## Infection/Inflammation

## PROPIONIBACTERIUM ACNES ASSOCIATED WITH INFLAMMATION IN RADICAL PROSTATECTOMY SPECIMENS: A POSSIBLE LINK TO CANCER EVOLUTION?

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#### ABSTRACT

Purpose: Inflammation is commonly observed in the prostate gland and has been implicated in the development of prostate cancer. The etiology of prostatic inflammation is unknown. However, the involvement of a carcinogenic infectious agent has been suggested.

Materials and Methods: Prostatic tissue from 34 consecutive patients with prostate cancer was cultured to detect the presence of bacterial agents. Prostatic inflammation was assessed by histological examination of wholemount tissue sections.

Results: The predominant microorganism detected was Propionibacterium acnes, found in 35% of prostate samples. A significantly higher degree of prostatic inflammation was observed in cases culture positive for P. acnes (p = 0.007). P. acnes was separated into 3 groups based on cell surface properties, phenotype and genetic grouping. All skin control isolates were classified as group 1 whereas most prostatic isolates were classified as groups 2 and 3.

Conclusions: P. acnes has been isolated from prostatic tissues in men who underwent radical prostatectomy for localized cancer and has been shown to be positively associated with prostatic inflammation. This inflammation may then be linked to the evolution of carcinoma. Furthermore, organisms infecting these patients with prostate cancer differ genetically and phenotypically from the commonly identified cutaneous P. acnes isolates, suggesting that specific subtypes may be involved in development of prostatic inflammation.

KEY WORDS: propionibacterium acnes, inflammation, prostatic neoplasms

Most radical prostatectomy and biopsy specimens are characterized by a multifocal pattern of chronic and acute inflammation that predominates in histologically benign areas. Chronic inflammation is represented by a lymphocyticmacrophage reaction surrounding atrophic glands, while the acute inflammation is characterized by neutrophil infiltration of the gland wall with accompanying epithelial disruption. This inflammation cannot be explained simply as an immune response to carcinoma since it is scattered throughout the entire organ rather than confined to the boundary of the tumor, and is commonly found in prostate glands devoid of malignancy.1

The etiology of this common and distinctive pattern of prostatic inflammation is unknown. However, similar findings are seen in Helicobacter pylori infection of the stomach, which is accompanied by inflammation, gastric atrophy and

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subsequent gastric carcinoma. Similarly the possibility of an infectious origin for prostate cancer has been suggested.<sup>2</sup> De Marzo et al proposed that prostate carcinoma originates from proliferative inflammatory atrophy, foci frequently seen to merge with regions of high grade dysplasia (prostatic intraepithelial neoplasia), a widely accepted precursor of prostatic carcinoma.<sup>2</sup> Therefore, we propose a microbial infection involving an indolent but persistent organism that is difficult to detect and difficult for the host to eradicate, as an etiological agent in prostatic inflammation and possibly the subsequent development of adenocarcinoma.

#### MATERIALS AND METHODS

Prostate tissue samples. Samples were collected from 34 consecutive radical prostatectomy specimens accessed between October 2002 and January 2003. The use of these tissues for research was approved (Hollywood Private Hospital Research Ethics Committee, HPH128). The study group consisted of 32 white European patients and 2 of Asian ethnic descent. All patients presented with increased prostate specific antigen (greater than 4 ng/ml) with cancer diagnosed on needle biopsy. No patient had clinical symptoms of current bacterial prostatitis and none were in acute urinary retention. The intact prostate glands were delivered to our pathology laboratory within 30 minutes of resection. The posterolateral aspect of both prostatic lobes was then incised superficially with a sterile scalpel blade, avoiding entry into the ejaculatory ducts, transition zone or prostatic urethra to minimize the risk of bacterial contamination from these sources. Samples of 100 to 200 mg of macroscopically normal tissue were taken from each lobe and finely macerated under sterile conditions before bacterial culture. Wholemount tissue sections from each case were examined by Gram stain, while routine hematoxylin and eosin stained sections were used to assess and quantitate the inflammation in the peripheral zone without knowledge of the subsequent microbial assavs (Appendix 1).

Culture and identification of bacteria from prostate tissue. Tissue specimens were incubated without agitation at 37C for up to 30 days in deep broth cultures (20 ml) of brain heart infusion broth (Oxoid Australia Ltd, West Heidelberg, Victoria, Australia) supplemented with 5% horse serum. Positive broth cultures were subcultured onto brain heart infusion agar (Oxoid Australia Ltd) with 5% horse serum at 37C in an atmosphere generation jar with CO<sub>2</sub> enrichment. These culture conditions were considered suitable for isolation of a wide range of pathogenic microorganisms, including fastidious and microaerophilic species. Microorganisms were identified by morphological characteristics and 16S rRNA gene sequencing as follows. Bacterial pellets were re-suspended in 128  $\mu$ l of sterile phosphate buffered saline and bacterial cell walls disrupted by bead beating using a mixer mill (MM301, Reicht, Germany) and acid washed 0.1 mm silica/zirconia beads (BioSpec Products, Inc., Bartlesville, Oklahoma) for 2 minutes at 30 Hz. DNA was extracted using the QIAamp DNA Mini Kit (Qiagen Pty Ltd, Clifton Hill, Australia) according to the manufacturer's tissue protocol.

Polymerase chain reaction (PCR) was performed with universal 16S rRNA gene primers (Appendix 2). Thermal cycling conditions were 10 minutes at 95C, 35 cycles of 30 seconds at 95C, 30 seconds at 59C and 1 minute at 72C, followed by 7-minute extension at 72C. Deep Vent DNA Polymerase<sup>TM</sup> (New England Biolabs, Beverly, Massachusetts) was used for universal bacterial primer amplifications because it contains little or no endogenous bacterial DNA. PCR products were sequenced and compared with those in GenBank® using the BLAST<sup>TM</sup> function. In terms of isolation and identification of cutaneous P. acnes, a total of 24 P. acnes isolates from the skin of 2 healthy males and 1 female volunteer were obtained using the swabbing technique described by McGinley et al,3 and identified as previously described for prostatic isolates.

Typing of P. acnes. Pulsed Field DNA Analysis: Genomic DNA from *P. acnes* (all prostatic and 6 selected cutaneous isolates) was analyzed by a previously described method.<sup>4</sup> DNA was run on a FIGE Mapper<sup>TM</sup> field inversion system (Bio-Rad, Hercules, California) on a 1% pulse field agarose gel in  $0.45 \times \text{tris-borate-edetic}$  acid with forward and reverse voltages of 180 and 120 volts with 0.1 to 2 seconds linear switch times for 16 hours.

Salt Aggregation Test (SAT): SATs to determine bacterial

cell surface hydrophobicity were performed as previously described on all *P. acnes* isolates evaluated by pulsed field gel analysis.<sup>5</sup> Stationary phase *P. acnes* broth cultures (1.5 ml) were re-suspended in 700  $\mu$ l of 0.02 M sodium phosphate buffer (pH 6.8) and 25  $\mu$ l aliquots were mixed on slides for 2 minutes with equal volumes of ammonium acetate at various concentrations (0.5 to 8.0 M). The lowest concentration of salt giving visible bacterial clumping was taken as the SAT score. An aliquot of bacterial cells without added salt was used as a negative control to test for auto-aggregation.

Transcarboxylase 12S Gene Sequence Analysis: Primers MMF and MMR (Appendix 2) were used to amplify a 633 bp region of the P. acnes methylmalonyl-CoA carboxyltransferase subunit 12S monomer or transcarboxylase 12S (Tc 12S) gene involved in production of propionate. Thermal cycling conditions were 15 minutes at 95C, 35 cycles of 30 seconds at 94C, 1 minute at 55C and 1 minute at 72C, followed by 7-minute extension at 72C. HotStarTaq DNA Polymerase (Qiagen Pty Ltd) was used for PCR with primers MMF and MMR. PCR products were sequenced using the BigDye Terminator® mix (Applied Biosystems, Foster City, California) and an automated 377 DNA sequencer. All prostatic and cutaneous isolates were analyzed.

Statistical analysis. Association between the presence of inflammation and culture of *P. acnes* was determined using Fisher's exact test. The unpaired t test was used to compare the extent of inflammation between patient groups positive or negative for culture of P. acnes. In all cases 2-tailed p < 0.05 indicated statistical significance.

#### RESULTS

Bacterial culture of prostate tissue. Positive bacterial cultures were obtained from 19 of 34 (56%) patients with prostate cancer. The predominant microorganism was P. acnes found in 12 (35%) of all cases. Less frequent isolates included coagulase negative staphylococcus and only single isolates of other species (table 1). Staphylococcus cultures became tur-

Table 1. Bacteria isolated from prostatic tissue of 34 unselected patients with prostate cancer

Organism	No. Pts* (%)
No bacterial growth	15 (44)
P. acnes	12 (35)
S. epidermidis	6 (18)
S. warneri	1 (3)
S. saccharolyticus	1 (3)
Lactobacillus iners	1 (3)
Corynebacterium sp.	1 (3)
Bacillus cereus	1 (3)
Bacillus subtilis	1 (3)

\* In 4 cases P. acnes grew in 1 sample and a different microorganism (S. epidermidis, L. iners or B. subtilis) grew in the other sample, in 1 case P. acnes grew in both samples and in 1 case 2 different species (P. acnes and Corynebacterium sp.) were isolated from a single sample.

Parameter	No Bacterial Growth	Other Bacteria Cultured*	P. acnes Cultu
No. pts	15	7	12
No. acute (grade 1–3) inflammation (%)	3 (20)	2 (29)	9 (75)
p Value (Fisher's exact test)		1.0	0.007†

Mean % glands with acute inflammation 2.7 3.8 p Value (unpaired t test)  $0.007 \ddagger$ 0.7 No. chronic (grade 1–3) inflammation present (%)§ 5 (71) 6(40)11 (92) p Value 0.4 0.01 Mean % glands with chronic inflammation 53 7.5 14 2 p Value 0.5 0.01

Table 2. Association between bacterial culture results and inflammation in radical prostatectomy specimens

<sup>\*</sup> Includes only cases negative for growth of P. acnes in both duplicate culture samples.

<sup>†</sup> Each group is compared back to the group negative for bacterial culture.

<sup>‡</sup> Each group is compared back to the group negative for bacterial culture.

<sup>§</sup> No chronic grade 3 inflammation (granulomatous prostatitis) was observed.

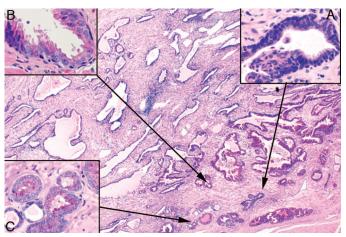


Fig. 1. Section of whole mount prostatectomy specimen (H & E, reduced from  $\times 10)$  showing background of glandular atrophy associated with stromal aggregates of lymphoid cells and histiocytes (grade 1—chronic inflammation). Insets (reduced from  $\times 100)$  show acute (active) inflammation grade 2, with migration of neutrophils through gland wall (top right), concurrent high grade dysplasia (prostatic intraepithelial neoplasia, (top left) and invasive carcinoma (bottom).

bid within 24 to 48 hours whereas most *P. acnes* cultures took 8 to 15 days of incubation to show visible growth. This suggests that any *P. acnes* organisms present in mixed culture

Group 2

Group 3

Group 4

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Fig. 2. Pulsed field gel analysis of *P. acnes* isolates. DNA banding patterns following digestion with *Spe*I (*A*) and DNA banding patterns following digestion with *Not*I (*B*). Groupings are based on similarity of DNA banding patterns, with group 1 containing all cutaneous isolates (A1-R5) and 2 prostatic isolates (2703 and 2972). Remaining 10 prostatic *P. acnes* isolates form groups 2 to 4.

with fast growing bacteria would have been overgrown and likely missed. Subcultures of *P. acnes* took 24 to 48 hours to produce visible growth, confirming that this bacterium is a slow growing organism and present in small numbers in the primary culture.

Association between bacterial culture and prostatic histology. No correlation was observed between bacterial culture results and clinicopathological factors such as patient age, tumor site (transition vs peripheral zone), cancer multifocality, grade or pathological stage (data not shown). Foci of acute inflammation were observed in 14 of 34 (41%) radical prostatectomy specimens while focal chronic inflammation occurred in 22 of 34 (65%) specimens (fig. 1). Analysis of inflammatory foci by tissue Gram stain did not detect bacteria in any case. However, statistically significant associations were observed between the presence and the extent of acute and chronic inflammation in the radical prostatectomy specimen, and the detection of *P. acnes* by culture (table 2). Cases which yielded S. epidermidis, S. warneri or Corynebacterium sp. showed no evidence of acute inflammation and no increase in degree of chronic inflammation compared with culture negative cases.

Comparison of P. acnes isolates from human prostate and skin. Genomic DNA Analysis: Genomic DNA extracted from selected cutaneous and all prostatic P. acnes was compared by pulsed field gel DNA electrophoresis. Analysis of the DNA banding patterns revealed 3 main groups of P. acnes (fig. 2). Group 1 consisted of all 6 cutaneous isolates and 2 prostatic isolates. Of the remaining prostatic P. acnes 9 could be divided into group 2 (5 isolates) and group 3 (4 isolates), whereas 1 isolate (02–2766) differed from all others and was assigned to group 4. These results suggest that most P. acnes cultured from the prostate are genetically distinct from those colonizing human skin.

Growth Characteristics and Cell Surface Properties: Groupings obtained by pulsed field gel DNA analysis correlated with differences in growth characteristics and cell surface properties. *P. acnes* from group 1 formed aggregates and settled into a granular sediment with clear supernatant when cultured to a high density in liquid medium without agitation (fig. 3). In contrast, *P. acnes* from groups 2 to 4 grew

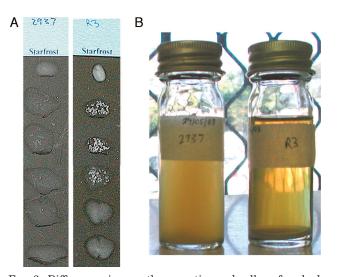


FIG. 3. Differences in growth properties and cell surface hydrophobicity for *P. acnes* of group 1 compared with group 2. SATs (*A*) show droplets of bacterial culture in increasing salt concentrations of 0.25 to 4 M as well as no salt negative control (top). Group 1 isolate (R3) has complete aggregation in 1 M salt, indicating hydrophobic cell surface compared with group 2 isolate (2937) with slight degree of aggregation in 2 to 4 M salt. Cultures grown in liquid medium to high density without agitation (*B*) show group 1 isolate (R3) as granular sediment with clear supernatant compared with group 2 isolate (2937) which has fine sediment with turbid supernatant.

as a slight fine sediment with a turbid supernatant shown by Gram staining to contain suspended cells. Analysis of cell surface properties using SAT tests revealed that group 1 *P. acnes* were hydrophobic (complete aggregation in 1 M salt) whereas group 2, 3 and 4 isolates were hydrophilic, showing only a minor degree of aggregation in 2 to 4 M salt (fig. 3). The 18 cutaneous isolates not characterized by pulsed field gel analysis and SAT tests also showed group 1 growth characteristics.

Transcarboxylase 12S Gene Sequence Analysis: Analysis of the Tc 12S gene sequences in all our cultured isolates revealed 2 distinct sequence types (designated sequence A and sequence B) that differ at 10 specific single base positions within the 633 bp amplified region (fig. 4). All *P. acnes* from groups 1 and 2 were found to have sequence A, whereas *P. acnes* from group 3 had sequence B. Prostatic isolate 02–2766 (group 4) had a Tc 12S sequence intermediate between A and B with several additional single base differences.

Taken together these results indicate that our *P. acnes* isolates fall into 3 main groups. Group 1 (mainly cutaneous isolates) has Tc 12S sequence A and a hydrophobic cell surface, group 2 (prostatic isolates) has Tc 12S sequence A and a hydrophilic cell surface, and group 3 (prostatic isolates) has Tc 12S sequence B and a hydrophilic cell surface. This classification correlates with genetic groupings obtained by pulsed field gel DNA analysis. The finding of a single isolate that differed by pulsed field analysis and Tc 12S sequence suggests that other less common groups may also exist.

#### DISCUSSION

The data presented here show for the first time to our knowledge that a single categorical bacterial species, *P. ac-*

SeqA SeqB	AGGGSAAGGTCAAGCTGTGGGGGTGGTGGGCCAGATGGCCATCATTGCTGGGCCCTGGG AGGGSAAGGTCAAGCTGTCAGGGGTGGTGGCCCAGATGGCCATTATTGCTGGCCCTGGG	60 60
boqb	* *	00
SeqA SeqB	OGGOGGOSOCIOCIATIOOOCIGOOCIGAOCIACTICATCATCATCATGAOGAAGAAGGOOC OGGTIGGOSOCIOCITATIOOOCIGOOCIGAOCIACOTACOTACATCATCATCATCATCATCATCATCATCATCATCATCA	120 120
sequ	*	120
SeqA.	ACATGTTCATTAOGGGCCCGGGGTCATCAAGTCGGTTACCGGTGAGGAGGTGACTGCTG ACATGTTCATTACGGGCCCCGGGGTCATCAAATCGGTTACCGGAGGTGACTGCTG	180 180
SeqB	* *	190
SeqA	$\verb"AOGACCIGGGIGGGGATGCGCACAIGTCCACCICGGGCAATAICCACITCGIGGCCG"$	240
SeqB	ACGACCTGGGTGCGGCATGCGCACATGTCCACCTCGGGCAATATCCACTTCGTGGCCG	240
SeqA	$\hbox{\tt AAGATGAOGAOGCAGTGCTCATOGOGCAGAAGTTGCTGAGCTTOCTGCOGCAAAACA}$	300
SeqB	AAGATGAOGAOGCAGTGCTCATOGOGCAGAAGTTGCTGAGCTTOCTGOOGCAAAACA	300
SeqA	ACACTGAGGACGOCCAGATCTOCCAACCCCAATGACGATGTCTOCCCGCAGCCTGAGCTGC	360
SeqB	ACACTGAGGACGOCCAGATCTCCAATGCCAATGTCTCCCCGCAGCCTGAGCTGC	360
SeqA SeaB	GOGACATOGITOOGCTGGATGGTAAGAAGGGCTAOCGAOGTOOGOGAOGTCATCTOCAAGA GOGACATOGITOOGCTGGAOGGTAAGAAGGGCTAOCGAOGTOOGOGAOGTCATTTTOCAAGA	420 420
•	* *	
SeqA SeqB	TOSTOSACTGGGGGGACTACCTAGAGGTCAAGGCCGSTTTGGGCGACCAACATCSTCACCG TOSTOSACTGGGGGGACTACCTAGAGGTCAAGGCCGGTTTGGGCGACCAACATCATCACCGC	480 480
sequ	*	400
SeqA	OCTITIGOCOGGGTCAATGGTTGGTACOGTGGCGATCGTGGCCAACCAGCCGAAGGTGATGT CCTTCGCCCGGGTCAATGGTCGTTACCGTCGGCCATCGTGGCCCAACCAGCCGAAGGTGATGT	540 540
SeqB	*	J4U
SeqA SeqB	OGGGTTGCCTTGACATCAATGCTTCGGACAAGGCTGCCGAGTTCATTACCT 591 CGGGTTGCCTTGACATTAATGCTTCGGACAAGGCTGCCGAGTTCATTACCT 591	
	+	

FIG. 4. Sequence variants of P. acnes transcarboxylase 12S gene. Sequence A (P. acnes groups 1 and 2) and sequence B (P. acnes group 3) differ at 10 specific single base positions (each indicated by asterisk). Sequence B is polymorphic at 1 base position (arrow) where either C or T may be present.

nes, infects a considerable proportion of prostate glands removed at radical prostatectomy. More significantly, this is a slow growing microbe that is difficult to isolate by culture and is easily overgrown by other bacteria, which may account for previous failures in identification. The presence of these prostatic *P. acnes* was strongly correlated with histological features of inflammation, which until now have not been explained.

The bacteriology of prostatic tissue has been previously investigated with culture of open prostatectomy samples and transurethral curettings.<sup>6-8</sup> These studies identified mainly urinary pathogens including Escherichia coli and Streptococcus faecalis. Although our finding of S. epidermidis in 18% of prostatectomy specimens approximates the reported incidence in the aforementioned studies, we did not isolate any intestinal flora and our predominant isolate was P. acnes, a bacterium not previously cultured from patients with prostate cancer. The slow growth of this organism allows for easy overgrowth by common microbes which may account for previous failure to identify it in the prostate gland. It should also be noted that previous studies revealing organisms other than P. acnes were conducted on men with obstructive symptoms, many with concurrent urinary tract infections.<sup>6,7</sup> In contrast, patients in the present study were undergoing radical prostatectomy for proven carcinoma, and none were in urinary retention or had evidence of urinary tract infection. Furthermore, culture conditions in the previous studies were not optimal for detection of *P. acnes* since they used either aerobic culture on blood agar<sup>7</sup> or an anaerobic culture incubation of only 7 days.8 The lack of concordance between our duplicate culture results suggests a highly focal distribution of bacteria, which contributes further to the difficulty of bacterial detection.

Previous studies using molecular techniques have detected bacterial DNA in prostatic tissue from 5% to 89% of patients with prostate cancer<sup>9–12</sup> but identification by sequencing was only performed in 9 with the species E. coli, Stenotrophomonas sp., Bacteroides sp., Lactobacillus crispatus and Ureaplasma urealyticum present. Three of the previously mentioned studies did not include mechanical disruption in the extraction technique, possibly explaining why they failed to detect P. acnes DNA in biopsies from patients with prostate cancer.  $^{9-11}$  The *P. acnes* cell wall is resistant to digestion by lysozyme and we have found that mechanical disruption such as bead beating is mandatory for successful DNA extraction from small numbers of *P. acnes* (data not shown). The 4th study did include bead beating but admits that the sensitivity limit of the DNA extraction/PCR assay (50 cfu per PCR reaction) may not be sufficient to detect low numbers of bacteria.  $^{12}$  The long incubation times for our primary P. acnes cultures to show visible growth suggest that P. acnes is present in low numbers, which would contribute to the difficulty in detecting it within human tissue samples using molecular techniques.

Our repeated failure to detect P. acnes on direct tissue Gram stain has also been noted by other authors investigating culture positive *P. acnes* infections. <sup>13</sup> Failure to detect *P.* acnes directly in tissue sections almost certainly reflects the low numbers of infecting bacteria but may also be partly due to poor uptake of the Gram stain by these microorganisms in vivo as a result of changes to the bacterial wall induced by the immune response. 13 However, our finding of a significant positive association between culture of P. acnes and inflammation in prostatectomy specimens does provide an indirect link between this bacterium and prostatic inflammation. P. acnes is known to be a potent stimulus to the immune system, capable of producing an inflammatory response without direct tissue invasion by secretion of as yet unidentified soluble agents. 14 P. acnes is also highly resistant to killing and degradation by human neutrophils and monocytes, 14 a characteristic which allows it to establish long-term low

grade infections that may persist for years<sup>15</sup> to decades.<sup>16</sup> It has recently been linked to several other chronic inflammatory conditions including sarcoidosis and sciatica. A similar prolonged infection and inflammation of the prostate gland may prove carcinogenic in some cases.

Although *P. acnes* has not previously been isolated from patients with prostate cancer, it has been cultured from urethral swabs and prostate biopsies from approximately 2% of healthy males. <sup>17,18</sup> Furthermore, cultures of prostate biopsies from men with chronic prostatitis have shown a slightly increased detection rate of propionibacteria (not identified to species level), found in 3.5% to 6% of patients. <sup>18,19</sup> However, propionibacteria were not considered pathogenic in these studies because other bacteria (coliforms, staphylococci and corynebacteria) were more frequently isolated. Our results show a considerably higher incidence of *P. acnes* in prostatic tissue from patients with prostate cancer compared with the incidences previously detected in healthy males or men with prostatitis.

It is likely that our *P. acnes* group 3 represents serotype II since we have subsequently found *P. acnes* serotype I (ATCC 6919) to have Tc 12S sequence A, whereas *P. acnes* serotype II (ATCC 11828) has Tc 12S sequence B. Group 3 occurred in prostatic tissue at a frequency similar to the 20% to 30% frequency reported for serotype II on human facial skin although we did not isolate any cutaneous strains with Tc 12S sequence B. This may reflect the fact that cutaneous strains were isolated on agar plates rather than liquid media. However, regardless of Tc 12S sequence or serotype the subtypes of *P. acnes* (groups 2 and 3) that predominate in prostatic tissue from patients with prostate cancer differ from common cutaneous isolates genetically as shown by pulsed field gel DNA analysis, and differ phenotypically in having hydrophilic cell surface properties.

The auto-aggregating properties of *P. acnes* isolated from human skin have been previously documented.<sup>20</sup> This aggregating behavior together with the characteristic growth properties in liquid medium and the hydrophobic surface properties, most likely reflect hydrophobic surface proteins involved in cell adherence which are maximally expressed in late exponential growth phase, as previously shown for streptococcal species.<sup>21</sup> Cell adherence and aggregation in skin isolates of P. acnes is promoted by sebum lipids, 22 suggesting that these properties are advantageous for colonization of human skin. Therefore, it is most unlikely that prostatic *P*. acnes with hydrophilic cell surfaces and lack of aggregating behavior (group 2 and 3) represent a skin contaminant introduced during surgery or subsequent tissue processing. They are more likely to be isolates adapted for growth in an environment without sebum lipids, for example the urinary tract and prostate gland. It is also interesting to note that a hydrophilic cell surface has been associated with resistance to phagocytosis and increased virulence.<sup>23</sup>

We have shown a positive association between P. acnes and prostatic inflammation which may be implicated in the development of prostate cancer. It is possible that prostatic inflammation may also be caused by other microorganisms which could not be identified by this study, for example obligate anaerobes or species which are difficult to culture under laboratory conditions. A second important limitation of this study is the lack of appropriate negative controls such as prostate tissue from patients without inflammation, atrophy and cancer. Such negative control tissue is difficult to obtain because prostatic inflammation is common even in patients undergoing treatment for benign prostate disease<sup>1</sup> and we do not have access to tissue samples from younger males, for example organ donors. Therefore, we are assessing an organ at the terminal phase of a carcinogenic process that spans many years. Assessment of prostate tissues at an earlier stage before development of atrophy, dysplasia and cancer may reveal increased microorganism numbers and inflammation. Due to difficulty in obtaining suitable tissue samples, future work will focus on indirect assessment of apparently healthy men at a younger age possibly through serological, urine or ejaculate analysis. Microorganism identification and typing can then be extrapolated to future cancer evolution and, if successful, could lead to a diagnostic test and prophylactic antibiotic regime that may alter the natural development of this common tumor.

#### CONCLUSIONS

*P. acnes* can be cultured from the prostatic tissues of a considerable proportion of prostate glands removed at prostatectomy and shows a positive association with prostatic inflammation. Furthermore, most *P. acnes* isolated from the prostate gland of patients with cancer differ phenotypically and genetically from common cutaneous isolates, suggesting that specific subtypes of *P. acnes* may be involved in development of prostatic inflammation, which may contribute to the development of carcinoma.

The Lions Club of City Beach provided equipment necessary to perform the study, and the Hollywood Hospital Research Foundation provided additional assistance.

# $\begin{array}{c} \text{APPENDIX 1:} \\ \text{CRITERIA USED TO GRADE THE DEGREE OF PROSTATIC} \\ \text{INFLAMMATION} \end{array}$

Acute Inflammation:

Grade 1—Isolated polymorphonuclear neutrophils (PMN) in lining epithelium and clusters of PMN in gland lumens.

Grade 2—Large numbers of PMN migrating through gland walls with epithelial disruption.

Grade 3—Microabscesses and necrosis.

Chronic Inflammation:

Grade 1—Isolated lymphohistiocytic aggregates in the stroma surrounding glands.

Grade 2—Lymphoid follicles, displacement of glands by inflammatory aggregates.

Grade 3—Granulomatous prostatitis.

### APPENDIX 2:

PRIMERS USED FOR AMPLIFICATION OF THE BACTERIAL 16S rRNA AND TRANSCARBOXYLASE 12S GENE SEQUENCES

Primer	Sequence $(5'-3')$
*16S1F	TGAAGAGTTTGATCCTGGCTCAG
*16S1R	GGACTACCAGGGTATCTAAKCCTG
$\dagger 16 \mathrm{S2F}$	GTGCCAGCAGCCGCGGTRA
$\dagger 16 \mathrm{S2R}$	AGSCCCGGGAACGTATTCAC
MMF	CGGGTACGGCAAGATGTTCT
MMR	GGAATATTGAACGAGTCGCAGA

16S rRNA gene primers were modified from Relman et al.<sup>24</sup>

\* 16S1F and 16S1R give a PCR product of 801 bp. † 16S2F and 16S2R give a PCR product of 875 bp.

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