The Cutaneous Microbiome:
Update and Implications for Skin Barrier Repair

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Dr. Elias is in large part responsible for the present wealth of knowledge on both the structure and myriad functions of mammalian stratum corneum. His pioneering research since the 1970's has dispelled the myth of the stratum corneum as a "dead, keratinized, basket-weave" structure, to establish the iconic "brick and mortar" model. Through his efforts, the stratum corneum is now viewed as a metabolically active, two-compartment composite that functions as a biosensor. The resultant "outside-in" concept of the barrier as a prime mover in the pathogenesis of cutaneous disease has also been a highlight of Dr. Elias' work, with the paramount role of skin barrier dysfunction in disease pathogenesis now widely recognized.

The Human Microbiome – Background

The enormous variety of bacteria, viruses and yeasts that live on and in the human body, formerly termed the 'normal flora,' is now called the 'microbiome.' While each of us consists of about 40 trillion cells, the internal and external surfaces of our body, including the GI tract, oral and vaginal mucosae, lungs and skin, are coated with myriads of microbes -- in fact, over 3 times as many microorganisms as our own cells, accounting for over one quarter of our body mass. While the great majority (about 80%) of these microorganisms cannot be routinely cultured, thanks to high throughput sequencing of bacterial 16S ribosomal RNA[1], we are now beginning to comprehend the immense diversity of organisms that reside both on and in us, but many questions then arise: why are the microorganisms there, and why in such vast numbers and diversity? Are they inert, just 'going along for the ride,' or do they interact with underlying tissues? If so, how are they impacting us, and could they also influence human health? Indeed, abundant evidence now exists that these microbes influence us in many ways, including disease susceptibility, immunity, energy metabolism, and even our behavior.[2]

The Cutaneous Microbiome

What about the skin's microbial community? Though not as vast and diverse as that of the gut, our skin surface nonetheless hosts a plentiful array of microorganisms, many of which again cannot be routinely cultured. The microbiome that resides on the surface of normal human skin displays a high diversity and substantial interpersonal variation.[3] Moreover, these organisms do not remain restricted to the skin surface, but rather they extend deep into hair follicles, sweat glands, even reaching under the epidermis into the dermis.[2,4] While the range of organisms residing on normal adult skin tends to remain relatively unchanged, there are important differences in the flora of children vs. adult skin,[5] and distinctive group niches, such as body folds and the scalp.[3]

1. Co-localized in extracellular ('mortar') domains
2. Pathogens attempt to invade through stratum corneum (SC) extracellular domains (see Fig. 2)
3. Some permeability barrier lipids (e.g., free fatty acids) also exhibit potent antimicrobial activity
4. Antimicrobial peptides (AMP) localize to lamellar bodies (along with lipids), and are co-delivered to SC extracellular domains (see Fig. 1)
5. AMP production accelerates after permeability barrier disruption, paralleled by increases in lipid synthesis
6. At least one AMP (LL-37) is required for permeability barrier homeostasis
7. Restoration of permeability barrier function enhances AMP production (see Fig. 3).

Table 1: HOW PERMEABILITY & ANTIMICROBIAL BARRIERS ARE LINKED

Recent studies show that various members of the normal cutaneous flora may protect the host, defining them not as simple symbionts, but rather as mutualistic.[2] Indeed, the cutaneous microbiome exerts profound influences on host defenses (innate immunity) against environmental pathogens that attempt to colonize the skin, while also regulating inflammatory responses, as well as 'educating' adaptive (T cell-mediated) immune responses.

Atopic Dermatitis (AD) and the Cutaneous Microbiome

Even prior to the microbiome era, it was well known that host-microbe relationships play a fundamental role in the pathogenesis of atopic dermatitis (AD). AD skin is susceptible to colonization and invasion by multiple types of pathogens, including viruses that cause warts and molluscum, as well as Candida species. But it is the link of disease flares to recent colonization by S. aureus that is particularly strong.[6,8] Moreover, both the elevation in surface pH that results from the barrier abnormality, as well as the inflammation in AD favors the growth of S. aureus.

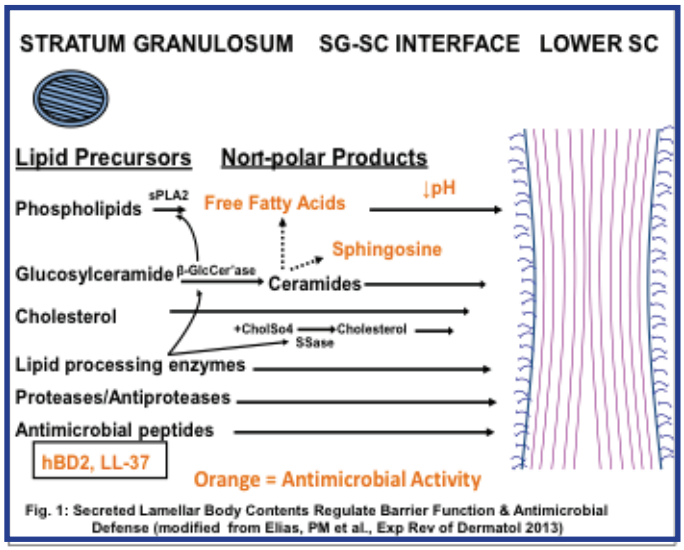

Fig. 1: Secreted Lamellar Body Contents Regulate Barrier Function & Antimicrobial Defense (modified from Elias, PM et al., Exp Rev of Dermatol 2013)

Fig. 1 Stratum Granulosum SG-SC Interface

The defective barrier in AD in turn allows pathogenic organisms to colonize and invade across the skin surface[11](Table 1). Indeed, recent RNA sequencing studies have confirmed that immediately prior to flares, the microbiome in AD changes dramatically, a change termed 'dysbiosis'[8]. Most strikingly, the microbiome becomes far less diverse, as the normal flora is increasingly replaced by S. aureus, the most frequent trigger of disease flares in AD.[6-8] Recent studies show that these organisms preferentially colonize sites of disease predilection, such as the body folds and facial area. Although how microbial 'dysbiosis' drives AD is incompletely understood, following successful treatment, the normal flora reappear, and S. aureus colonization rapidly declines.[7] Recent studies by Rich Gallo's group at UC San Diego are helping to clarify how the integrity of the skin barrier regulates S. aureus colonization, initiating the abnormal Th2 inflammation in AD.[11] They showed first that a competent permeability barrier is impervious to invasion by pathogens; i.e., it resists colonization and invasion by S. aureus, while conversely, a flawed permeability barrier, as occurs in filaggrin-associated AD, becomes susceptible to colonization and invasion by S. aureus, a universal trigger of disease flares in AD. The Gallo group then showed that the barrier abnormality not only favors the growth of S. aureus, but also the development of Th-2 inflammation. Finally, they showed that a ceramide-dominant, triple-lipid formulation of physiologic lipids: i) restored the barrier, ii) reduced Th-2 inflammation, and iii) decreased S. aureus colonization.

How Barrier Repair Therapy Enhances Antimicrobial Defense in AD

Based upon the above, several therapeutic strategies could be beneficial in AD. A'probiotic' approach, analogous to the consumption of yogurt, could be beneficial.[12,13] Emollient use also has been shown to reduce S. aureus colonization, and to normalize the microbiome in AD.[14] In addition, strategies that

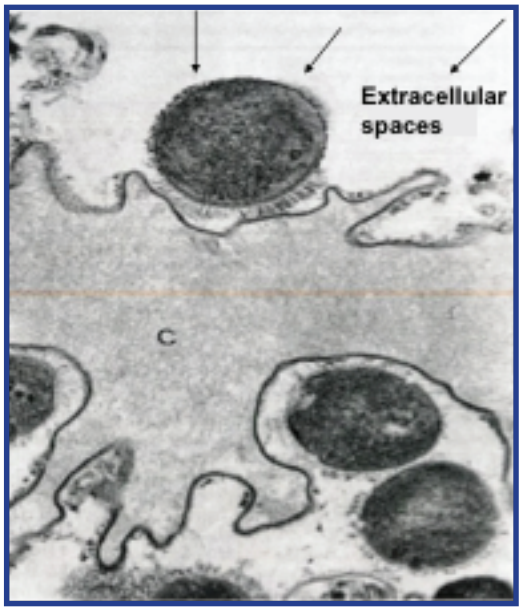

Fig. 2 (from Elias, PM, Semin Immunopath, 2007).Transmission electron microscopic study of biopsy specimen from culture-positive Staphylococcus aureus skin infection. Gram positive cocci may be seen intercalating between corneocytes (C) within the stratum corneum, i.e., via extracellular spaces occupied by the stratum corneum lipids. Note that corneocytes do not appear damaged and are not traversed by bacteria (42,000×).

enhance LL-37 levels could buttress host immunity against S. aureus.[15] Finally, our studies have shown that restoration of the permeability barrier with normalization of the acidic pH of the stratum corneum is also a logical approach. As we soon will see, the marketed high concentration, triple -lipid mixture encompasses all three of these approaches, including normalization of AMP production. Let's briefly review the basis for ceramide-dominant barrier repair therapy for AD. Even when AD is associated with mutations in structural proteins, like filaggrin, the barrier problem is always in the lipid 'mortar'[10], but how does that happen? Two key mechanisms—first, the mutated proteins interfere with the secretion of the 3 key lipids that provide the barrier into the intercellular spaces of the stratum corneum (Fig.1). As these lipids decline, S. aureus is able to penetrate into the stratum corneum (Fig. 2). Second, the chronic barrier abnormality allows allergens to get across, eliciting allergic (Th2 cell-dominant) inflammation that is characteristic of AD.[11] These Th2 cells then secrete a suite of 'bad' cytokines that down-regulate both epidermal ceramide production, and the production of the key AMP, LL-37[9]. This is the basis for the 'ceramide-dominant barrier repair therapy.'

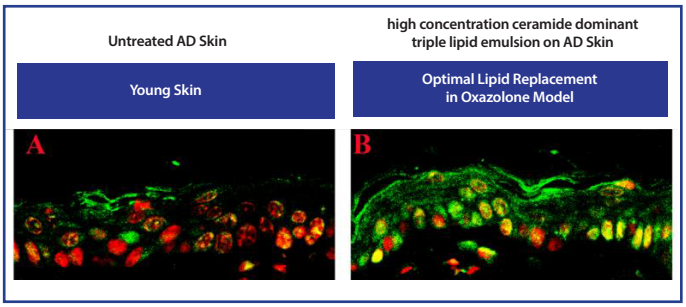

Fig. 3 Optimal Lipid Replacement with a high concentration, triple-lipid mixture enhances AMP Production. Topical high concentration, triple -lipid mixture enhances mRNA and protein levels for both hBD2 and LL-37 in normal human skin.

As noted, the Gallo group has now shown that this type of technology not only corrects the barrier abnormality, but it also blocks the entry of S. aureus and the production of the allergic 'bad' cytokines.[11] These results support the concept that permeability barrier function and epidermal antimicrobial defense are intertwined and co-regulated processes. Moreover, we previously showed that topical applications of high concentration ceramide dominant triple lipid emulsion also stimulate production of LL-37 by the epidermis[19] (Fig.3 and Fig.4). Thus, this formulation, by correcting the barrier, should potently reduce'allergic' (Th2-dominant) inflammation, while also inhibiting colonization and invasion by S. aureus.

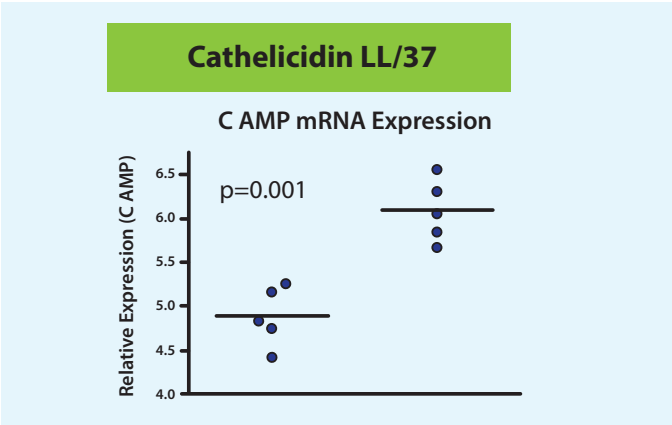

Fig.4 Cathelicidin Expression following triple lipid emulsion application.

It also is important to remember the additional contributions to antimicrobial defense provided by the acidic pH of the marketed triple-lipid formulation. Many studies have shown that S. aureus grows well at the high (neutral) pH of skin that is characteristic of AD, due in part to the faulty barrier.[10] Importantly, this pathogen grows poorly in the acidic pH of the normal stratum corneum. Note the anti-inflammatory benefits of the low pH in the properly formulated lipid emulsions, due to inhibition of the enzymes (kallikreins) that nitiate inflammation.[17] Finally, in separate studies we showed that the free fatty acids in the properly formulated lipid emulsions potently inhibit the growth of S. aureus.[18]

Further Clinical Implications

An abnormal barrier and an elevated surface pH are universal characteristics of almost all inflammatory dermatoses, including all of the adult eczemas and psoriasis. It is also worth noting that a barrier abnormality and pathogen colonization also occur commonly in many other inflammatory dermatoses, including seborrheic dermatitis, stasis dermatitis, and rosacea. There is also recent evidence that acne vulgaris is a disease of the follicular barrier. But further studies are needed to determine whether skin barrier repair will prove useful for the treatment of these disorders.

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