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Pruritus of Healing Wounds: why "Scabs" Itch

Herbert B Allen*, Brittany Heffner, Trisha Dasgupta, Carrie Ann Cusack, Bhaswati Sen and Suresh G Joshi

Department of Dermatology, Drexel University College of Medicine, Philadelphia, Pennsylvania, USA

***Corresponding author:** Herbert B. Allen, Department of Dermatology, Drexel University College of Medicine, 219 N. Broad St., 4th floor, Philadelphia, PA 19107, Pennsylvania, USA, Tel: 215-762-5550; Fax: 215-762-5570; E-mail: hallen@drexelmed.edu

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Abstract

We demonstrate herein the novel finding of occluded sweat ducts in healing wounds. We also show these occlusions are from biofilms because they are periodic acid Schiff positive which indicates they are polysaccharides and Congo red positive which shows they contain amyloid, which forms the infrastructure of biofilms. Further, from the skin immediately adjacent to the wounds, we have cultured *staphylococci*, all of which have the capability of forming biofilms as indicated by a colorimetric assay. These findings are similar to the findings in eczema, and we believe trigger the same response of the innate immune system in healing wounds, just as in eczema. The activated immune system (Toll-like receptor 2) then initiates the pathway leading to pruritus.

Keywords: Itch; Healing; Pruritus; Wounds

Introduction

In previous works, we documented the presence of biofilm-occluded sweat ducts in the lesions of atopic dermatitis [1,2]. Gram positive bacteria, including *Staphylococcus aureus* and *Staphylococcus epidermidis*, were discovered to be responsible for the production of these biofilms. We also investigated the innate immune system response to the biofilm occlusions with Toll-like receptor 2 (TLR2) because of the affinity of TLR2 for gram positive organisms, and its activation was present in all samples selected for immunohistochemical analysis in the previous study. Congo red staining was employed to demonstrate the presence of amyloid in biofilms because amyloid forms the infrastructure of biofilms [3]. its presence further supported our conclusion that staphylococcal biofilms are responsible for sweat duct occlusions in atopic dermatitis.

In the present work, we applied the concepts derived, as above, from the evaluation of pruritus in atopic dermatitis and applied them to the itch commonly described in scabs and healing wounds. The proteinase-activated receptor [2] pathway through TLR2 has been implicated in the pruritus of atopic dermatitis [4,5]. our hypothesis is that this same pathway is responsible for the pruritus of wounds. The presence of biofilm-occluded sweat ducts as well as *Staphylococcus aureus* and *Staphylococcus epidermidis* (capable of making those biofilms) in wounds support this hypothesis.

Very little research has been conducted regarding the etiology of the itch in healing wounds. It is an unsubstantiated myth that wound contraction is responsible for the pruritus. Most related literature discusses pruritus associated with burns, and neuropathic mechanisms are thought to be primarily responsible for the sensation [6]. However, burns are likely a separate entity from other wounds. Additionally, some research discusses the presence of biofilms in wounds in relation to inability to heal, but biofilm-occluded sweat ducts are not mentioned [7].

In order to investigate the itch of healing wounds, it is important to understand the pathophysiology of healing. Four main phases occur: hemostasis, inflammatory phase, proliferative phase, and remodelling [8]. Hemostasis involves the attraction of platelets to the wound to create a fibrin clot, which then serves as a provisional matrix for incoming cells. During the inflammatory phase, vasodilation occurs allowing the extravasation and migration of neutrophils, macrophages, and lymphocytes to the wound. These white blood cells phagocytize bacteria and debris. If this phase is prolonged by contamination or the presence of necrotic or foreign material, wound healing cannot proceed. The proliferative phase involves keratinocyte re-epithelialization, fibroplasia, angiogenesis, and wound contraction [9]. Fibroblasts proliferate and produce collagen to form granulation tissue. Some fibroblasts differentiate into myofibroblasts, gaining smooth muscle cell-like activity to bring together the edges of the wound. Finally, the remodeling phase occurs during which collagen III is replaced with collagen I, increasing the tensile strength of the scar tissue [10].

Materials and Methods

Sample selection and processing

Thirty-six samples from patients with re-excised biopsy sites were collected for pathology examination. These were processed according to routine procedures and were stained with hematoxylin and eosin (H&E), periodic acid Schiff (PAS), and Congo red. Subsequently the slides were studied by routine light microscopy; on occasion, as in AD, it was necessary to examine multiple sections. Controls for this study were the same as those utilized for the atopic dermatitis study2-ten samples were taken from inflamed skin (dermatological conditions including pityriasis rosea, tinea corporis, and psoriasis), and ten samples were taken from normal skin. These samples were evaluated for staining patterns to serve as positive and negative controls.

Ten samples from patients with crusts and wounds attending the Drexel University College of Medicine Dermatology Clinic were collected for microbiological culture using sterile swabs. Five of the patients were male, and the other five were female. The ages of the patients ranged from 26 to 73. Culture preparation followed standard procedures and included use of the Staphaurex test kit, Mannitol Salt

Agar plates, API-Staph phenotyping, colony direct species-specific polymerase chain reaction, and sensititer plate assay (TREK diagnostic systems). Safranin microtitre plate assay (XTT) was used to classify isolates as biofilm formers by their absorbance values [2].

Ethical approval

The Institutional Review Board of Drexel University College of Medicine has approved this study.

Results

Thirty-six of thirty-six (100%) of the pathology specimens from wounds showed occlusion of eccrine sweat ducts in the upper epidermis and proximal stratum corneum when stained with H+E, PAS, and Congo red. Controls (20/20) from inflamed and non-inflamed skin showed no occlusions (Figures 1-4).

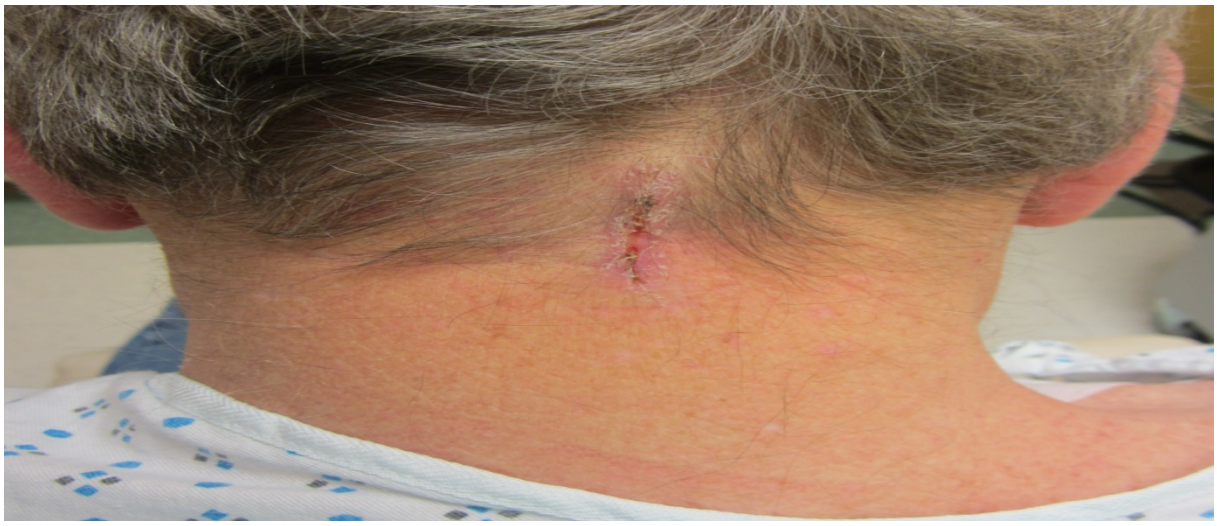

Figure 1: Healing wound (from a re-excision of a skin biopsy) is present on the posterior neck; cultures were taken from the skin adjacent to the suture line.

Of the 10 cultures, all were revealed to be *staphylococci* on API testing. 6/10 showed *S. epidermidis*, 3/10 showed *S. aureus* and 1/10 showed *S. tenuis* (Figure 5) 10/10 had a positive safranin assay Figure 6); all were multidrug resistant and 3/10 were Methicillin resistant.

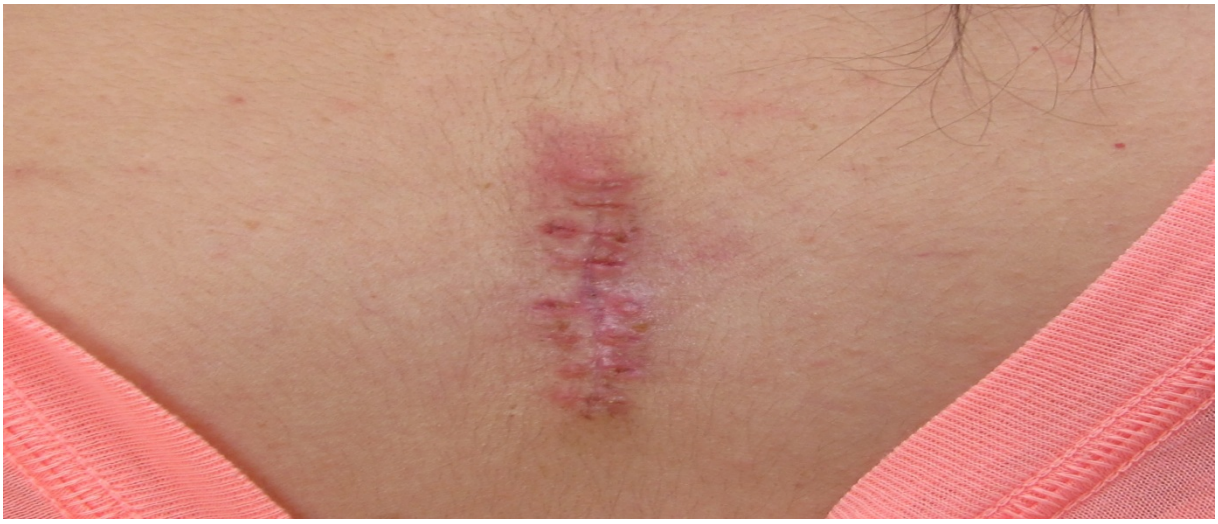

Figure 2: Healing wound on the back.

Discussion

Our findings in healing wounds are similar to that of atopic dermatitis. Namely, all isolates were *staphylococci* with a known capability of producing biofilms. These bacteria were the same as those isolated in atopic dermatitis lesions, except they mirrored normal skin flora more [2]. Thus, in wounds *S. epidermidis* was the predominant

species. Biofilm-producing bacteria were not found in nonlesional controls. Furthermore, all pathology samples of healing wounds showed biofilm-occluded sweat ducts, which was the pathology finding in atopic dermatitis lesions. (p=0.001).

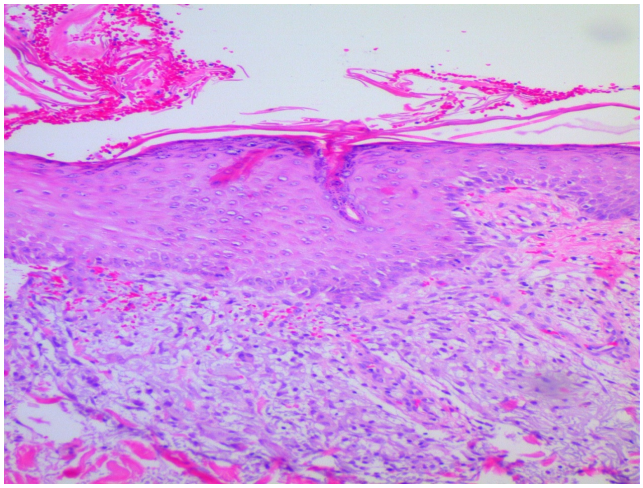

Figure 3: Pathology (from Figure. 1) revealing (from stratum corneum down: serous crust, occlusion of acrosyringium, mild acanthosis, forming scar in dermis). H+E 40X.

The pruritus of atopic dermatitis was investigated in a previous study [1]. *Staphylococci* were isolated from atopic dermatitis lesions, and these bacteria were tested for the *icaD* and *aap* genes that direct biofilm production. 97 % of the isolates were positive for these genes. In addition, the XTT assay showed all specimens were positive for biofilm production. In that previous study, we also examined the pathology and immunology of atopic dermatitis lesions. Ductal occlusions noted in pathology specimens stained positively with Congo red. Amyloid forms the infrastructure of biofilms, [2] therefore this positive staining demonstrates that biofilms are indeed present. Specimens were stained immunohistochemically for TLR-2 to evaluate the response of the innate immune system. Staining occurred periductally in the proximal stratum corneum where biofilm-occluded sweat ducts were located. Controls showed staining only in the basal zone of the epidermis.

With atopic dermatitis, we postulated that activation of TLR-2 led to two different inflammatory pathways, [11] one of which is responsible for the sensation of itch. The first is the MyD88 pathway, [12] which can be activated by almost every cell in the epidermis. The endpoint of this pathway is TNFa, a major regulator of the immune response to infection. The activation of TNFa is how TLR2, as a first responder, kills organisms. The second pathway involves the activation of PAR2 (protease-activating receptor 2) through kallikrein [13]. PAR2 is a pruritogenic molecule, and its activation has been shown to be increased in eczema.

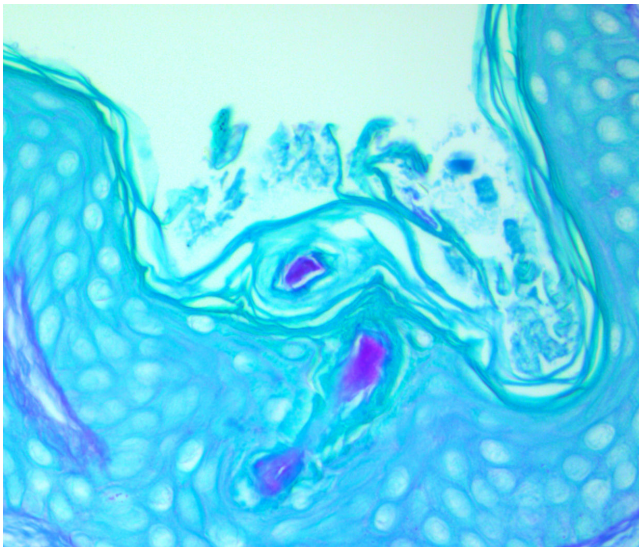

Figure 4: Pathology revealing occluded duct in the stratum corneum. PAS 40 X.

Our hypothesis was that the same pathway to pruritus is activated in healing wounds as in eczema. In the current study, all pathology specimens showed that biofilm-occluded sweat ducts were present in wounds. Moreover, all microbiological cultures showed staphylococcal bacteria capable of making those biofilms, as demonstrated in the positive XTT assay. These findings were similar to those in eczema. Further, these factors most likely activate TLR-2, leading to the initiation of the MyD88 and PAR2 pathways the same way they do in eczema. The activation of PAR2 provides a logical explanation for why healing wounds itch.

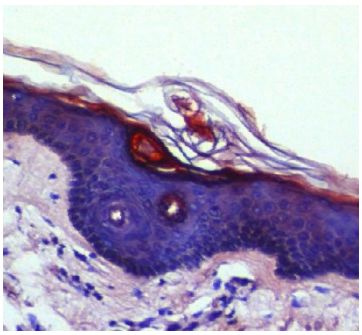

Figure 5: Pathology (from Fig. 2) demonstrating Congo red material in the sweat duct. Congo red 10 X.

We have discovered biofilm-occluded sweat ducts in several other dermatologic conditions, including tinea pedis, seborrheic dermatitis, and axillary granular parakeratosis [14]. These conditions, along with eczema, share a common feature contributing to the pathophysiology - the disruption of the stratum corneum. It is likely that the MyD88 and PAR2 pathways are activated in these conditions as well. Hydration is a major treatment modality for these diseases, with creams providing a barrier in lieu of the disrupted stratum corneum [15,16].

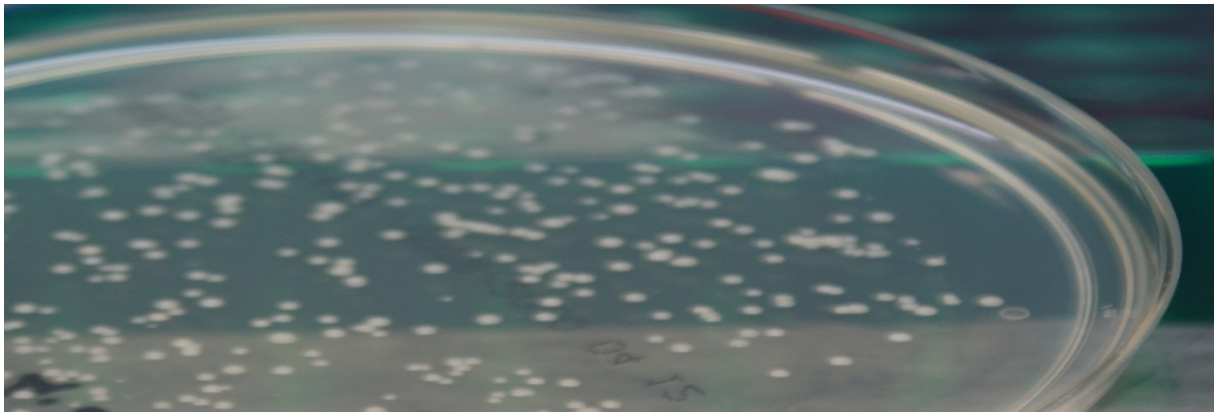

Figure 6: Culture of *Staphylococcus epidermidis*.

In the same way, healing wounds demonstrate a disruption of the stratum corneum. It would be interesting to investigate the effectiveness of occluding bandages in ameliorating pruritus. This would also provide moisture to the wound. If an anti-inflammasome were to be targeted for potential therapeutic effect, one might select anti-PAR2, but this seems truly unnecessary, inasmuch as hydration is so beneficial (Figure 7).

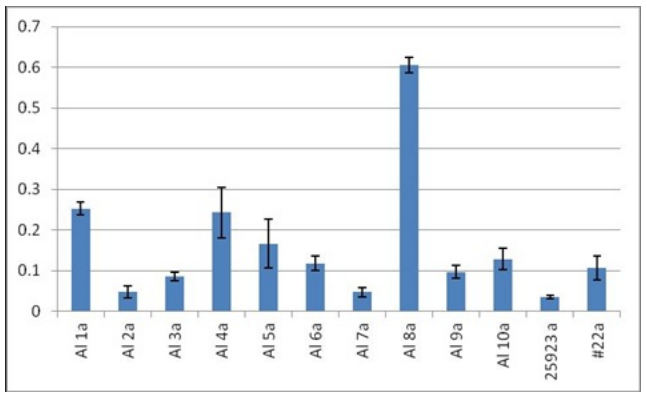

Figure 7: XTT transmissions showing each culture contained organisms capable of making biofilm; three were weak, but any positivity shows them capable of making biofilm.

One limitation of this study is the use of controls. If we used non-pruritic wounds or scabs as controls, false positives or false negatives would be likely to occur. A non-pruritic wound may have itched the week previous to sampling, or it may itch the subsequent week. Thus, we used the controls from the atopic dermatitis study. However, these controls are likely valid given that the etiology of pruritus in atopic dermatitis, and, as discussed above, healing wounds seem to be so closely aligned.

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