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Biofilms in Granuloma Annulare

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Abstract

In this preliminary study, we evaluate 10 skin specimens with a previous clinical and pathological diagnosis of granuloma annulare (GA) for biofilms with staining methods we have employed in skin and other diseases. Biofilms consist of two key ingredients: polysaccharides and amyloid. The stains used for polysaccharides were periodic acid Schiff (PAS) and colloidal iron and, for amyloid, Congo red. All ten specimens showed staining for both components revealing the presence of biofilms in GA. Because microbes create biofilms, GA, by definition, is a microbial disease, and it joins many other cutaneous diseases, such as psoriasis and eczema, with biofilms created by microbes. The identification of the specific microbe in GA awaits further investigation.

Keywords

Biofilms; Granuloma annulare; Skin diseases

Introduction

We have considered staining pathologically for biofilms in granuloma annulare (GA) because one half of the required process has been long been completed and accepted. That one half is staining for mucin which has been noted to be positive for more than 50 years [1]. The mucin in biofilms is composed of extracellular polysaccharides that surround the microbes [2]. The other one half is staining for amyloid with Congo red (CR); the amyloid acts as infrastructure for the biofilms [2]. With this approach, substantiated by microbiological assays, we have shown that biofilms are present in the skin in many other cutaneous diseases including atopic dermatitis, seborrheic dermatitis, tinea pedis, Doucas Kapetanakis, Meyerson's nevus, axillary granular parakeratosis, molluscum contagiosum (MC), all forms of eczema nummular, dyshidrotic, etc.), tinea versicolor, and healing wounds [3-6]. We have also identified skin diseases in which the pathological biofilms are found in other organs and not the skin. Specifically, these are psoriasis which has biofilms in the tonsils; leprosy, where the biofilms are in the liver, spleen, and kidney (and only in the skin as "globi" in late lepromatous leprosy); tertiary Lyme disease, where biofilms have been noted in the joints and brain (Lyme arthritis and Alzheimer's disease) [7-9].

The biofilms are created by different microbes: normal flora *staphylococci* in eczema, *streptococci* in psoriasis, *molluscum* virus in molluscum contagiosum, yeasts in tinea versicolor, mycobacteria in leprosy, and spirochetes in Lyme disease [3-5,7-9].

Inasmuch as we have been pursuing a "proof of concept" in this preliminary effort, we used biopsies that had been taken from the ringed, non-scaling plaques of GA that form the majority of the presentations of that disease. Not included were deep GA, disseminated GA or interstitial granulomatous dermatitis [1].

Methods

Ten specimens from 6F and 4M aged 6-22 were initially examined and diagnosed as GA both clinically and with routine pathology. Periodic acid Schiff (PAS), colloidal iron (which stains for acidic mucin), and CR stains were also performed on theses specimens. The findings were confirmed by 4 dermatopathologists. As controls, 10 specimens from healing wounds were examined with the same staining.

Results

On reexamination, all ten specimens showed the expected pathological findings of dermal necrobiotic granulomas surrounded by a lymphohistiocytic infiltrate. With PAS and colloidal iron, positive staining for mucin was shown in the necrobiotic zones of the lesions (Figures 1 & 2). Also, all ten showed positive staining with CR (Figure 3). Ten specimens from healing wounds showed changes only in the eccrine ductal occlusions as previously identified and not in the dermis as seen in GA (Figure 4).

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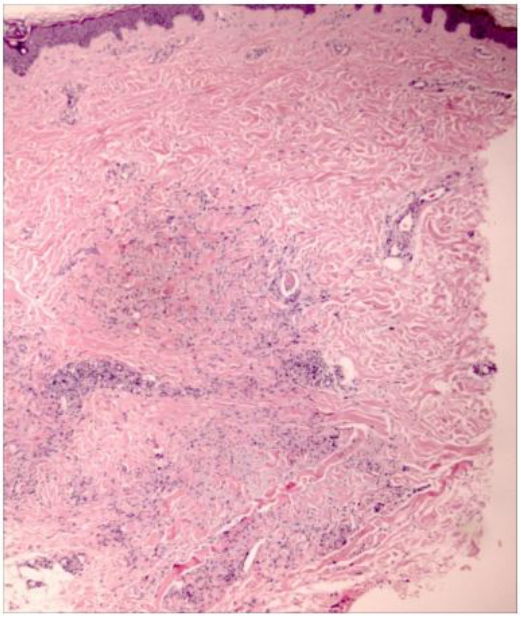

Figure 1: Granuloma annulare pathology-H+E stained section of skin showing necrobiotic granulomas in the dermis. Mucin in the granulomas shows gray-blue staining (5X)

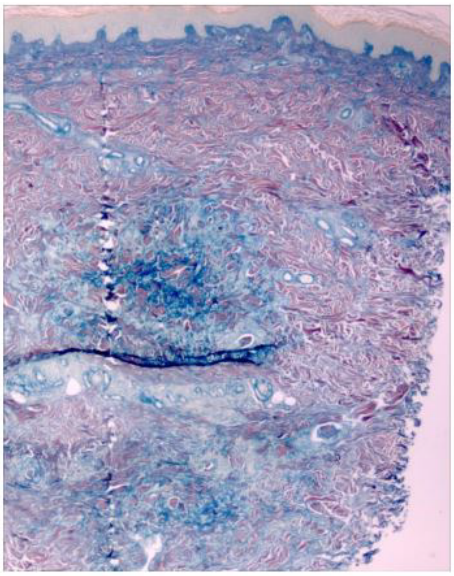

Figure 2: Acidic mucin in GA stained with colloidal iron-Positive staining for acidic mucin bright blue in necrobiotic granulomas (5X)

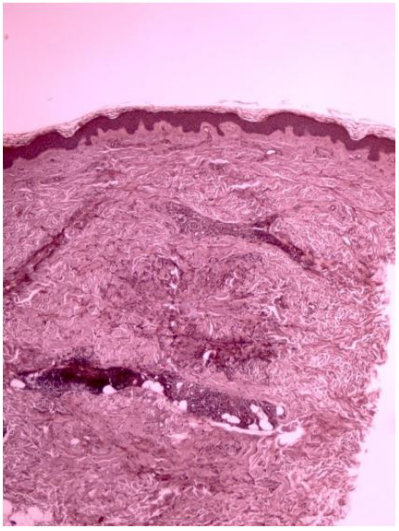

Figure 3: GA stained with Congo red-Congo red staining in the same necrobiotic granulomas as colloidal iron (5X)

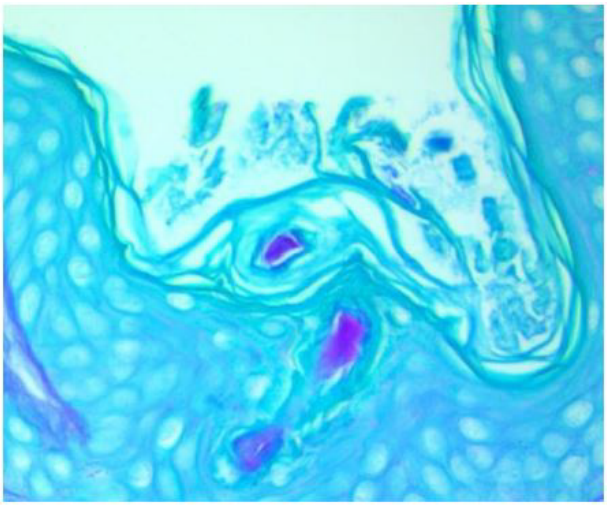

Figure 4: Healing wound stained with PAS-PAS stains the biofilm occluding the sweat duct in a healing wound. This is the pathology in eczema also that leads to pruritus (40X)

Discussion

By pathological assessments, similar to those which we have utilized in our previous studies, we have identified the presence of biofilms in the necrobiotic granulomas of GA. Positive staining for mucin in the foci of necrobiosis, has been noted for over 50 years [1]; the staining for amyloid (the other key ingredient in biofilms) is a novel finding. Amyloid forms the infrastructure of biofilms and has been seen in the many diseases outlined earlier in this work.

Biofilms are caused by microbes; moreover, 90% of microbes in nature live in biofilms [10]. They reside in that state in order to withstand environmental stressors [10]. In the body, microbes create biofilms to repel the immune system and antimicrobials in addition to the environmental stressors (hypo/hyperosmolarity, salt, water, etc) [11]. The "slime" (mucin) coating that surrounds the organisms inside the biofilm affords the overall protection. Antibiotics cannot penetrate the "slime", nor can the immune system. Often, in the attempt to penetrate the coating and kill the microbes, the immune system kills the surrounding tissue instead [12]. This is postulated to be a mechanism for the tissue in tertiary Lyme disease.

We have found biofilms in GA, but we have not found a specific microbe with this very preliminary study. Also, the disease is mostly asymptomatic; this was seen in tinea versicolor where the biofilms were present only in the stratum corneum, hidden in that location from the innate immune system. It was also seen in MC where the biofilms are found intracellularly, and thus are not visible to the immune system. Such is not the case in eczema and psoriasis; in eczema, the first response to the *staphylococcal* biofilm occluding sweat ducts is from the innate immune system molecule Toll-like receptor [2]. In psoriasis, that molecule is also present as is the adaptive immune system's *streptococcal* specific IgG [13,14].

Consequently, GA behaves much like TV and MC as it clinically produces little or no symptomology. The reason(s) for this are unclear: is the presence of a more acidic mucinous component a factor in limiting the activity of TLR2? This question can be answered

in future work. Biofilms made by other microbes have specific attachment sites for TLR2 even if they are created by gram negative organisms. Does the lymphohistiocytic infiltrate surrounding the necrobiotic collagen have a dampening effect on the immune response? Or, does the frequently associated vasculopathy have some effect? What is clear is that GA is a biofilm-related disease, and, biofilms are generated by microbes. Some heretofore unidentified microbe is very likely causing the disease.

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