Forms of epithelial differentiation of draining sinus in acne inversa (hidradenitis suppurativa)

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Summary

The draining sinus is a late complication of several forms of severe acne, leading to extensive, periodically inflamed lesions that are undermined by a system of fistulas, supposed to be of follicular origin. We investigated the expression of various cytokeratins (CKs) and desmosomal proteins in the draining sinus of acne inversa (hidradenitis suppurativa) using monoclonal antibodies in immunohistochemistry on paraffin-embedded sections. We were able to define three different phenotypes of stratified squamous epithelia covering the sinus tracts. Type I epithelium was cornifying and characterized by the presence of CK 10, desmogleins 1–3 and desmocollins 1–3 in an epidermis-like pattern. Type II epithelium was non-cornifying, negative for CK 10 and positive for CK 13. It was negative for desmocollin 1 but strongly immunopositive for desmoglein 1 suprabasally and for desmoglein 2 in the basal cells. Type III epithelium was non-cornifying and strongly inflamed. It was marked by the presence of CK 7, CK 19 and desmoglein 2 and the absence of CK 10, desmoglein 1 and desmocollin 1. In both type II and III epithelium, desmoglein 3, desmocollin 2 and desmocollin 3 showed an inverted staining pattern as compared with normal epidermis and type I epithelium. Desmoglein 2 and CK 5/14 always remained restricted to the basal cell layer. Antibodies against CK 6 and CK 13/15/16 were immunopositive in all three phenotypes and CK 17 was predominantly immunolocalized to suprabasal layers of type II and III epithelium. The three phenotypes are characterized as pathological stratified squamous epithelia reflecting the dynamic process of inflammation, proliferation and stratification taking place in acne inversa.

Key words: acne inversa, cytoskeleton, desmosome, epidermis, hidradenitis suppurativa, inflammation, squamous epithelium

Acne inversa, also known as hidradenitis suppurativa and acne tetrad,1–3 like acne vulgaris, is believed to be caused by follicular obstruction. Bacterial infection results in a rupture of the follicular canal and subsequent granulating inflammation.4 The draining sinus is a late complication of acne inversa, leading to extensive, periodically inflamed lesions that are undermined by a system of fistulas. They extend predominantly into the dermis in a leaf-like pattern and are lined by a variably thickened stratified epithelium. Surrounding collagenous or fatty tissue does not appear to be compressed, suggesting infiltrative rather than expansive growth.5–7

Cytokeratins (CKs), the main components of the epithelial intermediate filament cytoskeleton, are well-established differentiation markers of epithelial cells.8–15 Desmosomes are adhesive intercellular junctions that connect the CKs to the cell membrane. Desmogleins (Dsgs) and desmocollins (Dscs) are the main desmosomal transmembrane proteins. They belong to the cadherin family of calcium-dependent cell adhesion molecules. Three different isoforms of Dsg and Dsc, termed Dsg1–3 and Dsc1–3, can be distinguished. Their cytoplasmic portions are associated with a variety of plaque proteins, including common ones such as desmoplakin (DP) I and plakoglobin (PG), and also cell type-specific ones such as DP II and plakophilin (PP) 1 and 2.16–21 The desmosomal cadherins show a differentiation-specific expression in epithelia and can

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therefore serve, in addition to the CKs, as an independent system of differentiation markers. As isoform-specific antibodies have as yet been only incompletely available, information about the distribution of desmosomal cadherins in pathological epithelia is still scarce. We examined the epithelial differentiation of draining sinus in acne inversa using monospecific CK protein antibodies and desmosomal protein antibodies in order to study the influence of chronic inflammation and hyperproliferation on cytoskeletal and desmosomal proteins.

Materials and methods

We examined skin samples from 15 patients obtained during surgical removal of chronically inflamed lesions from the axilla and the groin. One part of the samples was routinely formalin-fixed and paraffin-embedded, and the other part was snap-frozen in liquid nitrogen and stored at −70°C until use. Only samples histologically diagnosed as acne inversa were used for immunohistochemistry.

For most antibodies, pretreatment using microwave oven heating (three or four times, 5 min, 600 W) for some antibodies, followed by incubation with 0.001% trypsin, was necessary to restore antigen recognition from 3- to 4-µm thick paraffin sections. The following primary antibodies were used: monoclonal antibodies (MoAbs) DP 1+2–2.15 and DP 1–2.17 against DP I+II,28 PG 5.129 and PG 11E4 (Dr M.J.Wheelock, Toledo, OH, U.S.A.) against PG, Dsg1E-P124 and Dsg1E-P23 against Dsg1,22 Dsg2E-G129 and Dsg2E-G96 against Dsg2,18 Dsg3-G194 and 5G11 against Dsg3,22 Dsc1-U100 against Dsc1,19 DC-Rab 36 (rabbit polyclonal) against Dsc2,10 MoAb Dsc3-U114 against Dsc3,19 PP1-9E7 and PP1-5C2 against PP 1,31 PP2-150 against PP 2,20 Ks2-342-7.1 against CK 2e (Dr L.Langbein, Heidelberg, Germany), 6B10 against CK 4,32 AE 14 against CK 5,33 Ks6.KA12 against CK 6,34 OV-TL 12/3015 and KS7-18 against CK 7,27 CAM 5.2 against CK 8,36 H9TY1 (guinea-pig polyclonal) against CK 9 (Dr L.Langbein), MoAbs K8-6037 and DE-K10 against CK 10,38 Ks8-12 against CK 13+15+16,35 Ks13-1 against CK 13,39 D27 against CK 13,12 LL001 against CK 14,40 Ks17.E3 against CK 17,41 Ks19-1 against CK 19,42 IT-Ks20-10 against CK 209 and MIB 1 against Ki-67.43

Sections were blocked in 10% normal horse serum in phosphate-buffered-saline (PBS). Incubation time for the primary antibody was 1 h at 37°C and for the secondary antibody (biotinylated horse antibodies against mouse IgG and biotinylated goat antibodies against rabbit or guinea-pig IgG, all from Vector, Burlingame, CA, U.S.A.) 30 min at room temperature in a Shandon Sequenza apparatus (Shandon-Life Sciences International, Frankfurt/Main, Germany). The avidin–biotin complex method (ABC Elite kit) was used according to the manufacturer’s instructions (Vector) with 3,3′-diaminobenzidine and H2O2 being employed for the staining reactions. For control purposes, immunohistochemical stainings were performed on acetone-fixed cryostat sections cut from the snap-frozen tissue specimens, employing the indirect immunoperoxidase method.44 In the case of the Dsc MoAbs, prior to their application, sections were incubated for 30 min with 5% goat serum in PBS containing 0.2% Triton X-100 and 2% dried milk powder.

Results

On histological examination, the samples chosen showed different stages of sinus tract formation with variable degrees of inflammatory activity. Cornifying stratified squamous epithelium (type I) could be distinguished from non-cornifying epithelia, the latter being either barely (type II) or strongly inflamed (type III). Strongly inflamed areas often bordered ulcerative epithelial defects. The epithelium close to the openings of the sinus tracts was cornifying (type I) in all specimens examined. In two cases, free hair shafts were found in the sinus and in the surrounding dermis, without apparent connection to the epithelium. In two patients, reticulate and filiform downgrowths resembling Dowling–Degos disease were found.45,46 Based on the above-mentioned histological subdivision, immunohistochemical analysis of cytoskeletal and desmosomal differentiation markers enabled definition of three different phenotypes (types I–III) of stratified squamous epithelia covering the sinus tracts. The results are summarized in Table 1.

MoAb Ks8-60 against CK 10 (weakly also against CK 1) and MoAb DE-K10 against CK 10 strongly labelled the suprabasal and granular layers of the orthokeratotic and hyperkeratotic parts of the epithelium (type I) while the non-cornifying and inflamed parts (type II and type III) remained negative (Fig. 1a). MoAbs AE 14 against CK 5 and LL001 against CK 14 stained most basal cells of the sinus tract epithelium (Fig. 2a). In some parts staining was very weak. CK 2e, CK 4 and CK 9 could not be detected at all. MoAb Ks6.KA12 against CK 6 and MoAb Ks17.E3 against CK 17 strongly stained suprabasal layers of the non-cornifying parts (types II and III).
of the sinus tracts. Cornifying parts (type I) showed a variable staining (Figs 1b and 2d) correlated to the proliferation activity as indicated by staining of basal and parabasal cells with MIB 1 antibody (not shown). CK 17 immunostaining was slightly less extended than CK 6 reactivity. MoAb Ks8’12 against CK 13, 15 and 16 strongly immunolabelled the suprabasal cell layers of all parts of the sinus, while MoAb 2D7, specific for CK 13, showed a positive reaction predominantly in the non-cornifying, weakly inflamed part of the sinus (type II) (Fig. 1c). Strongly inflamed parts of the epithelium, which were not cornifying (type III), were intensely immunolabelled by MoAb Ks19’1 against CK 19 (Fig. 2b) and in the intermediate and uppermost layers by MoAbs OV-TL 12/30 and Ks7’18 against CK 7 (Fig. 2c). The other simple epithelial CKs, i.e. CK 8 and CK 20, remained negative in all phenotypes. Two cases showed focal accumulations of CK 8-positive and CK 20-positive Merkel cells in the sinus.

The desmosomal plaque proteins DP I/II, PG and PP 1 were immunolocalized to all living layers of the sinus tract epithelium. Only in CK 19-positive parts, i.e. in non-cornifying inflamed parts (type III), desmosomal staining was patchy and weak (not shown). PP 2 was not detected. Differential staining patterns were obtained for the various desmosomal cadherins. Dsg1-specific antibodies immunostained all suprabasal cells in the hyperkeratotic parts (type I) and weakly inflamed non-cornifying parts of the sinus (type II). No Dsg1 staining was seen in CK 19-positive parts (type III). Dsg2 antibodies showed only a very weak staining of the basal layer of the overlying epidermis and hyperkeratotic parts of the sinus (type I), while the non-cornifying parts (types II and III) were characterized by a strong labelling of the basal layer. MoAb 5G11 against Dsg3 immunolabelled basal and lower suprabasal layers of the hyperkeratotic parts (type I) while in the non-cornifying parts (types II and III) all layers were positive (Fig. 3).

Upper layers of the hyperkeratotic parts of the sinus (type I) were clearly immunopositive for MoAb Dsc1-U100 against Dsc1. The staining intensity correlated well with the degree of cornification and was less extended than CK 10 antibody reactivity. The

Table 1. Staining patterns in the epidermis and the sinus epithelium of acne inversa

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Epidermis</th>
<th>Type I cornifying</th>
<th>Type II non-cornifying</th>
<th>Type III non-cornifying, strongly inflamed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desmoplakin 1+2</td>
<td>++</td>
<td>+</td>
<td>+</td>
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<td>Plakoglobin</td>
<td>++</td>
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<td>Plakophilin 1</td>
<td>++</td>
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<td>Plakophilin 2</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Desmoglein 1</td>
<td>++(B, S)</td>
<td>+</td>
<td>++(S)</td>
<td>+</td>
</tr>
<tr>
<td>Desmoglein 2</td>
<td>++</td>
<td>+</td>
<td>++(B)</td>
<td>+</td>
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<tr>
<td>Desmoglein 3</td>
<td>++(B, P)</td>
<td>+</td>
<td>++(B, P)</td>
<td>+</td>
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<tr>
<td>Desmocollin 1</td>
<td>++(upper S, G)</td>
<td>+</td>
<td>++(upper S, G)</td>
<td>–</td>
</tr>
<tr>
<td>Desmocollin 2</td>
<td>++(+B, +S)</td>
<td>+</td>
<td>++(+B, +S)</td>
<td>+</td>
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<tr>
<td>Desmocollin 3</td>
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<td>++(B, P, +S)</td>
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<tr>
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<td>–</td>
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<td>+</td>
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<td>CK 20</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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<tr>
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<td>+</td>
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<td>++(B, P)</td>
<td>++(B, P)</td>
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</table>

CK, cytokeratin; B, basal layer; G, granular layer; P, parabasal layer; S, suprabasal layer; without indication, all layers; ++, strong staining; +, weak staining; ±, weak or no staining; –, no staining.

*CK 9 is positive in palmpantar epidermis.
non-cornifying parts (types II and III) were immunonegative. The Dsc2 polyclonal antibody which in normal epidermis and the hyperkeratotic parts (type I) preferentially stained desmosomes of the basal and lower suprabasal layer, extended its reactivity to all layers in the non-cornifying parts (types II and III). MoAb Dsc3-U114 against Dsc3, which labels evenly all living layers of the scalp epidermis and type I epithelium, was predominantly immunolocalized to the basal and lowest suprabasal layers in the non-cornifying parts (types II and III). The inflamed areas (type III and, to a lesser extent, type II) showed an altered staining pattern for Dsc2 and Dsc3, caused by an intercellular oedema (Fig. 4).

Discussion

On the basis of the present results, the stratified squamous epithelium covering the draining sinus of acne inversa can be divided into three different phenotypes with characteristic features concerning both morphology and marker proteins: type I, cornifying with orthokeratotic and parahyperkeratotic parts, and characterized by the presence of CK 10, Dsg1–3 and Dsc1–3 in an almost epidermis-like pattern; type II: non-cornifying and moderately inflamed, negative for CK 10 and positive for CK 13, and negative for Dsc1 but strongly immunopositive for Dsg1 suprabasally and Dsg2 in the basal cells; and type III: non-cornifying and strongly inflamed, and marked by the presence of CK 7, CK 19 and Dsg2 and the absence of CK 10, Dsg1 and Dsc1. An intercellular oedema leading to an altered staining pattern of antidesmosomal antibodies and a strong infiltration with leucocytes are indicators of the highly inflammatory process.

The non-cornifying character of type II and III epithelia as opposed to type I is underlined by the absence of the terminal differentiation markers CK 10 and Dsc1, and by the strong expression of Dsg2 in the basal layer. Dsg3, Dsc2 and Dsc3 show an inverted staining pattern as compared with normal epidermis

Figure 1. The three different phenotypes of pathologically altered epithelium covering the draining sinus in acne inversa. (a) Antibody DE-K10 against cytokeratin (CK) 10 stains orthokeratotic to parahyperkeratotic parts designated type I. (b) Parallel section to (a), stained with Ks6.KA12 against CK 6. Both phenotypes I and II are labelled. (c) CK 13 is predominantly positive in type II epithelium. Note the lack of cornification and moderate inflammatory activity in the positive parts. (d) Transition from type II to type III is marked by the sudden onset of CK 19 immunoreactivity and strong inflammatory reaction. D, dermis; L, lumen of the sinus. Bars: a–c, 200 μm; d, 100 μm.
in type II and III epithelia, i.e. Dsc2 and Dsg3 are present in all layers while Dsc3 is restricted to the basal and parabasal layer. In normal epidermis and type I epithelium, the opposite pattern is present. The inflammatory character of type III epithelium as opposed to type I and II is marked by the presence of CK 7 and 19 and the absence of Dsg1. All three types are clearly distinct from interfollicular epidermis because of the absence of CK 2e and the presence of CK 6 and CK 13/15/16. Although there are similarities in the CK patterns with several other normal stratified squamous epithelium27 and in some aspects also with fetal epidermis,45 there is in no case complete accordance, reflecting the fact that sinus tract epithelia have undergone pathologically altered differentiation programmes. The three phenotypes were not in all cases sharply defined, and transitional stages were often found. This reflects the dynamic and pathologically altered processes of growth and inflammation in the sinus epithelia. A similar situation was reported previously in inflammatory processes of the human gingiva. As in the draining sinus of acne inversa, the strongly inflamed parts of the gingiva were reported to be marked by an upregulation of CK 19 and downregulation of CK 1/10.59,50 Unlike in the gingiva, we found in all CK 19-positive sinus epithelia also a variably strong reaction for CK 7 in the intermediate and uppermost cell layers. This difference, however, might be due to different sensitivities of the particular CK 7 antibodies used. We were not able to demonstrate a positive reaction for CK 4, even though our antibody 6B10, reacted strongly in positive controls and the usual type I CK partner of CK 4, CK 13, was found to be expressed in type II epithelium.

CK 19 is commonly found in simple epithelia, the basal cells of non-cornifying stratified squamous epithelia like in the oral cavity, in the outer root sheath of the hair follicle and in different carcinomas.47,51–54 The strongly inflamed CK 19-positive parts of the sinus epithelium showed no signs of terminal squamous differentiation, with nuclei present in the highest suprabasal layers and absence of keratoahyalin granules. Instead, they resembled epidermal keratinocytes grown in organotypic culture, which can be induced to build a non-cornifying epithelium and to express CK 19 together with CKs 4, 13 and 7, by the addition of retinoic acid to the culture medium.55,56 It would be interesting to examine the expression of retinoic acid and retinoid X receptors in the different epithelial phenotypes of the draining sinus. Retinoids are known to induce a differentiation shift in keratinocytes which thereby acquire certain features of simple glandular epithelia.56 Type III epithelium expressed some markers of such epithelia while keratinocyte maturation markers were reduced. This may be interpreted as a kind of metaplasia towards glandular differentiation.

A previous report on the distribution of desmosomal proteins in acne vulgaris25 showed a reduced staining intensity of Dsc3, Dsg1 and Dsg2 antibodies, while the other isoforms were not analysed. We have been able to use the complete set of isoform-specific antibodies against all desmosomal cadherins known so far. Studies from various laboratories have shown that the different members of this family of cell–cell adhesion molecules are expressed in different characteristic patterns depending on the epithelial cell type and state of differentiation and maturation.16,18,19,21,56 The highest complexity of desmosomal cadherins is elaborated in stratified squamous epithelia, with Dsg1 and particularly Dsc1 being linked to the terminal differentiation of keratinocytes. Dsg2, a constituent of desmosomes of most types of epithelia, is restricted within stratified squamous epithelia to the undifferentiated basal cells.18 However, it is expressed more abundantly, including in suprabasal layers, in fetal
stratified epithelium of the oesophagus (L.Schwetlick et al. personal communication). This is an example of the dynamic changes in the expression patterns of desmosomal cadherins which take place during embryonic development of stratified squamous epithelia until the mature state is reached.\textsuperscript{21,22} In the adult, suprabasal extension of Dsg2 is also a feature of the primitive stratified squamous epithelium seen in immature squamous metaplasia of the uterine cervix (L.Schwetlick et al. personal communication). Our finding of the restriction to the basal layer of Dsg2 even in the most altered type III sinus tract epithelium indicates that despite heavy inflammation, the degree of immaturity is not as pronounced as in early fetal stage or immature squamous metaplasia. The differential immunohistochemical data obtained for the six desmosomal cadherins allowed us to discriminate between the different epithelial subtypes and to show that not only the degree of keratinization and proliferation but also the inflammatory activity influences the expression patterns of the Dsc and Dsg isoforms. To what extent different isoforms result in differential adhesive properties of desmosomes remains to be elucidated.\textsuperscript{57}

The relationship of the sinus epithelium to the hair follicle and to apocrine glands has been a matter of debate for a long time.\textsuperscript{2} As we have shown, its differentiation characteristics are clearly distinct from those of the normal subinfundibular outer root sheath of the hair follicle. CK 5/14 and Dsg2 always remain restricted to the basal cell layer of the sinus epithelium, while in the subinfundibular outer root sheath they are also expressed in the suprabasal cell layers. Type I epithelium, which was found close to the opening of the sinus in all specimens examined, showed strong similarities to the upper pilosebaceous duct from which the inflammatory process seems to emerge. In both normal pilosebaceous duct epithelium and cornifying type I sinus epithelium, CK 5 and CK 14 are restricted to the basal layer, CK 10 and Dsc1 antibodies label suprabasal cells, Dsg1 and Dsg3 are present in an epidermis-like pattern and CK 2e is absent. This is in agreement with the theory that acne inversa lesions are caused by follicular plugging and subsequent rupture of the follicle epithelia.\textsuperscript{58} Residual fully keratinized hair shafts found in the dermis and the sinus lumen of several specimens could be remnants of the ruptured follicles. However, similarities in the patterns of differentiation markers are no definite proof of a histogenetic relationship as
modulations of cellular differentiation may occur, e.g. under environmental influences such as inflammation. Thus, the question whether the sinus epithelium is derived from the infundibulum or the deeper hair follicle cannot be fully clarified by analyses of such markers. The interesting point is that all three types of sinus epithelium identified by our marker studies apparently lack a normal counterpart and thus can be regarded as pathological phenotypes. It might be rewarding to investigate whether the presented data are of value in differentiating acne inversa lesions from other causes of fistulas like Crohn’s disease, which has been reported to be associated with acne inversa.59,60

In conclusion, the epithelial lining of the draining sinus in acne inversa is heterogeneous and comprises three different phenotypes of pathologically altered stratified squamous epithelia defined by the expression of special combinations of CKs and desmosomal proteins. Desmosomal cadherins and CKs have proved to be useful in independently characterizing subtypes of different epithelia.

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