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Isolation of capsulate anaerobic bacteria from orofacial abscesses

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Summary. The presence of capsulate *Bacteroides* spp. and anaerobic gram-positive cocci was investigated in pus specimens from 182 children with chronic orofacial infections or abscesses and in pharyngeal swabs from 26 children without inflammation. Of 216 *Bacteroides* spp. and anaerobic cocci isolated from clinical infections, 170 (79%) were capsulate, compared with 34 (35%) of 96 isolates from normal pharyngeal flora ($p < 0{\cdot}001$). The commonest organisms found to be capsulate more often from infected patients than from controls belonged to the *B. melaninogenicus* group. The possible evolution of encapsulation in these organisms and their importance in mixed orofacial infections are discussed.

Introduction

Aspirates from orofacial infections adjacent to mucous membrane surfaces generally contain a complex bacterial population; the relative importance of the various bacteria isolated is not clear. Several studies have demonstrated the pathogenicity of capsulate anaerobic bacteria, and capsulate strains of *Bacteroides* spp. and anaerobic gram-positive cocci appear to be more virulent than non-capsulated forms of the same organisms (Onderdonk *et al.*, 1977; Brook and Walker, 1983; Brook *et al.*, 1983; Brook *et al.*, 1984; Brook and Walker, 1984*a* and *b*). The pathogenicity of capsulate *B. melaninogenicus* in tonsillitis was also suggested recently (Brook and Gober, 1983).

In the present study the role of capsulate strains of *Bacteroides* spp. and anaerobic gram-positive cocci in orofacial abscesses and in chronic inflammation of the upper respiratory tract was evaluated by comparing the isolation rate of capsulate organisms for patients with chronic inflammation with the isolation rate of these organisms from the pharynx of normal individuals.

Patients and methods

Patients

Specimens were obtained from 182 children with orofacial infections and from 26 children who served as controls. The control group had non-infectious medical conditions, and had not received antimicrobial therapy during the previous month. Both groups were similar in age, race, and sex. The mean age was 9 years and 2 months (range 3–17 years), and 112 were male.

Forty-eight patients had chronic otitis media, 45 cervical lymphadenitis, 37 chronic sinusitis, 24 chronic mastoiditis, 16 peritonsillar abscesses, and 12 had periapical abscesses. These infectious processes had lasted for at least 10 days before sample collections.

Specimen collection

Cultures from infected sites were obtained during surgery, which was performed through intact skin (chronic sinusitis and mastoiditis), or by percutaneous aspiration of the abscess cavity after scrubbing the skin or mucous surface with providone-iodine (cervical lymphadenitis, periapical abscess), or by aspiration of an abscess before drainage (peritonsillar abscess), or by aspiration of ear fluid through a perforation in the tympanic membrane (chronic otitis media). Samples were aspirated directly into a syringe that was immediately sealed, or a swab was dipped into the pus and then placed in an anaerobic transport medium (Port-A-Col, BBL Microbiological Systems, Cockeysville, MD). The specimens were transported to the bacteriology laboratory and inoculated on to culture media within 10–30 min after collection.

Cultures from the control group were obtained by swabbing the surface of both tonsils with a sterile cotton swab that was immediately placed in anaerobic transport medium. The specimens were inoculated on to media within 15 min of collection.

Bacteriological procedures

All cultures were processed for aerobic and anaerobic organisms. Sheep blood (5%), 'chocolate', and MacConkey Agar plates (BBL, Cockeysville, MD, USA) were inoculated for the isolation of aerobic organisms. The plates were incubated at 37°C in an aerobic atmosphere

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(MacConkey) or in air plus CO_2 (blood and chocolate), and examined after 24 and 48 h. For anaerobic culture, the specimens were plated on to pre-reduced vitamin K_1-enriched Brucella blood agar, a blood-agar plate containing kanamycin 75 mg/L and vancomycin 7·5 mg/L and a blood-agar plate containing phenylethyl alcohol, and into enriched thioglycolate broth (Difco Laboratories, Detroit, MI, USA). These media were incubated inside an anaerobic jar (BBL) at 37°C and examined after 48 and 96 h. The thioglycolate broth was incubated for 14 days. Aerobic and anaerobic bacteria were identified by conventional methods (Sutter *et al.*, 1980; Lennette *et al.*, 1980). The presence of a capsule in *Bacteroides* spp. and anaerobic cocci was determined by Hiss's capsule stain (Lennette *et al.*, 1980) and by electronmicroscopy after staining with ruthenium red (Kasper, 1976). Ruthenium red staining demonstrated a homogenous polysaccharide capsule that was external to the cell wall. Statistical analysis was done by χ^2 test.

Results

Abscess contents

A total of 634 bacterial isolates (426 anaerobes and 208 aerobes) was obtained from the 182 clinical specimens (2·3 anaerobes and 1·1 aerobes per infected site). The number of isolates from infected sites varied from one to seven. The detailed microbiology of these specimens was similar to previously reported data (Brook, 1980*a* and *b*, 1981*a*, *b* and *c*; Brook *et al.*, 1981) and is therefore not presented here.

Normal pharyngeal flora

A total of 206 bacterial isolates (112 anaerobes and 94 aerobes) was obtained from the 26 normal pharyngeal specimens (4·3 anaerobes and 3·6 aerobes per specimen). *B. melaninogenicus* and anaerobic gram-positive cocci were isolated from all individuals.

Isolation of capsulate organisms

Of 216 isolates of *Bacteroides* spp. and anaerobic gram-positive cocci from clinical infections, 170 (79%) were capsulate, compared with 34 (35%) of 96 from normal pharyngeal flora ($p<0·001$) (table). The most significant difference was in the rate of encapsulation of the *B. melaninogenicus* group; 67 (84%) of the 80 *Bacteroides* isolates from infected sites were capsulate, compared with 8 (23%) of 35 from controls ($p<0·001$). Similar but less significant differences were observed with other anaerobic isolates. Analysis of the data according to clinical type of infection also revealed statistically significant differences in the total numbers of capsulate anaerobic isolates from each site of infection (table).

Discussion

The high isolation rate of capsulate anaerobic bacteria from orofacial abscesses, compared with their prevalence in the normal pharyngeal flora, suggests a pathogenic role for these organisms. Several recent studies have demonstrated the pathogenicity of capsulate anaerobes and their ability to induce abscesses when present alone. Onderdonk *et al.* (1976 and 1977) correlated the virulence of strains of *B. fragilis* with the presence of a capsule and Simon *et al.* (1982) described decreased opsonophagocytic killing by neutrophils of capsulate *B. fragilis*. Capsular material from *B. melaninogenicus* also inhibits phagocytosis by leukocytes and phagocytic killing of other micro-organisms in an in-vitro system (Okuda and Takazoe, 1973). The implication that capsular polysaccharide is associated with virulence is not unique to *Bacteroides* spp. The capsular material of *Streptococcus pneumoniae* has been shown to inhibit surface phagocytosis (Wood and Smith, 1949).

In recent studies (Brook *et al.*, 1983 and 1984; Brook and Walker, 1984*a* and *b*) of the relative importance of *Bacteroides* spp. and anaerobic gram-positive cocci in abscess formation in an animal model, non-capsulate organisms failed to induce abscesses. Many strains with minimal numbers of capsulate organisms (<1%), when inoculated with other aerobic and anaerobic bacteria, survived in abscesses and their progeny were capsulate. These capsulate isolates thereafter induced abscesses when injected alone. The selection of capsulate anaerobes occurred in the presence of other capsulate or non-capsulate abscess-forming organisms. This phenomenon may explain how non-capsulate non-pathogenic organisms, which are predominant in the normal flora, can become pathogens. This observation may also explain the importance of anaerobic organisms in chronic infections and in abscess formation, because the process of emergence of capsulate strains took 10–14 days (Brook *et al.*, 1983).

Detection of a capsule in a clinical isolate may indicate that the organism has a pathogenic role in the infection. The demonstration of the importance of capsulate organisms in mixed infections may justify directing therapy in such infections against these potential pathogens. Moreover early and vigorous antimicrobial therapy, directed at the aerobic and anaerobic bacteria present in mixed

Table. Capsulate anaerobic bacteria in children with abscesses and chronic inflammation and in controls

Clinical diagnosis (number of samples)	Number of strains isolated*					
	B. melaninogenicus group	*B. oralis*	*B. fragilis* group	*Peptococcus* spp.	*Peptostreptococcus* spp.	Total
Chronic otitis media (48)	15/19	4/6	7/10	13/17	6/8	45/60(75%)§
Chronic mastoiditis (24)	9/11	2/2	3/3	8/11	3/4	25/31(81%)§
Chronic sinusitis (37)	10/14	3/5	0	11/14	5/6	29/39(74%)§
Peritonsillar abscess (16)	21/23	3/5	0	2/5	14/17	40/50(80%)§
Periapical abscess (12)	8/9	3/3	0	9/9	1/3	21/24(87%)§
Cervical lymphadenitis (45)	4/4	0	0	4/5	2/3	10/12(83%)‡
Total infections (182)	67/80(84%)§	15/21(71%)†	10/13(77%)	47/61(77%)‡	31/41(76%)†	170/216(79%)
Control pharyngeal cultures (26)	8/35(23%)	4/13(31%)	0	14/30(47%)	8/18(44%)	34/96(35%)

* Capsulate/total (%).

† $p < 0{\cdot}05$, ‡ $p < 0{\cdot}005$, § $p < 0{\cdot}001$ compared with control.

infections, may abort the infection before the capsulate bacteria emerge to contribute to the chronicity of the infection.

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