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Eccrine Sweat Duct Occlusion by Staphylococcal-Derived Biofilms: An Unexpected Signature Finding of Eczema in Dermatologic Diseases

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Abstract

We believe that axillary *granular parakeratosis*, *tinea pedis*, and *Seborrheic dermatitis* are likely to be variants of eczema. All had culturable staphylococci capable of producing biofilms, as in eczema. This was confirmed on XTT assays, Congo red cultures, and PCR for gene analysis of the biofilm-forming icaD and aap genes. The pathology showed eccrine ductal occlusion that was noted on H+E and PAS stains (PAS stains positively the extracellular polysaccharide substance that makes up the bulk of the biofilm.) Toll-like receptor 2 (TLR 2) activities was found in the stratum corneum adjacent to the sweat ducts and not in its control location in the basal zone. The stratum corneum seemingly becomes altered by the fungi, yeasts, or tiny granules instead of by the filaggrin gene (1st in the double hit phenomenon); and, the sweat ducts become occluded causing activation of TLR 2. This leads to activation of the innate immune system (2nd hit), just as in eczema. Further, treatment with the mild topical corticoids and/or moisturizers helps restore the integrity of the skin.

Keywords: Biofilms; Eczema; *Seborrheic dermatitis*; *Tinea pedis*

Introduction

Atopic dermatitis (AD), or eczema, is thought to originate as a double-hit phenomenon to which both genetics and environment contribute [1]. Genetic variation at the filaggrin gene that leads to the production of a faulty stratum corneum is the likely "first hit" in AD [2]. The surprising environmental "second hit" appears to be staphylococcal biofilms that block eccrine sweat ducts [1,3]. Duct occlusion by such biofilms is a consistent finding in eczematous lesions, and has been shown to elicit a strong innate immune response via TLR2 activation, which is thought to cause the inflammation and pruritus characteristic of eczema [1,4-5]. Despite the ubiquitous nature of biofilms, surprisingly little is known about their role in promoting disease. These clusters of bacteria, surrounded by an extracellular polymeric matrix, protect the bacteria and enable their adherence to a surface. Additionally, biofilms provide nutrients and a milieu conducive to cell-cell interactions. Biofilms have been identified in many disorders, including inflammatory bowel disease [6] and colon cancer [7]. Most notably, they play an important role in chronic wounds [8,9]. The presence of a biofilm provides antibiotic resistance, creates a state of persistent inflammation [10] and delays wound healing by interfering with keratinocyte proliferation and migration [11]. Still, the significance of biofilms as a precursor to dermatologic disease is underexplored. Indeed, biofilms may in fact constitute a "second hit" in a wide range of dermatologic and non-dermatologic diseases.

Axillary granular parakeratosis, or just granular parakeratosis, is a rare disease with an unknown etiology. Clinically, it presents with a pruritic hyperpigmented or dull red hyperkeratotic plaque and is typically found in one or both axillae. While many etiologies have been considered, there is a disproportionately high incidence in young-middle aged women who use deodorant or antiperspirant [12]. Treatment with topical corticoids generally relieves pruritus and resolves the rash. Histopathology reveals a myriad of small granules in the proximal stratum corneum, a unique and defining feature. Epidermal hyperkeratosis, irregular acanthosis, variable spongiosis, and a dermal lymphohistiocytic infiltrate are also present [13].

Tinea pedis, commonly known as "foot fungus" or "athlete's foot," is a pruritic rash from which fungi can be cultured and/or visualized on potassium hydroxide examination. The most common dermatophyte isolate from these lesions is *Trichophyton rubrum*, an asexual species that is easily identifiable in culture [14]. Acute *tinea pedis* can be intensely pruritic, causing painful fissures and erosions. In contrast, the chronic form is minimally pruritic and itches primarily when it transforms locally into the acute vesicular variant. Various topicals are advocated for the treatment of *tinea pedis*; including topical azoles, tolnaftate, ciclopirox, terbinafine, and undecylenic acid. No topical agent can claim complete success in treating either form of the disease. Treatment often provides only transient regression of pruritic lesions. Oral medications; griseofulvin, terbinafine, and the oral azoles, offer similarly inconsistent success in clearing the eruption, and offer a cure in only 35% of cases [15].

The "double hit" hypothesis, in which biofilm-occluded sweat ducts promote eczematous lesions, does not appear to be unique to AD, but is in fact observed across multiple disease entities. In this paper we discuss this unanticipated, common finding in lesional skin samples from patients with *Seborrheic dermatitis*, *granular parakeratosis* and *tinea pedis*. Our results suggest a common environmental "second hit" in Seborrheic dermatitis as well as two diseases previously not considered eczematous in nature-granular *parakeratosis* and *tinea pedis*. While a "first hit" has not been identified for these diseases, these observations suggest a much broader impact for staphylococcal biofilms in the evolution of skin diseases.

	Atopic dermatitis	*Seborrheic dermatitis*	*Granular parakeratosis*	Tinea pedis
# of specimens	40	13	3	6
S. aureus	17	8	2	4
S. epidermidis	8	2	0	1
Other staphylococci	15	3	1	1
XTT assay	40/40 [34/40 strong]	13/13	3/3	6/6
Congo red culture	40/40	13/13	3/3	6/6
IcaD gene	37/40	12/12	3/3	6/6
aap gene	1/40	1/1	0/0	0/0
H and E stain	36/36	13/13	3/3	3/3
PAS stain	36/36	13/13	3/3	3/3
Congo red stain	36/36	0/0	0/0	1/1
TLR2 immunostain	10/10	1/1	0/0	1/1

Table 1: Microbiology and pathology findings (Atopic dermatitis patients previously presented; shown for comparison). Rows 1,2 3-staphylococcal speciation; rows 4,5-biofilm assays; rows 6,7-genetic analyses; rows 8,9,10,11-pathology. Other identified staphylococci species included *hominis*, *xylosus*, *warneri*, *simulans*, *caprae*, *chromogens*, *auricularis*, and *capitis*. Multiple sections were occasionally required to observe occluded ducts on histopathologic examination.

Methods

Ethical approval

This research protocol has been reviewed and approved by the Institutional Review Board of the Drexel University College of Medicine.

Sample collection and processing

Sixty-two total lesional specimens were obtained from patients of the outpatient dermatology clinic at Drexel University College of Medicine. The samples were subjected to microbiologic processing, culturing, species recognition, analysis with XTT [2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H -tetrazolium-5-carboxanilide] (a colorimetric assay which determines the capability of an organism to make biofilm in vitro and to be multi-drug resistant), and genetic analysis for the known biofilm-producing genes (IcaD and aap). All patients also underwent biopsy of lesional skin (n=62). Clinical diagnoses of the following conditions-atopic dermatitis/eczema (n=40), *Seborrheic dermatitis* (n=13), *granular parakeratosis* (n=3), and *tinea pedis* (n=6)-were confirmed by histopathologic evaluation with routine hematoxylin and eosin (H and E) and periodic acid-Schiff (PAS) stains. Thirty-seven of these specimens were further evaluated with Congo red preparation and twelve had immunostaining for TLR2 activation. Methods employed in specimen evaluation are identical to those as described in detail in our previously published work on atopic dermatitis [1,16].

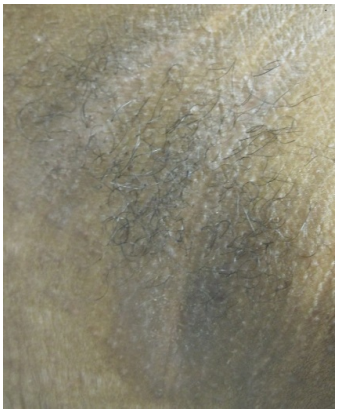

Figure 1: Axillary *granular parakeratosis* hyperpigmented scaling plaque in axilla.

Results

Microbiologic and histopathologic evaluation, as categorized by dermatologic entity, is presented in Table 1. Of the sixty-two lesional specimens, all sixty-two (100%) yielded positive cultures for biofilm-producing staphylococci. In addition, biofilm-producing genes were identified in all analyzed specimens. Histopathologic examination of all biopsied lesions revealed occlusion of eccrine sweat ducts on both H and E and PAS staining. Congo red staining uniformly demonstrated the presence of amyloid (which forms the infrastructure of biofilms) [17] in eccrine sweat ducts. In addition, we re-demonstrated activation of TLR2 by immunostaining in ten out of ten (100%) lesions of atopic dermatitis (that had previously shown TLR2 activation as well). Finally, a lesion of Seborrheic dermatitis as well as a lesion of *tinea pedis* also revealed activation of TLR2.

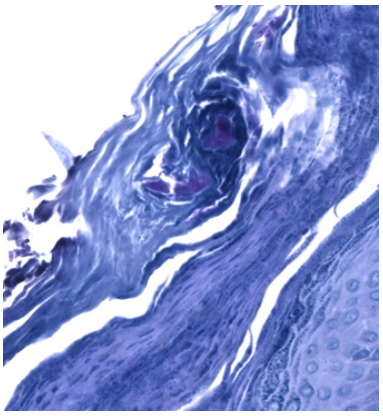

Figure 2: Axillary *granular parakeratosis* (PAS, 40X). PAS-positive material is noted in the upper acrosyringium (arrow). Many granules are noted in the stratum corneum, and mild epidermal spongiosis is present.

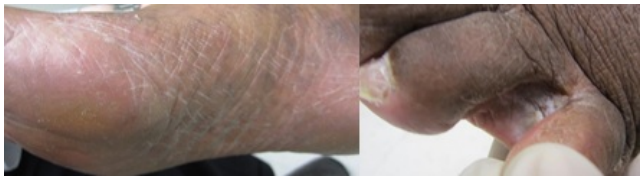

Figure 3: *Tinea pedis* scaling on sole and scaling and maceration between toes.

Granular parakeratosis

Our examination of multiple sections of three specimens of axillary *granular parakeratosis* was consistent with the typical histologic findings noted above (Figures 1 and 2). Additionally, we confirmed the presence of occluded sweat ducts in the upper epidermis and proximal *Stratum corneum*. Occlusive material was PAS positive, and no fungal organisms were present.

Tinea pedis

Among our six patients with a diagnosis of *tinea pedis*, all lesional skin specimens revealed positive cultures for both *Trichophyton rubrum* and *staphylococci* capable of producing biofilms (not presented in methods). XTT assays showed that these *staphylococci* were both biofilm-producing and multi-drug resistant (Figure 3).

Biopsies were performed on two patients to confirm the diagnosis of *Tinea pedis* and to rule out psoriasis and dyshidrotic eczema. These showed the fungi in the stratum corneum and occlusion of the acrosyringia (Figure 4) along with TLR2 activation at the sites of the ductal obstruction (Figure 5) rather than in the basal area as seen in control skin.

Seborrheic dermatitis

Seborrheic dermatitis (Figure 6) is distributed bimodally in children and adults. Our Seborrheic dermatitis patients had presentations typical for their respective age groups. While the children, ages 3 months to 6 years, presented with erythema and scaling in their scalp, the adults, ages 19 to 67 years, had scaling with variable erythema in the scalp, eyebrows, and in the nasolabial folds. Peri-auricular plaques were common, while chest, axillary, and groin involvement were uncommon. All patients had positive cultures for staphylococci capable of making biofilms. This was corroborated by gram stains of skin scrapings and by XTT analysis of these *staphylococci*, which showed that all of the organisms were capable of making biofilms, and that all were multi-drug-resistant.

Biopsy specimens showed occluded sweat ducts with hematoxylin and eosin, PAS and Congo red stains (Figures 7 and 8). Biopsies from adult patients were significant for the presence of yeast in the *stratum corneum*.

Discussion

Unexpectedly, we found histopathologic characteristics of eczema in granular parakeratosis, *tinea pedis* and *Seborrheic dermatitis*. In our examination of lesional specimens from these three conditions we found biofilm producing staphylococci. All of the biopsied specimens yielded cultures which grew *staphylococci* capable of making biofilms and all had biopsies demonstrating biofilm-occluded eccrine sweat ducts, just as observed previously in lesions of atopic dermatitis as well as in additional examinations of the eczema-associated lesions in Meyerson's nevus and Doukas Kapatenakis.

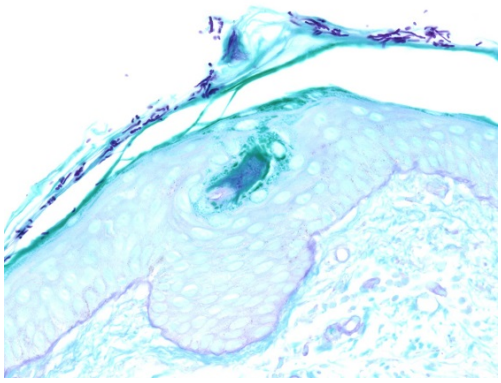

Figure 4: *Tinea pedis* PAS stain 40X Hyphae present in the stratum corneum. Duct occlusion evident.

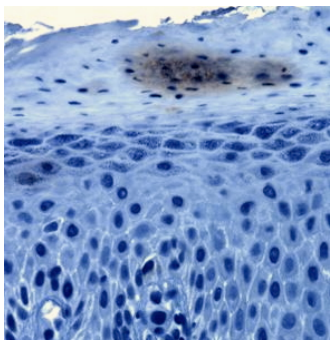

Figure 5: *Tinea pedis* (immunostain). Activation of TLR2 is noted in the stratum corneum; this is adjacent to the occlusions noted on H +E and PAS and Congo red staining. The control location for TLR2 is in the basal layer.

Just as biofilm formation by AD-associated staphylococci almost certainly plays a major role in the occlusion of sweat ducts that leads to inflammation and pruritus associated with AD, biofilm occlusion of sweat ducts leads to the pruritus and inflammation associated with the conditions of granular parakeratosis, *tinea pedis* and *Seborrheic dermatitis*. While this study suggests the second or environmental hit in each of these conditions is associated with staphylococci colonization and the occlusion of sweat ducts by biofilms, we suspect that the first hit that compromises the stratum corneum is distinct in each condition.

The clinical and histological features seen in our specimens of granular parakeratosis bear a striking resemblance to those observed in eczematous lesions. Clinically both present as a pruritic rash that resolves with corticosteroids, while histologically, both are characterized by epidermal hyperkeratosis and spongiosis.

Additionally we have now demonstrated the presence of staphylococcal-derived biofilms occluding sweat ducts in both lesions. These similarities not only suggest a previously undefined eczematous component to granular parakeratosis, but also suggest that biofilms play a central role in the evolution of these lesions as a likely 'second hit." Similar associations have been observed in miliaria [18]. The origin of the pathognomonic granules is unknown. Whether they represent a reactive entity [19,20] or perhaps an inherited disorder of cornification, they are not known to be a feature in other varieties of eczema. Perhaps their presence is evidence of a unique "first hit" resulting in a disruption of the normal barrier function of the stratum corneum as seen with filaggrin in AD.

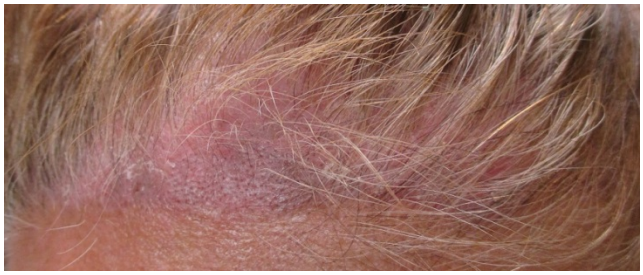

Figure 6: A scaling dull red plaque is present in the anterior scalp. It was also present in the eyebrows and post auricular areas.

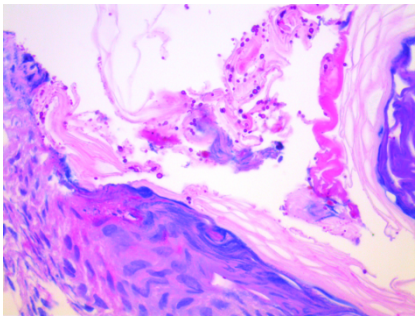

Figure 7: Seborrheic dermatitis (H+E 40X). Many PAS positive spores in stratum corneum; PAS-positive ductal occlusion (arrow) in the S*tratum granulosum*.

As with granular parakeratosis, our findings suggest a previously undescribed eczematous component to *Tinea pedis* [21]. Perhaps the fungal infection itself is responsible for disruption of the stratum corneum serving as the "first hit". This seems unlikely as previous research suggests that the stratum corneum barrier function remains intact despite dermatophytosis [22]. Alternatively an undiscovered "first hit" may permit fungal infection followed by sweat duct occlusion by staphylococcus biofilm leading to pruritus and inflammation.

Interestingly, as one might anticipate in eczematous disease, all three of the conditions discussed respond to treatment with low- or medium-potency topical corticoids. Acute *tinea pedis* appears to respond rapidly to ceramide-containing cream-more rapidly, in fact, than to antifungal creams (8-12 hours versus 36-72 hours for antifungals) [21]. This response is similar to that described by O'Brien upon applying lanolin to lesions of miliaria with subsequent clearing of lesions and resumption of sweat production [23]. Seborrheic dermatitis is also effectively treated with topical steroids [24]. As in eczema, gentle bathing and skin care habits also appear to have an ameliorating effect on these conditions.

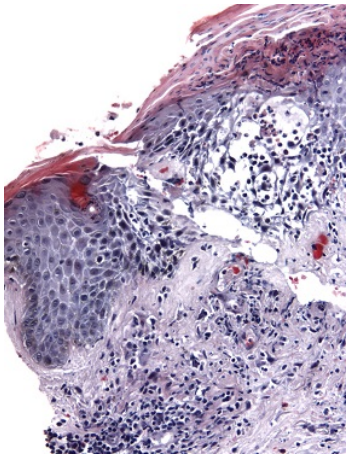

Figure 8: *Seborrheic dermatitis* (Congo red, 40X). There is a red-staining occlusion (arrow) in the duct in the granular zone. Congo red stains amyloid, which forms the infrastructure of biofilms. Spongiosis is present; a vesicle is also present on the right side of the image.

It is important here to note that our belief is not that eczema is borne solely from the occlusion of sweat ducts by staphylococcal-derived biofilm, but as elucidated in our previous work, that eczema develops secondary to a "double-hit phenomenon." [25] In atopic dermatitis, the first hit is a genetic one-in the form of filaggrin (or other gene) deficiency. It is the biofilm-occluded sweat ducts that constitute an environmental second hit which in turn permits activation of disease. Yet none of the entities among *Granular parakeratosis*, *tinea pedis*, and *Seborrheic dermatitis* are associated with a genetic filaggrin deficiency. What then accounts for the first hit in each of these three diseases? All are unified by the presence of factors that may lead to disruption of the stratum corneum-mimicking the faulty horny layer found in eczema. Specifically, these disruptive factors are as follows: granules in granular parakeratosis, fungi in *tinea pedis*, and yeast organisms (especially *Malassezia oleosa*) in adult-onset *Seborrheic dermatitis*. It has been reported that certain *Malassezia* species produce oleic acid, a molecule that, in experimental models, has been shown to alter the properties of the *Stratum corneum* [25]. Thus, in these diseases, the genetic hit is replaced by another environmental hit that results in a similar defect in the *Stratum corneum*.

Taken together, the microbiologic and pathologic findings of our investigation support that the presence of staphylococcal-derived biofilms in eccrine sweat ducts is consistently a second hit leading to a dermatitis like presentation. Furthermore, the occlusion of sweat ducts in the presence of biofilm-producing staphylococci in specimens of *granular parakeratosis*, *tinea pedis*, and adult-onset Seborrheic dermatitis warrants consideration that these diseases may indeed represent previously unrecognized variants of eczema.

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