Lipid raft-enriched stem cell-like keratinocytes in the epidermis, hair follicles and sinus tracts in hidradenitis suppurativa


Abstract: Hidradenitis suppurativa (HS) is a disease, that causes considerable morbidity in patients. A histological hallmark of the disorder is the formation of sinus tracts in the dermis and the subcutis. Biologically, they represent a poorly understood phenomenon involving the infiltrative growth of proliferating non-malignant keratinocytes. Lipid domains in plasma membranes (lipid rafts) play a role in the function of growth factors and are suspected of having a pathogenic role in cell migration and invasive growth.

Using HS as a model, the presence of lipid rafts was studied using cholera toxin conjugated with FITC (CTx-FITC) and anti α1 integrin (CD29)-CyChrome conjugate fluorescence staining of unfixed and acetone-fixed cryostat sections of lesional and paralesional skin samples. The double-labeled skin samples were observed in the confocal laser-scanning fluorescence microscope. Samples were obtained from five patients with HS. The lesional epidermis of HS contained three populations of keratinocytes: CD29brightCTxdim, CD29dimCTxbright and a third hitherto unseen population containing double-positive CD29brightCTxbright cells. The CD29brightCTxdim population resembles the earlier described epidermal stem-like cells, while the CD29dimCTxbright basal keratinocytes overlap with the transit-amplifying cell pool. The new population of double-positive CD29brightCTxbright cells was localized on the slopes of the papillas, focally in the suprabasal epidermal layers, in some hair follicles and in the majority of sinus tracts. Such double-positive cells have not previously been encountered by us in normal epidermis and hair follicles.

Using HS as a model, it is suggested that the keratinocytes involved in sinus tract formation are CD29brightCTxbright cells. Owing to the physical proximity of the cells, it is hypothesised that the described CD29brightCTxbright cells result from an increased expression of CD29 on the CTxbright cells. It is likely that the double-positive CD29brightCTxbright cells emerge due to the influence of local inflammatory cytokines.

Sinus tract formation may represent an aberrant epidermal repair response executed by the activated CD29brightCTxbright keratinocytes capable of non-malignant infiltrative growth in the dermis and subcutis.

Key words: hidradenitis suppurativa – keratinocytes – Lipid rafts – sinus tracts

Robert Gniadecki1 and Gregor B. E. Jemec2

1Department of Dermatology, Bispebjerg Hospital, Copenhagen, Denmark;
2Division of Dermatology, Department of Medicine, Roskilde Hospital, Roskilde, Denmark

Introduction

Hidradenitis suppurativa (HS) is a follicular inflammatory skin disease affecting mostly the axillary and perineal skins (1). As apocrine glands are abundant in these regions, HS was initially considered to be caused by the comedonal occlusion of the glandular duct. This theory has, however, not been supported by recent data which suggest that the disease is initiated by a poral occlusion of the follicle resulting in frank folliculitis (2). An important feature of HS is the formation of sinus tracts in the dermis and subcutis, which is thought to occur secondary to follicular inflammation and/or rupture and infiltrative growth of follicle cells in the dermis and subcutis (3). Histologically, the keratinocytes of the sinus tracts appear to grow into the surrounding tissues without any absence of compression of adjacent
structures. Development of sinus tracts is an important pathogenic step, as it is a hallmark of a chronic recurrent course of the disease.

From a biological standpoint, the formation of sinus tracts presents a fascinating, yet poorly understood, phenomenon involving the infiltrative growth of proliferating non-malignant keratinocytes in the dermis and subcutis. Unfortunately, very little information is available concerning the characteristics of the keratinocytes engaged in tract formation. It is conceivable that these cells are metabolically activated into a state enabling proliferation and migration.

Research of the last decade showed that the lipids in plasma membranes are organized into discrete microdomains, so-called lipid rafts (4). Our recent studies revealed that lipid rafts are also present in keratinocytes and play a role in the regulation of their metabolic activity and proliferation (5,6).

In normal human skin, the proliferative activity is confined to the keratinocytes in the basal cell layer. These cells are further subdivided into a stem cell-like population characterized by high expression of CD29 (β1 integrin) and low proliferative activity and the transit-amplifying cell population with low CD29 expression but high proliferative activity (7,8). Our data indicate that transit-amplifying cells are enriched in lipid rafts and the presence of rafts correlated with the mitotic activity of the cells (6). This is understandable in view of the fact that membrane lipid microdomains are the point of insertion of several growth factor receptors, including the epidermal growth factor receptor that is crucial for cell growth and migration (9). Moreover, studies on cancer cell lines revealed the presence of lipid rafts on the leading edge of migrating cells, underscoring the importance of these membrane structures for cell motility.

This report is the first one showing a possible importance of lipid rafts for skin disease.

Materials and methods
In keratinocytes, the lipid rafts are readily demonstrated by the fluorescein-tagged B-subunit of cholera toxin (CTx-FITC) (5). Using the CTx-FITC fluorescence staining, we labeled the unfixed and acetone-fixed cryostat sections of the skin obtained from the lesional and paralesional skins from five patients with HS undergoing routine surgical treatment (2 axillary/3 genitofemoral lesions, m/f: 1/4, age 18–36 years, duration of disease 2–16 years). Moreover, the same sections were stained with the antiβ1 integrin (CD29) antibody directly conjugated with CyChrome (Pharmingen BD Bioscences, Franklin Lakes, NJ, USA). Control buttock skin was sampled from healthy volunteers (n = 4; age 20, 29, 34, and 36 years), and clinically and histologically normal skin was sampled from various locations (arm, trunk, buttock, and lower extremity) of patients with dermatitis and bullous skin was sampled from various locations (arm, trunk, buttock, 20, 29, 34, and 36 years), and clinically and histologically normal skin was sampled from healthy volunteers (n = 4; age 20, 29, 34, and 36 years). Conclusively, phenomenon involving the infiltrative growth of proliferating non-malignant keratinocytes in the dermis and subcutis. Unfortunately, very little information is available concerning the characteristics of the keratinocytes engaged in tract formation. It is conceivable that these cells are metabolically activated into a state enabling proliferation and migration.

Research of the last decade showed that the lipids in plasma membranes are organized into discrete microdomains, so-called lipid rafts (4). Our recent studies revealed that lipid rafts are also present in keratinocytes and play a role in the regulation of their metabolic activity and proliferation (5,6).

In normal human skin, the proliferative activity is confined to the keratinocytes in the basal cell layer. These cells are further subdivided into a stem cell-like population characterized by high expression of CD29 (β1 integrin) and low proliferative activity and the transit-amplifying cell population with low CD29 expression but high proliferative activity (7,8). Our data indicate that transit-amplifying cells are enriched in lipid rafts and the presence of rafts correlated with the mitotic activity of the cells (6). This is understandable in view of the fact that membrane lipid microdomains are the point of insertion of several growth factor receptors, including the epidermal growth factor receptor that is crucial for cell growth and migration (9). Moreover, studies on cancer cell lines revealed the presence of lipid rafts on the leading edge of migrating cells, underscoring the importance of these membrane structures for cell motility.

This report is the first one showing a possible importance of lipid rafts for skin disease.

Results
In histologically normal epidermis of perilesional skin in HS CD29 and CTx-FITC stained mainly the cells in the basal layer and attenuated rapidly towards the spinous layer in the histologically normal epidermis of perilesional skin in HS. As observed earlier in healthy skin, (6) these markers define two non-overlapping subpopulations of keratinocytes: CD29brightCTxdim cells at the tips of dermal papillae and the CD29dimCTxbright cells localized in rete ridges (Fig. 1a). The first population resembles the earlier described epidermal stem-like cells, both with respect to their anatomical distribution and to the high CD29 labeling (7). Thus, it seems likely that the CD29dimCTxbright basal keratinocytes overlap with the transit-amplifying cell pool, i.e. the cells that in normal epidermis proliferate at a higher rate than the stem cells and that migrate to the outer epidermal layers while undergoing terminal differentiation (7).

In the lesional epidermis of HS, we found, in addition to the expected CD29brightCTxdim and CD29dimCTxbright cells, a third population containing the double-positive CD29brightCTxbright cells. The latter cells were often localized on the slopes of the papillas or focally in the suprabasal epidermal layers (Fig. 1b). Cells with similar characeristics were found in some hair follicles (Fig. 1c) and in a majority of sinus tracts, especially in the layers facing the dermis (Fig. 1d–e). We would like to stress here that such double-positive cells have not been encountered by us in normal epidermis and hair follicles, despite extensive research comprising the biopsies from over 30 individuals (RG-unpublished, data on file).

Discussion
Sinus tract formation is an important feature of HS, and a significant contributor to morbidity in other diseases such as inflammatory bowel disease or acne. Data on the possible pathogenesis of sinus tracts, however, remain scarce. In consequence, the therapeutic options remain similarly limited at present.

Lipid rafts (membrane microdomains composed of cholesterol and gangliosides) have recently been described and appear to have potentially relevant functions in the formation of sinus tracts. The lipid rafts provide anchor points for growth factor receptors and are expressed on migrating cells such as keratinocytes involved in wound healing.

Using HS as a model of sinus tract formation, it is suggested that the keratinocytes involved are CD29brightCTxbright cells. It is unclear whether the described CD29brightCTxbright cells emerge from the CD29+ stem-like cells due to the membrane enrichment in lipid rafts or due to the increased
expression of CD29 on the CTxbright cells. We would like to stress that our data are purely descriptive and it is unclear whether the described CD29brightCTxbright cells, emerge from the CD29+ stem-like cells due to the membrane enrichment in lipid rafts or due to the increased expression of CD29 on the CTxbright cells. We favor the last hypothesis in view of the fact that the double-positive keratinocytes were often seen either in deep rete ridges or on the slopes of the papillae, i.e. the regions normally occupied by the transient amplifying cells. The fact that the CD29bright CTxbright cells were also found suprabasally may indicate that induction of both CD29 and the rafts may take place in the differentiating cells, as recently described in healing epidermis (10). It is likely that the double-positive CD29brightCTxbright cells emerge due to the influence of local inflammatory cytokines.

It has long been known that a mechanical damage of the deeper parts of the hair follicles in the Rhino mouse causes sinus tract formation (11). We wish to propose that, in HS, the CD29brightCTxbright cells derived from the epidermis and/or hair follicles migrate from the sites of epithelial damage into the dermis forming sinus tracts. In some sections of the lesional HS skin we found epidermal CD29brightCTxbright cells in a continuum with the underlying sinus tracts (Fig. 1e), an observation that supports this hypothesis. Furthermore, transgenic mice overexpressing β1 integrin (CD29) in the epidermis develop a psoriasiform phenotype characterized by inflammation, epidermal hyperproliferation, and perturbed differentiation (12,13). The CD29bright CTxbright phenotype may be a marker of activated cells, migratory cells, like those seen in cultured keratinocytes or in vivo during wound healing (10). Thus, sinus tract formation may represent an aberrant epidermal repair response executed by the activated CD29brightCTxbright keratinocytes capable of non-malignant infiltrative growth in the dermis and subcutis.

References