Preliminary findings suggest hidradenitis suppurativa may be due to defective follicular support

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Accepted for publication
9 January 2013

Funding sources
None.

Conflicts of interest
None declared.

DOI 10.1111/bjd.12233

Summary

Background The initial pathology in hidradenitis suppurativa (HS)/acne inversa takes place in the folliculopilosebaceous unit (FPSU) and its surrounding tissue. The process involves follicular hyperkeratosis, inflammation and perifolliculitis. Identification of the exact origin of inflammation may shed new light on the pathogenesis and aetiology of the disease.

Objectives To study the morphology of the basement membrane zone (BMZ) in patients with HS.

Methods In total, 65 operative specimens from 20 patients diagnosed with HS were cut stepwise. Within each specimen, the focus was set on heavily involved HS regions (centre) and clinically uninvolved regions (border). All specimens were stained with periodic acid–Schiff (PAS) to visualize the epithelial support structures of the FPSU (i.e. the BMZ), the sinus tracts (STs) and the interfollicular basement membrane (BM). The intensity of BMZ PAS staining was graded from 0 to 4+.

Results Compared with the axillary skin of human controls, the sebofollicular junction in patients with HS was found to be almost devoid of PAS-positive material (grade 0/1+) in both the border and centre lesions of HS, whereas STs and BMs showed uniformly grade 2–3+ positivity irrespective of any inflammation present. The distribution of inflammatory cells around the sebofollicular junction occurred predominantly in areas of BMZ thinning.

Conclusions The BMZ PAS positivity of clinically uninvolved FPSUs of patients with HS appears to be wispy or not present at all. It is speculated that this may explain the apparent fragility of the sebofollicular junction. There is an increased concentration of inflammatory cells adjacent to these areas, while inflammatory cells are scarce in areas where the PAS-positive material is intact. It is hypothesized that the PAS gap identifies (i) areas susceptible to leakage, trauma and rupture, leading to release of materials that trigger inflammatory mediators, and (ii) the seeding of the dermis with free-living stem cells generating benign but invasive epithelialized sinuses, spreading horizontally in and below the dermis.
point – of inflammation may therefore shed new light on the pathogenesis and aetiology of the disease.

Mechanical trauma is implicated as a possible aetiopathological factor in HS. Combined with the fact that inflammation starts deep in the follicle and continues laterally, producing aggressive intradermal and subcutaneous extensions in particular, this suggests the possibility of a weakness in the wall of the FPSU, which we propose to study by describing the gross morphology of the basement membrane zone (BMZ) in patients with HS.

**Material and methods**

Operative specimens were obtained by wide excision at the Department of Dermatology and Venereology at Martin-Luther-University Halle-Wittenberg, Germany, and at the Department of Dermatology, Roskilde Hospital, Health Sciences Faculty, University of Copenhagen, Denmark, between 2009 and 2012, from 20 patients with diagnosed HS [16 women and four men, mean age 39–45 years (range 22–55 years)]. The specimens, from typical HS locations, were cut stepwise. The first specimen (i) was obtained from the centre of the lesion, and the second (ii) from the border of the excised area (clinically healthy skin). If the distance between these two regions was more than 30 mm, a third specimen (iii) was collected in between. This method provided a total of 65 specimens (25 from Germany, 40 from Denmark). We examined clinically unaffected skin and affected skin.

An additional single specimen was examined: a 5-mm punch biopsy of an HS lesion < 48 h old from the inframammary area of a 32-year-old woman, representing the earliest clinically symptomatic lesion in order to provide further insight into the life of lesions (uninvolved, subclinical, early symptomatic). All sections were fixed in 4% formaldehyde and embedded in paraffin. All were stained with haematoxylin and eosin and periodic acid–Schiff (PAS). The PAS reaction was used to visualize the epithelial support structures (i.e. the BMZ) investing the FPSU, as well as the STs and the interfollicular basement membrane (BM). The 65 slides were blinded and randomized. All areas of all FPSUs present, as well as the STs and BMs, were scanned from top to bottom and were compared from slide to slide, with attention being given to finding and describing variations in histomorphology. The intensity of positive PAS staining was classified from grade 0 to 4+. PAS staining of grade 4+ was defined as strong staining evidence at objective 2× and 4× magnification; 3+ as strong staining evidence at objective 10×; 2+ as moderate staining evidence at objective 20×; 1+ as weak staining evidence at objective 40×; and 0 as no staining intensity at objective 40× magnification. Grade 4+ indicated the brightest staining, as seen in the material investing the eccrine and apocrine glands.

Results

**Interfollicular epidermal basement membrane**

A PAS-positive-stained epidermal BM was a constant feature beneath the interfollicular epidermis. It was continuous and showed uniform (3+) positivity (Table 1, Fig. 1a). Nowhere in the slides examined was there any evidence of thinning, fragmentation or loss of continuity of subepidermal PAS-positive BM. These observations apply to both the border and central regions of HS.

**Control group**

The PAS-positive material investing the FPSU and forming the BM in the control group showed consistent and uniform 3–4+ positivity (Table 2, Fig. 2i).

**Folliculopilosebaceous unit**

In all 65 slides the seboc follicular junction of the FPSU in patients with HS was found to be almost devoid of PAS-positive material (Table 1). In several of these seboc follicular junction ‘PAS gap’ areas there were increased concentrations of chronic inflammatory cells that were generally present only where the PAS positivity was wispy or missing entirely; this was seen in the centre and border regions of HS (Figs 1b,c and 2c: central areas; Figs 1d–f and 2a: border regions). This significance is underlined by the absence of inflammatory cells in nearby areas where the PAS material is intact (Fig. 2a,c).

**Sinus-tract formation**

The PAS-positive material surrounding the ST formations arises early in their organization, and is uniformly dense and homogeneous grade 2–3+ (Table 1, Fig. 2d,e). It is noteworthy that, in the midst of the aggressive and destructive cellular inflammatory response, the newly generated structures that eventually produce the epithelium-lined sinuses of HS show unequivocal homogeneous (grade 2–3+) PAS positivity (Fig. 2f,g).

**Early hidradenitis suppurativa lesion**

The seboc follicular junction of the FPSU in an early HS lesion (< 48 h old) was found to be almost devoid (grade 0/1+) of PAS-positive material (Fig. 2b).
The BMZ stains a uniform bright pink with the PAS process. The PAS-deficient areas furthermore show an apparently incomplete (sebaceous glands missing) sinuses. Sinus formations are scarce.

**Discussion**

The BMZ stains a uniform bright pink with the PAS process. The morphology of the PAS-positive BMZ appears altered in the area of the FPSU where the sebaceous duct joins the folliculopilosebaceous units proper. However, artefacts may play a role in qualitative and semiquantitative histological studies. It is suggested that the large number of slides examined and the general identification of the expected PAS-positive (grade 3+) epidermal BM (in the interfollicular epithelium and the surrounding other adnexae) support the validity of the observations.

The PAS-deficient areas furthermore show an apparently increased concentration of chronic inflammatory cells, the character of which is described elsewhere. The significance of these infiltrates is underlined by the absence of inflammatory cells in adjacent areas where the PAS material is intact. The PAS-negative gap is thought less likely to be a secondary effect of inflammation because it is found exclusively in the seb follicular junction area, whereas obviously intact PAS positivity is noted in several highly inflamed apocrine gland areas (Fig. 2h). This supports the interpretation that the BM thinning is a primary event rather than secondary to inflammation [Fig. 2a (border HS region) and Fig. 2c (centre HS region)].

The collagen IV filaments of the BMZ provide mechanical support and are ensheathed in glycoproteins that, because they contain the carbohydrate component, provide an unmistakable marker for the collagen. In addition, proteoglycans are anchored into the membrane by an unknown mechanism, providing additional PAS positivity. Collagen IV also provides a scaffold for other structural macromolecules. Laminin–entactin/nidogen glycoprotein complexes self-associate into less-ordered aggregates, and laminin also binds to sulfated glycolipids, providing additional sources of PAS positivity. We suspect that PAS staining may be a useful initial marker giving insight into the BMZ structure as a whole. Thus our study suggests the presence of a structural defect of the BMZ in HS. Further studies will be needed to identify the exact background in detail, for example focusing on collagen IV, laminin, integrin, nidogen, perlecan or dystroglycan by means of immunohistochemistry, confocal laser microscopy and electron microscopy.

The dermoeipidermal BMZ that makes up the interface between the horizontal sheet of epidermal basal cells and the underlying dermis is complex. The structure comprises a web of protein fibres, specialized glycoproteins with extra carbohydrates called proteoglycans, and glycolipids. The structure more closely resembles felt fabric than gelatin. It may therefore be hypothesized that the ‘PAS-negative gap’ indicates a more tenu-
ous structure that, when damaged by physical trauma, permits the release into the dermis of intrafollicular materials that act as stimulants of the innate immune system and antigens for the adaptive immune system, thereby stimulating the inflammatory activity. This might explain the surprising existence of small amounts of keratin fragments in early HS lesions reported by van der Zee et al., material one would normally expect in later, clinically remarkable HS lesions, due to rupture of an inflammatory and mechanically stressed dilated hyperplastic follicle, leading to foreign-body reactions.

Table 2. Control group (nonhidradenitis suppurativa morphology): classification/intensity of periodic acid–Schiff (PAS) reaction positivity (grade 0–4+) in a control group, in the epidermal interfollicular basement membranes (BMs) and the folliculopilosebaceous units (FPSUs) compared with an internal slide control (PAS staining of eccrine and apocrine glands)

<table>
<thead>
<tr>
<th>Specimen ID</th>
<th>Age (years)/sex</th>
<th>Diagnosis</th>
<th>Location</th>
<th>BM (PAS)</th>
<th>FPSU (PAS)</th>
<th>Control (PAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1344-09</td>
<td>58/M</td>
<td>Naevus</td>
<td>Axilla</td>
<td>3+/4+</td>
<td>3+/4+</td>
<td>4+</td>
</tr>
<tr>
<td>2133-09</td>
<td>42/M</td>
<td>Naevus</td>
<td>Axilla</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>6562-09</td>
<td>72/M</td>
<td>Scar</td>
<td>Axilla</td>
<td>3+</td>
<td>3+/4+</td>
<td>4+</td>
</tr>
<tr>
<td>126-10</td>
<td>38/F</td>
<td>Naevus</td>
<td>Axilla</td>
<td>4+</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td>384-10</td>
<td>67/M</td>
<td>Fibroepithelioma</td>
<td>Axilla</td>
<td>3+</td>
<td>3+</td>
<td>ns</td>
</tr>
<tr>
<td>1161-10</td>
<td>68/F</td>
<td>Fibroepithelioma</td>
<td>Axilla</td>
<td>3+/4+</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>1251-10</td>
<td>48/M</td>
<td>Fibroepithelioma</td>
<td>Axilla</td>
<td>3+/4+</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>2408-10</td>
<td>43/F</td>
<td>Naevus</td>
<td>Axilla</td>
<td>4+</td>
<td>3+</td>
<td>4+</td>
</tr>
<tr>
<td>3537-10</td>
<td>51/M</td>
<td>Dermatofibroma</td>
<td>Axilla</td>
<td>3+</td>
<td>3+</td>
<td>4+</td>
</tr>
</tbody>
</table>

M, male; F, female; ns, not seen.

Specialization of different BMZs is achieved through the presence of tissue-specific isoforms of laminin and collagen IV, and of particular proteoglycan populations, by differences in assembly between different membranes, and by the presence of accessory proteins in some specialized BMs. These structural differences may prove to be important in the explanation of the defect that is postulated here as a possible base cause of HS, and may relate our findings to earlier observations.

Local tissue-specific and location-specific impairment of BMZ formation has been demonstrated in genetically modified
In the absence of nidogens these animals show normal BMZ formation beneath the epidermis, while BMZ formation in the dermal capillaries is severely impaired, showing an irregular, patchy distribution and a dramatically reduced deposition of collagen IV, perlecan and particularly laminin-411, indicating that very localized and specific genetically determined defects in BMZ formation can occur. A search for a parallel genetic defect responsible for expressing such loss of functionality in patients with HS is therefore of interest, especially in view of the fact that, for example, nidogen is localized on chromosome 1q43, in proximity to a possible gene locus (1p21.1–1q25.3) that has been described in HS. Nidogen is also close to the region of presenilin, a subunit of the γ-secretase that seems to be involved in HS, at least in its familial type.

The epithelium-lined STs of HS show unequivocally homogeneous grade 2–3+ PAS positivity, indicating that these structures are the fully functioning keratinocytic product of pluripotential stem cells, from the follicular bulge area of the ruptured FPSU as suggested by Gniadecki and Jemec. In summary, PAS staining of the FPSU in patients with HS demonstrates morphological defects that may be interpreted as flaws in the support structure of the neck of the ductus seboglandularis and may explain the apparent fragility of the seb follicular junction of the FPSU. This in turn may render the FPSU susceptible to leakage, trauma and rupture, producing a situation in which shearing forces lead to release of materials (keratin fragments and other materials) that trigger inflammatory mediators, and the seeding of the dermis with free-living stem cells that populate the gelatinous proliferative mass found in these lesions. This activity generates benign but aggressive horizontally invasive epithelialized sinuses. These in turn perpetuate the scarring process that characterizes HS.
What's already known about this topic?

• The inflammation that destroys the folliculopilosebaceous unit (FPSU) in hidradenitis suppurativa (HS), or acne inversa, appears to start in the follicular portion of the unit.
• Recent work demonstrates (i) a psoriasiform pattern in the interfollicular epidermis, (ii) inflammatory activity concentrated in the perifollicular area, (iii) loss of volume and disappearance of sebaceous glands, and (iv) invasive sinus tracts, apparently the product of stem cells originating from the follicular bulge area.

What does this study add?

• The periodic acid–Schiff (PAS)-positive equivalent of the FPSU in HS is missing, wispy or fragmented in patients with HS, particularly near the sebofollicular junction in early, border and centre lesions of HS compared with control skin.
• In contrast, the PAS positivity is strong and homogeneous at the dermoepidermal junction, and surrounding both the apocrine and eccrine glands and ducts in the dermis of patients with HS, irrespective of adjacent inflammation.

Acknowledgments

The authors wish to thank Mrs Christel Lindhof and Mrs Anja Lippert (Halle-Saale, Germany) for excellent technical assistance; Dr Jan von der Werth (St Leonards on Sea, U.K.) for supplying the initial tissue that stimulated this investigation and Dr Birgit Nürnberg (Roskilde, Denmark) for supplying additional material.

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