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.1-3 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.4 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 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.5,6

**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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Received 5 April 1990; revised version accepted 15 Aug. 1990.

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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_{10} 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 mm² (table II). All three facultative anaerobic bacteria

<table>
<thead>
<tr>
<th>P. acnes strain no.</th>
<th>Mean (SD) abscess size (mm²; n = 15) when <em>P. acnes</em> was inoculated</th>
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<tbody>
<tr>
<td></td>
<td>alone with</td>
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<td></td>
<td><em>S. aureus</em></td>
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<tr>
<td>None</td>
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<tr>
<td>1</td>
<td>NA*</td>
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<td>2</td>
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<td>5</td>
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<tr>
<td>6</td>
<td>48±9</td>
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<td>7</td>
<td>63±14</td>
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<td>8†</td>
<td>53±11</td>
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<td>9†</td>
<td>81±16</td>
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<td>10†</td>
<td>75±12</td>
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<td>11†</td>
<td>86±20</td>
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</table>

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 mm². 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 mm² (table II). Those induced by *P. acnes* strains 6–11 and facultative species reached a surface area of 128–241 mm². 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 log₁₀ cfu and 6·2 log₁₀ 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 of bacteria in host tissues. *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, meningitis associated with neurosurgical prosthesis, skin infection, septic arthritis and osteomyelitis. *P. acnes* is also known to be associated with acne. Although an unusual pathogen, pure cultures of *Propionibacterium* spp. have been isolated from brain abscesses, empyema, parotid and dental infections, pulmonary infections, and peritonitis. However, the mechanism that allows 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. 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...
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, stimulation of lysosomal enzymes release from human neutrophils, and production of serum-independent neutrophil chemotactic factors. 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.

The laboratory assistance of J. Perry, T. Elliott and E. Frazier, the editorial assistance of M. Greenville, and the secretarial help of Minerva G. Barreiro are greatly appreciated.

The opinions and assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

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.

References