Biofilms in Rheumatoid Arthritis Nodules: A Novel Clue Relating to Microbial Origin

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ABSTRACT
Biofilms have been found in many cutaneous diseases and play an important role in disease pathogenesis of atopic dermatitis, psoriasis, and tinea versicolor. Recently, necrobiotic granulomas in granuloma annulare were shown to contain biofilms, which led us to investigate whether biofilms are also present in the necrobiotic granulomas of rheumatoid arthritis (RA). In this brief report, we evaluated eight skin nodules from five patients with known rheumatoid arthritis. We used pathology staining to identify two key components of biofilms: polysaccharides (using periodic acid Schiff [PAS] and colloidal iron [CFe]) and amyloid (using Congo Red). We also used the immune histochemical stain CD 282 to evaluate whether Toll-like receptor 2 was present. The PAS, CFe, and Congo red stains were positive, indicating biofilms were present. CFe positivity is an indicator that the biofilms have an acidic matrix; gram negative organisms are known to thrive in that milieu. CD 282 was negative except in one lesion that was penetrating through the epidermis and one which was weakly positive. Since microbes are responsible for creating biofilms, our findings suggest that microbes, especially gram negatives, are involved in RA pathogenesis.

Keywords
Biofilms, Chronic diseases, Specimens, S. aureus.

Introduction
We have previously found biofilms in many chronic diseases including atopic dermatitis (AD) and psoriasis (the biofilms in psoriasis were in the tonsils, not the skin) [1,2]. More recently, we have identified biofilms in the necrobiotic granulomas of granuloma annulare (GA) [3].

The microbes responsible in AD were normal flora staphylococci (including S. epidermidis and S. aureus), and, in psoriasis, streptococci [1,2]. We have applied the same histological staining to rheumatoid arthritis (RA) nodules and have found similar staining patterns to GA [3]. This adds to the growing list of microbial biofilms seen in both cutaneous and internal diseases.

Methods
We obtained 8 specimens (nodules) for pathological processing from 5 patients (3 males, 2 females, aged 52-71) with RA. One patient provided three lesions. We utilized routine hematoxylin and eosin which yielded the diagnosis of necrobiotic granulomas. Further, we employed periodic acid Schiff (PAS), colloidal iron (CFe), and Congo red stains to identify potential biofilms [1-3]. CD 282, which stains Toll-like receptor 2 (TLR2), [1-3] determined whether that molecule was present.

Results
All specimens were positive for necrobiotic granulomas, and all contained biofilms as evidenced by positive staining for PAS, CFe, and Congo red (Figure 1-3).

The PAS/CFe identified the polysaccharide mass of the biofilm; the positive Congo red identified amyloid which serves as an infrastructure for biofilms [1]. The RA nodules’ staining pattern differed from AD and psoriasis by demonstrating a positive CFe (this was the pattern previously seen with the necrobiotic granulomas in GA) [3]. One specimen, which showed a penetrating lesion, demonstrated positive TLR2 staining, and one other specimen showed weak staining with TLR2.
Discussion

In this work, we have made the novel observation of biofilms in the necrobiotic granulomas of RA nodules. Biofilms, by definition, imply the presence of microbes and this has been shown in AD and psoriasis [1,2]. We have also made the novel observation of a different staining pattern in the nodules (compared to AD and psoriasis), namely the positive staining with CFe. This may provide an important clue as to the nature of the, as yet, unidentified microbes involved with making these biofilms because gram negative organisms prefer an acidic medium, and this is very likely present in the observed biofilms because of the positive CFe. The gram-negative periodontal bacterium, Porphyromonas gingivalis, has been epidemiologically linked to RA. This bacterium possesses a unique PPAD enzyme that citrullinates host proteins, triggering production of anti-citrullinated protein antibodies (CCP), an important serologic marker in RA [4,5].

*P. gingivalis* may be an important component of the biofilm, and the presence of this gram-negative negative bacterium is consistent with positive CFe staining. It may also be the gram-negative component of a larger biofilm community similar to that found in dental plaque. *S. mutans* is the leading organism to attach to the tooth; next, porphyromonas joins the community and last, treponemal species join the agglutination. These treponemal spirochetes are implicated in multiple other internal diseases including Alzheimer’s disease [6].

CD 282 was positive in one RA nodule that was penetrating (through the epidermis) and weakly positive in another. The TLR2 positive in the penetrating lesion was explainable because of the disruption of the epidermis; the other was similar to the weak staining in one lesion in the necrobiotic granulomas in GA [3]. Negative CD 282 is somewhat expected inasmuch as most of the skin lesions in both diseases are asymptomatic. The most important inference derived from the presence of biofilms in the RA nodules is it that implies that microbes are present in the biofilms (Figure 4), these are possibly gram negatives and possibly Porphyromonas gingivalis, but their identity needs to be positively determined.

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