

# Pathogenicity of *Propionibacterium acnes* in mixed infections with facultative bacteria

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**Summary.** Single and mixed infections with 11 clinical isolates of *Propionibacterium acnes* and three facultative bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*) were studied in a subcutaneous abscess model in mice. Abscesses were induced by pure cultures of six of 11 strains of *P. acnes* and by the three facultative bacteria. The abscesses produced by each of the six “virulent” *P. acnes* isolates mixed with *S. aureus*, *E. coli* or *K. pneumoniae* were larger than those induced by the single organisms in 16 of the 18 combinations. There was a significant increase in the numbers of the six *P. acnes* strains in 13 of the 18 bacterial mixtures and in the numbers of the facultative bacteria in 17 of the 18 combinations. These data illustrate the potential virulence of some *P. acnes* strains and their synergic capacity with facultative bacteria.

## Introduction

*Propionibacterium acnes* is a non-spore-forming, anaerobic, gram-positive bacillus that normally inhabits the skin and mucous membranes.<sup>1–3</sup> It is a common contaminant of blood and body fluid cultures and is generally considered to be non-pathogenic to man, although it has been identified in pure and mixed cultures as a cause of serious infections, especially in conjunction with foreign body implants.<sup>4</sup> Little attention has been paid to the direct virulence of *P. acnes* and to its importance in mixed infection. In this study, a subcutaneous (s.c.) abscess model in mice was used to evaluate the virulence of *P. acnes* isolates and their relationship with concomitant bacteria in mixed infections.

## Materials and methods

### Bacterial strains

All test strains were recent clinical isolates from patients admitted to the Naval Hospital, Bethesda, MD. They included 11 isolates of *P. acnes* from various clinical sources (table I) and one isolate each of *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*. A decision was made as to whether these isolates represented bacterial contaminants, or were significant clinical isolates causing infections by reviewing the patients’ medical records and microbiology laboratory data. The criteria used were, isolation (a) in pure culture from a surgical specimen

Table I. Clinical data relevant to the *P. acnes* isolates studied

Isolate no.	Source	Other organisms also isolated
1	Joint	None
2	Pleural fluid	None
3	Frontal sinus	<i>Bacteroides ureolyticus</i>
4	Skin nodule	<i>Peptostreptococcus micros</i> <i>Clostridium clostridioforme</i>
5	Abdomen	<i>Bacteroides fragilis</i>
6	Abdomen	<i>Bacteroides fragilis</i> <i>Fusobacterium varium</i>
7*	Blood	None
8*	Blood	None
9*	Maxillary sinus	None
10*	Soft tissue abscess	<i>S. aureus</i>
11*	Blood	None

\* Isolates regarded as clinically significant.

obtained during an open surgical procedure with clinical evidence of infection at that site, (b) from a body site normally sterile, along with at least one positive blood culture, or (c) from at least two positive blood cultures. Based on these criteria, only five strains of *P. acnes* were designated significant isolates (strain nos. 7–11). The strains of *S. aureus*, *E. coli* and *K. pneumoniae* used were from intra-abdominal abscesses, and were considered to be significant isolates. All the bacteria studied were identified by conventional methods.<sup>5,6</sup>

### Animals

Female B6D2F1 mice, 20–25 g, were obtained from Jackson Laboratories (Bar Harbor, ME, USA). They were held in quarantine for 2 weeks and representative samples were examined microbiologically to ensure

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the absence of specific bacteria and common murine diseases. Up to nine mice were housed in sanitised 46 × 24 × 15-cm polycarbonate boxes with a filter cover (Micro Isolator, Lab Products, Inc.). They were given commercial rodent chow and acidified (pH 2.5) water freely, and used when 8–19 weeks old.

### Inoculum

The test bacteria were inoculated onto Schädler Blood Agar with Brain-Heart Infusion Base (BHI; Difco) and incubated in anaerobic conditions at 37°C for 24 h. Cotton-wool swabs were used to transfer colonies from the plates to normal saline to give suspensions equivalent to a MacFarland standard 10. Viable counts (cfu/ml) were determined by pour-plate counts in BHI agar with vitamin K<sub>1</sub> 10 µg/ml and haemin 5 µg/ml.

### Measurement of abscess size and ability to induce abscesses

The inoculum used to induce abscesses was 0.1 ml of the appropriate bacterial suspension, containing total counts of 10<sup>9</sup> cfu/ml of each organism in saline, given by s.c. injection in the medial aspect of the right thigh. The doses were chosen to achieve minimal mortality and maximal abscess formation.

The ability of isolates to form an abscess was determined by s.c. inoculation of 10<sup>8</sup> cfu of each isolate alone (10<sup>8</sup> cfu in 0.1 ml of saline) or mixtures 10<sup>8</sup> cfu of each isolate in 0.2 ml of saline) into groups of 15 mice. The animals were observed for 42 days, and the abscess sizes were determined by external measurement without killing the animals. The experiments were repeated on three occasions.

In tests for quantitation of bacteria in abscesses, abscesses were examined at necropsy on the fifth day after inoculation because some lesions, if left longer, became fragile, and easily ruptured. Although the volume of the abscesses could not be determined accurately, their sizes were compared by measuring two perpendicular diameters representing maximum length and width. Assuming that the abscess is an irregular prolate spheroid, the product of the length and width is proportional to the outer surface of the abscess. This figure, expressed in mm<sup>2</sup>, was arbitrarily selected to measure and compare abscess size.

### Microbial quantitation in abscesses

Each strain of *P. acnes* alone and in combination with each of the three facultative bacteria, was tested in 12 mice with the inocula described previously. Six randomly selected mice were killed on the fifth day after inoculation by cervical dislocation and the abscesses were removed aseptically. Two of the abscesses were fixed in 10% formalin for histopathological examination and the other four were homogenised for culture. After embedding in paraffin wax,

sections (5- and 10-µm thick) were cut and stained with haematoxylin and eosin. Abscesses were homogenised in an anaerobic glove box with a ground-glass tissue grinder containing 1 ml of sterile saline.

Tenfold serial dilutions of the homogenates were made with sterile saline, and 0.1 ml of each dilution was spread in triplicate on enriched BHI blood agar. Colonies were counted after aerobic or anaerobic incubation at 37°C for 48 h to determine the number of each species in the abscess. Numbers of bacteria were expressed as log<sub>10</sub> cfu abscess. Characteristic colonies of all organisms were subcultured and identified by Gram's stain and biochemical tests.

The other six mice were observed for a total of 42 days. Experiments with each mixture of bacteria were performed on three separate occasions. The Mann-Whitney Wilcoxon test was used for statistical analysis.

### Results

Histopathological examination of the abscesses caused by the *P. acnes* isolates or other bacteria, or the combinations, 5 days after inoculation revealed collections of fibrous encapsulated material that contained bacteria and polymorphonuclear leucocytes.

### Abscesses induced by single organisms

Six of the 11 *P. acnes* isolates induced abscesses when inoculated alone (table II, isolates 6–11); five of these six were considered to be clinically significant isolates (isolates 7–11) (table I). Abscess surface area 5 days after inoculation of *P. acnes* was 39–116 mm<sup>2</sup> (table II). All three facultative anaerobic bacteria

**Table II.** Abscess size 5 days after inoculation of mice with 10<sup>8</sup> cfu of *P. acnes* alone or with one of three other bacterial species

<i>P. acnes</i> strain no.	Mean (SD) abscess size (mm <sup>2</sup> , n = 15) when <i>P. acnes</i> was inoculated			
	alone	with <i>S. aureus</i>	with <i>E. coli</i>	with <i>K. pneumoniae</i>
None	...	110 (32)	128 (36)	122 (23)
1	NA <sup>b</sup>	114 (22)	133 (17)	140 (23)
2	NA	125 (28)	118 (23)	136 (16)
3	NA	108 (16)	131 (17)	140 (28)
4	NA	118 (15)	138 (15)	153 (36)
5	NA	98 (18)	144 (19)	114 (23)
6	48 ± 9	196 (17)*	176 (32)*	162 (19)*
7†	63 ± 14	172 (31)*	175 (31)*	226 (27)*
8†	53 ± 11	169 (30)*	130 (16)	175 (26)*
9†	81 ± 16	138 (28)	174 (30)*	188 (41)*
10†	75 ± 12	159 (18)*	128 (28)*	198 (38)*
11†	86 ± 20	190 (23)*	241 (20)*	203 (33)*

NA, No abscess formed.

\* Significant difference between mean size of abscesses caused by *P. acnes* or other bacteria alone (p < 0.05).

† Isolates regarded as clinically significant.

induced abscesses when injected alone, and their abscess surface area 5 days after inoculation was 78–164 mm<sup>2</sup>. Abscesses induced by *P. acnes* alone were observed within 24–48 h in 92% of the animals given isolates 6–11, and reached maximal abscess surface areas by 7 days. Lesions due to *P. acnes* regressed spontaneously by 14–28 days without draining. Abscesses developed in 95% of animals inoculated with the facultative bacteria, and 31% of these drained spontaneously on days 7–12; the others regressed by 21–28 days. None of the mice died.

### *Abscesses induced by mixtures of P. acnes and facultative species*

After injection of mixtures of *P. acnes* and facultative species, abscesses developed within 24–48 h in 95% of animals with all combinations. Abscesses induced by combinations of *P. acnes* strains 1–5 and facultative species reached a surface area of 98–153 mm<sup>2</sup> (table II). Those induced by *P. acnes* strains 6–11 and facultative species reached a surface area of 128–241 mm<sup>2</sup>. Of these abscesses, 42% drained spontaneously on days 7–13, and the others regressed by days 20–35. When *E. coli* or *K. pneumoniae* was injected with *P. acnes* strains 8, 10 and 11, 45% of mice died after 7–10 days. Abscesses induced by mixtures of *P. acnes* strains 6–11 and facultative bacteria were larger than those induced by either strain alone in 16 of 18 combinations (table II).

### *Quantitation of bacteria in abscesses*

The average number of organisms in abscesses induced by *P. acnes* strains 6–11 varied between 3.8 log<sub>10</sub> cfu and 6.2 log<sub>10</sub> cfu (table III). A significant

increase in the numbers of *P. acnes* organisms of strains 6–11 was found in four of the six combinations with *S. aureus*, in five of six with *E. coli*, and in four of six with *K. pneumoniae*. A significant increase in the number of *S. aureus* and *K. pneumoniae* organisms occurred in all combinations with *P. acnes* strains 6–11, and of *E. coli* organisms in all but one of the mixtures.

### Discussion

This study illustrates the potential pathogenicity of *P. acnes* strains and their synergy with *S. aureus*, *E. coli* and *K. pneumoniae*. These facultative organisms were chosen for inoculation with *P. acnes* because they are frequently isolated with them from wound and other specimens in our laboratories. A good correlation was observed between the apparent clinical significance of *P. acnes* isolates and the observed mutual enhancement *in vivo* of their own growth and the growth of their bacterial partners in mixed infection. These data suggest that many *P. acnes* isolates, once they participate in an infectious process, can contribute to abscess formation and enhance the growth of abscesses through synergy with other bacterial species in a mixed infection. Synergy was demonstrated in this study by two methods—(i) by determining the abscess sizes, and (ii) by enumerating the number of organisms in the abscesses. Although the latter method was more accurate for assessing abscess formation, both techniques resulted in significant differences between lesions produced by bacterial mixtures. Several hypotheses have been proposed to explain microbial synergy, e.g., mutual protection from phagocytosis and intracellular killing<sup>7</sup> production of essential growth factors<sup>8</sup> and lowering of redox potentials in host tissues.<sup>9</sup>

*P. acnes* is generally considered to be a common contaminant in clinical specimens, although it has been found to cause endocarditis in implanted cardiac valves,<sup>10, 11</sup> meningitis associated with neurosurgical prosthesis,<sup>12, 13</sup> skin infection,<sup>14</sup> septic arthritis<sup>15</sup> and osteomyelitis.<sup>16</sup> *P. acnes* is also known to be associated with acne.<sup>17</sup> Although an unusual pathogen, pure cultures of *Propionibacterium* spp. have been isolated from brain abscesses,<sup>4, 17–19</sup> empyema,<sup>20–22</sup> parotid and dental infections,<sup>23</sup> pulmonary infections,<sup>24</sup> and peritonitis.<sup>25</sup> However, the mechanism that allow some strains to produce clinical illness as well as abscesses in mice and enhance growth of other bacteria are as yet undetermined.

In a 10-year review of infection in the Naval Hospital Bethesda, 94 (12%) of 816 clinical isolates of *P. acnes* were found to be associated with significant infections.<sup>26</sup> These included 15 isolates from blood, 10 from lymph glands, eight from abscesses, seven from joints and six each from cysts and sinuses. The most common predisposing condition was the presence

**Table III.** Total numbers of *P. acnes* and other bacteria in abscesses 5 days after inoculation of mice

<i>P. acnes</i> strain no.	Mean (SE) log <sub>10</sub> cfu of strains recovered from abscesses			
	<i>P. acnes</i> alone	<i>P. acnes</i> / <i>S. aureus</i>	<i>P. acnes</i> / <i>E. coli</i>	<i>P. acnes</i> / <i>K. pneumoniae</i>
None	...	8.0 (1.0)*	9.2 (0.8)*	8.1 (0.2)*
1	0	0/9.1*	0.6/9.4	0.8/8.5
2	0	0/8.4	0/7.8	1.2/7.6
3	0	0.8/9.3	0/8.6	0/7.5
4	0	0.5/9.0	0.3/9.5	0/9.0
5	0	0/7.9	0/10.0	0.6/8.4
6	4.6 (0.8)	5.3/10.1†	5.6/11.1†	6.3†/10.8†
7†	5.0 (1.1)	7.8†/9.3†	8.0†/10.9†	6.9†/11.2†
8†	3.8 (0.4)	6.4†/10.8†	6.0†/10.3†	4.6/12.1†
9†	6.6 (0.7)	7.4/10.5†	8.3†/12.1†	7.7/11.6†
10†	6.2 (0.7)	8.4†/11.3†	8.3†/11.8†	8.0†/10.4†
11†	5.9 (0.8)	7.6†/11.0†	7.8†/11.4†	8.3†/12.0†

\* Viable count of facultative species inoculated alone.

† Significant difference between viable counts in single and mixed infection ( $p < 0.05$ ).

‡ Isolate regarded as clinically significant.

of a foreign body (27) followed by diabetes (12) and malignancy and immunodeficiency (seven each).

*Propionibacterium* spp. possess immunostimulatory mechanisms, such as the activation of complement,<sup>27</sup> stimulation of lysosomal enzymes release from human neutrophils,<sup>28</sup> and production of serum-independent neutrophil chemotactic factors.<sup>29</sup> These mechanisms may play an important role in pathogenesis. The data presented in this study demonstrate the potential pathogenic quality of some *P. acnes* strains and highlight the need to assess carefully clinical isolates

of *P. acnes* and determine their significance in each case.

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The experiments conducted herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals", Institute of Laboratory Animal Resources, National Research Council, NIH Publ. No. 85-23.

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