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Propionibacteria in Patients with Acne vulgaris and in Healthy Persons* **

M. Gehse[1], U. Höffler[1], M. Gloor[2], and G. Pulverer[1]

[1] Hygiene-Institut der Universität Köln (Direktor: Prof. Dr. G. Pulverer), Goldenfelsstr. 19–21, D-5000 Köln 41, Federal Republic of Germany

[2] Hautklinik der Universität Heidelberg (Direktor: Prof. Dr. D. Petzoldt), Voßstr. 2, D-6900 Heidelberg 1

Summary. A total of 375 anaerobic and microaerophilic coryneform rods, isolated from the pilosebaceous ducts of 26 healthy persons (71 strains) and from comedones (93 strains), pustules (107 strains), and the unaffected skin (104 strains) of 36 acne patients were classified according to the species key in Bergey's manual, the biotyping scheme of Pulverer and Ko, the serotyping schedule of Höffler et al., and the phage typing schedule of Jong et al. The statistical evaluation demonstrated certain differences in the frequencies of the *Propionibacterium* species and types between the different groups tested. Thus, the species *P. granulosum* was isolated only from acne patients (50.0% of patients examined) and was found more frequently in comedones and pustules than in unaffected follicles in acne patients. The majority of *P. granulosum* strains belonged to serotype II (95). Biotype A propionibacteria were more frequently found in strains from healthy controls (52.1% of strains) than in strains from comedones (17.2%), pustules (27.1%) and unaffected skin (38.5%) of the acne patients. The results of phagetyping showed that the *P. acnes* lysotype I was more frequent in acne patients (total: 73.2% of strains), especially in the inflamed pustules (88.5%), than in healthy controls (55.1%).

Key words: Acne vulgaris – *Propionibacteria* – Pilosebaceous duct bacteria – Skin-surface bacteria

Acne vulgaris is a worldwide disease, which mainly affects juveniles between 14 and 18 years of age. An essential pathogenetic factor in this disease is the proliferation-retention-hyperkeratosis [20] of the infundibulum of the follicles, which promotes the development of comedones. Also responsible for the development of acne may be – hereditary and hormonal factors, seborrhea [1, 3, 20], changes in the contents of the sebaceous glands [3, 20], immunologic reactions [11, 20], and last but not least the presence of bacteria, especially propionibacteria.

Recently, several studies have been published describing the numbers and species of bacteria found in patients affected by acne and in healthy controls. Different results according to differences in obtaining material, the site of affected regions, and the variable age of persons affected have resulted in different judgements as to the pathogenic cause of propionibacteria in the development of acne [2, 13–18, 24]. Up to now, differences have not been demonstrated between *Propionibacterium* bio-, sero-, and phagetypes of acne patients and healthy controls. Investigations by Lentze et al. [13] proved that there was an abundance of certain types of propionibacteria in acne patients. The present investigation, therefore, aimed at differentiating and typing the anaerobic and microaerophilic coryneform bacteria in the sebaceous follicles of healthy human skin in comparison with those in unaffected follicles, comedones, and pustules of acne patients.

Material and Methods

Clinical Data

A total of 36 patients with severe acne vulgaris were investigated who always showed a large number of comedones and in most cases many inflamed papules and pustules. Cases of very severe acne with cysts and large nodules were not included. The average age was 19.1 years. Healthy persons ($n = 26$) were also included in the study. The two groups largely corresponded with regard to age, sex, and the time of investigation (between November 1979 and February 1980). Subjects tested were not treated with antimicrobial agents, either topically or systemically, for at least 4 weeks before the beginning of the investigations. The tested subjects did not wash their face on the day before the beginning of the trial.

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Offprint requests to: Priv. Doz. Dr. U. Höffler (address see above)

Bacterial Strains

A total of 375 strains of microaerophilic and anaerobic coryneform bacteria were examined. Strains were isolated from patients and healthy persons on reinforced clostridial agar (RCA, Biologische Arbeitsgemeinschaft, Lich, FRG) using the GasPak-system (Becton, Dickinson GmbH, Heidelberg, FRG) as previously described [4, 12]. From each specimen, three bacterial colonies were isolated in pure culture and stored on A-agar [7, 10] by means of the Fortner method [6] for the following differentiation and typing procedure.

1. Strains from Unaffected Follicles of Acne Patients. A total of 104 strains were isolated from the pilosebaceous ducts of the nonaffected skin of 32 acne patients [4, 12] with the cyanoacrylate technique according to Holland et al. [9]. One drop of cyanoacrylate gel was applied to the skin and a glass sampler was pressed upon it. After drying, the sampler was abruptly removed so that parts of the follicles remained attached. This procedure was repeated on the same site. Follicle portions were mechanically disrupted as originally described [9].

2. Strains from Comedones. A total of 93 strains were isolated from noninflamed comedones of 31 acne patients. Comedo material was obtained with a sterile comedo extractor. The material was diluted in reinforced clostridial medium (RCM, Biologische Arbeitsgemeinschaft, Lich, FRG), with the addition of Tween 80 (1% v/v), and homogenized [9, 12].

3. Strains from Pustules. A total of 107 strains were isolated from fresh pustules of 33 persons. Pustule material was obtained by sterile expression and processed as described above.

4. Strains from Healthy Controls. A total of 71 strains were isolated from the pilosebaceous ducts of the skin of healthy controls by the cyanoacrylate technique as described by Holland et al. [9].

Differentiation of the Isolates

1. General Differentiation. Most of the subcultivation and differentiation procedures were carried out in GasPak jars. All isolates were checked for colony morphology, Gram staining, catalase reaction, and absence of aerobic growth using standard methods [6, 13, 19].

2. Biochemical Differentiation. Biochemical differentiation was done using the Minitek differentiation system for anaerobes in GasPak jars (Becton, Dickinson GmbH, Heidelberg, FRG). The following reactions were tested: Acid formation from inositol, maltose, mannitol and sorbitol, esculin hydrolysis, nitrate reduction, and indole formation from tryptophane. In questionable cases, acid formation was measured by means of test strips (limit pH values 5.5). In addition, gelatinase (proteinase) production was checked on agar plates [23]. All strains were differentiated according to the key for propionibacteria in Bergey's manual [19] as well as the biotyping schedules described originally by Pulverer and Ko [13, 22] and extended by Höffler et al. [6].

3. Gas Chromatography. In doubtful cases, the propionic acid formation in peptone yeast extract glucose (PYG) broth was checked by gas-liquid-chromatography in a Packard gas chromatograph model 427 according to Holdeman et al. [8].

4. Serologic Differentiation. Serologic differentiation was performed as previously described [6, 7, 13, 22].

5. Phage-Typing. Phage-typing was carried out according to our previously published method [6, 10, 13].

Statistical Evaluations

Statistical analysis was carried out in the Rechenzentrum der Universität Köln using the Statistical Package for the Social Sciences (SPSS 8). Different groups of strains were paired via the chi-square test with a prescribed significance level of $\alpha = 0.05$.

Results

All isolates initially selected according to the microscopic and colony morphology on RCA agar belonged to the family of Propionibacteriaceae [19]. Since unknown biotypes and phage types were found, it was necessary to extend the previous schedules [6, 10, 22]. There was one new biotype, designated biotype *Q* (acid formation from inositol and mannitol was negative, from maltose and sorbitol positive), and there were 19 new phage types, designated XVIII to XXXVII (details to be published elsewhere).

The results of the species diagnostics are presented in Table 1. Of the 375 strains, 373 proved to be *P. acnes* or *P.* granulosum, the remaining two strains being *P. avidum.* Strains belonging to the species *P. granulosum* were isolated from acne patients only (65/304 = 21.4% of strains from patients compared with 0/71 in the control group). Of strains from the unaffected follicles of acne patients, 8/104 (7.7%) were classified as *P. granulosum.* This ratio increased in the groups of comedones and pustules to 28/93 (30.1%) and 29/107 (27.1%), respectively. Thus, *P. granulosum* could be isolated from 50.0% of all patients with acne vulgaris. Furthermore, *P. granulosum* was isolated in pure culture (Table 2) from the comedones of 16.1% of the patients and from the pustules of 15.2%. Of patients with comedones, 25.8% showed *P. acnes* combined with *P. granulosum* in their comedones, and 21.2% of patients with pustules showed the same mixed culture in their pustules.

From Fig. 1, the frequencies of propionibacteria biotypes in different samples can be seen. Biotype A was the most common biotype in healthy persons; it constituted 52.1% of all strains. Biotype E was found in 21.1%, and biotype D and all other biotypes were each found in less than 10%. In contrast, only 17.2% of strains from comedones belonged to biotype A, 27.1% were from pustules, and 38.5% from the unaffected skin of acne patients (mean 28.0%; difference is statistically significant). Biotype C was found in 18.8% of acne strains, but in none of the comparison strains.

Figure 2 shows the frequencies of different propionibacteria serotypes. Of the propionibacteria strains from healthy human skin, 97.2% proved to be *P. acnes* serotype KB, compared with 78.6% of strains from acne patients. The *P. granulosum* serotype 2 (95) was the dominant type in this species.

Figure 3 shows the frequencies of different phage types within the species *P. acnes.* The phage set used

Table 1. Species-differentiation of 375 propionibacterial strains isolated from comedones, pustules, and the unaffected follicles of acne patients and from healthy controls

Group	Number of persons	Number of persons with propionibacterial isolates	Number of isolates		Number of isolates			Number of patients with isolation of		
			Total	Per person	*P. acnes*	*P. granulosum*	*P. avidum*	*P. acnes*	*P. granulosum*	*P. avidum*
Acne patients										
Comedones		31	93	3.0	65[b] (69.9%)	28[b] (30.1%)		26 (83.9%)	13 (41.9%)	0
Pustules		33	107	3.24	78[a] (72.9%)	29[a] (27.1%)		28 (84.8%)	12 (36.4%)	0
Unaffected infundibula of acne patients		32	104	3.25	96[a,b] (92.3%)	8[a,b] (7.7%)		32 (100.0%)	6 (18.7%)	0
Total	36	–	304	–	239 (78.6%)	65 (21.4%)	0 (0%)	36[c] (100.0%)	18[c] (50.0%)	0
Healthy controls	26	26	71	2.73	69 (97.2%)	0 (0%)	2 (2.8%)	26[c] (100.0%)	0[c] (0%)	1 (3.8%)
Overall totals	62	–	375	–	308	65	2	–	–	–

[a,b,c] Chi-square test – significant

Table 2. Frequencies of pure and mixed cultures of different propionibacterial species isolated from comedones, pustules, and the unaffected follicles of acne patients and from healthy controls

Group	Number of persons with propionibacterial isolates	Frequencies of pure cultures of		Frequencies of mixed cultures of *P. acnes* and *P. granulosum*
		P. acnes	*P. granulosum*	
Comedones	31	18 (58.1%)	5 (16.1%)	8 (25.8%)
Pustules	33	21 (63.6%)	5 (15.2%)	7 (21.1%)
Unaffected infundibula of acne patients	32	26 (81.3%)	0	6 (18.7%)
Healthy controls	26	26 (100%)	0	0

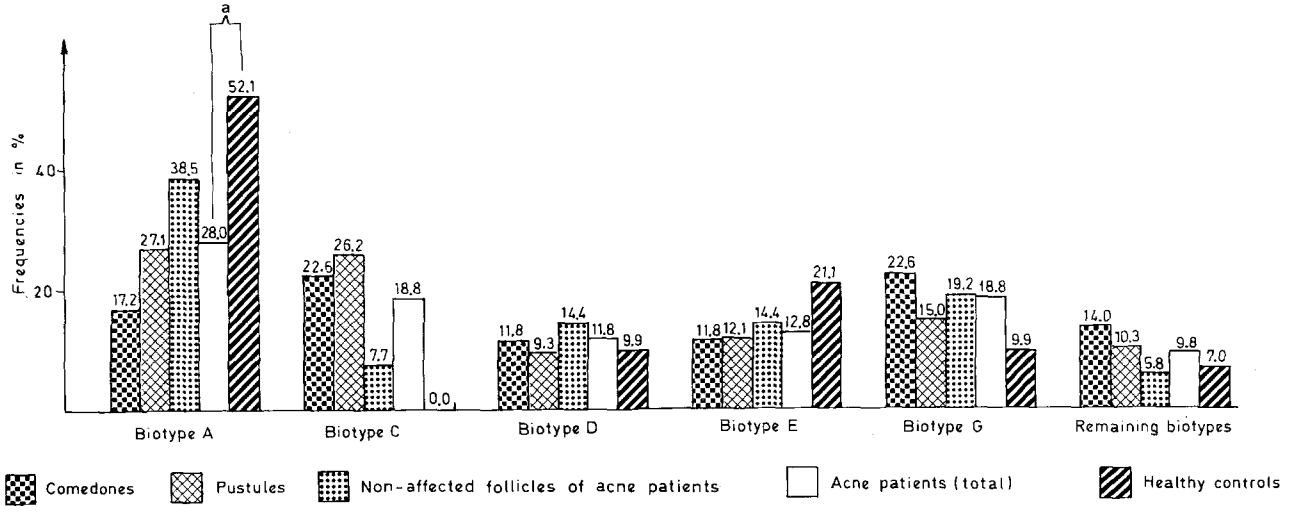

Fig. 1. Biotyping of all propionibacteria. Frequencies of biotypes according to Pulverer and Ko [22] of all propionibacteria isolated from comedones, pustules, and the unaffected follicles of acne patients and healthy controls. *a*, chi-square test – significant

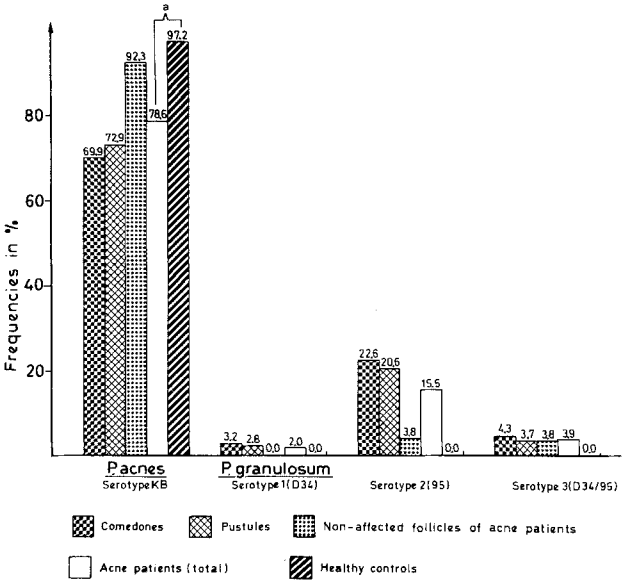

Fig. 2. Serotyping of all propionibacteria. Frequencies of serotypes according to Höffler et al. [7] of all 375 propionibacteria isolated from comedones, pustules, and the unaffected follicles of acne patients and healthy controls, *a*, chi-square test – significant

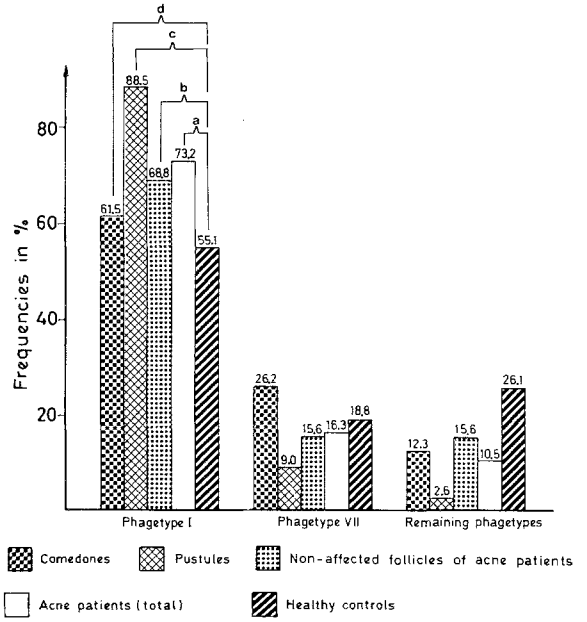

Fig. 3. Phage typing of *P. acnes*. Frequencies of phage types according to Jong et al. [10] of 308 *P. acnes* strains isolated from comedones, pustules, and the unaffected follicles of acne patients and healthy controls. *a*, *c*, chi-square test – significant; *b*, *d*, chi-square test – not significant

once more turned out to be specific for this species. In all, 65 *P. granulosum* strains and two *P. avidum* strains could not be lysed by the 13 virulent *P. acnes* phages. Of the *P. acnes* strains of the controls, 55.1% belonged to the phage type I, whereas 73.2% of all *P. acnes* strains isolated from acne patients could be classified into this group. Furthermore, it is notable, that over 88% of the *P. acnes* strains in the inflamed pustules were phage-type I.

Discussion

The present investigation on the classification and typing of 375 *Propionibacterium* isolates derived from pustules, comedones, and the unaffected skin of acne patients and from the pilosebaceous ducts of healthy controls produced the interesting result that *P. granulosum* could be differentiated in acne patients only (50% of patients examined). This species, furthermore, was more frequently found in acne lesions than in the unaffected follicles. Similar results were obtained by Leyden et al. [15], who when using the scrub method found *P. granulosum* in 42.8% of patients affected with acne and in only 2.5% of healthy individuals. It must be pointed out, however, that in their study species identification was done only by colony morphology and lysis by only one bacteriophage. Gloor and Franke also found a significant increase in the amount of *P. granulosum* in acne patients [2]. In their investigation, this species was demonstrated in only 6 of 48 controls, but in 9 of 24 patients affected with acne. Marples et al. [16] found less *P. granulosum* in the contents of comedones from slightly affected patients than in patients with severe acne. However, Whiteside and Voss [24] obtained contrasting results and suggested that *P. acnes* is more probably involved in the pathogenesis of acne than is *P. granulosum*. McGinley et al. [17] demonstrated that *P. granulosum* exists in 26% of healthy individuals on the forehead and in 85% on the alae nasi. The mean density of the population was about 10% of the bacterial count of *P. acnes*.

Differences in the frequencies of *P. granulosum* do not only exist between acne patients and unaffected persons but are also see when comparing different samples of patients with acne. As our investigation shows, *P. granulosum* was demonstrable in only 18.7% of the unaffected skin of acne patients, but in 36.4% of pustules and 41.9% of comedones (Table 1).

Serologic examinations showed no difference in the distribution of *P. granulosum* serotypes in our test groups. In each of the groups studied serotype 2 (95) was predominant. Similar results were also demonstrated by Lentze et al. [13]. Altogether, the system of serotyping applied proved excellent in verifying the diagnosis of the species involved. Significant differences in the distribution of some biotypes within the various groups existed not only between *P. acnes* and *P. granulosum*, but also within *P. acnes* strains. The possibility of correlating fermentation reactions with the production of extracellular enzymes and toxins remains to be determined.

We were able to demonstrate by phage-typing a significant increase in lysotype I *P. acnes* strains in acne patients as compared to healthy controls, which is similar to the results of Lentze et al. [13]. The reason for this remains unclear. No correlation between biotype and phage type could be demonstrated by Jong et al. [10].

A few investigations have been presented with regard to the differences between propionibacteria species in chemoattractant activities [21] and the production of lipase [24] or porphyrines [1]. Höffler [5] showed an increase in the activity of deoxyribonuclease and phospholipase (lecithinase) in strains of *P. granulosum* compared with related species of anaerobic coryneforms. Further investigations will be undertaken concerning the production of extracellular enzymes by the *Propionibacterium* strains of the different groups examined in our study.

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