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BIOFILMS IN NECROBIOTIC GRANULOMAS

Herbert B. Allen, MD[1,2 *], Iain Noel Incarnacion, MS[2], Alice A. Roberts, MD PhD[2]

1 Drexel University College of Medicine, Dept of Dermatology
2 Eastern Virginia Medical School

ABSTRACT

Biofilms, which indicate the presence of microbes, are extremely common and have been found in many cutaneous and systemic diseases. Systemic diseases include arthritis, gout, arteriosclerosis, and Alzheimer's disease. In dermatopathology, biofilms have been previously demonstrated in multiple dermatologic conditions, including atopic dermatitis, psoriasis, and leprosy. More recently, biofilms have been identified in two of the three benign necrobiotic granulomas, which are altered collagen and elastic fibers surrounded by granulomatous inflammation. Namely, these two are granuloma annulare (GA) and rheumatoid arthritis nodules (RA). Here we include the finding of biofilms in specimens of necrobiosis lipoidica (NL), completing the triad of benign necrobiotic granulomas.

Introduction

We have recently observed the presence of biofilms in the dermatopathological specimens from lesions of Necrobiosis lipoidica (NL). NL was previously known as Necrobiosis lipoidica diabeticorum (NLD); but, because diabetes was present in 40% or fewer patients, the name was changed. This novel finding documents the presence of biofilms in the triad of benign necrobiotic granulomas: granuloma annulare (GA), rheumatoid arthritis nodules (RA), and now NL.[1,2] Biofilms, which indicate the presence of microbes, are extremely common (more than 90% of the microbes in nature live in this state) and have been found in many cutaneous and systemic diseases. Among the skin diseases are eczema (in all its various presentations), psoriasis, leprosy, tinea versicolor, acute tinea pedis, molluscum contagiosum, and squamous cell carcinoma in situ in pigmented transplant patients.[3-8] The systemic diseases include arthritis, gout (tophi), arteriosclerosis, and Alzheimer's disease.[10-12]

Methods

Skin specimens from the lower legs of four females, ages 48-67, were processed routinely with hematoxylin and eosin. The leading clinical differential diagnosis was NL. Periodic acid Schiff (PAS) that stains mucopolysaccharides, colloidal iron (CFe) that stains acid mucopolysaccharides particularly hyaluronic acid, Congo red (CR) that stains amyloid, and CD282 that stains Toll-like receptor 2 (TLR 2) were all carried out. The polysaccharides form the bulk of biofilms that coat the microbes within. The amyloid forms the infrastructure of the biofilms. TLR 2 is a first-responder innate immune system molecule.

Results

All three of the specimens showed the expected changes on routine H+E staining. (Fig. 1) These included layered collagen, lymphohistiocytic inflammation, occasional plasma cells and giant cells, and focal necrobiotic collagen. The necrobiosis in NL was not nearly as well-defined as in GA and RA nodules.

PAS showed minimal to no changes: CFe showed positivity in the areas of inflammation, and CR staining was similar. (Figs. 2,3) These changes followed the staining patterns seen in GA and RA nodules. CD 282 was negative.

The pattern differed from that seen in eczema, psoriasis, leprosy and TV in which the PAS showed positivity.[3-6] This implies that the necrobiotic granulomas are associated with different microbes. Eczema is associated with normal flora skin staphylococci and psoriasis with streptococcus pyogenes.[3,4] Acidic biofilms have been found together with gram negative organisms as opposed to the gram-positive ones mentioned. The mechanism regarding gram negatives was discussed in a previous study.[2]

Discussion

Finding biofilms in NL completes the novel discovery of biofilms in each of the triad of necrobiotic granulomas. These cutaneous

disorders join the rather lengthy list of skin diseases associated with biofilms. The biofilms can be extracellular as in eczema and TV and intra (and extracellular as in psoriasis. The extracellular biofilms in eczema interact with the innate immune system molecule TLR 2 leading to disease in the genetically susceptible patients. TLR 2 and streptococcal specific IgG have been noted in psoriasis likely in response to extra and intracellular presence of the biofilms.[3,4,6] Consequently, the innate and adaptive immune systems are involved in genetically susceptible psoriasis patients. (Both eczema and psoriasis are associated with a double hit phenomenon.) The biofilms in TV are extracellular and are present in the acellular stratum corneum in the skin and thus generate no immune reaction. The lesions of TV are manifest only by color change and skin peeling.

The necrobiotic granulomas are asymptomatic and thus generate minimal immune reaction. A question that arises concerns which comes first: does the necrobiosis precede the biofilm? Or, do the biofilms lead to the necrobiosis? The same questions arise in the deposition diseases that we have studied, namely arteriosclerosis and gout (tophi).[10-11] In those diseases, do cholesterol and uric acid precede the biofilms? Our sense leads us to postulate the biofilms are first and the substances join the community, but this has not been determined.

What has been shown is that many other microbes join the biofilm of the initial organism. Biofilms have attachment (receptor) sites for other organisms.[13] Consequently, many organisms have been associated with arteriosclerosis and Alzheimer's disease. These include bacteria, and viruses.[14] As has been said previously, the microbe(s) responsible for the biofilms in the necrobiotic granulomas have not been identified.

Various items cause microbes to form biofilms: in eczema, the salt and water in sweat cause the staphylococci to form biofilms in the sweat ducts.[3] The hyperosmolar serum in diabetes causes biofilms to form in Alzheimer's disease brains.[18] The hyperosmolar serum may have been responsible for the relationship of diabetes and NL. The formation of NL plaques on the lower legs may have been at sites of trauma or microtrauma in diabetics. Trauma around joints may also help in the formation of RA nodules. All of this needs further evaluation and study.

Fig. 1 Pathology of lower leg lesion (H+E)

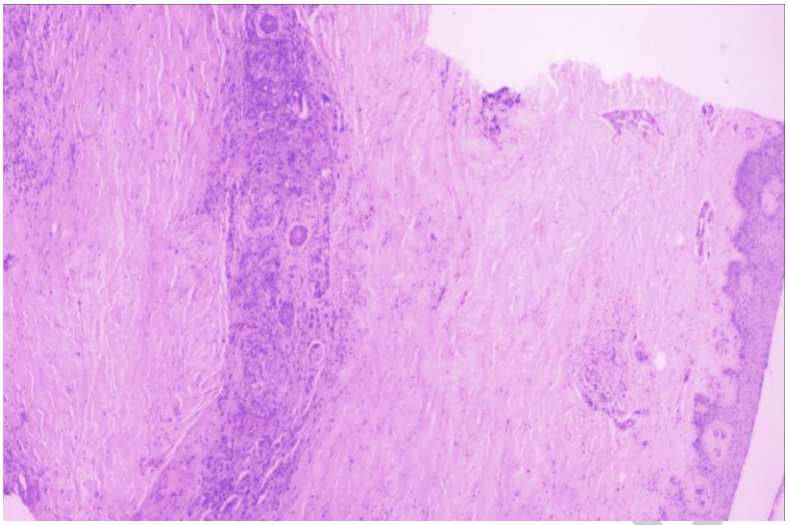

Fig. 1 Features of NL are seen: linear deposition of collagen in the dermis, lymphohistiocytic infiltrate in the mid dermis, focal necrobiosis within the lymphohistiocytic infiltrate (noted by the tinctorial change. 5X

Fig. 2 Pathology of lower leg lesion (Congo red) on left; lung control on right

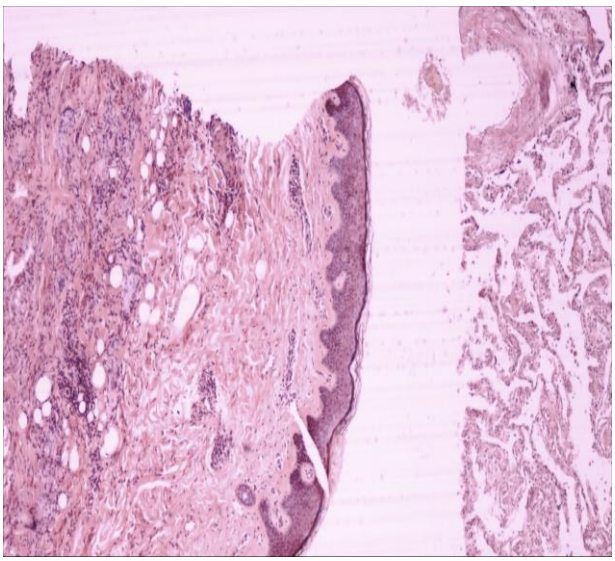

Fig. 2 Positive staining of Congo red in specimen (denotes amyloid which forms the infrastructure of biofilm). 10X

Fig. 3 Colloidal iron staining of lower leg lesion

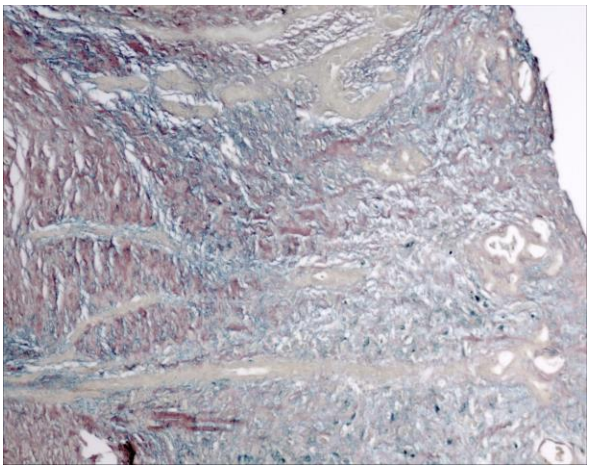

Fig. 3 Positive blue-green staining noted amidst the infiltrate; this represents hyaluronic acid which makes up the mass of the biofilm. 10X

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