

Psoriasis, Atopic Dermatitis, Lyme Disease and Tinea Versicolor: All caused by Microbes but none a Classic Infection

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Abstract

There is a possibility for a role of biofilms in the pathogenesis and chronicity of atopic dermatitis, plaque psoriasis, Lyme disease and tinea versicolor. In atopic dermatitis, plaque psoriasis and Lyme disease, there is evidence to suggest that biofilm-mediated Toll-like-receptor 2 recruitment and activation of the MyD88 pathway is essential. There is also evidence to suggest involvement of the adaptive immune system. Conversely, tinea versicolor does not seem to activate innate nor adaptive immunity, presumably an explanation for its more benign and generally asymptomatic course. Existing evidence for biofilms in the aforementioned diseases is reviewed, and possible links to immune responses, or lack thereof, are postulated. All of this is in sharp contrast with acute microbial infections.

Keywords: Atopic dermatitis; Lyme disease; Infection

Introduction

The immune system can be categorized into three lines of defense to protect against infection: (1) external barriers, (2) the innate immune response, and (3) the adaptive immune response. External defense mechanisms include mechanical barriers, chemical barriers and bacterial interference [1]. The second line of defense is the innate immune system. An important component of this system is toll-like receptors (TLRs), a class of pattern-recognition receptors (PRRs) that identify components of pathogens called pathogen-associated molecular patterns (PAMPs) in an effort to eliminate invading threats [2,3]. When a more robust response is necessary, the innate system can stimulate adaptive immunity, which aids innate components in eliminating pathogens and establishes long-term memory in the case of subsequent re-exposures [4]. Adaptive immunity includes both humoral and cell mediated responses, which are largely mediated by B- and T-cells, respectively [4]. Activation of the innate and adaptive immune systems with humoral and cellular involvement results in the classic signs of infection: dolor (pain), rubor (redness), calor (heat) and tumor (swelling), with subsequent *functio laesa* (loss of function).

Some pathogens can evade elimination by the immune system through the formation of biofilms. Biofilms are groups of microorganisms attached to biologic or non-biologic surfaces in a matrix of extracellular polysaccharides, amyloid, DNA, and adhesive fibers. Formation occurs in response to environmental cues and is largely driven by quorum sensing, a type of signaling that enables microbes to monitor and respond to their population densities. The biofilm protects microbes from the immune system and antimicrobial therapy, thus acting as a refuge for individual cells and providing a source of persistent infection [5]. Here, we review evidence that Psoriasis, Atopic Dermatitis, Lyme disease and Tinea Versicolor are all chronic microbial states in which organisms evade eradication by the immune system via formation of biofilms.

Atopic Dermatitis

The etiology of atopic dermatitis is thought to be a double-hit phenomenon in which filaggrin gene mutations disrupt barrier function and predispose affected individuals to an unknown environmental insult. Work from Allen et al. has identified *Staphylococcal* biofilms as a likely second hit [6,7]. The 2014 study examined lesional and non-lesional skin scrapings and biopsies from 40 atopic dermatitis patients and 20 control patients (10 samples from inflamed, nonatopic skin and 10 samples from non-inflamed, nonatopic skin). Nonatopic controls were used because atopic dermatitis patients are known to have barrier dysfunction on both lesional and non-lesional skin. Results revealed that 93% of samples (37 out of 40) from affected patients and 95% of samples from control patients contained multi-drug resistant *staphylococci*, with *S. aureus* (42%) and *S. epidermidis* (20%) as the predominant species in affected patients. Additionally, 38 out of 40 atopic samples and 20 out of 20 control samples were shown to have biofilm positive staphylococci both through phenotypic or genotypic testing via XTT [2,3-bis-(2-methoxy-4-nitro-5-sulphenyl)-2H-tetrazolium-5-carboxanilide] or polymerase chain reaction (PCR), respectively.

Pathologically, eccrine duct occlusion was visualized with hematoxylin and eosin (H&E) in 100% of atopic specimens, but none of the control specimens. Occluding material stained positively with Congo red and periodic acid-Schiff (PAS), indicating presence of amyloid and extracellular polysaccharides, correspondingly. Immunohistochemical (IHC) analysis revealed TLR2 activation in the stratum corneum adjacent to ductal occlusion in lesional samples, versus the stratum basale only in the controls ($P=0.001$, X^2).

The authors believe that biofilms form in the eccrine ducts due to the H₂O and NaCl content, which have previously been shown to induce biofilm formation [8]. Their findings lead to the hypothesis that TLR2 is activated and recruited from its physiologic location in the stratum basale to the stratum corneum in response to the organisms and the biofilm within eccrine ducts. Once there, it futilely attempts to penetrate the biofilm through activation of the MyD88 pathway, which

generates well-characterized mediators of pruritus (i.e., proteinase-activated receptor 2), and spongiosis (i.e., tumor necrosis factor), the main symptom and the main pathologic finding of atopic dermatitis, respectively. Although occluded eccrine ducts are normally associated with miliaria [9], Allen et al. proposed that in combination with defective filaggrin, occlusion of eccrine ducts results in atopic dermatitis. In the double hit model, defective filaggrin serves as the genetic component and *staphylococcal* biofilm serves as the environmental component.

While the concept of biofilms in atopic dermatitis is relatively new, antimicrobial and biofilm dispersing agents have been included in the treatment regimen for atopic dermatitis for decades. Emollients, a mainstay of treatment for atopic dermatitis, contain such ingredients, including petrolatum [10]. Future studies will be necessary to investigate whether antimicrobials and biofilm dispersers should play a bigger role in the treatment of this common skin disorder.

Psoriasis

Psoriasis is traditionally described as an immune mediated disease, and current biologic therapies are aimed at dampening this pathway. While effective, they do not address the source of the inflammatory response, which is currently unknown. Historic and emerging evidence suggest that *Streptococcus pyogenes* (*S. pyogenes*), also known as Group A *Streptococcus* (GAS), may play a direct role. Epidemiologic data shows that the prevalence of GAS in the environment correlates with the incidence of psoriasis, and in environments lacking GAS, such as Australia before the arrival of British prisoners, no cases of psoriasis can be found [11]. In 1955, Norrlind found a higher average antistreptolysin (ASO) titer in psoriatic patients compared to normal patients (54.5% versus 7.3%). He also observed that about two thirds of patients with psoriasis, especially guttate psoriasis, reported preceding streptococcal upper respiratory tract infection [12]. The presence of GAS in guttate psoriasis is now well characterized, and penicillin is utilized in the treatment of this psoriatic subtype [13].

Norrlind also noted that chronic plaque psoriasis may be exacerbated by streptococcal pharyngitis [12], a finding that has been corroborated in more recent studies [14]. However, a direct association between GAS and plaque psoriasis has been more difficult to delineate since the bacteria is not readily cultured and affected patients don't always develop an elevated ASO titer. Two theories for the lack of evidence of GAS presence in chronic plaque psoriasis have been proposed based on known properties of the bacteria: (1) internalization [15] and (2) biofilm formation [16]. In both of the proposed scenarios (internalization and biofilm formation), GAS would be able to establish a persistent infection within or attached to tonsillar epithelial cells and evade the immune system for extended periods. Extravasation from host cells or detachment from biofilm may occur, but with only a narrow window of culturability before recolonization or biofilm establishment. Evidence for this notion comes from the work of El-Rachkidy et al., which showed that a substantial portion of IgG-reactive proteins from cultured *S. pyogenes* are secreted from the bacteria [17]. When the secreted protein fraction of *S. pyogenes* was used in an ELISA to measure IgG titers, it was significantly higher in patients with chronic plaque psoriasis compared to age and sex matched healthy controls ($p=0.0009$) [17]. These findings not only help substantiate the association between GAS and psoriasis, but also suggest involvement of the adaptive immune system in the mechanism of disease.

Of course, not all patients with GAS infections develop psoriasis. This was reflected in El-Rachkidy's data [17], in which some control patients displayed titers as high as those with psoriasis. A likely explanation for that is, like atopic dermatitis, psoriasis is a double-hit phenomenon. The first hit may be genetic and the second may be Streptococcal internalization or biofilm. If true, the control patients with high IgG titers did not possess the necessary predisposition to develop clinical disease.

Allen et al. found TLR 2 in the upper dermal capillaries [16]. They postulated the source for TLR 2 to be the tonsillar biofilms.

Intriguingly, there have been reports of efficacy with tonsillectomy for amelioration of psoriatic disease [18]. In 2005, Saxena and Dogra published a two-year open study on the use of long-term benzathine penicillin for the treatment of chronic plaque psoriasis. Intramuscular injection of 1.2 million units of benzathine penicillin was administered every two weeks for 24 weeks and then monthly. Significant improvement in Psoriasis Area and Severity Index (PASI) score was evident after 12 weeks of treatment and maintained at 2 years [19]. Comparable results were found in a single blind randomized case control trial of long-term "pulse" azithromycin [20]. The results from these studies seem promising; however, larger, randomized control trials should further examine the application of anti-streptococcal agents in the treatment of psoriasis.

Lyme Disease

Lyme disease is caused by the spirochete *Borrelia burgdorferi*, which is transmitted by the Ixodes tick. There are three stages of infection. Early infection is characterized by erythema migrans (EM) rash. EM classically appears as a "bull's eye", however, there are multiple clinical presentations [21]. Early disseminated infection, or stage 2, occurs within weeks of the initial rash and may involve the nervous system, joints and/or heart [22]. Left untreated, the disease can progress to stage 3, late disseminated infection, in weeks to months. This stage is characterized by persistent arthritis, acrodermatitis, or neuroborreliosis [23]. Neuroborreliosis, which indicates involvement of the nervous system, is seen in up to 40% of infected patients [24].

The variable presentation of EM is thought to contribute to underreporting of Lyme disease. The true prevalence of the disease may be more than 10 to 30 times greater than the number of confirmed cases per year, based on CDC estimates and nationwide surveys [25]. This may be partly attributed to or compounded by the fact that standard diagnostic tests (ELISA and antibody titers) are not very sensitive, particularly for early infection [26,27]. A more sensitive C6 peptide ELISA exists [28,29] but is not routinely used. Moreover, the CDC criteria for diagnosis of LD do not cover patients with encephalopathy, polyneuropathy, neuropsychiatric disease, or chronic manifestations [30]. Thus the prevalence of Lyme disease may be much higher than current estimates.

Although antibiotic therapy is highly effective for resolution of erythema migrans, up to 50% of patients complain of persistent subjective symptoms lasting for more than 6 months after treatment [31-33]. This entity is called post-treatment Lyme disease syndrome (PTLDS), and the primary identified risk factor is delayed diagnosis [33]. Animal studies in beagle dogs, mice and rhesus macaques have provided evidence of persistence of spirochetal DNA after antibiotic therapy [34-36]. It is hypothesized that there is only a small window to effectively eradicate *Borrelia burgdorferi* with antibiotics [37]. If so, many patients who delay seeking treatment, are overlooked due to

atypical EM presentation, or who don't fall into CDC criteria could be receiving treatment after it is too late (or not at all).

Sapi et al. found substantial evidence that *Borrelia burgdorferi* form biofilms *in vivo* [38], which may play a role in their survival beyond treatment and contribute to the ability to establish chronic infection. More recently, evidence of spirochetal biofilm formation in brains of Alzheimer's disease has been revealed [39]. Dental spirochetes and *Borrelia burgdorferi* spirochetes have both been implicated [40-42]. Using the same methods employed to investigate atopic dermatitis; Allen et al. showed biofilm presence and TLR2 activation in hippocampal specimens from Alzheimer Disease patients. Interestingly, the presence of biofilm co-localized with deposition of β -amyloid, [43] the aggregates of which form the pathologic plaques of Alzheimer disease. This correlated well with the findings of Macdonald [44]. It was postulated that, as in atopic dermatitis, TLR2 is stimulated by the presence of spirochetal biofilm and activates the MyD88 pathway. In addition to producing TNF α , the MyD88 pathway also produces NF κ B, which is known to upregulate beta-secretase, the enzyme that cleaves amyloid precursor protein (APP) into beta-amyloid [45,46] Allen et al. proposed that the following cascade of events could be responsible for the hallmark beta-amyloid of Alzheimer's disease: *Borrelia* infection \rightarrow biofilm formation \rightarrow TLR2 recruitment \rightarrow MyD88 pathway activation \rightarrow NF κ B production \rightarrow β -secretase up-regulation \rightarrow increased cleavage of APP into β -amyloid [43]. Another theory suggests that TLR2 is attracted to the "curli" fibers of the biofilm [47], rather than the spirochetes. Additional studies will be necessary to clarify the precise mechanism of recruitment of TLR2.

Tinea Versicolor

The etiology of tinea versicolor is *Malassezia furfur*, a lipophilic yeast that is part of the normal skin flora. Clinical infection occurs when the yeast assumes a pathogenic state in the stratum corneum of the epidermis, which generally results in asymptomatic hypopigmented or hyper-pigmented scaling lesions on the upper trunk. Hypopigmentation is the most common clinical manifestation, which occurs secondary to tyrosinase inhibition by azelaic acid produced by *M. furfur* despite treatment; infections are often chronic [48].

Based on previous work on atopic dermatitis and the known ability of fungi and yeasts to form biofilms, Allen et al. hypothesized that *Malassezia* biofilm formation could be responsible for chronic tinea versicolor [49]. To investigate this, skin scrapings from 24 patients with active tinea versicolor were collected for KOH preparation, crystal violet staining, and direct inoculation on an antibiotic-containing culture medium. Twenty samples from non-affected skin of the same patients were collected as controls. After the confirming the typical "spaghetti and meatball" appearance of hyphae and spores in affected samples, crystal violet staining was performed, revealing aggregates of organisms, which were interpreted to be indicative of biofilm. Twenty out of 24 samples grew positive cultures, all of which produced slime or other variations of biofilm [49].

Allen et al. suggested that the pathogenesis of tinea versicolor may follow a double-hit hypothesis, as was proposed for atopic dermatitis and psoriasis. The first hit may be genetic (i.e., immunosuppression, hyperhidrosis or other hereditary predisposition) and the second hit, *Malassezia furfur*. However, unlike atopic dermatitis or psoriasis, they concluded that there is no pathologic activation of inflammation, hence the total lack of pruritus or other symptoms. Metabolites of the

yeast itself are known to down-regulate inflammation, which may play a role in the lack of immune activation. Another possibility is that the biofilm is suspended in the stratum corneum or attached to the corneocytes and is thus unable to elicit a response, since corneocytes are not known to possess TLRs.

Discussion

Recent work has provided evidence for a role of biofilms in the pathogenesis of atopic dermatitis, chronic plaque psoriasis, Lyme disease and tinea versicolor. In each disease, the biofilms are made by different organisms. With regard to atopic dermatitis, chronic plaque psoriasis and Lyme disease, the evidence suggests a key role for the innate immune system, specifically TLR2 and the MyD88 pathway [6,16,43]. Conversely, tinea versicolor may not be able to stimulate an innate immune response due to its location within the stratum corneum- it is possible that cutaneous biofilms only initiate a response once the stratum granulosum (where epidermal cells are found) has been penetrated.

Data from El-Rachkidy et al. also suggests a possible role for the adaptive immune system in chronic plaque psoriasis [17]. Adaptive immunity is more robust than the innate immune system. This is clearly seen when contrasting an adaptive-mediated to a non-adaptive mediated disease or injury. In the case of cerebrovascular accident (CVA), the blood brain barrier is breached, allowing adaptive immune components such as immunoglobulin and T-cells to cross. Not surprisingly, the course of Alzheimer's disease after CVA is rapid (1-3 years), versus the typical course, which is much longer [50]. Future studies should aim to verify whether Streptococcal biofilm and the "soluble" TLR 2 stimulates adaptive immunity in chronic plaque psoriasis and quantify whether involvement of the adaptive system is reflected in disease onset, severity or course. Likewise, perhaps breach of the epidermis via scratching in atopic dermatitis enables an adaptive response to Staphylococcal biofilms, and in turn, results in a more severe presentation. This would be an interesting explanation for "the itch that rashes".

Despite the evidence reviewed here, there is much work to be done in the field of biofilm-mediated diseases to validate their role and investigate biofilm-targeted therapies. Large studies with vigorous study designs should be employed to reveal the possible key role that biofilms play in the pathogenesis of cutaneous and other diseases.

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