Phagocytosis of bacterial strains isolated from acute dentoalveolar abscess

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Summary. The phagocytosis by human polymorphonuclear leucocytes of 37 bacterial strains identified as *Streptococcus milleri* (10 strains), strictly anaerobic gram-positive cocci (10) *Prevotella intermedia* (6), *Pr. oralis* (5) and *Fusobacterium nucleatum* (6) was investigated *in vitro*. The ingestion of *S. milleri* and strictly anaerobic gram-positive cocci was significantly greater ($p < 0.001$) than that of strains of *Prevotella* spp. and *F. nucleatum*. The degree of uptake of capsulate and non-capsulate strains did not differ.

Introduction

The microflora of acute dentoalveolar abscess usually comprises three or four bacterial species from a wide variety of genera, including facultative anaerobic gram-positive cocci, strictly anaerobic gram-positive cocci and strictly anaerobic gram-negative bacilli.¹ Strict anaerobes tend to predominate in the mixed flora of acute dentoalveolar abscess;² clinical isolates of *Streptococcus milleri*, strictly anaerobic gram-positive cocci, *Porphyromonas* and *Prevotella* spp. (both *Bacteroides* spp.) and *Fusobacterium nucleatum* have proved pathogenic in an animal model.³ However, the bacterial species encountered are usually regarded as members of the commensal oral flora⁴ and because of this it is unclear which of their potential pathogenicity factors cause abscess formation. It has been suggested that the possession of a capsule may be partly responsible for the pathogenicity of strictly anaerobic gram-positive cocci, *Bacteroides fragilis* and *P. asaccharolytica*.⁵ Capsulate strains of the *B. fragilis* group and *Pr. melaninogenica* isolated from various body sites have proved to be more pathogenic than non-capsulate forms^{6–9} but there is little information available as regards the role of strict anaerobes isolated from orofacial abscess. Capsulate strains of strictly anaerobic gram-positive cocci and *Prevotella* spp. have been cultured from periapical abscess in children⁶ but information relating to adult infection is lacking. Since a capsule is known to protect bacterial strains from phagocytosis by host polymorphonuclear leucocytes (PMNL)^{5,10} the aim of the present study was to examine the phagocytosis *in vitro* of capsulate and non-capsulate strains of the predominant bacterial

species isolated from acute dentoalveolar abscess in adults.

Materials and methods

Bacteria

Bacterial strains were isolated from pus samples aspirated from acute dentoalveolar abscess in adults as described previously.² The 37 isolates selected for study were *S. milleri* (10 isolates), strictly anaerobic gram-positive cocci (10), *Pr. intermedia* (6), *Pr. oralis* (5) and *F. nucleatum* (6). All isolates were lyophilised within three subcultures of primary isolation.

Lyophilised cultures were reconstituted in Anaerobic Blood Broth (Gibco) and inoculated on to Columbia Blood Agar (Oxoid) before incubation at 37°C in an anaerobic chamber (Don Whitley Scientific Ltd, Shipley, West Yorkshire). Purity of growth was checked and c. 40 colonies were inoculated into 20 ml of anaerobic blood broth before anaerobic incubation at 37°C for 48 h. Subsequent cultures were centrifuged at 1000 *g* for 10 min and the pellets were washed and resuspended in modified Hanks's Balanced Salts Solution containing gelatin 0.1% (Gel-HBSS). The bacterial suspension was standardised spectrophotometrically to give a viable count of 1×10^7 cfu/ml.

Capsular staining

The presence of a capsule was established for each isolate with Hiss's stain¹¹ and by negative staining with India ink.¹²

Opsonisation

Fresh human plasma was collected from five healthy volunteers, pooled and stored in 5-ml volumes at –70°C. For opsonisation 1 ml of a bacterial sus-

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Fig. 1. The percentage of PMNL containing bacteria in phagocytic experiments with A, 10 strains of *S. milleri*; B, 10 strains of strictly anaerobic gram-positive cocci, and C, 17 strains of gram-negative anaerobic bacilli—*Pr. intermedia* (6), *Pr. oralis* (5) and *F. nucleatum* (6)—isolated from acute dentoalveolar abscess. Capsulate strain ○; non-capsulate strain ●; mean -----.

pension was mixed with an equal volume of plasma before incubation in an orbital incubator (Fison Scientific Equipment, Loughborough, Leics) at 100-rpm for 45 min at 37°C. The preparation was then centrifuged at 1000 *g* for 10 min and the bacterial pellet was resuspended in 2 ml of Gel-HBSS to produce a concentration of 5×10^6 cfu/ml.

Leucocyte preparation

Heparinised venous blood freshly obtained from one of the five healthy volunteers was mixed with dextran (mol. wt 150000; 6% w/v in saline 0.85%) and left for 30 min at 20°C to allow sedimentation of the erythrocytes. The supernate was then layered over Histopaque-1077 (Sigma Diagnostics) and centrifuged at 150 *g* for 30 min. The leucocyte-rich pellet was

Fig. 2. Percentage uptake by PMNL of bacterial strains: A, 10 strains of *S. milleri*; B, 10 strains of strictly anaerobic gram-positive cocci and C, 17 strains of gram-negative bacilli—*Pr. intermedia* (6), *Pr. oralis* (5) and *F. nucleatum* (6) isolated from acute dentoalveolar abscess. Capsulate strain ○; non-capsulate strain ●; mean -----.

washed with ammonium chloride 0.87% to lyse any remaining erythrocytes. The preparation was then centrifuged at 150 *g* for 5 min, washed and finally resuspended in Gel-HBSS at a concentration of 1×10^5 PMNL/ml as determined with a haemocytometer. The viability of the PMNL was confirmed at this stage by trypan blue exclusion;¹³ if this was > 95% the preparation was used immediately.

Phagocytosis assay

Equal volumes (1 ml) of opsonised bacterial suspension and PMNL suspensions were mixed and placed in an orbital incubator at 100 rpm and incubated at 37°C for 45 min.

Duplicate 100- μ l amounts were removed and placed on to glass slides with a Cytospin 2 cytocentrifuge

(Shandon Scientific Ltd, Runcorn, Cheshire) set at 500 rpm for 5 min. Preparations were fixed and stained by Leishman's method, as for a blood film.¹⁴ Cytopreparations were examined by light microscopy and a total of 100 randomly selected PMNL were examined for the presence of bacteria. (Previous standardisation analysis had shown that a count of 100 PMNL gave reliable and reproducible results.) The number of PMNL containing bacteria (PMNL count) and the number of bacteria within the PMNL (bacterial count) were recorded. The percentage uptake of bacteria was calculated as follows.

$$\frac{\text{Number of PMNL in suspension} \times (\text{PMNL count}/100) \times (\text{bacterial count}/100)}{\text{Number of bacteria in suspension}} \times 100$$

Each bacterial strain was tested three times on separate occasions with a different source of PMNL in each experiment. Results were expressed as the mean of the three independent observations.

Statistical analysis

Student's *t* test was used to assess the significance of the results.

Results

In experiments with *S. milleri* 63–92% (mean 83%) of PMNL were found to contain bacteria (fig. 1) and with strains of strictly anaerobic gram-positive bacteria 65–90% (mean 78%). Differences between these two groups of organisms were not statistically significant. The percentage of PMNL containing strictly anaerobic gram-negative bacilli was 6–56%, mean 26%. Statistical analyses showed that significantly fewer PMNL contained strains of strictly anaerobic gram-negative bacilli (*Pr. intermedia*, *Pr. oralis* or *F. nucleatum*) than strains of either *S. milleri* ($p < 0.001$) or strictly anaerobic gram-positive cocci ($p < 0.001$). There was no difference between the ingestion of capsulate or non-capsulate strains.

The percentage uptake of *S. milleri* (mean 15%) and strictly anaerobic gram-positive cocci (mean 13%) was similar (fig. 2), although that of strictly anaerobic gram-negative bacilli (mean 1%) was significantly less than that of either *S. milleri* ($p < 0.001$) or strictly anaerobic gram-positive cocci ($p < 0.001$). There was

no difference in the degree of uptake of capsulate strains compared to non-capsulate strains.

Discussion

The results of this study indicate that human PMNL are less able to phagocytose strains of *Pr. intermedia*, *Pr. oralis* and *F. nucleatum* isolated from acute dentoalveolar abscess than strains of *S. milleri* or strictly anaerobic gram-positive cocci, but as it is known that the amount of serum required for opsonisation of gram-negative bacteria is greater than that for gram-positive organisms,¹⁵ this may account for differences in patterns of phagocytosis. However, it was considered that the 50% v/v used in the present study was adequate to opsonise the spectrum of species studied and that any differences in phagocytosis could be attributed to factors associated with the bacterial strains themselves.

The possession of a capsule may in part explain the apparent pathogenicity of bacteria isolated from orofacial infections because of the inhibition of phagocytosis by leucocytes, but in the present study no difference in phagocytosis between the 10 non-capsulate strains and 27 capsulate strains was demonstrated. A capsule may not be involved primarily in resistance to phagocytosis but could influence other activities such as functions in cells. Also, it may be that in mixed infections the capsule from one species acts synergically with a second species. Capsular polysaccharides and liposaccharides of strictly anaerobic gram-negative bacilli are known to inhibit the phagocytosis of facultative gram-positive cocci in varying degrees,¹⁵ and this phenomenon may be important in acute dentoalveolar abscess, since the microbial flora often comprises a combination of strictly anaerobic gram-negative bacilli and facultative or strictly anaerobic gram-positive cocci.¹ Furthermore, strains of strictly anaerobic gram-negative bacilli isolated from acute dentoalveolar abscess have been found to enhance the pathogenicity of strains of *S. milleri* and strictly anaerobic gram-positive cocci when inoculated into animals.³ Experiments on phagocytosis *in vitro* to assess the differential uptake of each component of a mixture of strains of *Prevotella* spp. and strictly anaerobic gram-positive cocci could help to explain the behaviour of the microflora in acute dentoalveolar abscess.

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