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Research Article

Altered Expression of Epithelial Proteins in Hidradenitis Suppurativa

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ABSTRACT

Hidradenitis Suppurativa (HS) is a chronic inflammatory skin condition characterized by intradermal rupture of occluded pilosebaceous units which releases corneocytes and apocrine gland contents into the dermal compartment thereby eliciting a marked chronic inflammatory response. The direct cause of the severe and long-standing lesions in HS has not been elucidated. Using immunohistochemistry assays on formalin-fixed paraffin-embedded tissue sections, the current study assayed 27 of the more than 50 proteins directly responsible for corneocyte maturation and shedding in an attempt to describe alterations in protein expression which may be partially responsible for the firmly-cemented comedone plugs. Increased expression was found in A2ML1, CK 5/6, desmocollin 2/3, desmoglein 2, elafin, envoplakin, EphA2, filaggrin, involucrin, KLK6, KLK7, and plakophilin III, while decrease expression was seen in CK 1/10 and LEKTI. Together, these data suggest a multifactorial cause/effect which may explain the highly variable clinical course apparent in persons with HS.

Keywords: Corneocytes; Cytokeratin; DSC; DSG; Etiology; Comedone; IHC; CDSN

INTRODUCTION

The original supposition that Hidradenitis Suppurativa (HS) originates in apocrine glands has been successfully disputed. However, because the most frequent anatomical locations of lesions in HS are areas rich in apocrine glands, their secretions likely influence HS development. Apocrine glands become active during puberty which is when the earliest manifestations of HS become apparent [1] suggesting the two events are not unrelated. The HS population varies based on country and method of data collection but overall, there are 2X–3X more women affected as compared with men, and there is a decrease in lesion formation in women after menopause [1,2] which suggests that hormones may initiate lesional activity. Dark-skinned persons are more frequently affected than those of lighter skin and the mean age of diagnosis (not of onset) is 31 ± 11 years [2] .The majority of HS lesions are found in intertriginous areas of apocrine-bearing skin, specifically the axillary, inframammary, inguinal, groin, and buttock regions [2]. There are several very distinct physiological differences in intertriginous skin regions which may be involved in the development of HS lesions and include factors such as moisture content, pH, skin flora, temperature, mechanical friction, oxygen content, and skin thickness [3,4].

The presence of multiple, large comedones, or 'blackhead' pores occluded with hyperkeratotic squamous cells and glandular secretions, differentiate HS from a bacteria-induced dermal infection [5]. Comedone formation is often exacerbated by increased heat and humidity, [6] both characteristics which are found in the most common HS anatomic sites. Another clinical characteristic is painful scar-forming erythematous dermal nodules, often with sinus tract formation and discharge. The clinical presentation along with nodule recurrence and location of lesions are the three criteria which allow for definitive diagnosis [5]. Histologic evaluation supports the clinical interpretation of HS but is not required for diagnosis. Because human serum which contains antibodies to the stratum corneum [7,8] is kept physically isolated from stratified squamous epithelium under normal conditions *via* a basement membrane and multiple layers of squamous epithelial cells, the antibodies remain latent and do not stimulate an immunological reaction under normal circumstances. During follicular rupture, however, cells from the stratum corneum are directly exposed to the dermal compartment and a massive and rapid chronic inflammatory response is generated. Surrounding collagen and elastin are degraded as plasma cells exit local blood vessels [9]. The chronic inflammatory response is aided by an influx of granulocytes and increase in macrophages as the invading epithelium is broken down [10]. Complete degradation of epithelial sheets is a slow process as the epithelium continually attempts to regenerate while simultaneously being attacked by the immune system.

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The most effective long-term treatment to date for severe HS lesions is wide surgical excision, but even then, a 14% recurrence rate was found [11]. Removal of most hair follicles and underlying apocrine glands in commonly affected areas leaves fewer targets for future lesion development. Although apocrine glands themselves are not the affected entities in HS lesions, their secretions which change skin pH, and thereby increase or decrease enzymatic reactions could, in fact, be responsible for the alterations which produce the abnormal accumulation of keratinocytes leading to the overexpansion of the hair follicle and the eventual ruptured basement membrane.

Hidradenitis lesions begin in pilosebaceous units when degenerating squamous cells admixed with glandular secretions plug the area surrounding a hair shaft and result in a comedone formation. In persons unaffected by HS, formation of a comedone is a transient occurrence, [6] often hormonally related, and resolves without intervention by spontaneous degeneration and expulsion of the pore contents. For reasons thus far unidentified, the comedone resolution mechanism is altered in HS. Corneocyte shedding is impaired as underlying maturing squamous cells continue to add to the plug, thereby stretching the walls and base of the hair follicle and leading to eventual rupture of the basement membrane. Stratified squamous epithelium supported by resilient dermal collagen provides significant stretching and tearing resistance, and rupture of the sides or base of the pilosebaceous unit due to accumulation of pore contents suggests altered structure or function of one of the structural components. Possibilities include a structurally unsound basement membrane, weakened support from dermal collagen, or particularly resilient cohesive properties of the sebaceous/corneocyte plug. While any of the aforementioned causes may be individually or collectively responsible for the initiation of HS lesions, the literature does not describe any other clinical symptoms related to impaired collagen production or dermal integrity. Indeed, histologic review reveals non-affected skin layers in persons with HS are indistinguishable from those of persons without HS. Additionally, in early HS lesions prior to rupture of the basement membrane, the hair follicle compartment may distend to >10X the diameter of the original follicle which advocates for a resilient epidermis and basement membrane and suggests that adhesive corneocyte properties may be the primary contributing factor to HS lesions.

The goal of this study was to evaluate protein expression in epithelial HS lesions as they compare with similar skin in persons without HS using Immunohistochemical (IHC) assays. While most HS studies focus on relief of symptoms, reduction in the inflammatory response, or treating lesions, there have been few published works which attempt to identify the indirect causative mechanism. Of the >50 epithelial proteins specific to integument epithelial maturation and shedding, [12] this study examined 27 proteins which could be responsible for the suspected hyper-adhesive properties of HS comedone keratinocytes.

MATERIALS AND METHODS

This project was approved by the University of Tennessee Health Science Center Institutional Review Board (19-07040-NHSR). Formalin-fixed paraffin-embedded tissues were acquired from the shared tissue resource center and included 10 pathologist-confirmed cases of HS and 10 inframammary/axillary Normal Skin (NS) samples obtained during routine breast reduction surgery. In 9 of the 10 cases of HS, there were Migrating Sheets of Squamous Epithelium (MSSE) in the dermal compartment due to rupture of distended hair follicles. The tenth case exhibited distended but intact follicle only. Serial sections were cut at 4 µm, mounted onto adhesive slides, and placed into an oven at 60^{0}C for 24 hours. After deparaffinization and rehydration of tissue sections through several changes of xylene and absolute alcohol, and final rinses in 95% ethanol followed by deionized water, sections were immersed in antigen retrieval solutions optimal for antibodies assayed (Table 1). Antigenic sites were decloaked 5 minutes at 120°C using a Biocare Decloaking Chamber (Biocare Medical, Pacheco, CA, USA). Once cooled, the antigen retrieval buffers were replaced with 1X Envision™ Flex Wash buffer, Tris-Buffered Saline (TBS) (DM831, Dako/Agilent, Santa Clara, USA) before application of primary antibody (Table 1) at room temperature for 20 minutes. Immunohistochemistry assays were performed with Bond™ Polymer Refine Red Detection Kit (DS9390, Leica Biosystems, Buffalo Grove, IL, USA) using manual benchtop methods. Briefly, TBS rinses were used on the tissue sections after primary antibody incubation, followed by the kit post primary reagent for 20 minutes. After rinsing the sections with TBS, sections were incubated with kit polymer for 30 minutes followed again by TBS rinses. Deionized (DI) water was used to rinse buffer off prior to red chromogen development, and to rinse chromogen after application. Sections were counterstained in the kit hematoxylin for 1 minute, rinsed with DI water, TBS and DI water. Sections were allowed to air dry prior to brief immersion in xylene and then were coverslipped using a resinous mounting medium. All positive and negative controls were deemed acceptable before assay evaluation on patient tissues. Images were captured using an Olympus BX45 light microscope (Olympus Corp, Tokyo, Japan) and CellSens software (Olympus).

Table 1: Antibodies used for immunohistochemistry assays in HS.

Antibody	Manufacturer	Catalog Number	pH of Antigen Retrieval	Antibody Dilution
Alpha-2-Macroglobulin-Like Protein 1 (A2ML1)	Santa Cruz Biotechnology, Dallas, TX, USA	Sc-393082	High	1:100
Acid Sphingomyelinase (ASM)	Santa Cruz Biotechnology	Sc-293189	High	1:50
Beta-catenin	Cell Marque/Sigma Aldrich, Rocklin, CA, USA	Sc-224M-18	Low	1:1
Beta-glucosidase	Santa Cruz Biotechnology	Sc-365745	High	1:100
Cathepsin D	Santa Cruz Biotechnology	Sc-377299	High	1:100
Corneodesmosin	Novus Biologicals, Centennial, CO, USA	NBP2-47501	Low	1:50

Cytokeratin 1/10 (CK 1/10)	Santa Cruz Biotechnology	Sc-53251	High	1:100
Cytokeratin 5/6 (CK 5/6)	Roche/Ventana, Indianapolis, IN	790-4554	Low	1:4
Desmocollin 1	Santa Cruz Biotechnology	Sc-398590	High	1:100
Desmocollin 2/3	Santa Cruz Biotechnology	Sc-53485	High	1:60
Desmoglein 1	Santa Cruz Biotechnology	Sc-137164	High	1:200
Desmoglein 2	Santa Cruz Biotechnology	Sc-365856	High	1:100
Desmoplakin I/II	Santa Cruz Biotechnology	Sc-390975	High	1:50
E-cadherin	Cell Marque/Sigma Aldrich	AC-0003	High	1:100
Elafin	Santa Cruz Biotechnology	Sc-398075	High	1:100
Envoplakin	Santa Cruz Biotechnology	Sc-137033	High	1:100
Ephrin type-A receptor 2 (EphA2)	Santa Cruz Biotechnology	Sc-398832	High	1:40
Filaggrin	Santa Cruz Biotechnology	Sc-66192	High	1:50
Gamma-catenin	Santa Cruz Biotechnology	Sc-514130	High	1:50
Group IVD phospholipase A2	Santa Cruz Biotechnology	Sc-398758	High	1:100
Involucrin	Santa Cruz Biotechnology	Sc-21748	High	1:400
Kallikrein 6 (KLK6)	Santa Cruz Biotechnology	Sc-374564	High	1:100
Kallikrein 7 (KLK7)	Santa Cruz Biotechnology	Sc-514447	High	1:100
LEKTI	Santa Cruz Biotechnology	Sc-137109	High	1:100
Periplakin	Santa Cruz Biotechnology	Sc-365530	High	1:100
Plakophilin II	Santa Cruz Biotechnology	Sc-393711	High	1:100
Plakophilin III	Santa Cruz Biotechnology	Sc-166655	High	1:100

Note: Where High=pH 8.5 retrieval solution (EnVision FLEX Target Retrieval Solution High pH 50X (K800421-2, Agilent/Dako, USA)). Low=pH 6.0 retrieval solution (IHC Antigen Retrieval Solution (10X) (00-4955-58, Invitrogen, USA))

RESULTS

Immunohistochemistry assays comparing protein expression between HS and NS exhibited no differences for the following antibodies: ASM, beta-catenin, beta-glucosidase, cathepsin D, corneodesmosin, desmocollin 1, desmoglein 1, desmoplakin I/II, E-cadherin, gamma-catenin, group IVD phospholipase A2, involucrin, periplakin, and plakophilin II. In contrast, approximately half of the antibodies assayed did show partial to complete deviation in HS epithelial elements as compared with those in NS (Table 2).

Table 2: Protein expression in HS lesions compared with that of NS.

Antibody	SE and MSSE like NS	Change in SE	Change in MSSE
Alpha-2-Macroglobulin-Like Protein 1 (A2ML1)	1/10 (10%)	Increased in 8/9 (89%)	Increased in 8/9 (89%)
Acid Sphingomyelinase (ASM)	10/10 (100%)	No change	No change
Beta-catenin	10/10 (100%)	No change	No change
Beta-glucosidase	10/10 (100%)	No change	No change
Cathepsin D	10/10 (100%)	No change	No change
Corneodesmosin	10/10 (100%)	No change	No change
Cytokeratin 1/10 (CK 1/10)	1/10 (10%)	Decreased in 7/10 (70%)	Decreased in 9/10 (90%)
Cytokeratin 5/6 (CK 5/6)	0/10 (0%)	Increased in 10/10 (100%)	Increased in 9/9 (100%)
Desmocollin 1	10/10 (100%)	No change	No change
Desmocollin 2/3	4/10 (40%)	Increased in 4/10 (40%)	Increased in 4/9 (44%)
Desmoglein 1	10/10 (100%)	No change	No change
Desmoglein 2	1/10 (10%)	Increased in 8/10 (80%)	Mild increase in 8/9 (89%)
Desmoplakin I/II	10/10 (100%)	No change	No change
E-cadherin	10/10 (100%	No change	No change
Elafin	0/10 (0%)	Moderately increased in 10/10 (100%)	Markedly increased in 9/9 (100%)
Envoplakin	1/10 (10%)	Increased in 9/10 (90%)	Increased in 5/9 (56%)

Ephrin type-A receptor 2 (EphA2)	3/10 (30%)	Increased in 6/10 (60%)	Increased in 5/9 (56%)
Filaggrin	3/10 (30%)	No change	Increased in 7/10 (70%)
Gamma-catenin	10/10 (100%)	No change	No change
Group IVD phospholipase A2	10/10 (100%)	No change	No change
Involucrin	1/10 (10%)	Increased in 9/10 (90%)	Increased in 9/9 (100%)
Kallikrein 6 (KLK6)	2/10 (20%)	Increased in 4/10 (40%)	Increased in 8/9 (89%)
Kallikrein 7 (KLK7)	1/10 (10%)	No change	Increased in 8/9 (89%)
LEKTI	1/10 (10%)	Decreased in 3/10 (30%)	Decreased in 7/9 (78%)
Periplakin	10/10 (100%)	No change	No change
Plakophilin II	10/10 (100%)	No change	No change
Plakophilin III	3/10 (30%)	Increased in 5/10 (50%)	Increased in 4/9 (44%)

Abbrevations: HS: Hidradenitis Suppurativa; NS: Normal Skin; SE: Surface Epithelium in HS lesions; MSSE: Migrating Sheets of Squamous Epithelium in the dermal compartment in HS.

A2ML1 (Alpha-2 Macroglobulin-Like 1)

Present in the Stratum Granulosum (SG) of NS, the overall intensity of expression was greater in HS epithelium. Expression in HS often began in the bottommost Stratum Spinosum (SS) layer and was strongly present in all SS and SG layers. Some uninvolved surface epithelium in HS appeared similar to NS expression. However, HS epithelium overlying inflammation showed increased intensity and increased cell depth including all layers of the SS. In the MSSE, expression intensity was marked.

CK 1/10 (Cytokeratins 1 and 10)

All suprabasal layers were boldly labeled in NS. In the majority of HS lesions, however, there was loss of CK1/10 in the Stratum Corneum (SC), a strong reduction in expression in the other layers and very often complete absence in MSSE (Figure 1).

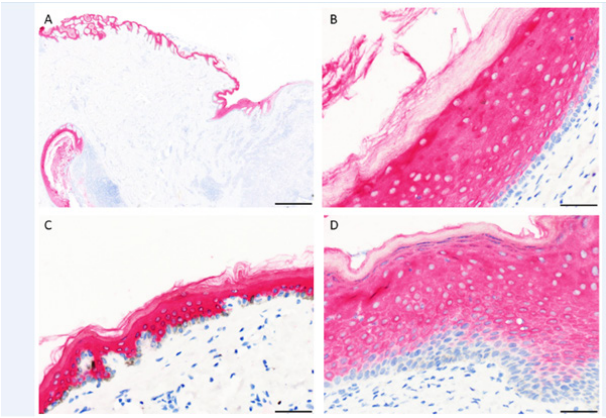

Figure 1: Cytokeratin 1/10 expression determined by immunohistochemistry assay in (A,B,D) HS lesion and (C) normal skin. Although markedly present in suprabasal epithelial layers in normal skin (C), cytokeratin 1/10 is moderately decreased in (B) suprabasal layers of involved surface epithelium overlying HS lesion and (D) in MSSE. Scale bar (A)=1 mm. Scale bar (B-D)=50 µm.

CK 5/6 (Cytokeratins 5 and 6)

In NS, CK 5/6 labeled the Stratum Basale (SB) layer and the lower layers of the SS boldly and then decreased throughout the SS and was very weak to absent in the maturing cells of the SG. However, marked differences were noted in HS epithelium. In areas overlying inflammation, the CK 5/6 expression was homogeneously bold throughout all epithelial layers except SC. In some of the migrating epithelium, the CK 5/6 expression increased in the more mature layers.

Desmocollin 2/3

Although no expression was seen in NS there was weak expression of desmocollin 2/3 in HS epithelium in approximately half the cases and strong expression in one case.

Desmoglein 2

Desmoglein 2 was absent in NS except weakly expressed in the basal layer of hair follicles. However, it was weakly present in HS skin overlying inflammation, and weakly to moderately present in all MSSE (Figure 2).

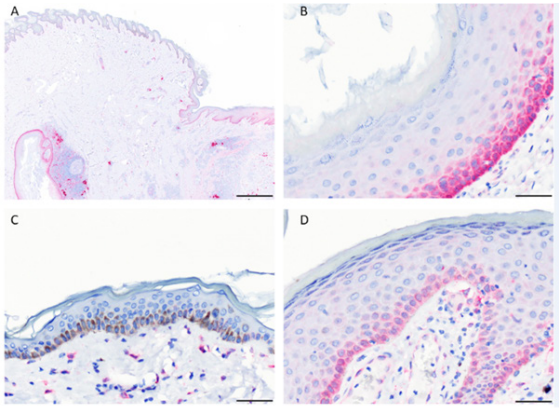

Figure 2: Desmoglein 2 expression determined by immunohistochemistry assay in (A,B,D) HS lesion and (C) normal skin. Although not present in normal skin (C), desmoglein 2 is increased in (B) basal layer of involved surface epithelium overlying HS lesion and (D) in MSSE. Scale bar (A)=1 mm. Scale bar (B-D)=50 µm.

Elafin

In NS, elafin was virtually absent. When seen, it was found focally and minutely in the SG layer only. In HS uninvolved surface epithelium, elafin presented in a similar manner as that seen in NS. However, in surface epithelium overlying areas of inflammation, in keratin-filled follicles, and in MSSE, elafin production was not confined to the SG, but was also present in the upper half of the SS and was frequently also present in the SC where the cells were incarcerated in ruptured or unruptured follicles. Elafin was markedly expressed in all MSSE (Figure 3).

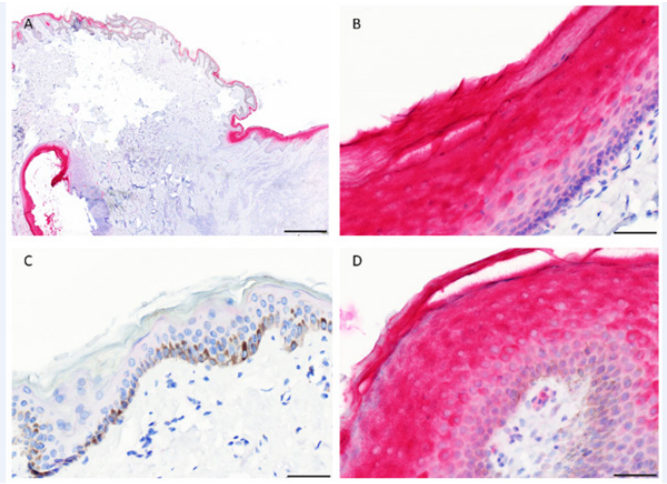

Figure 3: Elafin expression determined by immunohistochemistry assay in (A,B,D) HS lesion and (C) normal skin. Although not present in normal skin (C), elafin is increased in (B) suprabasal layer of involved surface epithelium overlying HS lesion and (D) in MSSE. Scale bar (A)=1 mm. Scale bar (B-D)=50 µm.

Envoplakin

In NS, envoplakin was uniformly present on all squamous cell membranes from SB through SG. It was not present in the SC. In HS lesional tissues, envoplakin was increased in surface epithelium and in MSSE. The increased expression was seen in the SS and SG (Figure 4).

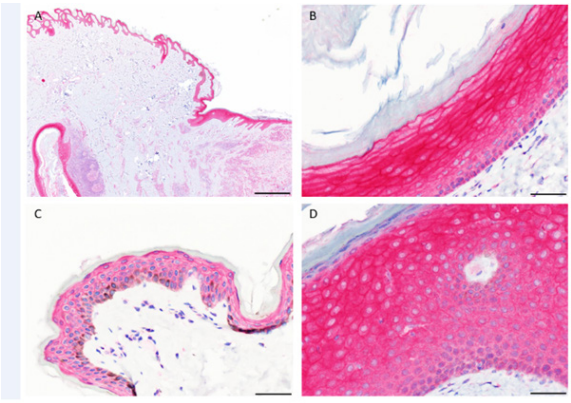

Figure 4: Envoplakin expression determined by immunohistochemistry assay in (A,B,D) HS lesion and (C) normal skin. Although present in all nucleated epithelial layers in normal skin (C), envoplakin is increased in (B) stratum spinosum and stratum corneum of involved surface epithelium overlying HS lesion and (D) in MSSE. Scale bar (A)=1 mm. Scale bar (B-D)=50 µm.

EphA2 (Ephrin type-A receptor 2)

In NS, all epithelial layers except SC were labeled weakly and homogeneously. In some HS tissues, the EphA2 expression mimiced that seen in NS, but in other sections, EphA2 expression was mildly increased, and in other specimens, expression was markedly increased.

Filaggrin

Found in all layers of the SG, and in lowest layers of SC in NS, filaggrin in HS was increased. The SG in HS was thicker so more cells express filaggrin. In keratinocytes trapped in follicles or in the dermal compartment, the entrapped corneocytes were typically very strongly labeled suggesting that filaggrin breakdown was delayed, possibly due to differences in oxygen exposure, light, pH differences, etc (Figure 5).

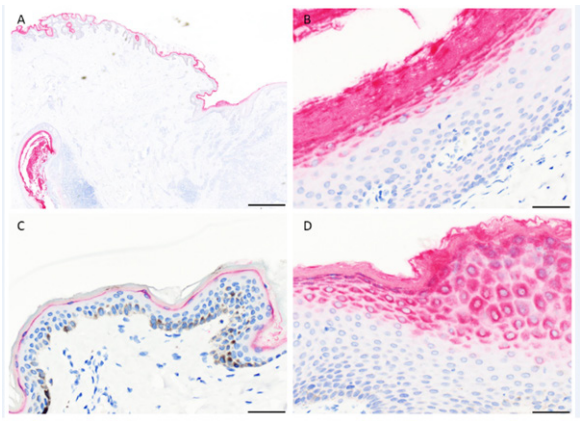

Figure 5: Filaggrin expression determined by immunohistochemistry assay in (A,B,D) HS lesion and (C) normal skin. Although present only in the stratum granulosum in normal skin (C), filaggrin is increased in (B) portions of the upper stratum spinosum, stratum granulosum, and stratum corneum of involved surface epithelium overlying HS lesion and (D) in MSSE. Scale bar (A)=1 mm. Scale bar (B-D)=50 µm.

Involucrin

In NS, involucrin was boldly seen in the SG and moderately present in the upper SS. It was weak to absent in the SB and lower SS. In HS skin overlying inflammation, in distended keratin-filled follicles, and in migrating epithelium, involucrin expression was increased to cover all layers from SB through SG.

KLK6 (Kallikrein Related Peptidase 6)

KLK6 exhibited weak to absent expression in all NS epithelium except in SG of hair follicles. No expression was noted in surface epithelium. In most HS skin, the expression was also weak to absent. However, in areas of epithelial surface ulceration in HS, there was expression in SG, and within MSSE, there was distinct labeling of the SG and occasionally uppermost SS.

KLK7 (Kallikrein Related Peptidase 7)

Found in SG and SC in NS, KLK7 expression was increased, beginning in the SS in HS surface epithelium overlying inflammation, in distended follicles, and was particularly prominent in MSSE.

LEKTI (Lympho-Epithelial-Kazal-type-1 Inhibitor)

Lympho-Epithelial Kazal-type inhibitor was boldly present in all epithelial layers other than SC in NS. In HS, the surface epithelium expression of LEKTI was similar to or slightly reduced as compared with NS. In most of the MSSE, LEKTI expression was moderately reduced.

Plakophilin III

In NS, all layers of the epithelium from SB through SG were weakly positive. In HS, there was variable expression with a slight increase in protein labeling in involved surface epithelium and/or MSSE. In one sample, the increase in protein labeling was pronounced.

DISCUSSION

The stratified squamous cells of skin epithelium form 4 main layers (Figure 6a): The Stratum Basale (SB) is the cuboidal bottom-most layer housing the dividing cells which replenish the cell layers. The Stratum Spinosum (SS) overlies the SB and forms the thickest layer of viable cells. The Stratum Granulosum (SG) layers are 1-3 cells thick, comprise cells which are flat and represent the topmost viable cell layer. The Stratum Corneum (SC) forms the topmost layer and is composed of cell membranes, a few intermediate filament proteins, several cell adhesion proteins, and cellular breakdown products from the anucleated squamous cells. The SC provides a critical barrier function to prevent water loss and provides protection against mechanical, chemical, and microbiological assaults [13] and replaces itself gradually over a 2 to 4-week period. The rate of cell addition by the SB balances the rate of cell loss from the SC [13]. Hyperkeratosis is the term given to excess layers of corneocytes on the skin surface and may be due to excess proliferation of the underlying basal cells or may be termed "Retention Hyperkeratosis" whereby normal accumulation of squamous cell layers is not dismantled in a timely manner [14]. The relatively invisible shedding of individual corneocytes from the SC is tightly and enzymatically controlled [13]. An example of an H and E stained skin section from a patient with HS is shown in Figure 6b to demonstrate the significant inflammatory reaction as well as the intradermal migrating epithelial sheets characteristic of HS lesions.

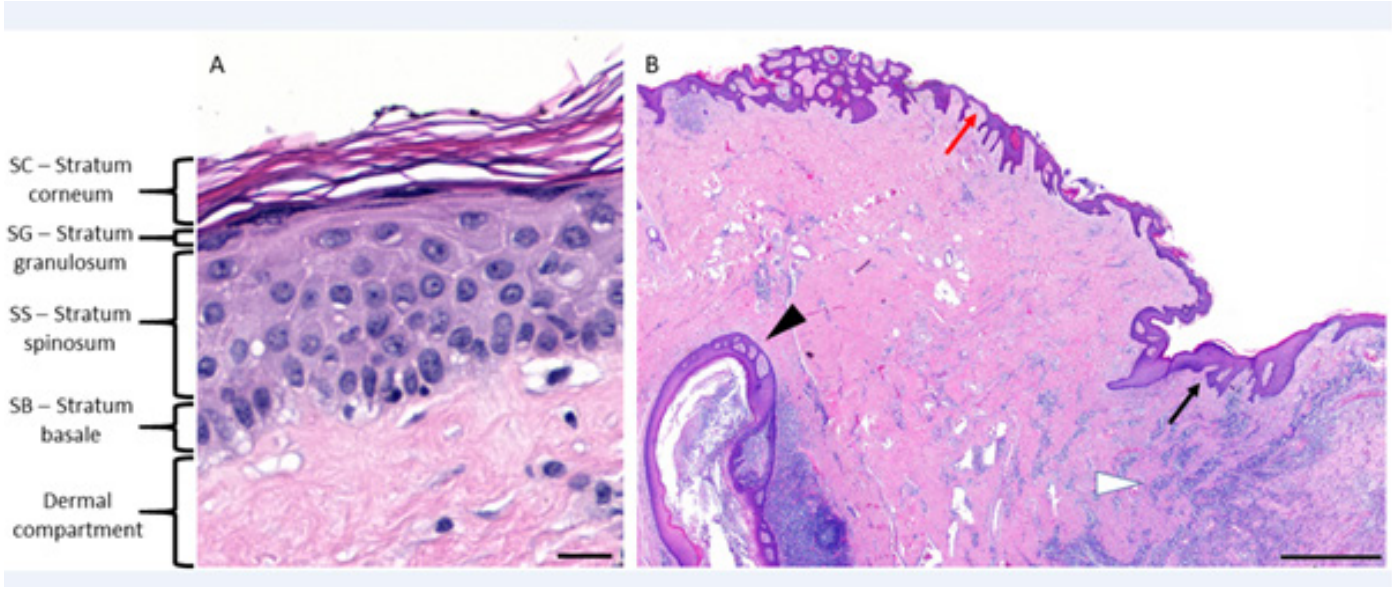

Figure 6: Hematoxylin and eosin stained section of (A) normal skin and (B) skin of patient with hidradenitis suppurativa. (A) Normal layers of epithelial maturation. From least mature: Stratum Basale (SB)–the germinal layer, Stratum Spinosum (SS)–the thickest cell layer with multiple "spiny" processes from desmosomal connections, Stratum Granulosum (SG)–characterized by dark purple cytoplasmic keratohyaline granules and the final layer of cells with viable nuclei, Stratum Corneum (SC)–the uppermost surface layer composed of anucleated corneocytes. (B) Uninvolved surface skin in a patient with HS (red arrow), involved surface epithelium (black arrow) overlying inflammation (white arrowhead), and portions of a Migrating Sheet of Squamous Epithelium (MSSE) (black arrowhead) in the dermal compartment. Scale bar (A)=20 μm. Scale bar (B)=1 mm.

Desmosomes are intercellular complexes which form points of adhesion between adjacent cells and are critical for providing the strength to resist abrasive forces on the epidermis [15]. Seen clearly on hematoxylin and eosin stain preparations with light microscopy in the SS layer of the epithelium, desmosomes are composed of 3 types of proteins: An armadillo family of proteins, plakins and desmosomal cadherins [16,17]. The armadillo protein family includes plakophilins 1-3, plakoglobin (γ-catenin), β-catenin, and p120 catenin [15,16]. The plakin family includes desmoplakin I, desmoplakin II, envoplakin, periplakin, and plectin [16,17]. Desmocollin and desmoglein are part of the cadherin family, calcium-dependent cell adhesion molecules which form the intercellular contacts of desmosomes [15,18] There are 4 desmoglein proteins (1-4) and 3 desmocollin proteins (1-3) [15]. Identical plaques anchored by integral membrane proteins desmocollin and desmoglein associate with the intermediate filament protein network [15]. Although no differences in expression of desmocollin 1, desmoglein 1, desmoplakin I/II, beta-catenin, gamma-catenin, periplakin, or plakophilin were noted in this study, both desmocollin 2/3 and desmoglein 2 as well as plakophilin II were found to be increased in HS epithelium suggesting that adhesive properties between squamous cells may be increased. Envoplakin and periplakin cross-link involucrin and corneodesmosin to stabilize corneocytes [16,19]. Both envoplakin and involucrin were found to be increased in most HS lesions. E-cadherin, a calcium-dependent transmembrane adhesion protein found in all layers of the epidermis and responsible for many cell adhesive properties [20] was not found to be altered in expression in this study.

During terminal differentiation of corneocytes, lamellar bodies which are membrane-enclosed transport vesicles in cells of the SG, discharge their contents at specific intervals to regulate the initial adhesion of corneocytes and then later dissociation of the same cells [21]. Corneodesmosin (CDSN), Kallikrein-Related Peptidases (KLKs), and Lympho-Epithelial Kalzal-type Related Inhibitor (LEKTI) proteins are all components of lamellar bodies in the upper epidermis [21,22]. Corneodesmosin is the

major compositional protein formed when skin desmosomes evolve in the SC, and when combined with desmocollin 1 and desmoglein 1, they together function as an adhesive to cement corneocytes into a compact barrier [21,23]. KLK proteins each have enzymatic activities and work together to cleave very specific members of the desmocollin and desmoglein families as well as CDSN [21].

The mechanisms needed to form the firm, semi-impermeable SC and provide protection from abrasion must, at some point, be undone to allow for shedding of individual corneocytes. Actions to degrade compacted corneocytes are mediated primarily by the actions of serine, cysteine, threonine, glutamate and metallo-proteases [24]. Although human skin has a near neutral pH in the basal layers extending through most of the SG, the pH drops quickly throughout the layers of the SC [19,25] and normal skin surface constantly exposed to air has a pH of 4.5–5.0. The surface of intertriginous skin, however, maintains a higher pH [19]: The inframammary region in females is 5.8, axillary regions range 5.8–6.6, the vulvar region is 6.0, and inguinal areas are 6.2. Most diseases of skin are associated with increased surface pH [19,26] and many lipid-processing reactions in the upper epidermal layers including the enzymes responsible for corneocyte shedding are inhibited by pH increases [27]. Typically, persons with increased skin melanin production have increased SC acidity and therefor superior skin barrier function, [19,28] but as most persons afflicted with HS have dark skin, the intrinsic acidification mechanisms may not be sufficient to counteract the alkaline nature of aberrant protein expression.

Stratum corneum chymotryptic enzyme, also known as human kallikrein 7 protein, KLK7, or hK7, is one of a group of 15 genes/proteins which belong to the human kallikrein family with gene designations KLK1-KLK15 [29,30]. KLK7 has been identified in human skin along with KLK5, KLK6, KLK8, KLK9, KLK11 and KLK13 and is, in part, responsible for the enzymatic cleavage of individual corneocytes from the SC and is influenced by steroidal hormones [29-31]. KLK7 has been shown to cleave both corneodesmosin and desmocollin 1 in maturing skin [32]. Many skin KLKs are secreted in the apocrine and sweat glands allowing for a uniform distribution of these proteins both within the hair follicle lumen and across the surface epithelium [30]. In the current study, LEKTI, an inhibitor of KLK enzymes [21,33,34] was found to be decreased in one-third of affected HS surface epithelium and in most MSSE. With decreased expression of LEKTI, KLK6 and KLK7 were unsurprisingly overexpressed which would logically suggest a concurrent finding of decreased corneodesmosin and desmocollin 1 expression which should provide a more rapid discohesion of individual corneocytes. However, CDSN and desmocollin 1 were not found to be reduced in HS lesions suggesting that the normal actions of KLK6 and KLK7 may have been altered.

In the SC, the intercellular layers of lipids are comprised of 45%-50% ceramides, 20%-25% cholesterol, and 10%-15% free fatty acids [35]. Secretory Phospholipases A2 (SPLA2) comprises a large group of enzymes which break down cell membrane phospholipids. Several sPLA2s are located in the epidermis. The breakdown of membrane phospholipids by sPLA2 produces fatty acids which help to acidify the SC layer [19,36] and which along with ceramide [16,37] provides the cement for ageing corneocytes in the SC layers. Along with sPLA2 enzymes, Acid Sphingomyelinase (ASM) and β-glucocerebrosidase activities in the SG and SC change the pH of the stratum corneum from neutral to acidic. Both ASM and β-glucocerebrosidase function optimally at pH 5 and exhibit reduced activity when the pH in the SC rises to neutral as seen in several inflammatory conditions [38]. Acid sphingomyelinase hydrolyzes sphingomyelin to phosphocholine and ceramide. The literature shows that ceramide synthetase 2 was found to be downregulated while ceramide synthetase 5 was found to be upregulated in HS skin tissues compared with non-lesional skin tissues suggesting that de novo ceramide production is downregulated in HS tissues [37]. Neither ASM, group IVD phospholipase A2, nor beta-glucosidase were found to have altered expression in the current study. Cathepsin D, a breakdown product of ASM, is an aspartatic protease in the stratum granulosum which by activating transglutaminase 1 allows for the cross-linking of involucrin and loricrin to the corneocyte cell envelope [14]. In mice deficient for cathepsin D, a reduction in involucrin, loricrin, and filaggrin was also noted [14]. Although cathepsin D cleaves CDSN [21], the current experiments failed to show a change of expression in cathepsin D in HS skin.

Elafin, also known as proteinase inhibitor 3, elastase-specific inhibitor, and skin-derived antileukoprotease/protease, is typically not expressed in normal skin. It is can be found in the upper stratum spinosum, [39] may form part of the cornified cell envelope, and inhibits KLK7 [21]. Elafin expression has been linked to signals released from neutrophils and is thus found in inflammatory lesions [40]. In keeping with these concepts, elafin in the current study was found to be moderately increased in affected HS surface epithelium and markedly increased in MSSE. Elafin contributes to the corneocyte cell envelope, in part, by forming cross links with loricrin [23,39] which is first expressed in the SG layer of stratified squamous epithelium. Loricrin, in turn, is known to associate with cytokeratins to enhance adhesive properties of epidermal cells [41].

Originally linked to desmosomal structures during maturation, only a few cytokeratins including CK1, CK2e, and CK10 resist degradation, bind with remnants of desmoplakin and envoplakin, and may be found in the SC [16]. Compared with NS, the expression of CK 1/10 in the current study was found to be reduced in affected HS skin as well as in MSSE. Conversely, CK 5/6, a marker often used for identification of poorly-differentiated squamous cell carcinoma due to its ability to successfully label stratified squamous epithelium was found to be upregulated in all HS lesional epidermis, both on skin surface and intradermally.

Alpha-2-macroglobulin [42] and Alpha-2-Macroglobulin-Like Protein 1 (A2ML1) [12] are barrier function proteins found in human skin. Genetic mutations in A2ML1 which lead to loss of function have been associated with increased incidence of otitis media [43]. In the present study, increased expression of A2ML1 was found in both surface epithelium and in MSSE in persons with HS.

Filaggrin, generated as profilaggrin in keratohyaline granules in the stratum granulosum, is secreted into the extracellular spaces between maturing corneocytes where it is broken down into amino acids and their derivatives which provide the hygroscopic elements to allow skin to remain hydrated [44]. Filaggrin undergoes proteolysis to histidine and then further to acidic breakdown products of histidine but is inhibited by high humidity, [19] a characteristic found in HS lesions. Surprisingly,

filaggrin was found to be increased in the majority of MSSE in HS lesions. Increased expression of Ephrin type-A receptor 2 (EphA2), a receptor tyrosine kinase, has been associated with a poorer prognosis in oesophageal carcinoma [45]. In the current study, increased expression of EphA2 was found in approximately half of HS lesions.

One significant limitation when evaluating phospholipase or other enzymatic activity in this study is that fixed tissues typically exhibit reduced or absent enzymatic reactivity when compared to activity seen in fresh tissues [46]. While many of the enzymatic antigenic sites assayed with IHC methods did result in positive marking of some tissue elements, it is possible that other assayed enzymes were destroyed by the fixation and processing modifications on tissues leading to possible false negative or falsely decreased expression results. A second relevant limitation is that several antigenic sites assayed in the SC layer where cell remnants are tightly cemented together may have been unavailable for labelling due to inaccessibility of antibodies and IHC reagents [47]. A third limitation of this study is found in interpretation of cause and effect of proteins in HS lesions. While much confidence resides in the interpretation of increased/decreased protein expression in HS lesions on the tissue sections investigated, it cannot be exclusively determined whether certain proteins are the causative reasons for HS lesion development, or whether their aberrant expression is a natural downstream response after formation of HS lesions. A fourth and final limitation of this study is in the sample size. Ten HS samples are unlikely to be representative of the entire HS population.

With over 3 billion nucleotides and 20,000–25,000 genes in the human genome, it is expected that each person has on average 2 proteins which are mutated, producing varying degrees of disease [48]. Some mutations are silent insofar that the substituted nucleotide(s) produce minimal change to the protein structure and function. Often, even a nonsense mutation which truncates a needed protein may produce minimal disease if the body has collateral mechanisms which provide a function similar to the missing protein. While gene mutations may be responsible for increased/decreased protein expression in persons with HS, it is more likely that epigenetic changes regulate the changes in expression seen in this study. Additionally, many diseases produce a varied clinical picture based on the physiology of individual subjects. Moreover, two people with the same manifestations of a disorder may dispute the disease severity due to differences in perception. In patients with clear clinical HS symptoms, the severity may be dependent upon one or more genetic mutations, epigenetic changes, or may depend, in part, upon the specific physiological make-up of the individual and how well his/her body responds to signaling pathways.

CONCLUSION

In this study, compared to normal apocrine-bearing skin, HS lesional skin displayed altered expression of A2ML1, CK 1/10, CK 5/6, desmocollin 2/3, desmoglein 2, elafin, envoplakin, EphA2, filaggrin, involucrin, KLK6, KLK7, LEKTI, and plakophilin III. Because aberrant expression was found in 14 epithelial proteins in HS lesions, this study suggests that multiple gene defects or epigenetic changes could be responsible for the variation of severity in persons afflicted with HS.

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AUTHOR CONTRIBUTIONS

SC was responsible for study design, experiments, and manuscript drafting.

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