No link between rosacea and *Propionibacterium acnes*

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Rosacea is a chronic skin disease affecting worldwide up to 45 million people (1). Rosacea is suggested to be a mainly cutaneous vascular and inflammatory disorder (2). However, cause and pathogenesis of inflammatory changes are still unclear (3). Several infectious agents have been hypothesized to contribute to the promotion of inflammation, namely *Demodex* mites (4), *Helicobacter pylori* (3) or *Staphylococcus epidermidis* (1). To date, microbial involvement in the pathogenesis of rosacea is poorly understood, although antibiotic treatment can be beneficial (2). *Propionibacterium acnes* is a Gram-positive, anaerobic rod and forms part of the normal microbiota of human skin (5). *P. acnes* is considered an opportunistic pathogen, mainly in connection with acne vulgaris (6) and implant associated infection (7). Genome sequence analysis of *P. acnes* has revealed several putative gene products for inflammatory factors that might be involved in *P. acnes*-related diseases (8). Acne and rosacea are both inflammatory conditions and respond to similar treatment regimens. As *P. acnes* has an important role in acne inflammation, it may also have a pathogenic role in rosacea. We designed a case-record archival study to investigate the presence of *P. acnes* in different locations within the skin of rosacea patients. To the best of our knowledge, no study visualizing *P. acnes* in the skin of rosacea patients has been performed.

**MATERIALS AND METHODS**

**Patients**

Rosacea patients with papulopustular variant or subtype 2 (9) were identified by case records and corresponding archival tissues containing facial biopsies were retrieved from the Umeå University Hospital biobank. The patient cohort was clearly biased toward diagnostically difficult patients as biopsies were required to exclude concomitant malignancies. A treatment history was not available.
for a majority of patients. Eighty-two rosacea patients (48 women and 34 males) were enrolled in the retrospective study. Median age of the patients was 53.5 years (range 18–84 years). The control group comprised 25 patients (13 females and 12 males) with different skin disorders (benign sebaceous gland hyperplasia (2), trichoepithelioma (5), seborrhoeic keratosis (4), nevus (5), folliculitis (3), lichenoid eruption (1), basal-cell carcinoma (1), haemangioma (1) and normal skin (3)]. Median age of the control group was 39 years (range 26–83 years). Patients in the rosacea group were significantly older than patients in the control group (p = 0.0351; unpaired Student test). Incisional or puncture facial biopsies were available for rosacea patients, while control specimens were obtained from the face (17), neck (2) and back (6). The number of hair follicles was calculated in every biopsy specimen.

**Immunostaining**

Archival skin biopsies were analysed. After antigen retrieval the samples were stained with a *P. acnes*-specific monoclonal antibody QUBPa3 (10) followed by labelling with anti-mouse IgG-FITC conjugate. The immunofluorescence assay was validated on both *in vitro* grown *P. acnes* and skin specimens from acne vulgaris patients (10, 11). As little as three bacteria can be visualized in a single hair follicle. All the samples were stained with DAPI (4,6'-diamidino-2-phenylindole), which detects the DNA of both bacterial and host cells. Confocal laser scanning microscopic imaging was performed using a Leica confocal microscope (TCS-SPE, Leica Microsystems CMS GmbH, Mannheim, Germany).

**Statistical assay**

Student’s *t*-test and Fisher’s exact test were used for statistical analysis.

**RESULTS**

Skin biopsies from 82 rosacea patients and 25 controls were analysed. The number of hair follicles in patient biopsies (median 3, range 1–8) was lower than in control subjects (median 4, range 1–21) (p = 0.0001; unpaired Student test).

Seven rosacea biopsies (8.5%) and six control samples (24%) scored positive for *P. acnes* (p = 0.0729; two-tailed Fisher’s exact test). In three rosacea samples, *P. acnes* was observed as matrix-encased bacterial clusters attached to the hair follicle walls (Fig. 1), the latter being characteristic of biofilm (12, 13). Bacterial microcolonies in hair follicles as well as a biofilm in the stratum corneum were seen in three and one patient respectively. Positive control samples showed the presence of *P. acnes* as biofilm and microcolonies in both hair follicles and stratum corneum. In two control subjects, *P. acnes* biofilm was evident in both hair follicle and stratum corneum. None of the three control subjects with folliculitis was positive for *P. acnes*. Age distribution for *P. acnes*-positive rosacea patients and control subjects is shown in Table 1. *P. acnes*-positive and -negative rosacea samples contained a comparable number of hair follicles (median 3, range 2–6 vs median 3, range 1–8). In contrast, *P. acnes*-positive control samples contained three times higher the number of hair follicles than negative control samples (median 9, range 2–11 vs median 3, range 1–21), although not statistically significant (p = 0.3006; unpaired Student test). *P. acnes* was seen in median 33% (range 20–100%) of follicles in positive rosacea patients and median 27% (range 9–100%) of positive control samples. The DAPI staining did not reveal any other bacterial or yeast cells in *P. acnes*-positive and -negative follicles. We further evaluated whether there is a possible link between *P. acnes* and histological inflammation in rosacea in four *P. acnes* biofilm-positive patients and four gender and age matched *P. acnes*-negative rosacea patients. Two of the biofilm-positive rosacea samples showed evidence of granuloma in haematoxylin/eosin staining (Fig. 2).

**DISCUSSION**

We have for the first time investigated the presence of *P. acnes* in skin compartments of patients with rosacea. *P. acnes* was only observed in approximately 1 in 10 patients. *P. acnes* colonization decreases with age (14), and the median age of the patients investigated in this study was 53.5 years. This is in keeping with our observation that the younger control group of patients with non-rosacea disorders (median age 39 years) had a 2.5-fold higher *P. acnes* detection rate. Nonetheless, *P. acnes*-positive rosacea and non-rosacea patients were observed in middle aged and older age groups.
Given the variations of hair follicle parameters in different body sites (15) we took into consideration the number of hair follicles present in skin biopsies. The similar hair follicle counts in P. acnes-positive and -negative patients exclude the possibility that the numbers of follicles influenced the P. acnes detection rate in the rosacea patients. The higher detection rate in the control group, however, may be associated with a significantly higher number of follicles per biopsy or the age difference between the two groups. Interestingly, P. acnes was absent from the hair follicles of the majority of individuals in both groups. Whether this reflects a highly uneven distribution of P. acnes hair follicle colonization across different skin areas or an absence of follicle colonization in these individuals remains to be determined. In half the P. acnes-positive rosacea patients the bacterium was present as matrix-encased bacterial clusters either attached to the follicle wall or inside the stratum corneum. These features are indicative of biofilm formation (16). Inflammation was evaluated in a limited number of patients and two of the biofilm-positive rosacea samples showed evidence of granuloma. P. acnes associated granuloma formation has been described in humans (17) as well as mice (18). Whether P. acnes may induce granuloma formation in rosacea is not known and requires further investigation. Whether there is an association of one particular P. acnes phylotype (10) with granuloma formation is also unknown.

In conclusion, we can suggest that P. acnes is unlikely to play a major role in the pathogenesis of rosacea.

Fig. 1. Paraffin embedded skin biopsy section analysed by confocal laser scanning microscopy. Propionibacterium acnes was stained with anti-P. acnes monoclonal antibody (QUBPa3) and rabbit-anti mouse FITC-conjugated secondary antibody. (A) P. acnes biofilm within a hair follicle, scale bar 50 μm. (B) Close-up of the P. acnes biofilm shown in part A, scale bar 5 μm.

Fig. 2. Paraffin embedded skin biopsy section positive for Propionibacterium acnes biofilm analysed by haematoxylin/eosin staining showing granuloma.

Table 1. Age distribution of Propionibacterium acnes-positive subjects

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Rosacea (number of P. acnes-positive/total number)</th>
<th>Control (number of P. acnes-positive/total number)</th>
</tr>
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<tbody>
<tr>
<td>&lt;30</td>
<td>2/7</td>
<td>1/5</td>
</tr>
<tr>
<td>30–50</td>
<td>3/25</td>
<td>1/9</td>
</tr>
<tr>
<td>50–70</td>
<td>2/41</td>
<td>3/9</td>
</tr>
<tr>
<td>&gt;70</td>
<td>0/9</td>
<td>1/2</td>
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REFERENCES

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