A Solute Gradient in the Tear Meniscus. II. Implications for Lid Margin Disease, including Meibomian Gland Dysfunction

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ABSTRACT We have hypothesized previously that evaporation from the tears generates a solute gradient across the tear meniscus, which delivers hyperosmolar stress to the mucocutaneous junction (MCJ) of the lid margin. This is proposed as the basis for Marx's line, a line of staining with topically applied dyes that lies directly behind the MCJ. In this article, we consider the implications of this hypothesis for progressive damage to the lid margin as an age-related phenomenon, its amplification in dry eye states, and its possible role in the etiology of meibomian gland dysfunction (MGD). It is suggested that a hyperosmolar or related stimulus, acting behind the MCJ over a lifetime, promotes the anterior migration of the MCJ, which is a feature of the aging lid margin. This mechanism would be amplified in dry eye states, not only by reason of increased tear molarity at the meniscus apex but also by raising the concentration of inflammatory peptides at this site. This could explain the increased width and irregularity of Marx's line in dry eye. While the presence of stem cells at the lid margin may equip this region to respond to such stress, their depletion could be the basis of irreversible lid margin damage. It is further proposed, given the proximity of the MCJ to the meibomian gland orifices, that the solute gradient mechanism could play a role in the initiation of MGD by delivering hyperosmolar and inflammatory stresses to the terminal ducts and orifices of the glands. By the same token, the presence of a zone of increased epithelial permeability in this region may provide a back door route for the delivery of drugs in the treatment of MGD.

KEY WORDS dry eye, evaporation, fluorescein, galectin-3, lid margin, lissamine green, Marx's line, meibomian gland dysfunction, MGD, mucin, mucocutaneous junction, osmolarity, rose bengal, solute, tear meniscus

I. INTRODUCTION

In a preceding article, we hypothesized that evaporative water loss from the tear meniscus creates a gradient of solute across its profile, leading to increased tear molarity at the peripheral meniscus apex, over its insertion at the mucocutaneous junction (**MCJ**).[1] It was predicted that during the interblink, this hyperosmolarity would stress the underlying band of superficial, conjunctival epithelial cells directly behind the MCJ. This was proposed as the basis of Marx's line, a physiological line of staining with dyes such as fluorescein, lissamine green, and rose bengal, present throughout life.[2,3] Staining was proposed to reflect a deficiency of sealing molecules in the surface glycocalyx, such as MUC16 and galectin-3, which normally serve to exclude the entry of dyes into the surface epithelium.

In the present article, we suggest that this basic mechanism, acting over the life span, may be responsible for progressive damage to the lid margin and that the zone of superficial epithelial cells represented by Marx's line may play a special role in chronic lid margin disease and in the etiology of meibomian gland dysfunction (**MGD**).

II. AGING AND MARX'S LINE

In youth, Marx's line is thin and its contour smooth and continuous, the lines of the upper and lower lids joining temporally at the outer canthus and nasally, commonly passing anterior to the puncta. After about 50 years of age, the MCJ becomes increasingly irregular,[4-8] and in the presence of various forms of blepharitis, it may migrate either forward

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OUTLINE

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or backward.[4,9,10] Forward migration of the MCJ causes it to lie anterior to the meibomian orifices in a process sometimes referred to as *conjunctivalization* of the lid margin.[9,11] Posterior migration of the MCJ is a prominent feature of cicatricial conjunctivitis, but it occurs to a lesser degree as a primary disorder or secondary to skin disease.[9,10] Forward migration of the MCJ may also place the affected orifices deep to the tear meniscus, but with the orifices remaining on the occlusal surface of the lid margin. This distinguishes the condition from posterior migration, usually associated with cicatricial conjunctival disease, in which the orifices and terminal ducts, together with the MCJ, migrate posteriorly into the tarsal conjunctiva.

The quantitative aspects of forward migration have received attention in a study by Yamaguchi et al,[8] who graded the disposition of Marx's line in a group of 251 subjects without a history of ocular surface disease, using fluorescein dye (2 μl of 1%). Staining was observed in blue light without the use of a yellow barrier filter. The grading levels were: 0 = the line runs entirely on the conjunctival side of the meibomian orifices; 1 = parts of the line arch forward to touch the meibomian orifices; 2 = the line runs through the meibomian orifices; 3 = the line lies on the skin side of the meibomian orifices. Grading was performed in the inner, central, and outer thirds of the lower lid. Grading was reasonably consistent between observers, the lower lid grades were significantly correlated with upper lid grades, and grades were similar between men and women. An important finding was that the grade score increased with age, implying that Marx's line (and therefore the MCJ) moves forward with time. We interpreted this finding to reflect the consequences of lifelong stress to the MCJ induced by our proposed gradient mechanism.

The gradient hypothesis predicts several additional pathological consequences at the lid margin.

III. PATHOLOGICAL CONSEQUENCES OF THE GRADIENT HYPOTHESIS

A. Implications for Dry Eye Disease

A possible consequence of the gradient hypothesis concerns the influence of dry eye on the lid margins. Tear hyperosmolarity is accepted as a core damage mechanism in any form of dry eye, responsible for initiating inflammatory events and causing cell death at the ocular surface.[12] However, the implications of tear hyperosmolarity for damage to the lid margin has received no attention. We suggest that, based on considerations of the solute gradient hypothesis, lid margin damage might be accentuated in two ways.

First, the solute gradient hypothesis proposes a mechanism in which tear evaporation amplifies the concentration of solute at the peripheral meniscus apex. Therefore, in the presence of an existing tear hyperosmolarity, as occurs in dry eye disease, it may be predicted that the level of hyperosmolarity at the meniscus apex will rise above that present in the tear film or body of the meniscus. Experimental exposure of ocular surface epithelial cells to hyperosmolar conditions stimulates a cascade of inflammatory events within the cells, involving MAP kinases and NF-κB,[13-15] leading to the generation and release of inflammatory cytokines (interleukin [**IL**]-1α, IL-1β, tumor necrosis factor [**TNF**]-α) and matrix metalloproteinases (MMP-9)[16] into the tears in dry eye or dysfunctional tear syndrome. In addition, bax expression stimulates apoptosis through a mechanism involving cytochrome-C release and activation of caspase-3 and JNK and ERK signaling pathways.[17] In other studies, it has been shown that hyperosmolarity will induce cornification of human corneal epithelial cells.[18] Thus, it is to be anticipated that the amplification of tear molarity at the meniscus would amplify inflammatory events at the MCJ.

Second, another mechanism would be expected to operate. The gradient hypothesis predicts a rise in solute concentration at the peripheral apex of the tear meniscus to a level strongly influenced by the molecular radius of the solute molecule. The rise in solute concentrations depends sensitively on the diffusion coefficients of individual solutes and, thus, the transport of large molecules, such as mucins and proteins, away from the meniscus apex will be more restricted than that of small molecules, such as sodium ions. This would be anticipated to maintain a concentration gradient of pro-inflammatory proteins, such as IL-1β, IFNγ, TNF-α and MMPs, across the meniscus, with a higher concentration at the meniscus apex than in the body of the tears. The concentration difference would be significantly greater than that expected for salts.

We therefore predict that pathological broadening, irregularity, and anterior migration of Marx's line will occur in dry eye, over and above that expected on an age-related basis, due to the amplification of both tear molarity and of inflammatory protein concentrations at the meniscus apex. Furthermore, once Marx's line starts to broaden and encroach upon the meibomian glands, it would be inherently unstable, so that small imperfections and stochastic fluctuations would tend to amplify geometrical irregularities. These clinical changes would be accompanied by congruent pathological changes in the MCJ. Additionally, it has been shown that TNFα and also neutrophil elastase, both present in dry eye tears, can cause shedding of mucins, such as MUC 16, from the surface of human corneal epithelial cells in culture[19] and that MMP9 causes proteolysis on

tight junctional proteins such as ZO1.[20] These agents could contribute directly to an increase in rose bengal staining in such disorders.[19] Over time, these processes could create a "sink" for cell death close to the MCJ, leading to depletion of repair mechanisms at the lid margin and a broadening and accelerated migration of Marx's line. These events predict that Marx's line will broaden in the presence of dry eye disease, leading to a fusion of a pathological Marx's line with lid-wiper epitheliopathy, an important feature of both asymptomatic and, particularly, symptomatic dry eye.[20,21]

Reduced clearance of inflammatory mediators from the tears has been reported in other ocular surface disorders, and it would be anticipated that this would lead to pathological changes in Marx's line and the MCJ in these conditions, independent of the occurrence of dry eye.[22-24]

1. Aqueous-Deficient Dry Eye

Because the volume and profile of the tear meniscus is likely to differ between the two major forms of dry eye, there could be subtle differences in the delivery of damage to the lid margin in aqueous-deficient dry eye (**ADDE**) and evaporative dry eye (**EDE**). In ADDE, the tear hyperosmolarity characteristic of all forms of dry eye is the result of a deficiency of lacrimal tear production. The progressive fall in tear meniscus volume, which accompanies increasing severity of ADDE, would be predicted to lead to a progressive shallowing of the slope of the apical region of the meniscus and an amplification of the gradient mechanism. It could also result in a posterior recession of the meniscus apex, which could move the pinning point of the meniscus backward and change the chief locus of epithelial damage. This would both increase the level of hyperosmolarity and result in the posterior spread of hyperosmolarity over the occlusal surface of the marginal mucosa. This could be an additional explanation for broadening of Marx's line.

2. Evaporative Dry Eye

In EDE, the situation would be similar but not identical to that predicted for ADDE. On the one hand, as in ADDE, progression of the disease is accompanied by an increasing hyperosmolarity of the tears. On the basis of the gradient hypothesis, it would be expected that the hyperosmolarity would be amplified at the central apex of the tear meniscus, as in ADDE. However, it has been suggested that, in the early stages of EDE, the rise in tear osmolarity is offset by a compensatory increase in lacrimal secretion, induced reflexly through the operation of the lacrimal functional unit. Tear volume is not expected to fall in this early stage of EDE,[25, 26] and there the shallowing of the apical profile of the meniscus expected in ADDE would not occur. This could modify the evolution of hyperosmolarity at the MCJ.

B. Effects of Globe Immobility

The solute gradient hypothesis is dependent for its action on dynamics generated by evaporation from and surface tension within the tear fluid as it wets the hydrophilic occlusal conjunctiva of the lid margin. The menisci of the preocular tear compartment are unusual in that they have both a peripheral and a central apex, since, in the upstroke of the blink, as the menisci are formed, they separate almost immediately from the tear film, which remains isolated during the remainder of the interblink.[27, 28] This event can be observed in vivo when the tears are stained with fluorescein, as a "black line" of non-staining, which separates the two compartments.[27,29] The peripheral apices of the menisci thus abut the MCJ, while the central apices abut the black line. At first sight then, it would be expected that solute concentration would rise at each tear meniscus apex and that both the lid margin and the ocular surface of the globe and cornea would be at risk of exposure to hyperosmolar and other forms of solute-related stress. However, whereas the peripheral apices of the tear menisci are pinned to the occlusal mucosa at the MCJ, the black lines are related to regions of the cornea and conjunctiva, which vary in position during eye movement. Therefore, whatever level of hyperosmolarity is achieved in this region is spread over a wider epithelial area by the tear mixing achieved by the movements of the globe. We suggest that this is the reason that a solute gradient leads to the staining pattern of Marx's line at the MCJ, but, in normal circumstances, does not result in damage to the ocular surface.

However, these conditions would be expected to change in the setting of relative globe immobility, such as in supranuclear ophthalmoplegia[30] and endocrine exophthalmos,[31] and contribute to surface damage and staining over the globe in these conditions. In thyroid orbitopathy, the damage is exacerbated by the combination of proptosis, globe exposure, and globe immobility. It is relevant, too, that during contact lens wear, staining punctate epithelial erosions may occur on the cornea and bulbar conjunctiva, roughly in the horizontal meridian.[32-34] This is termed *3 o'clock, 9 o'clock staining* and has been attributed to a low blink frequency or blink amplitude,[33] or to the intersection of the lower tear meniscus with the contact lens meniscus. This suggests that the coincidence of the interacting menisci is able to amplify a hyperosmolar, meniscal edge stress. Since this interaction would occur over the lower surface of the globe when the eye is in the primary position, prolonged downgaze with a reduced blink rate during reading may be a factor in its occurrence.

Importantly, Yamaguchi et al found a positive correlation between the regional meibography scores and the regional Marx's line score and also with the quality of expressed meibum (graded on a 0-4 basis), implying an association with MGD.[8] In the presence of MGD, the line of stain may broaden and advance to involve the region of the meibomian glands,[8] or in the upper lid, with dry eye and contact lens wear, may broaden posteriorly to merge with lid-wiper epitheliopathy.[7,35] The lower lid may be similarly affected.[36]

C. An Etiological Role in Meibomian Gland Dysfunction

Noncicatricial MGD is a condition of obstruction of

the terminal ductules of the meibomian glands caused by a process of hyperkeratinization of the terminal duct epithelium.[10,37-39] To a lesser extent, it may reflect an increase in viscosity of meibum, the lipid secretion of the glands. It gives rise to a symptomatic condition in its own right, and because it leads to a reduction of meibum delivery to the tear film lipid layer and to qualitative changes in meibum composition, it causes instability of the tear film, which may lead to ocular surface damage and EDE. Noncicatricial MGD is the most common cause of EDE and may, in fact, be the most common form of dry eye.[40] A full account of the presumed mechanism of MGD is given elsewhere.[41] MGD may occur secondary to a broad range of skin diseases,[42] but it also occurs as a primary condition for which the etiology is unknown. At the present time, no hypothesis has been advanced to explain why the terminal ducts of the meibomian glands are targeted in this disease or what mediates the hyperkeratinization of the duct lining. The gradient hypothesis presented here and a consideration of the permeability properties of Marx's line may provide a clue.

It has been pointed out that Marx's line is located directly behind the meibomian gland orifices and, further, that the line is an expression of an increase in permeability affecting the rows of conjunctival epithelial cells directly behind the MCJ. This is the basis of the dye entry and staining that characterizes Marx's line. It may be inferred from this that the Marx's line zone offers an entry point, at least for anionic molecules of similar size to the dyes used in staining Marx's line (molecular mass: fluorescein 376, lissamine green 576 and rose bengal 1018 Da). Although the permeability of this zone to molecules of larger size is unknown, its location, precisely behind the terminal ducts and acini of the meibomian gland, make it reasonable to propose that the Marx's line zone of cells could facilitate the diffusion of potential toxins and inflammatory molecules into these tissues. It is therefore relevant that Yamaguchi et al found a positive association between the anteroposterior location of Marx's line and MGD.[8] It is also relevant that the first grade change is a forward migration of the line to involve the meibomian orifices and, hence, the region of the terminal ductule.

We therefore postulate that the juxtaposition of the MCJ and orifice region is relevant to the etiology of MGD through the action of inflammatory molecules targeting a susceptible site deep to the meniscus apex. The locus of Marx's line is here seen as a vulnerable region, giving access to the terminal ducts of the meibomian glands by a backdoor route. Because hyperkeratinization of the terminal meibomian ducts is a key event in MGD, then the cytokines of greatest interest would be IL-1β and IFNγ, since, experimentally, they are able to induce cornified envelope precursor proteins, such as the small, proline-rich repeat protein, SPRR1β, in epithelial cells.[43,44] The p38MAPK cascade is the common intermediate for these cytokines, acting through the recruitment of the transcription factors CREB (cAMP response element binding protein) and ZEB1 (zinc-fingered E-box binding homeobox 1).[43]

At present, although it may be assumed that molecules of at least the dimensions and charge of rose bengal may penetrate the epithelial surface in the region of Marx's line, there is no information as to the integrity of intercellular junctions in this region. The intracellular uptake of dyes into the cells of Marx's line implies transcellular diffusion but provides no information about access to paracellular pathways. It has been estimated, however, that the epithelium of intact conjunctiva is more permeable than that of the cornea and that the bulbar conjunctiva is an important route of entry for topically instilled drugs into the anterior segment of the eye.[45-47] Furthermore, perfusion studies in the rabbit have shown the bulbar conjunctiva to be permeable to mannitol (182 Da), inulin (5 kDa) and FITC dextran-20 (20 kDa) but not to FITC dextran equal to or larger than 40 kDa. The cornea was permeable to mannitol but not to inulin or dextran and was estimated to be 50 times less permeable than the conjunctiva.[47]

In other studies, also in the rabbit, it has been found that both the palpebral and bulbar conjunctiva are 15-25 times more permeable to water-soluble molecules than the corneal epithelium.[48] It might reasonably be expected that the permeability of the Marx's line epithelium is greater than that of intact epithelium of the conjunctiva proper, and would therefore permit the entry of peptides and other soluble proinflammatory molecules of at least 20 kDa. With this background in mind, it is noteworthy that the molecular masses of some peptide mediators of interest are: TNFα-17 kDa; IFNγ-17.1 kDa; IL-1 β-22 kDa with some lower molecular weight forms, and MMP9-92 kDa.

The solute gradient hypothesis predicts that such peptides would be concentrated in the region of the meniscus apex. It must be emphasized that the meibomian targeting mechanism proposed here is independent of the basis for the increased permeability in the region of Marx's line. It also follows from the arguments presented that, whether or not the permeability of the Marx's line region is relevant to the etiology of MGD, this region may represent an important route for the delivery of drugs in the treatment of MGD. There may be differences in access to the terminal meibomian ducts and tissue residence times, according to whether drugs are delivered as topical agents or within gels or ointments applied to the lid margin. In the future, there will be great interest in exploring the permeability of this region, using tracer dyes, such as lanthanum, horseradish peroxidase, and fluorescent dextrans, and in understanding the pharmacokinetics of these different modes of drug delivery.

III. SUMMARY AND CONCLUSIONS

In the preceding paper, we hypothesized that Marx's line, a feature of the normal lid margin, is due to the action of a solute-concentrating mechanism, driven by evaporative water loss from the tear meniscus.[1] We proposed that this generates a region of hyperosmolarity at the apex of the tear meniscus, causing epithelial damage directly behind the MCJ. We have further suggested that this mechanism,

operating over a lifetime, can explain progressive pathological changes to the MCJ associated with aging. We have elaborated the hypothesis in two ways, to suggest that, in dry eye, the obligatory presence of tear hyperosmolarity will amplify lid margin damage and that, in any form of ocular surface inflammatory disease, the solute gradient mechanism would serve to retain and concentrate inflammatory mediators at the MCJ and exacerbate lid margin damage in this region. Finally, we have proposed that the anatomical location of Marx's line could give inflammatory mediators access to the terminal ducts and orifices of the meibomian glands and play some role in the pathogenesis of MGD. By the same token, Marx's line may represent the most direct therapeutic route for the delivery of drugs in the treatment of MGD. There is some urgency to undertake pharmacokinetic studies in this region in order to understand in detail the barrier characteristics of this region. This has relevance both to the etiology of MGD and its treatment.

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