Bacterial biofilm in acute lesions of hidradenitis suppurativa

Authors: Ginette A. Okoye, Natalia Vlassova, Omolara Olowoyeye, Alessandra Agostinho, Garth James, Philip S. Stewart, Anthony Leung, Gerald Lazarus

Okoye GA, Vlassova N, Olowoyeye O, Agostinho A, James G, Stewart PS, Leung S, Lazarus G, “Bacterial biofilm in acute lesions of hidradenitis suppurativa," British Journal of Dermatology, 2017 Jan;176(1):241-243. doi: 10.1111/bjd.14805.

Bacterial biofilm in acute lesions of hidradenitis suppurativa

DEAR EDITOR, Hidradenitis suppurativa (HS) is a chronic inflam-matory disorder of the hair follicles, characterized by recurrent tender subcutaneous nodules, sinus tracts and scarring in regions of the body with a high density of apocrine glands. Epidemiology studies of HS have shown a range of prevalence from 0·053% to 4%.[1] The female : male ratio of HS is approximately 3 : 1,[2] and HS may be more common in peo-ple of African descent.[3–5]

Patients with HS generally present with acute flares, charac-terized by tender subcutaneous nodules that drain malodorous purulent material. Sinus tracts are considered chronic lesions of HS but can continue to drain purulent material indefinitely. Both acute and chronic lesions severely impair the quality of life of patients.[6,7]

Although the clinical presentation of HS is reminiscent of infection and multiple bacterial species have been isolated from HS lesions, the role of bacteria in the pathogenesis of HS is unclear.[8] Given the polymicrobial nature of HS lesions,[8] slow healing times and the inconsistent response to standard antibiotic doses, we hypothesized that bacterial biofilm forma-tion is a possible pathogenic pathway in the aberrant inflam-matory response seen in HS.

Hidradenitis suppurativa nodules and sinus tracts are pock-ets of necrotic tissue in a low oxygen tension environment, which provide an ideal setting for bacterial proliferation. Additionally, the structurally abnormal tissue of HS lesions may provide a substrate similar to foreign bodies and favour the formation of biofilms.[9] Many microorganisms have been isolated from HS lesions, in particular anaerobic bacteria, Staphylococcus aureus and coagulase-negative staphylococci (CNS).[8,9] Although generally considered to represent normal surface skin flora, CNS may play a pathogenic role in HS. CNS have been found deep in HS lesions,[9] and have the ability to form biofilms following attachment to the surfaces of foreign bod-ies.[9,10] Bacterial biofilms have been found in HS sinus tracts.[11] These chronic lesions are perfect environments for secondary development of biofilms. However, it is unknown if biofilms are primarily involved in HS pathogenesis.

The objective of this study was to evaluate acute HS lesions for the presence of bacterial biofilms by histopathology and a semiquantitative biofilm grading scale using epifluorescence microscopy.

After the Johns Hopkins institutional review board approved the study, participants were recruited from the dermatology clinics. After a 2-week washout period from oral and topical antibiotics, participants notified the study team when they developed at least one acute HS lesion (a new, nonruptured tender subcutaneous nodule). Tissue samples were obtained from the acute HS lesion and from clinically uninvolved skin in the same anatomical area. The skin was cleansed with a chlorhexidine swab before sampling to decrease the number of surface microorganisms. The entire acute lesion was removed using a sterile 8-mm punch biopsy tool for lesions smaller than 8 mm or by elliptical excision using a sterile scal-pel for lesions 8 mm or larger. The 8-mm punch biopsy tool only was used to sample the uninvolved skin.

The samples were frozen with liquid nitrogen prior to over-night shipment to the Center for Biofilm Engineering (CBE) at Montana State University. Upon arrival at the CBE, they were embedded in OCT (Tissue-Tek® Optimum Cutting Tempera-ture; Sakura Finetek, Torrance, CA, U.S.A.), frozen on dry ice and then stored at −70 °C. The entire sample was divided into three 'locations': side 1, middle and side 2. At least three nonsequential sections were examined for each location until at least one hair follicle was found in every sample. Five-milli-meter-thick sections were cut at −20 °C using a Leica CM1850 cryostat (Leica, Wetzlar, Germany), placed on Super-frost Plus microscope slides (Fisher Scientific, Pittsburgh, PA, U.S.A.) and stored at −70 °C. Sections of each specimen were stained using Sytox Green®- and Texas Red®-conjugated wheat germ agglutinin components of the ViaGram™ Red + Bacterial Gram Stain and Viability Kit (Life Technologies, Carlsbad, CA, U.SA.). Sections were examined using an Eclipse E-800 epifluorescence microscope (Nikon, Melville, NY, U.S.A.). Each section was scored based on the amount of bacteria/biofilm observed using a 6-point scale created at the CBE: 0 = no bacteria; 1 = single individual cells; 2 = small microcolonies (10–100 cells); 3 = large microcolonies (> 100 cells); 4 = continuous biofilm; 5 = thick continuous biofilm. Representative images of the biofilms were collected using a CoolSNAP EZ cooled charge-coupled device camera (Photo-metrics, Tucson, AZ, U.S.A.) and processed using MetaVue software (Molecular Devices, Sunnyvale, CA, U.S.A.).

A total of 10 participants were recruited, and they all com-pleted the study. The average age was 38·4 years. There were eight women; seven of the participants were white, two were black and one was Hispanic. There were three participants with Hurley stage I disease, six with stage II disease and one with stage III disease.

Table 1 Biofilm in lesional and nonlesional skin in hidradenitis suppurativa (HS)

Patient	Age (years)	Sex	Ethnicity	Hurley stage	Location	Involvement	Biofilm score
1	53	M	W	III	Left upper flank	HS lesion	2
					Right upper flank	Normal skin	0
2	40	F	W	I	Right inguinal fold	HS lesion	3
					Right mons	Normal skin	0
3	53	F	W	I	Right mons	HS lesion	0
					Left mons	Normal skin	0
4	31	M	W	II	Right axilla	HS lesion	0
					Left axilla	Normal skin	0
5	23	F	W	II	Left axilla	HS lesion	0
					Right axilla	Normal skin	0
6	47	F	W	II	Right inguinal fold	HS lesion	0
					Left inguinal fold	Normal skin	0
7	52	F	B	I	Right medial thigh	HS lesion	0
					Left anterior thigh	Normal skin	0
8	35	F	W	II	Right labia major	HS lesion	0
					Right mons	Normal skin	0
9	22	F	B	II	Left axilla	HS lesion	0
					Right axilla	Normal skin	0
10	28	F	H	II	Right axilla	HS lesion	0
					Left axilla	Normal skin	0

M, male; W, white; F, female; B, black; H, Hispanic.

Biofilms were not found in any of the uninvolved skin samples. Tissue samples of acute HS nodules showed structural abnormalities with histological evidence of obliterated follicles, dense inflammation and necrotic-appearing tissue. However, biofilms were found in only two of the acute HS lesions (Table 1; Fig. 1).

A biofilm is a complex aggregation of microorganisms in which cells adhere to a surface and form colonies. These cells are protected by an extracellular polysaccharide-rich matrix and are physiologically distinct from single cells of the same organism. Biofilm-associated bacteria replicate at slower rates and have reduced antibiotic susceptibility,[12] and are therefore much more difficult to eradicate with conventional antibiotic doses.

Studies on biofilms in chronic wounds have shown that biofilms alter the host inflammatory response and promote the release of proinflammatory cytokines.[13] Although biofilms were not found in the acute HS lesions in this study, they have been found in chronic lesions of HS (sinus tracts),[11] suggesting secondary involvement of bacteria and biofilms in the pathogenesis of HS, particularly in established lesions.

Acute lesions of HS have not been previously studied to assess for the presence of biofilm and the possibility of its role in early disease. The lesions sampled in this study represented acutely inflamed hair follicles in intertriginous areas. The absence of biofilm was an unexpected finding given the fact that the environment of intertriginous areas is highly favourable for bacterial proliferation and biofilm formation in the skin.[14] Iwase *et al.* demonstrated that skin commensals such as *Staphylococcus epidermidis* have the ability to inhibit biofilm formation. Perhaps the absence of biofilm in acute HS lesions is an indication of a high density of *S. epidermidis* in early HS.[15]

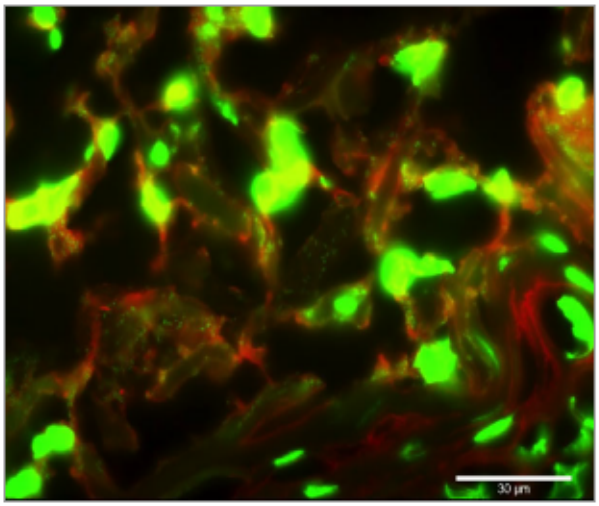

Fig 1. Infundibulum of a hair follicle within an acute hidradenitis suppurativa lesion from patient 2. Stained with a fluorescent Gram stain (ViaGram™ Red + Bacterial Gram Stain) and imaged with an epifluorescence microscope. Bacteria and the nuclei of mammalian cells stain green and connective tissue stains red. The small green dots represent large microcolonies of biofilm (grade 3).

The main limitation of this study is the small sample size. Additionally, HS lesions were excised from different anatomical locations. It is possible that different anatomical locations may demonstrate different bacterial flora and therefore different propensities for biofilm formation. It is also possible that the chlorhexidine used to remove bacteria from the epidermis prior to excising the HS nodules decreased the amount of biofilm in the deeper tissues.

Further studies are needed to characterize the possible role of bacterial biofilms in chronic HS lesions such as nonhealing draining nodules, sinus tracts and comedones. Patients with these lesions tend to have more severe disease with very few effective treatment options. If biofilms are found to play a role in chronic disease pathogenesis, more rational treatment decisions will be made for these patients, such as foregoing ineffective antibiotic therapy for earlier surgical excision of chronic HS lesions.

[1]Department of Dermatology, Johns Hopkins School of Medicine, 5200 Eastern Ave, Suite 2500, Baltimore, MD 21224, U.S.A.
[2]Department of Dermatology, University of Pittsburgh, Pittsburgh, PA, U.S.A.
[3]Center for Biofilm Engineering, Montana State University, 366 EPS Building, Bozeman, MT 50717-3980, U.S.A.

G.A. Okoye[1]
N. Vlassova[2]
O. Olowoyeye[1]
A. Agostinho[3]
G. James[3]
P.S. Stewart[3]
S. Leung[1]
G. Lazarus[1]

References

1 Jemec GB, Kimball AB. Hidradenitis suppurativa: epidemiology and scope of the problem. *J Am Acad Dermatol* 2015; **73** (5 Suppl. 1): S4–7.
2 Revuz J. Hidradenitis suppurativa. *J Eur Acad Dermatol Venereol* 2009; **23**:985–98.
3 Vlassova N, Kuhn D, Okoye GA. Hidradenitis suppurativa disproportionately affects African Americans: a single-center retrospective analysis. *Acta Derm Venereol* 2015; **95**:990–1.
4 Chaikin DC, Volz LR, Broderick G. An unusual presentation of hidradenitis suppurativa: case report and review of the literature. *Urology* 1994; **44**:606–8.
5 Anderson BB, Cadogan CA, Gangadharam D. Hidradenitis suppurativa of the perineum, scrotum, and gluteal area: presentation, complications, and treatment. *J Natl Med Assoc* 1982; **74**:999–1003.
6 Deckers IE, Kimball AB. The handicap of hidradenitis suppurativa. *Dermatol Clin* 2016; **34**:17–22.
7 Gooderham M, Papp K. The psychosocial impact of hidradenitis suppurativa. *J Am Acad Dermatol* 2015; **73** (5 Suppl. 1):S19–22.
8 Ring HC, Emtedtam L. The microbiology of hidradenitis suppurativa. *Dermatol Clin* 2016; **34**:29–35.
9 Lapins J, Jarstrand C, Emtestam L. Coagulase-negative staphylococci are the most common bacteria found in cultures from the deep portions of hidradenitis suppurativa lesions, as obtained by carbon dioxide laser surgery. *Br J Dermatol* 1999; **140**:90–5.
10 McCann MT, Gilmore BF, Gorman SP. *Staphylococcus epidermidis* device-related infections: pathogenesis and clinical management. *J Pharm Pharmacol* 2008; **60**:1551–71.
11 Kathju S, Lasko LA, Stoodley P. Considering hidradenitis suppurativa as a bacterial biofilm disease. *FEMS Immunol Med Microbiol* 2012; **65**:385–9.
12 Costerton W, Veeh R, Shirtliff M *et al*. The application of biofilm science to the study and control of chronic bacterial infections. *J Clin Invest* 2003; **112**:1466–77.
13 Percival SL, Bowler P, Woods EJ. Assessing the effect of an antimicrobial wound dressing on biofilms. *Wound Repair Regen* 2008; **16**:52–7.
14 Leyden JJ, McGinley KJ, Nordstrom KM, Webster GF. Skin microflora. *J Invest Dermatol* 1987; **88** (3 Suppl.):6S–7S.
15 Iwase T, Uehara Y, Shinji H *et al*. *Staphylococcus Epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. *Nature* 2010; **465**:346–9.

Funding sources: this study was funded by a Patient-Directed Investigation Grant from the Dermatology Foundation.

Conflicts of interest: none declared.