Coagglutination reactions between *Candida albicans* and oral bacteria

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Summary. An agglutination assay for detecting intermicrobial adherence between the cells of *Candida albicans* and various oral bacteria is described. Strains of *Streptococcus sanguis*, *S. salivarius*, *S. mutans*, *S. mitis*, *Fusobacterium nucleatum* and *Actinomyces viscosus* all coagglutinated with *C. albicans*. No interaction could be demonstrated between the cells of *Bacteroides melaninogenicus* and those of *C. albicans*. Preliminary investigations of these interactions suggest that binding of *F. nucleatum* and *A. viscosus* to *C. albicans* is mediated by bacterial proteins, possibly lectins. Other mechanisms must account for the binding of oral streptococci to *C. albicans*. The possible implications of these findings in relation to oral mucosal colonisation and oral candidal clearance are discussed.

Introduction

It is apparent that the initial adherence of micro-organisms to mucosal surfaces is an important first step in the pathogenesis of many infections (Ofek and Beachey, 1980). Prevention of adherence could prevent infection, but the mode of attachment of *Candida albicans* to host cells is incompletely understood. *In-vitro* investigations suggest a role for mannose-containing moieties on the surface of either *C. albicans* or epithelial cells as mediators of adherence to human epithelium (Sandin *et al.*, 1982; Lee and King, 1983). The pre-incubation of uro-epithelial and buccal epithelial cells with fimbriate strains of *Escherichia coli* or *Klebsiella pneumoniae* increased the subsequent attachment of *C. albicans* to epithelial cells, the whole process being inhibited by mannose (Centeno *et al.*, 1983). These data suggest that certain bacteria possess ligands that bind to both candidal and epithelial cells, and mediate adherence of the yeast by a 'bridging' action. Similar intermicrobial binding is important in the formation of dental plaque (Gibbons and Nygaard, 1970).

The possible role of this type of attachment in relation to oral colonisation by *C. albicans* has received little attention. Clearly the commensal oral bacteria are very different from those in the genito-urinary tract, and normally there is a total absence of mannose-sensitive fimbriate enterobacteria. This preliminary study was designed to screen oral micro-organisms for their ability to bind to cells of

C. albicans and form visible aggregates (coagglutination). The approach is similar to that employed to screen bacteria for mannose-specific lectin activity by the agglutination of yeasts (Mirelman *et al.*, 1980), a lectin being defined as a sugar binding protein of non-immune origin. If interactions between oral bacteria and *C. albicans* are demonstrated, the hypothesis that the bacteria are involved in candidal attachment merits further investigation.

Materials and methods

Bacteria

The strains of aerobic bacteria studied were *Streptococcus sanguis* NCTC 10558; *S. salivarius* NCTC 8606; *S. mitis* 3 NCTC 10712; *S. mutans* D282 NCTC 10832; *Staphylococcus aureus* NCTC 6571 and *Escherichia coli* NCTC 10418. All were grown on blood agar for 24 h at 37°C. The anaerobic bacteria studied were *Fusobacterium nucleatum* NGB 15-73; *Actinomyces viscosus* ATCC 15987 and *Bacteroides intermedius* NCTC 9336; these were grown on blood agar for 48 h at 37°C, in an atmosphere of H₂ 10%, CO₂ 10% and N₂ 80%.

Yeasts

Candida albicans NCPF 3281 was studied in the coagglutination experiments and was grown aerobically on Sabouraud agar for 24 h at 38°C. Sixty-six fresh clinical isolates of *C. albicans* were assayed exclusively for auto-agglutinating activity after culture in the same conditions.

Agglutination assay

In the coagglutination assay, both the yeast cells and the bacteria were suspended in potassium phosphate buffer (0.25 M, pH 7.4). A concentration of *C. albicans* 6×10^6 cfu/ml, tested with 6×10^7 bacteria/ml yielded the most consistent results. Samples (1.5 ml) of bacterial and yeast-cell suspensions were mixed in 7-ml screw-capped bottles. The bottles were sealed and rotated at 33 rpm on a tube rotator overnight at room temperature. Results were read by pipetting a small volume of each mixture on to a microscope slide and observing agglutination with the naked eye, or, when necessary, by wet mount microscopy. Negative control preparations in which *C. albicans* and the bacteria were suspended individually in buffer were always performed, and examined for autoagglutination.

Heat treatment of bacteria

Suspensions were placed in a waterbath at 85 °C for 30 min or 60 min.

Trypsin treatment

Bacteria were treated with Type II trypsin from porcine pancreas (Sigma Chemical Co. Ltd, Fancy Road, Poole, Dorset BH17 7NH). The trypsin was dissolved in Tris-HCl buffer (0.2 M, pH 7.4) at a concentration of 10 000 BAEE units/ml, and the bacteria were treated with this solution for 45 min in a water bath at 37 °C. The bacteria were washed once after trypsinisation before the agglutination assay.

Sodium metaperiodate oxidation of *C. albicans*

Cells of *C. albicans* were treated for 5 min with sodium metaperiodate, at concentrations of 1, 5 and 10 mg/ml in 0.25 M potassium-phosphate buffer, pH 7.4, at room temperature. The cells were then washed in potassium-phosphate buffer before the agglutination assay.

Results

In the buffer system described, 21% of 66 fresh isolates of *C. albicans* autoagglutinated. *C. albicans* 3281, in common with most test strains, did not autoagglutinate in these conditions. Of all the bacterial strains tested, only *Staph. aureus* and *B. intermedius* did not coagglutinate with *C. albicans* 3281 (table I).

Heat treated bacteria

The effects of heat treatment of the bacteria on subsequent coagglutination with *C. albicans* 3281 are summarised in table II. Heat treatment abolished the agglutinating activity of *E. coli*, *F. nucleatum* and *A. viscosus*, the two anaerobes being

Table I. Coagglutination of bacteria with *C. albicans* 3281

Bacterial strain	Result
<i>E. coli</i>	+
<i>Staph. aureus</i>	—
<i>S. sanguis</i>	+
<i>S. salivarius</i>	+
<i>S. mitis</i>	+
<i>B. intermedius</i>	—
<i>A. viscosus</i>	+
<i>F. nucleatum</i>	+

+ = Agglutination;
— = no agglutination.

Table II. Effect of heat treatment of bacteria on their subsequent coagglutination with *C. albicans* 3281

Bacterial strain	Agglutination after heat treatment		
	Room temperature	85 °C; 30 min	85 °C 60 min
<i>E. coli</i>	+	—	—
<i>F. nucleatum</i>	+	±	—
<i>A. viscosus</i>	+	±	—
<i>S. sanguis</i>	+	+	+
<i>S. salivarius</i>	+	+++	+++
<i>S. mitis</i>	+	+++	+++
<i>S. mutans</i> D282	+	+++	+++

+ to +++ = Agglutination of increasing strength; — = no agglutination.

Table III. Effect of trypsin treatment* of bacteria on their subsequent coagglutination with *Candida albicans* 3281

Bacterial strain	Agglutination of cells	
	non-trypsinised	trypsinised
<i>F. nucleatum</i>	+	—
<i>A. viscosus</i>	+	—
<i>S. mitis</i>	+	—
<i>S. salivarius</i>	+	—
<i>S. sanguis</i>	+	—
<i>S. mutans</i> D282	+	±

+ = Agglutination; ± = weak agglutination;
— = no agglutination.

* See Methods.

Table IV. Effect of periodate treatment* of *C. albicans* 3281 on subsequent coagglutination with *F. nucleatum* and *A. viscosus*

<i>C. albicans</i> preparation	Coagglutination with		Autoagglutination of <i>C. albicans</i>
	<i>F. nucleatum</i>	<i>A. viscosus</i>	
Untreated <i>C. albicans</i>	++	++	—
<i>C. albicans</i> treated with NaIO ₄ 1 mg/ml	++	++	+
<i>C. albicans</i> treated with NaIO ₄ 5 mg/ml	±	±	±
<i>C. albicans</i> treated with NaIO ₄ 10 mg/ml	±	±	±

+ or ++ = Agglutination; ± = weak agglutination; — = no agglutination.

* See *Methods*.

slightly less sensitive than *E. coli*. However, heating did not reduce the coagglutinating activity of the oral streptococci with *C. albicans*.

Trypsin treatment of bacteria

Trypsinisation of *F. nucleatum*, *A. viscosus* and all four strains of oral streptococci caused either complete inhibition, or a reduction, of subsequent coagglutination with *C. albicans* 3281 (table III).

Periodate treatment of *C. albicans* 3281

The effects of treatment of *C. albicans* 3281 with various concentrations of sodium metaperiodate on subsequent coagglutination with *F. nucleatum* and *A. viscosus* are shown in table IV. Treatment with

periodate 1 mg/ml resulted in autoagglutination of *C. albicans*, but at 5 and 10 mg/ml the autoagglutination was far less marked, and resulted in a greatly reduced coagglutination with these two anaerobic bacteria.

Table V shows that, in contrast, periodate oxidation of *C. albicans* 3281 had negligible effect on its coagglutination with the four strains of oral streptococci, with the possible exception of *S. sanguis*.

Discussion

This study shows that some strains of bacteria that are commonly present in the oral cavity bind to *C. albicans* and form visible aggregates. If the same bacteria bind to oral mucosal or hard tissue surfaces they could function as "bridges", to bind *C. albicans* to oral tissues.

Strains of *E. coli* and *Staph. aureus* were employed as positive and negative controls respectively. *E. coli* NCTC 10418 is a fimbriate strain that produces a mannose-sensitive agglutination of *C. albicans* or *Saccharomyces cerevisiae* (Bagg, 1982) and *Staph. aureus* NCTC 6571 does not coagglutinate with yeast cells (Mirelman *et al.*, 1980). These reports are confirmed by the present study. Although not part of the normal oral flora, *E. coli* may occur in the mouth as part of a replacement flora during antibiotic therapy or in immunocompromised states. In these instances, mediation of candidal adherence by *E. coli* could be important as a predisposing factor in the acute intra-oral candidoses often suffered by such patients.

B. intermedius was the only one of the oral bacteria tested that did not coagglutinate with *C. albicans*. Attachment of *B. asaccharolyticus* to oral surfaces has been studied (Slots and Gibbons, 1978)

Table V. Effect of periodate treatment* of *C. albicans* 3281 on subsequent coagglutination with oral streptococci

Bacterial strain	Coagglutination with	
	untreated <i>C. albicans</i>	periodate treated <i>C. albicans</i>
<i>S. mitis</i>	+	+
<i>S. sanguis</i>	+	±
<i>S. mutans</i> D282	+	+
<i>S. salivarius</i>	+	+
Autoagglutination of <i>C. albicans</i>	—	±

+ = agglutination; ± = weak agglutination; — = no agglutination.

* At a concentration of NaIO₄ 10 mg/ml; see *Methods*

and the presence of dental plaque containing *Actinomyces* spp. and other gram-positive bacteria may be essential for the subsequent attachment and colonisation by pigmented bacteroides. This exemplifies microbial 'bridging', the interactions between particular *Bacteroides* strains and other oral bacteria being highly specific (Kolenbrander *et al.*, 1985). However, *Bacteroides* strains comprise only a small percentage of the cultivable flora in samples of supra-gingival plaque, and they are present on the dorsum of the tongue and vestibular mucosa in very small numbers if at all (Kelstrup, 1966). Thus, even if *B. intermedius* had shown positive coagglutination with *C. albicans*, co-operation of this organism in oral mucosal colonisation by the yeast *in vivo* would have been unlikely.

Both *A. viscosus* and *F. nucleatum* coagglutinated with *C. albicans*. These species of bacteria possess lectins that recognise galactose-like residues (Mongiello and Falkler, 1979; Ellen *et al.*, 1980), and this mechanism may explain their agglutination with *C. albicans*, although sugar inhibition tests were inconclusive.

The bacteria which seem most likely to act as mediators of candidal adherence to oral mucosa are the oral streptococci. Different strains of oral streptococci bind preferentially to particular oral sites, and this correlates with their natural distribution in the mouth (Gibbons and van Houte, 1971). Gibbons and van Houte (1971) report that *S. salivarius* and *S. sanguis* were recovered in high proportions from cheek and tongue surfaces and, because these sites are often colonised by *C. albicans*, the oral streptococci appear to be ideally placed to act as "bridges" for candidal attachment.

Having shown that certain oral bacteria coagglutinate with *C. albicans*, physical and chemical treatments of both the bacteria and the yeast were performed in a preliminary study of the mechanisms involved. Agglutination of yeast cells by *E. coli* is mediated via bacterial protein (Eshdat *et al.*, 1978) and the coagglutinating activity of this organism was reduced by heat treatment. Heat treatment of *F. nucleatum* and *A. viscosus* also abolished coagglutination with *C. albicans*, consistent with binding by bacterial protein. Heat treatment of the oral streptococci did not reduce their coagglutinating activity, a finding which is incompatible with a proteinaceous adhesin.

Trypsin treatment of *A. viscosus* and *F. nucleatum* abolished coagglutination with *C. albicans*, and suggested the involvement of bacterial protein. The oral streptococcal coagglutination with *C. albicans* was also either abolished or greatly reduced by trypsinisation of the bacteria. Adherence to epithelial cells by *S. salivarius* and *S. mitis* is also trypsin-

sensitive (Gibbons *et al.*, 1972; Liljemark and Gibbons, 1972) and electronmicroscopy has shown that a trypsin-sensitive, fibrillar 'fuzzy coat' layer on the surface of oral streptococci is responsible for their adherence (Gibbons *et al.*, 1972). Thus the effect of trypsin in reducing coagglutination of oral streptococci with *C. albicans* may be mediated by a non-specific removal of this surface 'fuzzy coat'.

Since bacterial proteins appeared to be involved in coagglutination with *C. albicans*, a possible role for bacterial lectins was evident. Sodium metaperiodate oxidation cleaves the C-C bond between vicinal hydroxyl groups of sugars, and disrupts carbohydrate receptors. Oxidation of *C. albicans* 3281 with sodium metaperiodate at a concentration of 1 mg/ml produced strong autoagglutination whereas with 5 mg/ml and 10 mg/ml autoagglutination was much reduced. Periodate oxidation of *C. albicans* greatly reduced subsequent coagglutination with *F. nucleatum* and *A. viscosus*. This result suggests that carbohydrate residues on the yeast-cell surface may act as receptors for bacterial adhesins associated with these two organisms.

In contrast, the effect of periodate oxidation of *C. albicans* on subsequent coagglutination with oral streptococci was negligible and suggests a binding that is unlikely to involve carbohydrate receptors on the yeast-cell wall.

Clearly, binding between some oral bacteria and *C. albicans* occurs *in vitro*. The significance of this *in vivo* in relation to candidal attachment to the oral mucosa has not been assessed, but 'bacterial bridging' may have a role to play. The occurrence of acute intra-oral candidosis after broad-spectrum antibiotic therapy militates against a bacterial involvement in candidal attachment. However, *C. albicans* persists in the oral cavity in the presence of a normal oral flora and in this situation 'bacterial bridging' may be important in the normal carriage of *C. albicans*.

Finally, Liljemark *et al.* (1981) showed that adherence to hydroxyapatite by large aggregates of oral streptococci was reduced compared with that of individual cells or small aggregates. Similarly, large salivary aggregates of bacteria and yeast cells may be more readily cleared from the oral cavity than are individual cells, the coagglutination described therefore acting as a non-specific host defence mechanism. In-vivo studies will clearly be necessary to fully elucidate the true biological significance of the in-vitro findings reported in this paper.

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