Enzyme Production of Propionibacteria from Patients with Acne vulgaris and Healthy Persons

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375 strains of propionibacteria isolated from puslesions, comedones, and from normal skin of patients with acne vulgaris and from healthy persons have been examined for their enzymatic activity. In contrast to healthy individuals, protease and caseinase production of strains from acne patients was significantly lower. On the other hand, DNA'ase production of strains from acne lesions was increased, and lecithinase could be demonstrated in strains from acne patients only. Key words: P. acnes; Acne vulgaris; Enzymes, extracellular.

The relevance of bacteria in the complex etiology of acne vulgaris has been widely discussed. Some investigators demonstrated differences between acne patients and healthy persons concerning numbers, species and types of propionibacteria (1, 2). Furthermore, differences between various types of propionibacteria are known concerning their metabolic activities. Fanta et al. (3) demonstrated an increased production of porphyrines, and Höfller et al. (4) showed increased production and activity of neuraminidase in strains from patients affected by acne compared with strains from healthy controls. Differences in production of cytotaxin are demonstrable between different comedonal bacteria (5). Whiteside & Voss (6), however, were unable to demonstrate differences in lipolytic activity between strains from acne patients and normal skin.

The aim of this study was to determine whether there are differences in the production of five extracellular enzymes between propionibacteria strains isolated from patients with acne vulgaris and healthy persons.

MATERIAL AND METHODS

Clinical data of 36 patients with severe acne vulgaris and 26 healthy comparison persons, sampling techniques, and methods for isolation and differentiation of bacteria were described in our previously published studies (7, 8).

Bacterial strains

A total of 375 propionibacteria strains were examined. 104 strains were isolated from nonaffected follicles of acne patients with the cyanoacrylate technique as described earlier (7, 8). 93 strains were from non-inflamed comedones, 107 strains were from acute pustules, and 71 strains were from the pilosebaceous ducts of healthy persons (7).

Differentiation of the isolates

All isolates were classified by standard methods. Further biochemical differentiation was done by using the Minitek differentiation system for anaerobes in GasPak jars (Becton, Dickinson GmbH, Heidelberg, FRG). Biotyping, serological differentiation and phage typing was carried out as previously described (7, 8).
Enzyme tests

The bacteria were tested for the production of five enzymes by use of agar dilution techniques with one loopful of an 48 h old fresh culture of each strain in duplicate. Plates were incubated for 72 h at 37°C by using the Heraeus incubator VT/N2 (W.C. Heraeus GmbH, D-6450 Hanau, FRG; 85% N2, 10% H2 and 5% CO2 at normal atmospheric pressure), and the results were read immediately thereafter.

Protease (gelatinase) test. Production of protease (gelatinase) was tested with agar containing 30 g gelatine per 100 ml (7). After incubation the agar was flowed by sulfosalicylic acid (20% v/v) for 5 min.

Caseinase test. The contents of the casein test agar was as follows: Skim-milk powder (L 31; Oxoid Deutschland GmbH, D-4230 Wesel 1), 30 g; Trypton (Difco Laboratories, Michigan, USA), 10 g; Lab-Lemco (Oxoid L 29), 3 g; Yeast-extract (Difco), 1 g; NaH2PO4, 5 g; agar No. 4 (Oxoid), 14 g; aqua dest., 1 000 ml; pH 7.2. Results were read by observation for growth and clearing of medium.

Deoxyribonuclease test. The DNA’ase test agar No. CM 321 from Oxoid was used.

Lecithinase (Phospholipase) test. Production of lecithinase (phospholipase) was detected by incorporating egg yolk emulsion (10%, v/v) in plates with blood agar base (both from Oxoid) containing 0.6% NaCl.

Phosphatase test. The contents of the phosphatase test agar was as follows: Pepton (Difco), 5 g; Lab-Lemco (Oxoid L 29), 5 g; NaCl, 5 g; agar No. 4 (Oxoid), 14 g; aqua dest., 1 000 ml; 10 ml phenolphthaleindiphosphat in aqueous solution (1% v/v). Two different pH values (5.8 and 8.0) were tested.

Statistical evaluations

Statistical analysis was done in the Rechenzentrum of the University of Cologne by using the Statistical Package for the Social Sciences (SPSS 8). Different groups of strains were paired via chi-square-test with a prescribed significance level of α=0.05.

RESULTS

In Fig. 1 the frequencies of protease (gelatinase) and caseinase production of strains from comedones, pustules, non-affected follicles of acne patients, and from healthy controls are shown. Strains of healthy controls and unaffected skin of acne patients showed high
frequencies of protease production, whereas strains of acne comedones were significant less active ($\alpha = 0.0032$). In contrast to healthy individuals, caseinase activity of strains from acne patients is significantly lower ($\alpha = 0.0005$). Considering different lesions of acne patients it is evident that strains from nonaffected skin produce caseinase as often as strains from healthy individuals, but strains from comedones and pustules are significantly less active ($\alpha = 0.0002$).

Fig. 2 demonstrates the frequencies of DNA'ase production of different strains. The comparison between healthy controls and acne patients shows a distinct increase. All tested $P. \text{granulosum}$ strains produced DNA'ase, but only 11.7% of the $P. \text{acnes}$ strains.

As to be seen in Fig. 3, lecithinase could not be demonstrated in strains from healthy individuals, but in 17.4% of all strains from acne patients.

Fig. 4 shows the frequency of phosphatase production. No significant differences could be shown between different groups of strains; values differed from 88.7% (controls) to 96.8% (comedones).

**DISCUSSION**

Only little information about the production of enzymes by propionibacteria isolated from acne lesions and from healthy human skin was found in previous publications. Differences in lipase activity (6) and hyaluronate lyase (9) between strains from different origin could
not be demonstrated. However, neuraminidase activity could be discovered in a higher percentage of *P. acnes* strains isolated from acne lesions compared with strains from normal human skin (4). Neuraminidase-positive strains, moreover, produced statistically significantly higher amounts of the enzyme than isolates from normal skin (4).

The results of our study with 375 strains of propionibacteria indicate that isolates from healthy controls and the unaffected skin of acne patients are more often proteolytic active than isolates from comedones and pustules. On the other hand, strains from nonaffected skin are lower producers of DNA'ase and lecithinase than strains from acne lesions. These two latter enzymes were almost exclusively produced by the species *P. granulosum*, which in a former study (7) could be differentiated in acne patients only and was more frequently found in acne lesions than in the unaffected follicles of acne patients. By splitting cellular tissue detritus and skin surface lipids, it seems possible that these two enzymes of propionibacteria act as etiological factors in the complex pathogenesis of acne vulgaris.

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REFERENCES

Cholinergic Urticaria Shows Neutrophilic Inflammation

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Three patients with cholinergic urticaria were studied by biopsy and neutrophilic urticaria was observed in two. Direct immunofluorescence was negative in one patient, and monoclonal antibody studies identified a large population of OKM-1 antibody positive cells. Key words: Biopsy; Monoclonal antibody; Immunofluorescence. (Received January 28, 1985.)

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Cholinergic urticaria is a unique, whealing response to heat, exercise and at times, anxiety. The skin will form wheals with intradermal acetylcholine and congeners which show peripheral satellite whealing. Histamine release agents will exhaust the skin capacity for this reaction, and the H1 blocking agents will suppress a major portion of the reaction.

Histological examination of the skin of these wheals has been limited and of little help in explaining the phenomenon and its differences from the usual acute and chronic urticaria. Illig (1) biopsied 2 of 8 cases and found in both cases perivascular large round cells and in some sections predominant neutrophiles with occasional eosinophils. He concluded no difference existed between histologic changes of cholinergic urticaria and other forms of urticaria. James et al. (2) also did not find significant microscopic differences between types of physical urticaria.

METHODS

Biopsies obtained from three patients with cholinergic urticaria were taken from clinical wheals produced by exercise. A positive acetylcholine test was demonstrated in two patients. The patients had had their cholinergic whealing for 1½, 4 and 8 years respectively. Atopic disease was not present in these patients. Blood chemistry, hemogram, erythrocyte sedimentation rate, and serum protein electrophoresis values were normal in two patients. One of two patients had a CH50 of 37 U (normal 41-80), but C3 and C4 values were normal. The serum α-1 trypsin inhibitor in this patient was normal. One patient had limited laboratory examinations. Fifty hematoxylin and eosin sections and five aldehyde fuchsin-Giemsa and five alcian blue-PAS sections were available for microscopic study.

RESULTS

Two of the three patients showed neutrophilic urticarial histology: Polymorphonuclear leukocytes in and about the walls of the superficial subpapillary dermal venules (Fig. 1).