explore the involvement of inflammation in MGD.

Infection and Therapy

The pathophysiology of MGD is complex and whether bacterial infection is a cause of MGD remains controversial. It is well known that commensal bacteria such as coagulase-negative staphylococci (CNS), *Staphylococcus aureus*, and *Propionibacterium acnes* are related to and contribute to the pathologic course of chronic blepharitis.[41,398] In contrast, Gutgesell et al.[357] described that the inflammation related to bacterial infection was not an important factor in obstructive MGD, because only a minimal or absent inflammatory cell infiltration was found in histopathology. Bacterial infection can, in principle, focally destroy the meibomian gland structure as seen in hordeolum. Although such direct and active bacterial infection is reportedly not involved in the pathogenesis of MGD, bacterial products such as lipase and toxins (without infection) are still believed to be pathogenically relevant.[7,41] Dougherty and McCulley[41] reported that the greatest bacterial lipolytic activity was found in those patients with meibomian gland abnormality among six clinical groups of chronic blepharitis. Bacterial lipase could alter the lipid composition, influencing the physical characteristics of the tear film and causing evaporative dry eye. Effective antibiotics used in the treatment of MGD are tetracycline, doxycycline, and minocycline.[41,399,400] Tetracycline and doxycycline are used at doses that are not antimicrobial, but the dose of minocycline is. At the doses typically prescribed, the antibiotics are hypothesized to have suppressive effects on lipases and inflammation.

It has been well known that *Demodex* infestation can be associated with blepharitis.[401–403] To date, the pathogenic potential of these mites in MGD remains unclear.[7]

Functional Interactions in the Pathogenesis of MGD

Course of Structural Alterations of the Meibomian Gland in Obstructive MGD

The clinically more obvious consequence of obstructive MGD is in the occurrence of dry eye symptoms[5,6,8,10,12,49,51,261,404–406] because of a deficiency in the tear film lipid layer[2,5,43,407,408] and downstream events, such as a hyperosmolarity of the tears,[409–412] due to increased evaporation[12] and decreased TBUT,[413,414] among others, which result in a wetting deficiency with mechanical irritation through increased friction,[415] the onset of inflammatory cascades at the ocular surface,[397,416–418] and unstable visual acuity.[419] Altogether, this result represents a complex dysregulation of the functional anatomy of the ocular surface.[3,15,404,420,421] In addition, obstructive MGD results in an atrophic process in the glandular tissue itself inside the tarsal plates of the eyelids, determined by histopathologic investigations and conceivably also indicated by gland dropout.[10,53,355,362,422,423] Atrophic degeneration of the meibomian gland is clinically less obvious and conceivably underestimated, except when more sophisticated methods such as meibography[53,354,413,424–426] are applied.

The literature suggests a course of progression in obstructive MGD (Knop E, et al. *IOVS* 2009;50:ARVO E-Abstract 4833; Knop E, et al. IOVS 2010;51:ARVO E-Abstract 2366)[3,6,31,351,354,357,358,377,427] (Fig. 24) and that the pathology in the disease starts with an obstruction of the orifice and excretory ducts by hyperkeratinization and/or a pathologic, highly viscous meibum. The involvement of hyperkeratinization as a causative factor in obstructive MGD (1) was raised by clinical observations,[3] (2) was substantiated by histologic observations (Knop E, et al. *IOVS* 2009;50:ARVO E-Abstract 4833),[3,31,357,358] and by the finding of keratinized cell material in the opaque thickened meibum expressed from obstructed glands,[3] and (3) was further verified by the presence of keratinized cell material in the expressed meibum of patients in molecular biological and immunologic assays (Obata H, et al. *IOVS* 2002;43:ARVO E-Abstract 60).[385] Animal models of obstructive MGD also supported hyperkeratinization as a major causative factor.[351,354,377]

When the delivery of meibum onto the lid margin and tear film is blocked by an obstruction, meibum accumulates within the ductal system of the gland due to the continuing secretion from the secretory acini.[36] Persistent accumulation of meibum inside the obstructed glands conceivably results in a progressive increase in pressure inside the ductal system and thus in progressive widening of the ductal system. After a prolonged time, this increased internal pressure also extends into the secretory acini, via a widening of the small connecting ductules. The acini undergo atrophic changes, with a loss of secretory meibocytes[357] (Fig. 18) and eventually a squamous metaplasia that can result in full cornification of the epithelium of the ducts and acini.[31] The consequence of acinar atrophy is a secondary hyposecretion, and acinar atrophy is conceivably also the reason for the gland dropout seen on meibography. Acinar atrophy appears mainly as a pressure atrophy, because the dilatation of the meibomian gland is limited by the more rigid tarsal connective tissue,[31] although other factors that interfere with cell differentiation may also contribute to it.

It was pointed out in the earliest histopathologic description of meibomian gland dilatation in the human[31] and verified by later studies[3,357,358] that this process of obstructive atrophy of the meibomian gland occurs in the absence of overt inflammation and in the absence of inflammatory leukocytes in the glandular tissue. Although recent in vivo confocal investigation[395,396] points to a potential presence of periglandular inflammatory cells in certain cases, this technique provides only limited information compared with histopathology. In light of the rapidly increasing knowledge of ocular surface immunology and tissue homeostasis,[416,428,429] it can be hypothesized that at least subclinical inflammatory events contribute to the degenerative process. Inflammatory reactions are exerted by factors such as irritative lipid species (e.g., free fatty acids)[41] or by proinflammatory downstream agonists (e.g., phospholipase

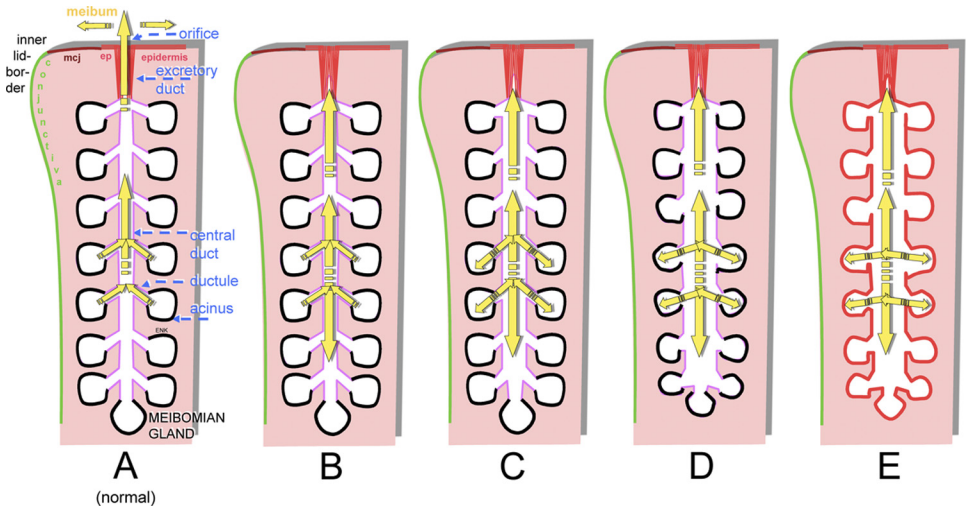

FIGURE 24. Course of structural alterations of the meibomian glands in obstructive MGD. Schematic drawing of a meibomian gland and the posterior lid margin. (**A**) Normal: In the normal meibomian gland, the secretory product (meibum, *yellow arrows*) that is produced inside the acini is transported through the connecting ductules into the central duct and is finally delivered through the excretory duct and orifice that is located within the keratinized epidermis (*red*) at the posterior lid border. The ductal system has an incipient stage of keratinization (*pink*). The acini are spherical to elongated, and the connecting ductules are narrow. (**B**) Obstruction: When the orifice and excretory duct are obstructed by hyperkeratinization of the epithelium and/or increased viscosity of the meibum, the delivery of meibum onto the lid margin is reduced or completely inhibited. (**C**) Additional dilatation: The continuing secretion of meibum in the acini generates an increasing pressure inside the glands that leads to a gradual dilatation, first of the central duct. (**D**) Additional atrophy: After a prolonged time, the increased pressure inside the gland leads to dilatation of the connecting ductules and a pressure atrophy of the acini with rarefaction of secretory meibocytes. This effect causes shrinkage of the whole acini that may represent the histopathologic equivalent of the clinically detectable gland dropout and results in a presumed secondary hyposecretion. (**E**) Additional cornification of the glandular epithelium: In late stages the whole ductal epithelium can become cornified and the meibocytes replaced by a stratified squamous cornified epithelium. Reprinted from Knop E, Knop N. [Meibomian glands. Part IV: Functional interactions in the pathogenesis of meibomian gland Dysfunction (MGD).] Meibom-Drüsen, Teil IV: Funktionelle Interaktionen in der Pathogenese der Dysfunktion (MGD). *Ophthalmologe.* 2009;106:980–987 with the kind permission of Springer Science and Business Media.

A2, leukotrienes, or arachidonic acid[430]) and by induced inflammatory cytokines, possibly triggered by MAP kinase[397] or other pathways. Such factors are also described in the obstructive alteration of the hair-associated sebaceous glands of the skin (acne).[107] The extent of the dilatation and atrophic changes in the gland structure conceivably depends on the grade of obstruction and on the duration of the process, which points to the necessity of a timely diagnosis and appropriate therapy of obstructive MGD.[6,389]

Interacting Pathways in the Pathogenesis of MGD

Core Mechanisms. Many underlying factors such as age, sex, hormonal disturbances, and environmental factors, as well as changes in the composition of meibum, contribute to the pathogenesis of obstructive MGD. These factors appear to form different pathways that interact in an interrelated sequence of events (Fig. 25) and also give rise to several vicious circles that reinforce the process and can aggravate the dysfunction if they are not limited by effective and timely therapeutic interventions.

Obstruction. Obstruction of the meibomian gland as a core mechanism of MGD leads to two limbs of downstream consequences, both of which result in a decreased availability of meibomian lipids at the lid margin and tear film and hence in an evaporative dry eye condition: (1) directly via a low *delivery* of oil onto the lid margin and (2) indirectly via a stasis of meibum inside the gland that results in several downstream events, such as increased pressure, resultant dilatation, and, eventually, acinar atrophy, which causes low *secretion*.

Hyperkeratinization. Various endogenous and exogenous factors (Fig. 25) that can influence the pathologic course of MGD lead to hyperkeratinization of the epithelium at the lid margin and meibomian gland, either directly or by a possible influence on cell differentiation.

Hyperkeratinization appears as the main pathomechanism of MGD, as indicated by a large body of literature (Knop E, et al. *IOVS* 2009;50:ARVO E-Abstract 4833).[3,31,351,354,357,358,377] This can be explained by the fact that the meibomian glands share strong similarities with the hair follicles of the cilia in embryologic development and structure.[26–28] The meibomian glands have preserved an incipient stage of keratinization in the form of keratohyalin granules throughout the luminal layer of the ductal epithelium in animals[32] and in the human.[30] As mentioned earlier in the article, these observations have led to the statement that the meibomian gland can be regarded as a hair follicle without a hair shaft.[24,431] The hyperkeratinization in obstructive MGD may therefore be caused by the removal of a developmental block that prevents progression of the natural incipient keratinization into full cornification. In disease, such as chronic blepharitis, the meibomian glands can still develop a hair, a condition known as distichiasis.[432,433] Hyperkeratinization may also be influenced by an aberrant differentiation or migration of stem cells. The migration and differentiation of stem cells generally assumes a higher degree of plasticity due to wounding[434] and probably also due to mechanical stress or to the occurrence of mediators of a subclinical inflammation. Such factors are shown to be influential in the development of acne in the hair-associated sebaceous glands.[107] Other factors shown to increase keratinization of the lid margin and meibomian gland are topical[354] or systemic[320,435] medications and chemical toxins.[365]

With advancing age, degenerative changes, including hyperkeratotic events, generally increase at the posterior lid margin and also affect the orifices of the meibomian glands by orifice narrowing.[348] Meibomian gland drop out, which conceivably reflects a loss of functional glandular tissue and represents an endstage of acinar atrophy, results in respective symptoms of ocular dryness that proceed strongly with aging.[53]

Hormonal influences on meibomian gland function are well described. Pathologic alterations (in particular of androgen action[406]) result in hyperkeratinization of the epithelium at the lid margin, obstructive MGD with a lack of meibum on the lid margin and on the tear film, an altered lipid profile, and associated dry eye symptoms.[105,257–260]

Increased Viscosity. Besides hyperkeratinization, increased viscosity of meibum is the other most important pathogenetic

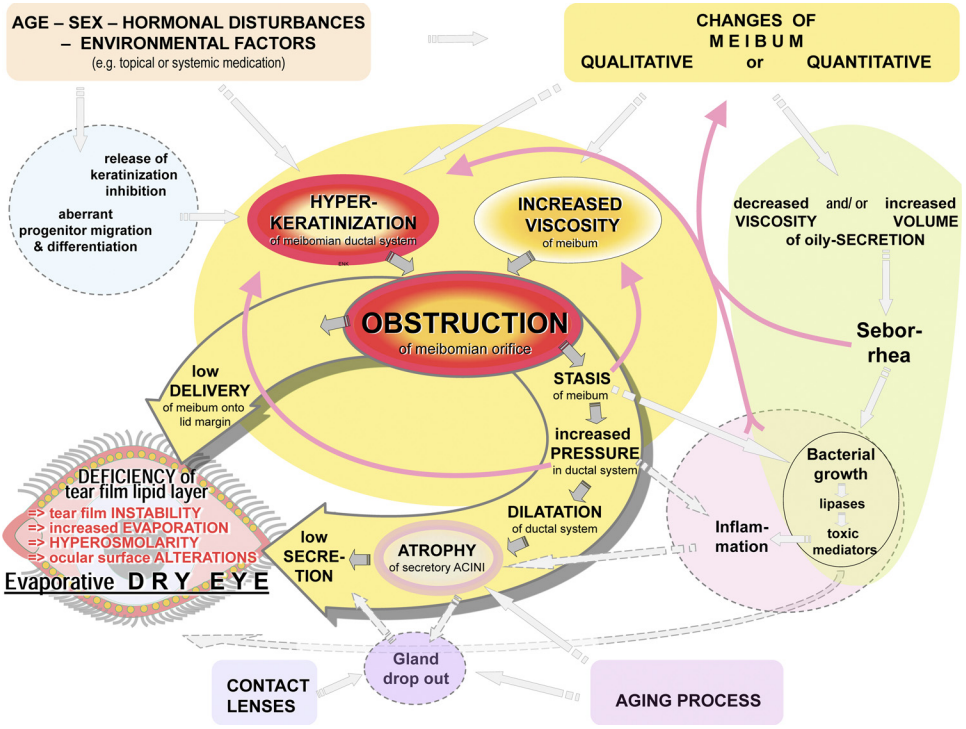

FIGURE 25. Pathways and proposed sequence of events that lead to self-enforcing vicious circles in MGD. Mechanisms and interactions (*arrows*) in MGD occur as a result of underlying causative factors (*colored square boxes* located in the periphery). The core mechanisms of gland obstruction due to ductal hyperkeratinization and increased viscosity of the meibomian oil (meibum) are shown in the center of the figure on a *yellow* underlay and result in two effector limbs (wide shadowed downstream *arrows*, also on *yellow* underlay). Associated functional complexes, such as progenitor cell differentiation, bacterial growth, inflammation, and seborrhea, are shown on color-shaded spherical zones around the core mechanisms. *Dashed arrows* depict likely interactions; functional complexes of likely but insufficiently clarified importance are shown in *dashed circles*. Vicious circles that result in a progressive process of dysfunction are indicated by *red bent arrows*. Hyperkeratinization of the epithelium of the excretory duct and orifice is the main factor that leads to obstruction of the meibomian glands. This effect is influenced by endogenous factors such as age, sex, and hormonal disturbances as well as by exogenous factors, such as topical medication. These may act, at least in part, via the release of a physiological inhibition of full keratinization and via an aberrant differentiation of progenitor cells. Increased viscosity of meibum through qualitative changes of its composition is the other important causative factor that contributes to the obstructive process. It may occur independently, because of the influence of endogenous or exogenous factors or a preexisting obstructive stasis of secretum. Obstruction leads on the one hand (left effector limb *arrow*) to the immediate clinically observable low delivery of meibum onto the lid margin and tear film that results in an evaporative dry eye condition. On the other hand (right effector limb *arrow*), obstruction also results in several consecutive negative effects directly inside the meibomian glands, because of an internal stasis of meibum. Stasis can be associated with increased viscosity of the meibum, which reinforces the obstruction in a vicious circle. The continuous secretory activity of the meibocytes leads to a progressive increase in pressure within the glands. This increased pressure can, in another vicious circle, induce an activation of the epithelial cells that reinforces hyperkeratinization. Pressure further leads to a dilatation, first of the ductal system and, after a prolonged time, also to atrophy of the acini, with rarefaction of their secretory meibocytes, and thus results in a secondary hyposecretion with low secretion of lipids. Atrophy may be the reason for the clinically detectable gland dropout, and CL wear is, by presently unknown mechanisms, associated with gland dropout. Stasis of meibum also promotes the growth of bacteria on the ocular surface and possibly inside the glands, usually pre-existing commensals, that produce lipid-degrading enzymes. Their action on the meibomian lipids leads to the production of toxic mediators, such as free fatty acids, that may initiate subclinical inflammatory reactions with release of inflammatory cytokines. Toxic and inflammatory mediators may promote subclinical inflammatory events inside the gland, in the periglandular conjunctiva, on the lid margin, and on the ocular surface, as suggested by observations in dermatology (e.g., in acne pathogenesis and skin irritation). Toxic mediators are also assumed to have negative effects on tear film stability. Furthermore, they can lead to qualitative changes in the composition of meibum that increase its viscosity or, through activation of epithelia on the lid margin and possibly inside the gland, they can reinforce keratinization. Altogether, these events can give rise to several vicious circles (*red arrows*) that increase the preexisting obstruction, if not limited by timely diagnosis and therapeutic intervention. There is evidence from a mouse model that acinar atrophy may also occur due to the aging process. If MGD occurs in conjunction with systemic skin diseases such as seborrheic dermatitis, possibly accompanied by blepharitis, an increased amount of oil (seborrhea) with decreased viscosity can be observed on the lid margin. Seborrheic blepharitis, similar to stasis, can be associated with increased bacterial growth and its downstream negative effects. The seborrheic oil has a different composition than that of normal meibum and thus may have negative effects on the tear film. All major mechanisms of the schematically depicted process are supported by findings in the literature. Reprinted from Knop E, Knop N. [Meibomian glands, Part IV: functional interactions in the pathogenesis of meibomian gland dysfunction (MGD).] Meibom-Drüsen, Teil IV: Funktionelle Interaktionen in der Pathogenese der Dysfunktion (MGD). *Ophthalmologe*. 2009;106:980–987 with the kind permission of Springer Science and Business Media.

factor in MGD. It is observed in all cases of obstructive MGD,[3,4,10,359,385,420,436,437] also occurs in animal models,[351,354,377] and may be primary[258] or secondary. A secondary change in obstructive MGD appears to be due to the stasis of meibum inside the ductal system of obstructed glands and to the potential influence of lipid degrading enzymes. Stasis of meibum thus can, in a vicious circle, further aggravate the obstruction. Highly viscous meibum is mixed with hyperkeratotic cell material, as seen in expressed pathologic human meibum prepared as smears[3] or in impression cytology (Obata H, et al. *IOVS* 2002;43:ARVO E-Abstract 60) (Fig. 21) and in histopathology,[3,356] as verified by molecular biology and immunohistochemistry.[385] Increased viscosity has also been observed inside the obstructed glands of animal models[338] (Fig. 20).

Increasing age, which brings about hormonal changes, is associated with increased frequency of changes in lipid composition, such as alterations of the polar and neutral lipid profiles.[105] Qualitative lipid changes may result in an increased viscosity of meibum, as observed in the decrease in monounsaturated fatty acid, specifically oleic acid, in patients with chronic blepharitis.[438] Since a decreased desaturation of lipid raises its melting point and hence leads to its thickening,[407] this phenomenon can reinforce an obstructive process and explains an elevated rate of obstruction in blepharitis. A loss of the polar lipids that are assumed to maintain the adherence of the superficial nonpolar lipid layer to the aqueous tear film[2,102,439] may contribute to an increase in tear film instability and evaporation in patients with MGD.

Associated Functional Complexes. Apart from the core mechanism of MGD as an obstructive process that is caused by hyperkeratinization of the meibomian duct and orifice, together with increased viscosity of meibum, there are also some associated functional complexes that interact with the pathogenesis.

Altered Cell Differentiation. An aberrant differentiation of stem cells and progenitor cells inside the meibomian glands or on the lid margin can result from alterations in the endogenous and exogenous underlying factors (such as age, sex, and hormone levels). In ductal cells, this change can lead to hyperkeratinization (as explained earlier) and may contribute to alterations in the lipid profile in the acinar cell. Alterations in cell differentiation can also generally be assumed to be involved in the aging process of the human and are shown in acinar atrophy of the mouse.[87] Aberrant cell differentiation is also responsible for general pathologic alterations of the meibomian gland.

Seborrhea. For a long time, disease affectations at the lid margin, including the meibomian gland, were simply termed blepharitis because a clear distinction between anterior and posterior blepharitis or MGD was not always made.[6,7] In their blepharitis classification, McCulley et al.[394] reported a large percentage of the seborrheic type of blepharitis. In most of these patients, it was associated with generalized skin disease, mainly seborrheic dermatitis, which affects areas of the skin rich in sebaceous glands. It frequently, but not always, presents with copious amounts of oil of low viscosity and is generally associated with hyperkeratinization.[440] This helps explain why seborrheic dermatitis may be associated with hyperkeratotic obstructive MGD. Seborrhea is assumed to give rise to several feedback mechanisms that can reinforce qualitative and quantitative changes in meibum and epithelial hyperkeratinization. An increased sebum production is also known to promote the obstructive disease of the skin sebaceous glands in acne.[148] In patients with chronic blepharitis and seborrhea, qualitative changes in lipid composition were observed, including a higher amount of monounsaturated fatty acids that lead to a decreased melting point and hence explain the fluid appearance of oil on the lid margin in this condition.[438] Seborrheic blepharitis, similar to obstructive MGD, is also associated with increased bacterial growth and hence shares the presence of bacterial lipid-modifying enzymes and respective alterations of the lipid composition as well as the downstream effects of toxic mediators and negative influences on the tissue and conceivably on the tear film.[398] We can therefore link seborrhea with increased bacterial growth and subclinical inflammation in the pathogenesis of blepharitis. Definitions for the various subtypes of blepharitis and MGD can be found in the Definition and Classification Report.

Influence of Bacteria: Commensal Bacterial Growth. MGD can be associated with an increased growth of bacteria on the lid margin, but conceivably inside the obstructed ductal system as well, which represents an ideal undisturbed niche full of nutrients. Evaluation of bacteriology in patients with chronic blepharitis and normal controls has revealed commensal bacteria, mainly of the species *S. aureus*, coagulase negative *Staphylococcus* spp., lipophilic *Corynebacterium* spp., with *P. acnes* as the most frequently isolated organism, found on 98% of lid margins.[42] The same species were cultured from 52% of freshly expressed meibum samples after lid margin cleaning with a sterile swab.[42] Even though this technique does not totally exclude the possibility of contamination of the freshly expressed meibum with bacteria from the lid margin, the findings in this study give a strong indication that commensal bacteria also may be naturally present inside the meibomian gland. It is important to note that this bacterial colonization does not represent an infection, but rather an increased growth of preexisting commensal species.[42,353] The observation of a high degree of association of MGD with all the seborrheic groups of blepharitis[394] led to the hypothesis that lipid abnormalities are a causative factor in the disease. In a later study, it was observed that the previously cultured bacterial species were able to degrade meibomian lipids by their lipid-degrading enzymes (lipases and esterases),[41] which modify the normal meibomian lipids and lead to an altered lipid spectrum.[441] This degrading influence resulted in significant changes in some free fatty acids. It is known that free fatty acids are irritants to epithelia, can penetrate the epidermal barrier, and can cause inflammation and hyperkeratinization.[386] They are also recognized as factors that promote the obstructive disease in skin sebaceous acne.[107,442,443] In particular, free fatty acids and fatty acid alcohols are thought to contribute significantly to chronic blepharitis, and it is assumed that they can result in irritation of the epithelium and stimulate keratinization.[444,445] Increased availability of cholesterol from cholesterol esterase activity can further promote bacterial growth.[444] The increased growth of commensal bacteria and the downstream presence of irritative lipid species and toxic mediators must be regarded as an influencing factor in MGD. These factors do not appear to present a primary cause of obstructive MGD, however, but rather a secondary phenomenon that becomes important due to a preexisting obstruction and stasis. Bacterial growth negatively reinforces hyperkeratinization and may play a role in qualitative and quantitative changes in the meibum.

Inflammatory Mediators. Increased bacterial growth is linked with subclinical inflammatory events through the release of lipid species, such as free fatty acids,[41] that may irritate and act in a proinflammatory manner on the tissue and potentially on the tear film as well. Increased amounts of phospholipase A2 are found in the meibum of patients with blepharitis.[446] Phospholipase A2 can induce the formation of arachidonic acid, an unsaturated fatty acid, from which prostaglandins and leukotrienes are synthesized. These factors have a central position in inflammatory processes. They can irritate and activate the ocular surface epithelium and it is speculated that they also result in tear film instability.[41,445] Activated epithelial cells then produce inflammatory cytokines such as TNF- and interleukin-α and -β and promote a subclinical inflammatory microenvironment.[4,41,407,430,447,448] Such inflammatory cytokines are also produced by normal and stressed sebocytes.[148,449,450] The addition of IL-1-α to isolated pilosebaceous units in vitro results in hypercornification,[451] as discussed by Zouboulis.[151] Similar events are described in the obstructive disease (acne) of the hair-associated sebaceous glands of the skin, as discussed by Kurokawa et al.[107] In acne, three distinctive events occur. First, desaturated fatty acids, lipoperoxides, and commensal bacteria such as *P. acnes* that also occur at the lid margin can induce the production of inflammatory cytokines by epithelial cells.[452] Second, inflammatory cytokines such as IL-1α activate epithelial cells and induce an alteration of normal epithelial differentiation toward increased proliferation and keratinization,[453] which together results in obstructive sebaceous gland disease. Third, peroxides, such as the lipoperoxides that arise during the pathologic modification of meibomian lipids, can act as ligands for the transcription factor PPAR-γ, which promotes cell maturation and lipid production. These effects may contribute to the seborrhea in acne but also in the seborrheic blepharitis associated with MGD.

Physiological Aging Process. An age-dependent degeneration of the human meibomian gland that may reflect physiological age-related changes is indicated by various observations. In the human, degenerative changes, including hyperkeratosis

and meibomian orifice narrowing,[348] generally increase at the posterior lid margin with advancing age and are assumed to induce obstructive MGD. Aging also results in changes in the composition of meibum that are reflected by alterations in the polar and neutral lipid profiles,[105] as explained earlier. Obstructive MGD may be responsible for the observed reduction by half in the number of actively secreting glands between the ages of 20 and 80 years,[50] as well as for the drastic loss of functional glandular tissue (gland dropout)[53] that is observed over the same age range and results in respective symptoms of ocular dryness. CL wear is found to cause alterations that resemble an increased aging process,[355] although the exact pathomechanism that causes the gland dropout is not yet clear.

Apart from the changes in the meibomian glands that can occur secondary to obstruction, there are also indications that age-dependent alterations may affect the glandular tissue's physiology directly.[387] In a mouse model, Nien et al.[87] observed several indications of reduced gland function in the sense of atrophic acinar changes due to an alteration of factors that are crucial for the differentiation and maturation of sebaceous glands. In old animals, a significant reduction in meibocyte mitosis is paralleled by a decrease in the size of the acini, by a relocation of the meibocyte maturation marker PPAR-γ, and by a decrease in lipid production. These alterations apparently occur without gland obstruction and hyperkeratinization. In contrast to the pathology in obstructive MGD, an increased number of bone marrow–derived cells occurs in the glandular tissue that are perceived as reflecting a cellular inflammatory reaction.

From these findings, it may be assumed that, similar to other organs in the body, the meibomian glands undergo a primary age-dependent form of degeneration that leads to a gradual decline in glandular function in contrast to a secondary pathologic obstructive MGD. In a mouse model,[87] the atrophic changes in the gland acini were apparently not accompanied by hyperkeratinization and obstruction of the ducts and orifices. These latter phenomena may represent useful criteria for differentiating between primary age-dependent degeneration and pathologic atrophic destruction of the glands due to obstructive MGD. On the other hand, since these atrophic changes in the mouse do not resemble the typical human pathology they may also represent species-specific differences compared with the typical human situation.

CL Wear. CL wear is a widespread environmental factor associated with MDG.[454] Although obstructive MGD was first described by Korb and Henriquez[3] as causative in patients with CL intolerance, several later studies did not provide an unequivocal opinion[359,436,454–457] on the association of obstructive MGD with CL wear compared with non-CL wear.[389] On the other hand, MGD therapy by lid hygiene was found by Paugh et al.[436] to be effective in improving CL intolerance in such patients. Ong and Larke[458] reported that the onset of CL wear results in an increased rate of MGD, independent of lens type, with blocked glands and abnormal meibum. They reported in a later study[359] that CL wearers had only a slightly increased prevalence of MGD. There are clear data by Mathers and Billborough[388] on a distinctly increased rate of MGD, with thickened meibum and gland dropout in patients showing CL-associated giant papillary conjunctivitis that may support the potential involvement of inflammatory mediators in the pathogenesis of MGD. More recent data by Arita et al.[355] indicate a high prevalence of gland dropout together with dry eye symptoms in CL wearers. Dropout correlated with the duration of lens wear but was independent of the lens type. Such patients in their early 30s already had a degree of gland dropout that resembled a normal population in their 80s,[53] and it was hence concluded that CL wear results in changes that accelerate the natural aging process of the meibomian gland. It may thus be assumed that CL wear is associated with alterations of the meibomian gland in the sense of MGD, although the exact pathomechanism is not yet clear and remains to be identified.

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