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Acidification in the epidermis and the role of secretory phospholipases

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The function of the epidermis is to form an effective barrier between the dry, external environment and the interior of the body. The barrier specifically resides within the extracellular lipid membranes of the stratum corneum (SC) and an acidic pH is necessary to maintain its competency against various insults. The purpose of this review is to explore the mechanisms which are postulated to contribute to the acidification of the stratum corneum, including both exogenous and endogenous sources. However, recent research as pointed to several endogenous mechanisms as the major source of acidification, including a sodium/proton pump (NHE1) and free fatty acid conversion from phospholipids by secretory phospholipase A2 (sPLA2). 

Introduction

Epidermis forms a barrier between the dry external environment and the interior of the body, preventing water and ions from exiting and toxins, antigens and bacteria from entering. Residing in the extracellular lipid membranes of the stratum corneum (SC), this vital barrier requires an acidic environment in order to remain functional and competent against various insults. The acid pH not only limits the growth of pathogenic skin flora, but also is required for the enzymatic lipid processing that results in an effective permeability barrier.

The mechanisms behind this “acid mantle” have long been debated. Originally, exogenous sources of acid were thought to cause this drop in SC pH. However, more recent evidence has emerged that identifies endogenous sources as the primary mode of SC acidification. A sodium/proton pump, NHE1, has been implicated in acidifying the border between stratum granulosum and SC. Another significant mechanism contributing to the acid mantle is free fatty acid conversion from phospholipid by a secretory phospholipase A2 (sPLA2). Lastly, urocanic acid generation via histidase is postulated to be another endogenous source of acid, although this has been disputed.

Studies of the neonatal acidification processes in murine epidermis demonstrate the central role sPLA2 plays in forming the acid mantle at the beginning of life. Maintenance of an acidic SC in adults, however, appears to require additional mechanisms.

This (secretory phospholipase A2 family) enzyme plays a significant and varied role in many mammalian cells and the individual localization and function of the various sPLA2 members identified in skin (sPLA2-Ia, -Ib, -IIC, -IID, -IIE, -IF, -III, -V, -X and -XII) still must be defined. More importantly, given the role of sPLA2s in inflammatory dermatoses such as atopic dermatitis and psoriasis, further discoveries could lead to novel, life-changing therapies.

Function of Acidification

The identification of the SC “acid mantle” was first recognized by Heuss in 1892. Subsequently, there have been numerous studies confirming this observation using progressively more sophisticated methods. Using tape stripping to remove sequential layers of the SC, Ohman and Valhquist demonstrated that SC pH was inhomogenous, distributed with a progressively neutral pH from the apex to the base of the SC. Later studies using fluorescent lifetime imaging revealed discrete microdomains of acidity within larger areas of neutral pH at the base of the SC. It is here that the process of SC acidification begins in the neonatal period, as well as the period after barrier abrogation. The increasing acidity seen in the upper SC layers derives from an increasing number of uniformly acidic microdomains rather than the increasing acidity of a fixed number of individual microdomains.

It has long been recognized that SC acidification functions as a defense against microbial invasion, as it was shown that an acidic pH inhibits the colonization of pathogens such as Staphylococcus aureus and Streptococcus pyogenes while encouraging the growth of normal skin flora. In addition, the loss of acidic pH coupled with the initiation of the inflammatory cycle in epidermal barrier disruptions can create a vicious cycle of inflamed and colonized skin. An alkaline environment, as in the urea-soaked skin of diaper dermatitis, is an important initiating factor of bacterial and yeast infections, due to the increased growth of pathogens and epidermal barrier abnormality.

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SC integrity, which is attributed to increased serine protease activity at a neutral pH. Inhibiting serine proteases maintains CD density, preserving the integrity of the SC. Together, these two pathways can explain why the loss of SC acidification leads to loss of SC integrity and cohesion, as well as the abnormality of epidermal lipid barrier function.

Mechanisms of Acidification

There are many mechanisms that have been postulated to contribute to the acid mantle of the skin (see Fig. 1). Originally, exogenous mechanisms have long been thought to be the main contributors to acidification. For example, eccrine gland-derived products, such as lactic acid, are one of many exogenous sources thought to further decrease SC pH. Free fatty acids of pilosebaceous origins also were thought to be an exogenous contributing mechanism. The significance of this mechanism is questionable, as a normal SC acid mantle is seen in asebia mice, which show a profound hypoplasia of sebaceous glands. Microbial metabolites also were postulated to contribute to an acidic pH. However, the microbial colonization of neonatal skin is not seen to increase concurrently with early acidification. In addition, acidification has been shown to begin from the deeper levels of the SC, not the surface, which would be expected if these exogenous pathways were indeed the source of the acid mantle. This evidence seems to minimize, if not exclude, these exogenous mechanisms from contributing to SC acidification.

More recently, studies have identified that endogenous mechanisms are essential in the formation of an acidic SC. Endogenous mechanisms such as the Sodium-Hydrogen antiporter (NHE-1) and activation of the sPLA₂ family of enzymes are the best-studied of these mechanisms. When either the sPLA₂ or NHE-1 pathways are compromised, the bulk SC pH rises, indicating that other acidifying mechanisms cannot completely compensate for them. NHE-1, one of the essential contributors to the acid mantle, is from a family of ion transporters that is important in intracellular regulation and maintenance. NHE-1 has been shown to be expressed in keratinocytes and melanocytes. Two key enzymes, β-glucocerebrosidase and acid sphingomyelinase, which metabolize spingomyelin and glucosylceramide to ceramide, require an acidic pH for optimal enzymatic activity. The activity of β-glucocerebrosidase is reduced in skin with a neutral pH and activity is restored after re-acidification of the epidermis. Abnormalities of SC integrity and cohesion also occur in a neutral pH environment. Accelerated corneodesmosome (CD) degradation accompanies this loss of glands. Microbial metabolites also were postulated to contribute to an acidic pH. However, the microbial colonization of neonatal skin is not seen to increase concurrently with early acidification. In addition, acidification has been shown to begin from the deeper levels of the SC, not the surface, which would be expected if these exogenous pathways were indeed the source of the acid mantle. This evidence seems to minimize, if not exclude, these exogenous mechanisms from contributing to SC acidification.

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Additionally, this important component of the acidification process is directly regulated by changes in external pH, as alkalinization is shown to be a major stimulus for NHE1 expression. Defects of acidification in the lower domains of aged epidermis have recently been attributed to decreased NHE1 expression. This pathway plays a significant role in contributing to the SC pH, possibly being the mechanism providing the initial acidification required for lipid processing and permeability barrier formation.

Phospholipid to free fatty acid conversion by sPLA₂ has emerged as another significant mechanism of lowering SC pH. PL disappear during cornification and the processing of PL results in a family of nonessential fatty acids within the SC interstices, which are required for normal barrier homeostasis. The specific phospholipases responsible for this phenomenon have not been fully elucidated, but the application of inhibitors of the 14 kDa family of secretory phospholipases result in a blockade of PL processing, altering epidermal barrier homeostasis. A more recent study has shown that the inhibition of sPLA₂ specifically alters barrier function via disrupting SC acidification, providing direct evidence in its role in contributing to the acid mantle. The consequences of the disruption of acidification, such as the loss of SC integrity, cohesion and delayed barrier recovery, are shown to occur with the blockade of the sPLA₂ pathway. Furthermore, these abnormalities can be prevented during sPLA₂ inhibition with the co-application of the end products of PL hydrolysis, non-essential FFA such as PA (C16:0) or stearic acid (18:0). An acidic pH alone is not sufficient to reverse the barrier abnormalities, showing that the PL-derived generation of FFA is not only significant as an acidification mechanism, but also important in the formation of the barrier (see Fig. 2).

Finally, in vitro studies have shown acidification via urocanic acid generation from histidine by the deaminating enzyme histidase. It is questionable whether this pathway is truly essential for acidification of SC. Although levels of histidase would be expected to rise in conjunction with neonatal SC acidification, a recent study has shown that this is not the case. Additionally, this study showed histidase deficient mice do not exhibit a higher pH compared to control mice and flaky tail mice (ft/ft), deficient in the processing of filaggrin, actually show a lower SC pH. Two other endogenous mechanisms of acidification, free fatty acid generation by isoforms of secretory phospholipase A₂ (sPLA₂) and sodium/proton pump antiporter-1 (NHE-1), are upregulated in ft/ft mice. This compensatory response is sufficient to appropriately acidify the SC, demonstrating that the filaggrin-histidine-urocanic acid cascade is not essential in the formation of the acid mantle.

The Importance of sPLA₂: Neonatal Studies

Among the variety of pathways that contribute to epidermal acidification, it can be shown that sPLA₂ conversion of PL to FFA is the most significant in neonatal SC acidification. Skin surface pH is neutral at birth in both humans and animals. Although a fully developed cornified envelope and abundant extracellular lamellar bilayers are present, the lack of acidification in neonatal skin is associated with, impaired barrier homeostasis has been shown in the neutral SC of neonates, even though basal barrier function has been shown to be normal.

Examining the mechanisms involved in the development of acidification in the first week of birth in neonatal rats elucidated that sPLA₂ is largest contributor to the drop in SC pH. sPLA₂ activity increases 66.4% between day 0 and 4 after birth and progressively extends from the deep layers of the SC to all SC layers by day 5. Application of a sPLA₂ inhibitor delayed the development of the acid mantle, accounting for a two-third to one pH unit of bulk acidification. Evidence points to sPLA₂ as the major pathway of acidification, whereas other mechanisms contribute and prevent the complete blockade of acidification. Interestingly, concurrent inhibition of NHE1 and sPLA₂ did not increase neonatal SC pH above levels achieved with either inhibitor alone, suggesting the existence of yet unidentified mechanisms of acidification.

Recently, several studies have shown that activation of LXR, a nuclear hormone receptor, can accelerate SC acidification in neonatal rodents, in part because of an increase in sPLA₂ activity. This accelerated drop in epidermal pH was accompanied by an improvement of SC integrity and barrier homeostasis. This study provides some insight to sPLA₂ regulation, although it is possible LXR can also be modulating other pathways of acidification.

 Peroxisome proliferators-activated receptors (PPARs) are members of the same subfamily of nuclear hormone receptors as LXR and isotypes PPARα, PPARβ/δ and PPARγ have all been shown to be expressed in keratinocytes. Topical treatment with PPAR activators not only stimulates keratinocyte differentiation, it improves permeability barrier homeostasis after an acute disruption. Topical PPARα application is shown to accelerate neonatal acidification, as well as accelerate the kinetics of barrier recovery following acute disruption by tape stripping. Activation of only the PPARα isoform was shown to increase sPLA₂ activity. Concurrent inhibition of sPLA₂ with the application of a PPARα activator blocked the ability of the PPARα activator to acidify the neonatal SC. However, the inhibitor of

Figure 2. The downstream effects of the phospholipid to free fatty acid pathway in the stratum corneum interstices. Lamellar bodies deliver both phospholipid and sPLA₂ to the SC. This pathway, mediated by sPLA₂, plays a functional, as well as structural role, in the maintenance of the barrier.
sPLA₂ also regulates Ca²⁺ influx; therefore, another mechanism could be preventing the PPARα activator from restoring acidifying processes. Although it seems like sPLA₂ plays a prominent role in acidification, there is still much to be uncovered about the specific regulation of this enzyme.

**Declining SC Acidification in Aging**

SC acidification is defective in both moderately aged humans and mice, although the contributing mechanisms are not fully elucidated. Studies have shown that in humans over 50 years old, the surface pH of SC increases progressively over time. This was also found in a mouse model (aged 12–15 months), where researchers were able to assess that the difference begins at the SG-SC interface and diverged further throughout the epidermis. Delayed barrier recovery rates are seen in aged mice in comparison to young mice and recovery rates in aged skin became comparable to young skin with external acidification treatment. Similar abnormalities seen in neonatal skin lacking an acidic SC are also seen in aged skin, including abnormal SC integrity and delayed formation of mature lamellae membranes. In contrast to studies on NHE1 in aging skin, loss of sPLA₂ activity and/or expression has not been adequately examined in aged epidermis.

However, NHE1 levels show a developmental decrease with aging and seem to contribute to the loss of acidification in aged epidermis. The actions of NHE1 are localized to the lower SC, which is contiguous with the acidification defect seen in aged epidermis. In addition, the protein levels of NHE1 are seen to decrease after birth, in contrast to increasing levels of sPLA₂ function postnataally. The evidence is highly suggestive of the role NHE1 plays in contributing to the loss of acidification in aging. However, the extent to which loss of NHE1 contributes to a higher pH still needs to be quantitated and in uncovering that, the specific role of sPLA₂ in the aging epidermis is not yet defined.

**The Secretory Phospholipase Family**

Secretory phospholipase A₂ is a subtype of the larger phospholipase A₂ family. These enzymes catalyze the hydrolysis of the sn-2 ester bond of phospholipid substrates, producing free fatty acids and lysophospholipids. These products have a wide variety of functions across many mammalian tissues, including the formation of eicosanoids, which are important mediators of vascular tone, tissue homeostasis and inflammation. There are 12 groups of phospholipases A₂ (I–XII) and are subdivided into different categories based on substrate specificities, cellular location and calcium sensitivity: group IV cytosolic phospholipases A₂, group VI calcium-independent phospholipases A₂, groups VII and VIII platelet-activating factor acetyl hydrolases and low molecular weight secreted phospholipases A₂ (sPLA₂-IB, -IIB, -IIC, -IID, -IIF, -III, -IV, -V, -X and -XII).

sPLA₂ play a part in normal skin function, maintaining barrier homeostasis through contributing to the acid mantle. However, it is still not clear exactly which of the many sPLA₂ subtypes is responsible for FFA release and subsequent acidification. There have been many studies attempting to shed some light on the many different roles of sPLA₂, but unfortunately, there are many contradictory reports and very little unrefuted findings in this area (see Table 1). One hurdle to establishing definitive data is the difference of sPLA₂ localization and function between humans and mice. For example, murine sPLA₂-IIA is expressed almost exclusively in the small intestine, whereas human sPLA₂-IIA is found in numerous tissues. This suggests that the enzyme in the human and mouse models are not functional orthologs of each other, therefore conclusions drawn in a murine model cannot necessarily apply to the human model.

In a study analyzing different subtypes expressed in the different layers of epidermis during various stages of development, murine epitermis expressed groups I, II, V, X and XII sPLA₂s, although expression between the basal and suprabasal layers was dependent on calcium conditions. sPLA₂-IIIF, -V and -XII were expressed suprabasally, according to in vivo immunohistochemical studies. Their presence in the upper SC suggested that they also might have a role in SC acidification, in addition to sPLA₂-IB that has previously been thought to function in that regard. However, the sPLA₂-IB inhibitor used in that study, p-bromophenacyl bromide, also alkylates the active site histidine that is conserved in all sPLA₂s and most likely inhibits multiple sPLA₂s. A more recent study has shown that in filaggrin-deficient ft/ft mice, sPLA₂-IIA is upregulated along with NHE1 expression, while sPLA₂ forms -IF and -X do not, contrasting with a previous study that demonstrated that sPLA₂-IIA does not contribute to acidification and barrier function and sPLA₂s-X is the main isoform to be secreted from keratinocytes. To assign more definitive roles to these isofoms, more enzyme-specific sPLA₂ antibodies and inhibitors are needed, which in part will enable the identification of specific sPLA₂ present within LB. Cytochemical studies show that sPLA₂ activity is codelivered with ceramide precursors and phospholipids (PL) in lamellar bodies at the SG-SC interface, but more specific EM localization and antibody studies are lacking.

In addition to SC acidification, members of the sPLA₂ family have been postulated to mediate inflammation, hyperproliferation and even stimulate melanocyte dendricity. The basal compartment exhibits a different expression profile from the suprabasal layers, showing the presence of sPLA₂-IIA, -IIF, -IV, -V and -XII. sPLA₂-IIIC is found throughout the epidermis. This result, as well as other studies in references 56 and 57, suggest that sPLA₂-IIA functions in the hyperproliferation of keratinocytes. sPLA₂-X has been shown to release arachidonic acid from adherent cells, suggesting this enzyme may play a role in eicosanoid formation. sPLA₂-X also functions in the release of lysophosphatidylcholine (LPC), stimulating melanocyte dendricity. sPLA₂-IIA and -IIF may also have the ability to release arachidonic acid; therefore it is hypothesized that these basally located sPLA₂ may play a role in inflammatory hyperproliferation.

A recent study has shown a possible inflammatory role for sPLA₂-III in skin, as transgenic mice overexpressing sPLA₂-III...
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exhibit severe dermatitis.\textsuperscript{60} It also showed upregulation of sPLA\textsubscript{2-IID} and -V in these transgenic mice, suggesting that these two isoforms are involved in inflammatory processes. Surprisingly, there was a marked absence of sPLA\textsubscript{2-X} in the skin of transgenic and normal mice, as previous studies suggested sPLA\textsubscript{2-X} is the major isoform expressed in mouse skin. This same study provided evidence of sPLA\textsubscript{2-III} in normal epidermis for the first time,\textsuperscript{51,60} in addition to its role in inflammation.

There is much to be discovered about the sPLA\textsubscript{2} family of enzymes. Hopefully, with the development of more specific inhibitors and more sensitive technology, their function and localization can be definitively pinpointed. With that knowledge, they can be used as therapeutic targets in many inflammatory diseases of the epidermis.

### sPLA\textsubscript{2} in Psoriasis and Atopic Dermatitis

The important role of sPLA\textsubscript{2}s in the epidermis is demonstrated by its involvement in atopic dermatitis and psoriasis. Psoriasis is a disorder characterized by epidermal hyperproliferation, altered keratinocyte maturation and inflammation. An early study showed that overexpression of sPLA\textsubscript{2-JIA} in the skin of transgenic mice result in hyperkeratosis and epidermal hyperplasia, resembling the classic psoriasis phenotype.\textsuperscript{61} More recently, studies have shown a dramatic change in the location

| sPLA\textsubscript{2} subtype | Function/localization in epidermis | Citation |
|-------------------------------|-----------------------------------|----------|
| IIB                           | Expressed in superbasal keratinocytes (SG-SC junction) (M/H) | Gurrierri, et al. 2003; Mazereeuw-Hautier, et al. 2000; Haas, et al. 2005 |
| IIA                           | Inflammation (M) | Li-Stiles, et al. 1998 |
| IIC                           | SC acidification (M/H) | Fluhr, et al. 2001; Mazereeuw-Hautier, et al. 2000; Mao-Qiang, et al. 1996 |
| IIA                           | Located in the upper SC (H) | Haas, et al. 2005 |
| IIA                           | Growth factor for keratinocytes (M/H) | Grass, et al. 1996; Rys-Sikora, et al. 2003 |
| IIA                           | Inflammation (M) | Sjursen, et al. 2000; Li-Stiles, et al. 1998; Kudo, et al. 1993; Vadas et al. 1993 |
| IIIE                          | No inflammatory role in vivo (M) | Sato, et al. 2009; Grass et al. 1996 |
| IID                           | PAF-mediated arachidonic acid release (H) | Jorgensen, et al. 2010 |
| IID                           | Expressed in keratinocytes throughout epidermis | Gurrierri, et al. 2003 |
| IID                           | Expressed in keratinocytes throughout epidermis (M) | Gurrierri, et al. 2003 |
| IID                           | Localized around nucleus (H) | Haas, et al. 2005 |
| IID                           | PAF-mediated arachidonic acid release (H) | Jorgensen, et al. 2010 |
| IIII                          | Inflammation (M) | Sato, et al. 2009; Grass, et al. 1996 |
| IIII                          | Expressed in keratinocytes (M) | Sato, et al. 2009 |
| IIII                          | Hyperproliferation (H) | Rys-Sikora, et al. 2003 |
| IIII                          | Expressed in basal layers (H) | Haas, et al. 2004 |
| IIII                          | PAF-mediated arachidonic acid release (H) | Jorgensen, et al. 2010 |
| IIII                          | Inflammation (M/H) | Sato, et al. 2009; Murakami, et al. 2002 |
| X                             | Major subtype, constitutively expressed (H) | Schadow, et al. 2001; Haas, et al. 2005 |
| X                             | No expression in skin (M) | Sato, et al. 2009 |
| X                             | Inflammation (eicosanoid formation) | Saiga, et al. 2001 |
| X                             | Stimulation of melanocyte dendricity (H) | Scott, et al. 2007 |
| XII                           | Expressed in superbasal keratinocytes (M) | Gurrierri, et al. 2003 |
| XII                           | Undetectable in dermis (H) | Haas, et al. 2005 |

*(M), murine; (H), Human.
Elevated pH and impaired barrier homeostasis, leading to a host of negative downstream consequences, including lipid depletion. It is not clear what the role of sPLA₃ plays in the pathogenesis of this disease, but it was found that sPLA₃, sPLA₁ and -IID are upregulated in the skin of mice with induced AD. Another study showed the upregulation of sPLA₃-IIE mRNA in epidermis of mice with AD, however, there are no protein studies yet available on this finding. These results suggest the role of these sPLA₃s in the inflammatory process in AD. However, it is yet to be seen whether their pathogenic mechanism contributes to lipid dysfunction, cytokine dysfunction via arachidonic acid release or perhaps even antimicrobial dysfunction.

More recent studies suggest that sPLA₂, sPLA-IIE or -V play a role in platelet-activating factor arachidonic acid release and could be potential targets for anti-inflammatory interventions in AD and other dermatoses.

Determining the in vivo biological functions of sPLA₃ is a daunting challenge. However, determining those functions can lead to novel and targeted treatments for atopic dermatitis and psoriasis, as well as other common inflammatory skin diseases.

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