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Lamellar Bodies: The Key to Cutaneous Barrier Function

Kenneth R. Feingold[1,2]

The two primary barrier functions of skin (permeability and microbial barriers) are provided by lipids and proteins delivered to the extracellular spaces of the stratum corneum by the secretion of lamellar bodies. Owing to their importance in this process, the mechanisms of and the factors regulating lamellar body formation must be better understood. Tarutani *et al.* (2012) provide data furthering the concept of the importance of the Golgi network in lamellar body formation and the necessity of acidification of the Golgi for normal function.

Journal of Investigative Dermatology (2012) **132,** 1951–1953. doi:10.1038/jid.2012.177

It has been recognized for many years that the cutaneous permeability barrier is abnormal in patients with atopic dermatitis and psoriasis, but this was considered a secondary defect owing to underlying immunological abnormalities and/or inflammation. However, recent studies have demonstrated that genetic abnormalities in filaggrin lead to defects in cutaneous permeability barrier function and predispose to the development of atopic dermatitis (Irvine *et al.*, 2011). These studies have rekindled interest in the abnormal cutaneous barrier as a primary defect (Irvine *et al.*, 2011). In addition, genetic studies have also suggested that the pathogenesis of psoriasis may similarly involve abnormalities in cutaneous barrier function (Capon *et al.*, 2012). Thus, cutaneous barrier function has left the backwaters of dermatology research to become an area of increasing interest and study.

Although the skin has many barrier properties, the two most important are creating a barrier against the movement of water and electrolytes (permeability barrier), and creating a barrier against the entry of microorganisms (antimicrobial barrier) (Elias and Choi, 2005). Both of these barriers are localized primarily to the stratum corneum layer of the epidermis (Elias and Choi, 2005). The cutaneous permeability barrier is mediated by extracellular lipids, cholesterol, free fatty acids, and ceramides, which form extracellular lipid–enriched lamellar membranes between the corneocytes that block the movement of water and electrolytes (Feingold, 2007). The antimicrobial barrier is mediated by both lipids, particularly free fatty acids, and antimicrobial peptides, such as the beta-defensins and cathelicidins, which also are localized to the extracellular spaces of the stratum corneum (Elias and Choi, 2005; Feingold, 2007). Of particular note, both the lipids that form the permeability barrier and the antimicrobial peptides are delivered to the extracellular spaces of the stratum corneum by the secretion of lamellar bodies (LBs; Elias and Choi, 2005; Feingold, 2007).

LBs are ovoid secretory organelles that are first observed in the upper stratum spinosum layer of the epidermis, with increasing numbers found in the stratum granulosum layer (Feingold, 2007). LBs contain phospholipids, glucosylceramides, sphingomyelin, and cholesterol (Feingold, 2007). In addition, numerous enzymes, including lipid hydrolases such as β glucocerebrosidase, acidic sphingomyelinase, secretory phospholipase A_2, and neutral lipases, and proteases such as chemotryptic enzymes (kallikreins) and cathepsins, are present in LBs (Feingold, 2007). In addition, enzyme inhibitors, such as the serine protease inhibitor, elafin, are also packaged into LBs. Moreover, antimicrobial peptides, such as human β-defensin 2 and the cathelicidin LL-37, are also present in LBs (Oren *et al.*, 2003; Braff *et al.*, 2005). Because of the central importance of LB in the development of a competent permeability and antimicrobial barrier, information on the mechanisms of LB formation and the factors that regulate the rate of formation are needed.

Previous studies by our group have shown that the incorporation of the lipid constituents of LBs and the enzymes in LBs occur coordinately and in parallel (Rassner *et al.*, 1999). After acute permeability barrier disruption, LBs are rapidly secreted by the stratum granulosum cells and new LBs quickly form (Rassner *et al.*, 1999; Feingold, 2007). The appearance of lipids and enzymes in these new LB occurs simultaneously (Rassner *et al.*, 1999). Inhibiting lipid synthesis prevents the delivery of lipids to LBs and also prevents the incorporation of enzymes into the LBs (Rassner *et al.*, 1999). Providing exogenous lipids restores the delivery of lipids to LBs and also leads to the incorporation of enzymes into LBs, indicating that lipids have a key role in facilitating the incorporation of the enzymes into LBs (Rassner *et al.*, 1999).

The expression of antimicrobial peptides in the epidermis is also co-regulated with permeability barrier function (Aberg *et al.*, 2008). Disruption of the permeability barrier stimulates antimicrobial peptide production (Aberg *et al.*, 2008). Moreover, in $CRAMP^{-/-}$ mice, the synthesis of LBs following acute barrier disruption is abnormal, leading to abnormalities in permeability barrier homeostasis (Aberg *et al.*, 2008). The density of LBs in stratum granulosum cells is normal, but the internal lamellar cargo of the LBs is often sparse in $CRAMP^{-/-}$ mice (Aberg *et al.*, 2008). This suggests that CRAMP has an important role in the incorporation and/or organization of lipids into LBs. Despite the paucity of lamellar contents in $CRAMP^{-/-}$ mice, other

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Clinical Implications

- Lamellar bodies have the central role in the formation of the permeability and antimicrobial barriers.
- Lamellar bodies are derived from the Golgi apparatus, and acidification of the Golgi is required for this process to proceed normally.
- Additional studies have suggested that the pathogenesis of psoriasis and atopic dermatitis may include primary abnormalities in cutaneous barrier function.

proteins (e.g., the hydrolytic enzyme, acid lipase) are packaged in LBs normally, indicating that the incorporation of these enzymes into LBs is not dependent on CRAMP (Aberg *et al.*, 2008). Interestingly, in this model, as compared with inhibiting lipid synthesis, the absence of lipid incorporation into LBs does not prevent the packaging of enzymes into LBs, suggesting that the formation of LBs may be a complex multistep process (Aberg *et al.*, 2008). Whether lipids also have a key role in facilitating the incorporation of antimicrobial peptides into LBs has not yet been determined.

The pathways that mediate the entry of lipids into LBs have not been fully elucidated. However, studies have shown an important role for ABCA12 (Akiyama, 2011). ABCA12 is a member of the ABCA subfamily of transporters, which are involved in the transport of a variety of lipids. Loss-of-function mutations in *ABCA12* have been shown to cause harlequin ichthyosis, and milder missense mutations cause a severe lamellar ichthyosis phenotype (Akiyama, 2011). *ABCA12* mutations lead to a decrease in LB contents, a decrease in lamellar membranes in the extracellular spaces of the stratum corneum, and loss-of-permeability barrier function, which in harlequin ichthyosis can result in neonatal lethality (Akiyama, 2011). Biochemically, the absence of ABCA12 is characterized by the accumulation of glucosylceramides with a reduction in ceramides (Akiyama, 2011). These studies demonstrate a critical role for ABCA12 in mediating the transport of glucosylceramides into LBs and the subsequent formation of mature LBs and normal barrier function. How cholesterol and phospholipids are transported into LBs remains to be elucidated. ABCG1, another member of the ATP-binding cassette superfamily, facilitates the transport of cholesterol across the plasma membrane to HDL particles. Our laboratory has observed that ABCG1-null mice display abnormal LB contents and secretion, leading to impaired lamellar bilayer formation, suggesting a potential role for ABCG1 in LB formation (Jiang *et al.*, 2010).

Morphological observations have demonstrated that nascent LBs appear to bud off the cisternae of the trans-Golgi network, a process that accelerates after acute barrier disruption when LB formation is accelerated (Elias *et al.*, 1998). In support of an important role of the Golgi, brefeldin A, which inhibits organellogenesis via the Golgi, blocks the formation of LB (Feingold, 2007). In this issue, Tarutani *et al.* (2012) provide additional data demonstrating the importance of the Golgi in the formation of LBs. This group recently identified a novel anion channel, Golgi pH Regulator (GPHR), which is critical for Golgi acidification. In the current article, they used a Cre-loxP system to disrupt the expression of GPHR, specifically in the skin. Although a number of cutaneous abnormalities were noted, of particular interest was a decrease in the expression of several lamellar body markers, including TGN46, KLK7, and sphingomyelinase. Moreover, electron microscopy revealed that the normal internal membrane structure of the lamellar bodies was markedly reduced, with some empty lamellar bodies visible. As expected, given the abnormal lamellar body appearance, the resultant bilayer lamellar structures in the extracellular spaces of the stratum corneum were decreased in number and abnormal in appearance. Moreover, transepidermal water loss was increased in mice deficient in GPHR, indicating an abnormal permeability barrier. These observations provide further evidence indicating that LBs are derived from the Golgi apparatus, and that acidification of the Golgi is required for this process to proceed normally.

Although this study further advances our understanding of LB formation, many questions remain. After acute barrier disruption, there is a marked acceleration in LB formation. What are the signaling pathways that lead to this increase? Is the rate of LB formation linked with the rate of LB secretion, and if so, what signaling pathways link these processes? Although ABCA12 is required for the transport of glucosylceramides into LBs, how are the other lipid constituents of LBs transported into LBs? What are the pathways by which enzymes and antimicrobial peptides are incorporated into LBs? How is the incorporation of these various components coordinated? Is the incorporation of the various components sequential or simultaneous, and is the incorporation of certain components dependent on other components? Given the central role of LBs in the formation of both the permeability and antimicrobial barrier, answering these and other questions may allow us to manipulate the formation of LBs and improve barrier function.

CONFLICT OF INTEREST

The author states no conflict of interest.

ACKNOWLEDGEMENTS

Over the years, our research has been supported by NIH grants, which were administered by the Northern California Institute for Research and Education, and with resources of the Veterans Affairs Medical Center, San Francisco, California.

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Melanocortin 1 Receptor Function: Shifting Gears from Determining Skin and Nevus Phenotype to Fetal Growth

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Variants in the *MC1R* gene influence skin pigmentation and thereby modulate risk of melanoma and basal and squamous cell carcinoma. In this issue, Kinsler *et al.* report an association between the *MC1R* genotype and the development of congenital melanocytic nevi. Further, higher birth weight was observed in carriers of *MC1R* variants, suggesting a role for the melanocortin network in fetal growth.

Journal of Investigative Dermatology (2012) **132,** 1953–1955. doi:10.1038/jid.2012.216

Melanocortin 1 receptor functioning and genetic variants

The human *MC1R* gene, located on chromosome 16q24.3, is highly polymorphic. The gene encodes for a G protein–coupled melanocortin 1 receptor that is expressed in melanocytes and other cell types that reside in the skin, including keratinocytes and fibroblasts, as well as cells of the immune system.

The major agonists of *MC1R* are cleavage products of proopiomelanocortin, and they include the melanocyte-stimulating hormones (MSHs) and adrenocorticotrophin. In particular, *MC1R* has high affinity for α-MSH, which acts in an autocrine and paracrine manner.

Activation of *MC1R* by α-MSH leads to an increase of cAMP, ultimately leading to complete activation of the promoter of the microphthalmia transcription factor (*MITF*) gene. MITF has a key role in the control of melanogenesis, leading to increased transcription of a range of genes including *TYR* and *TYRP1*, which are involved in the control of the relative and absolute amounts of eumelanin and pheomelanin pigment in the skin. The MITF transcription factor has many other transcriptional targets, including BCL2, MET, and HIF1A (Cheli *et al.*, 2010). Robbins *et al.* (1993) first described the association of *MC1R* variants and receptor functionality in modulating the fur color of mice. Soon thereafter, studies on the functionality of *MC1R* variants were carried out in humans. Population studies revealed that several *MC1R* variants are associated with red hair and fair skin, designated as red hair color (RHC) and fair skin variants. Interestingly, the variant alleles differ in their penetrance concerning the RHC phenotype (Sturm, 2002). The common variants R151C, R160W, and D294H and the rarely occurring variants D84E and R142H are strongly associated with the red hair phenotype in the Caucasian population, with odds ratios of 50- to 120-fold (referred to as (strong) R variants). The weak RHC alleles V60L, V92M, and R163Q have odds ratios for red hair between 2 and 6 and are therefore considered to be (weak) r variants. Soon after the correlations were observed between *MC1R* variants and the RHC phenotype, associations were observed between these variants and other skin-related phenotypes, such a risk of developing melanoma and non-melanoma skin cancer, as well as freckles (Sturm, 2002). Interestingly, associated odds ratios increased when more RHC variants were present in the genotype, with homozygous R variants conferring the highest risk.

Despite the fact that more than 100 variants in the *MC1R* gene have been identified to date, the consequences of these variants on the physiological function of *MC1R* have been defined only partially (Dessinioti *et al.*, 2011). RHC variants R151C, R160W, and D294H have been defined as strong RHC (R) variants based not only on their significant associations with specific phenotypic features, such as red hair, fair skin, and freckling, but also on their diminished receptor function, reduced response to α-MSH, and reduced functional coupling of *MC1R* to adenylate cyclase *in vitro*. The functional relevance of the weaker (low penetrant) RHC (r) variants, such as V60L, V92M, and R163Q, is still a matter of debate, with some reports pointing to a minor signaling impairment, and others showing a behavior similar to that of wild-type *MC1R* (Dessinioti *et al.*, 2011). One report suggested that the V92M substitution reduces the binding affinity of *MC1R* for α-MSH, whereas another found no

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