Effect of salivary secretory IgA on the adhesion of *Candida albicans* to polystyrene

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Attachment of *Candida albicans* to plastic materials of dental prostheses or to salivary macromolecules adsorbed on their surface is believed to be a critical event in the development of denture stomatitis. In an earlier study, it was shown that adhesion of *C. albicans* to polystyrene, a model system to study the adhesion of *C. albicans* to plastic materials, can be partially inhibited with an mAb directed against cell wall polysaccharides of *C. albicans*. In the present study, the role of whole saliva in the adhesion of *C. albicans* to polystyrene has been investigated, and three mAbs directed against epitopes of cell wall mannoproteins have been used to mimic the inhibitory effect observed with salivary secretory IgA (sIgA) on the adhesion of *C. albicans* to polystyrene. In the absence of whole saliva, adherence of *C. albicans* 3153 increased with germination. However, the presence of whole saliva enhanced the adhesion to polystyrene of *C. albicans* 3153 yeast cells but decreased the adhesion of germinated cells. The enhancement of adhesion of yeast cells to polystyrene mediated by saliva was confirmed with an agerminative mutant of *C. albicans* 3153. The inhibition of the adhesion of *C. albicans* 3153 germ tubes to polystyrene was due to the salivary sIgA since sIgA-depleted saliva enhanced the adhesion of *C. albicans* 3153 to polystyrene. The inhibitory effect mediated by sIgA was not related to the inhibition of germination but to the blockage of adhesins expressed on the cell wall surface of the germ tubes. The three mAbs studied reduced the adhesion of *C. albicans* 3153 to polystyrene at levels equivalent to those for purified sIgA. The highest reduction in the adhesion was obtained with the IgA mAb N3B. The best results were obtained when the three mAbs were combined. The results suggest that whole saliva plays a different role in the adhesion of *C. albicans* to polystyrene depending on the morphological phase of *C. albicans*. These results may give new insights into the conflicting role of saliva in the adhesion of *C. albicans* to plastic materials of dental prostheses.

Keywords: *Candida albicans*, adhesion, salivary secretory IgA, plastic, monoclonal antibodies

INTRODUCTION

Candida albicans is the fungus most frequently found in the human oral cavity, where it can be recovered from 2–62% of healthy individuals (Odds, 1988). However, in patients with immunodeficiencies and other predisposing conditions, *Candida* species can cause a variety of oral infections, including pseudomembranous

candidiasis, erythematous candidiasis, hyperplastic candidiasis, *Candida*-associated denture stomatitis, *Candida*-associated angular cheilitis, rhomboid glossitis and chronic mucocutaneous candidiasis (Delgado & Aguirre, 1997). Denture stomatitis is an inflammatory process affecting the oral mucosa of 25–65% of patients wearing removable dental prostheses (Budtz-Jørgensen, 1990). Several factors have been implicated in the aetiology of denture stomatitis, including denture-induced trauma, allergy, malnutrition, the use of oral antibiotics and fungal and bacterial infections (Arendorf

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Abbreviation: sIgA, secretory IgA.

& Walker, 1987). *C. albicans* is the fungal species most frequently isolated in patients with denture stomatitis (Budtz-Jørgensen, 1974) and, among other factors, adherence to plastic materials or to salivary macromolecules adsorbed on the surface of prosthetic devices is an important event in the ability of *C. albicans* to colonize dentures in the mouth (Vasilas *et al.*, 1992).

C. albicans can adhere to a variety of plastic materials, including the acrylic used to make dental prostheses (MacFarlane, 1990). Several factors are involved in the adhesion of *C. albicans* to plastic materials. Among them, surface hydrophobicity, electrostatic forces, presence of sucrose in the culture medium and germ tube formation seem to play a key role in the adhesion process (MacFarlane, 1990). Tronchin *et al.* (1988) and San Millán *et al.* (1996) have shown that the adhesion of *C. albicans* to polystyrene, a model system to study the adhesion of *C. albicans* to plastic materials, correlates with germ tube induction, since conditions which promote filamentation increased adhesion and those inhibiting germination decreased the adhesion. The use of agerminative and filamentous mutants has confirmed this observation (Hawser & Douglas, 1994; San Millán *et al.*, 1996). The increased ability of germ tubes to adhere to plastic materials has been correlated with the expression of a fibrillar layer on the surface of the cell wall which contains adhesins of >200, 200, 68 and 60 kDa (Tronchin *et al.*, 1988).

The adhesion of *C. albicans* to oral surfaces is modulated by different salivary components, including mucins (Edgerton *et al.*, 1993), other salivary proteins (Cannon *et al.*, 1995) and secretory IgA (sIgA). The effect of these components on the adhesion of *C. albicans* to oral surfaces may be very different since, while some of them can enhance adhesion, others can bring about inhibition (Epstein *et al.*, 1982; Vudhichamnong *et al.*, 1982; Edgerton *et al.*, 1993). Inhibition of adhesion of *C. albicans* to oral surfaces is thought to be an important strategy in preventing oral candidiasis. Various approaches have been resorted to in an attempt to inhibit *C. albicans* adhesion to host cells or to plastic surfaces. Such approaches include the use of *C. albicans* antigens, host proteins, antifungal agents and antibodies (Epstein *et al.*, 1982; Critchley & Douglas, 1987; Vuddhakul *et al.*, 1988; Edgerton *et al.*, 1993). Bendel *et al.* (1993) demonstrated inhibition of adhesion of *C. albicans* to buccal epithelial cells with an mAb against the β_2 -integrin subunit α_M , and Miyakawa *et al.* (1992) demonstrated reduced adherence of *C. albicans* to buccal epithelial cells using an mAb against factor 6, an epitope in the cell wall mannan responsible for serotype A specificity in *C. albicans*. We have previously shown that adhesion of *C. albicans* to polystyrene can be partially inhibited with an mAb directed against an epitope of antigen 6 expressed on the >200 kDa component of the fibrillar adhesins of *C. albicans* (San Millán *et al.*, 1996). In this report, we have studied the role of whole saliva in the adhesion of *C. albicans* to polystyrene and we have used three mAbs directed against epitopes of cell wall mannoproteins to mimic the

inhibitory effect observed with salivary sIgA on the adhesion of *C. albicans* to polystyrene.

METHODS

Fungal strains and culture conditions. *Candida albicans* serotype A (NCPF 3153), obtained from the National Collection of Pathogenic Fungi (NCPF, Bristol, UK), was used in most experiments. In one experiment, *C. albicans* CA2, a germ-tube-deficient mutant of the parental strain 3153 kindly supplied by Dr A. Cassone (Istituto Superiore di Sanita, Rome, Italy), *C. albicans* ATCC 90028, obtained from the American Type Culture Collection (ATCC, Manassas, VA) and *C. albicans* UPV-C2t, UPV-94392, UPV-94393 and UPV-94394 from the Universidad del País Vasco strain collection were also used. The strains were maintained at 4 °C on slants containing 20 g glucose, 10 g yeast extract and 20 g agar l⁻¹. For the adsorption of saliva with *C. albicans* NCPF 3153 cells, yeast cells and germ tubes were obtained in medium 199 (pH 6.7; Sigma) as previously described (Pontón *et al.*, 1996).

Saliva and mAbs. The experimental protocols were approved by the Institutional Review Board of the School of Medicine and Odontology at the University of Basque Country and all the subjects gave informed consent to participate. Unstimulated whole saliva from four healthy *C. albicans* carriers and non-carriers was collected. In every case, the saliva was centrifuged at 6000 g for 30 min at 4 °C and the supernatant was stored at -80 °C until used. The salivas were studied by both indirect immunofluorescence and spectrofluorometry assays against three *C. albicans* oral isolates (UPV-94392, UPV-94393 and UPV-94394). The pattern of reactivity of each saliva was the same with each strain so the most reactive salivas from three asymptomatic *C. albicans* oral carriers were used. The concentration of IgA in saliva was estimated by ELISA using purified human IgA (Sigma) as a control.

In one experiment, whole saliva was adsorbed with formalin-killed germ tubes or yeast cells of *C. albicans* NCPF 3153 suspended at 10¹⁰ cells (ml PBS)⁻¹. Adsorption was performed by mixing equal volumes of the adsorbing suspension and saliva. After incubation in a rotatory mixer for 2 h at room temperature, the organisms were removed by centrifugation. Supernatants were concentrated to the initial volume and stored at -80 °C until used. The *C. albicans* cells used in the adsorption of saliva were washed with PBS (7 mM Na₂HPO₄, 2.7 mM NaH₂PO₄ and 145 mM NaCl; pH 7.2) and components attached to the cell wall surface of *C. albicans* were then released by treatment with 2.5 M NaI in PBS for 1 h at room temperature. Cells were removed by centrifugation, and the supernatant was dialysed against PBS for 48 h at 4 °C. Eluted components were concentrated to the initial volume with polyethylene glycol 20000 and stored at -80 °C until used. The eluted components were analysed by SDS-PAGE as previously described (Pontón *et al.*, 1996). The total amount of protein loaded per lane was 5 µg.

To obtain saliva depleted of IgA, saliva samples were applied to an Affi-Gel 10 column (Bio-Rad) coupled to an anti-human IgA (Sigma). The saliva depleted of IgA was concentrated to the initial volume as described above and the depletion of IgA was assessed by SDS-PAGE and Western blotting with both a goat anti-human IgA and a goat anti-human secretory component (Pontón *et al.*, 1996). The purity of the IgA eluted from the affinity chromatography column was also checked by SDS-PAGE and Western blotting. In some experiments, an irrelevant IgA purified from human colostrum (Sigma) was used as a control. Two IgM mAbs (C7 and B9E) and one IgA

(N3B) were produced following standard methods with splenocytes from BALB/c mice immunized by subcutaneous injections of a partially purified antigen of 260 kDa from a germ tube extract (B9E and N3B) (Pontón *et al.*, 1993) or a cell wall stress mannoprotein of 200 kDa (C7) (Pontón *et al.*, 1996). mAbs were purified from ascites fluid by affinity chromatography on an anti-mouse IgM or an anti-mouse IgA (Sigma) coupled to an Affi-Gel 10 column, essentially as described for purifying sIgA. Purified antibodies were dialysed against PBS before use. Their reactivity against cell wall extracts from *C. albicans* was assessed by SDS-PAGE and Western blotting as previously described (Pontón *et al.*, 1993). The reactivity of mAb C7 was studied in DTT extracts after mild periodate oxidation [20 min at room temperature with 10 mM sodium metaperiodate (Sigma) in 100 mM acetate buffer, pH 5.3].

Adherence assays. Two adhesion assays were used. The visual assay was performed as previously described (San Millán *et al.*, 1996). To determine the effect of the saliva on adherence, the yeast cells were inoculated in medium 199 containing 520 µl whole saliva or sIgA-depleted saliva. In one experiment, the cells were grown in an Erlenmeyer flask containing medium 199. Germ tubes were induced at 37 °C with shaking and yeast cells were grown at 25 °C. After 40 and 140 min growth, the cells were washed with medium 199 at 4 °C, counted in a haemocytometer and transferred to polystyrene Petri dishes at a final concentration of 5×10^6 cells ml⁻¹ medium 199 or a mixture of medium 199 and saliva. The Petri dishes were incubated at 25 °C for 40 min and the adhesion was quantified as described above. The controls consisted of cells incubated in Petri dishes for 40 and 140 min at both 37 and 25 °C.

In the other assay, adhesion was quantified spectrofluorometrically. Yeast cells were inoculated in 350 µl medium 199, pH 6.7, at a final concentration of 1×10^6 cells ml⁻¹ and incubated at 37 °C in 24-well tissue culture polystyrene plates. After washing, the plates were stained with 0.1% calcofluor white in saline for 20 min at room temperature. Plates were washed again and the fluorescence was read in a spectrofluorometer (Fluoroskan Ascent; Labsystems) with an excitation filter of 358 nm and an emission filter of 460 nm. The controls used to examine possible interference from autofluorescence and non-specific binding consisted of wells without adherent cells and their background fluorescence was subtracted from that shown by the test wells. All values quoted represent mean figures derived from at least four independent assays. To determine the effect on adherence of the saliva, the purified secretory IgA and the mAbs, the yeast cells were inoculated in medium 199 containing 280 µl saliva or 20 µg of the purified sIgA or the mAb. In experiments where a combination of two or three mAbs were studied, 10 µg or 6.66 µg of each mAb was used, respectively.

Statistics. The ANOVA test was used to assess the significance of differences between means in adherence assays. Data were considered significant at $P < 0.05$.

RESULTS

Comparison of two methods to measure the adhesion of *C. albicans* to polystyrene

The kinetics of adhesion of *C. albicans* 3153 to polystyrene was initially studied by two different methods, a visual method in which the adhered cells were counted microscopically and a fluorometric

Fig. 1. Kinetics of filamentation and adhesion to polystyrene of *C. albicans* 3153 grown at 37 °C in the absence or presence of whole saliva. ●, Filamentation in the absence of whole saliva; ■, filamentation in the presence of whole saliva; ○, adhesion in the absence of whole saliva; □, adhesion in the presence of whole saliva. Data representing a typical experiment are expressed as the mean $\pm$ SEM of four determinations.

method in which the adhered cells were detected spectrofluorometrically. In both methods, adhesion of *C. albicans* 3153 yeast cells increased with the time of incubation, reaching a maximum at the end of the experiment (140 min). Similar results were obtained with a different strain of *C. albicans* (ATCC 90028; data not shown). The significant positive correlation ($P < 0.001$; $r = 0.97$) showed by both methods allowed us to use them interchangeably, depending on the characteristics of each experiment.

Influence of whole saliva on the adhesion of *C. albicans* to polystyrene

The kinetics of germination and adhesion to polystyrene of *C. albicans* 3153 was initially studied at 37 °C by the visual method in the absence of saliva (Fig. 1). Under these conditions, 20% of *C. albicans* 3153 cells showed short germ tubes (with a length approximately one or two times greater than that of the mother yeast) after 40 min incubation. Longer periods of incubation resulted in an increase in the percentage of germination and an extension in the length of the germ tubes. The maximum level of germination (96.8%) was reached at 140 min. At this time, the length of the germ tubes was approximately 10 times greater than that of the mother yeast. Adhesion of *C. albicans* 3153 to polystyrene increased with germination and it also reached a plateau (76.7%) at 100 min. In the presence of whole saliva, the germination of *C. albicans* 3153 was very similar to the control without saliva and no statistically significant differences were observed when germination in the presence and absence of whole saliva was compared (Fig. 1). In contrast to the adhesion to polystyrene in the absence of saliva, the presence of whole saliva enhanced the adhesion to polystyrene (43.7%) of *C. albicans* 3153 cells grown for 40 min at 37 °C but decreased the

Fig. 2. Kinetics of germination and adhesion to polystyrene of *C. albicans* 3153 grown at 37 °C in the absence or presence of sIgA-depleted saliva. ●, Filamentation in the absence of sIgA-depleted saliva; ■, filamentation in the presence of sIgA-depleted saliva; ○, adhesion in the absence of sIgA-depleted saliva; □, adhesion in the presence of sIgA-depleted saliva. Data representing a typical experiment are expressed as the mean $\pm$ SEM of four determinations.

adhesion of germinated cells by 68.6% at 140 min incubation.

To separate germination from adhesion, germ tubes were induced by incubating cells in an Erlenmeyer flask for 40 and 140 min at 37 °C. Under these conditions, 21.37% and 75.40% of *C. albicans* 3153 cells showed germ tubes after 40 and 140 min incubation, respectively. No significant autoaggregation in the germ tubes was observed. The cells were then transferred to polystyrene Petri dishes and allowed to adhere in the presence of whole saliva for 40 min at 25 °C to avoid further germ tube growth. As expected, whole saliva produced a statistically significant enhancement of adhesion to polystyrene of cells grown in Erlenmeyer flasks for 40 min ($38.46\% \pm 3.65\%$ versus $11.69\% \pm 1.12\%$ in the absence of saliva, $P < 0.0001$) but significantly reduced adhesion of cells grown in Erlenmeyer flasks for 140 min ($12.05\% \pm 0.16\%$ versus $77.32\% \pm 3.61\%$ in the absence of saliva, $P < 0.0001$).

Influence of sIgA-depleted saliva on the adhesion of *C. albicans* to polystyrene

The results obtained suggested that one or more components present in whole saliva was inhibiting the adhesion of *C. albicans* 3153 germ tubes to polystyrene. Since sIgA is one of the salivary components which are believed to be involved in the inhibition of the adhesion of micro-organisms to oral surfaces we depleted the saliva of sIgA and studied the ability of the sIgA-depleted saliva to inhibit the adhesion of *C. albicans* 3153 to polystyrene. As shown in Fig. 2, *C. albicans* 3153 germinated in the presence of sIgA-depleted saliva with similar kinetics to that shown by the cells in the absence of saliva. However, the presence of sIgA-depleted saliva in the adhesion experiments enhanced the adhesion of *C. albicans* 3153 to polystyrene when compared to the

Table 1. Adhesion to polystyrene of the agerminative mutant *C. albicans* CA2 in the presence of whole saliva from three donors

Data representing a typical experiment are expressed as the mean $\pm$ SEM of four determinations.

Time (min)	Adhesion (%)			
	Control	Saliva 1	Saliva 2	Saliva 3
40	0.3 ± 0.1	8.0 ± 0.3	22.6 ± 1.0	2.1 ± 0.1
60	0.5 ± 0.1	18.8 ± 0.2	43.0 ± 0.8	7.3 ± 0.3
80	0.4 ± 0.2	12.3 ± 0.5	36.2 ± 0.3	6.9 ± 0.1
100	0.5 ± 0.1	3.2 ± 0.7	47.1 ± 1.1	2.2 ± 0.2
120	0.9 ± 0.2	4.8 ± 0.1	49.3 ± 0.9	1.2 ± 0.1
140	0.3 ± 0.1	2.8 ± 0.8	47.5 ± 1.2	2.5 ± 0.1

adhesion of the cells in the absence of saliva, especially during the first 80 min of incubation. Adhesion and germination of *C. albicans* 3153 were not affected by the presence of IgA purified from human colostrum.

Adhesion of *C. albicans* to polystyrene in the absence of filamentation

The enhancement of adhesion to polystyrene shown by the cells incubated for 40–60 min in the presence of sIgA-depleted saliva or for 40 min in the presence of whole saliva suggested that the saliva was able to increase the adhesion of *C. albicans* 3153 cells when they were in the yeast phase or presented short germ tubes. To test this possibility, we studied the adhesion of *C. albicans* CA2, an agerminative mutant of *C. albicans* 3153, to polystyrene in the presence and absence of whole saliva. As expected, in the absence of saliva the agerminative mutant showed very low adhesion to polystyrene (Table 1). However, the saliva of three different donors produced a statistically significant increase in the adhesion of the mutant to polystyrene at all times studied ($P < 0.0001$).

Inhibition of adhesion of *C. albicans* to polystyrene by salivary components eluted from the surface of *C. albicans* cells

sIgA is thought to block the adhesion of micro-organisms to the oral cavity by binding the microbial surface thus blocking the receptors present on the cell wall. To assess whether the effect of saliva on the adhesion of *C. albicans* to polystyrene was due to the coating of the fungal cell wall surface, we incubated whole saliva with *C. albicans* 3153 cells and, after removing the *C. albicans* cells, we tested the capacity of the adsorbed saliva and that of the materials adsorbed to the cell surface after their elution from it to block the adhesion of *C. albicans* germ tubes to polystyrene. Analysis by SDS-PAGE of the eluted components showed two bands of 59 and 63 kDa (Fig. 3). When compared to the control without saliva or salivary

Fig. 3. Coomassie staining of a 10% polyacrylamide gel loaded with salivary components eluted from the surface of *C. albicans* germ tubes (lane 1) and blastospores (lane 2).

Fig. 4. Western blots of a 10% polyacrylamide gel loaded with DTT extracts from germ tubes, stained with whole saliva (lane 1) and mAbs N3B (lane 2), B9E (lane 3) and C7 (lane 4). The antigenic extract in lane 4 was treated with 10 mM sodium metaperiodate.

components, whole saliva significantly inhibited the adhesion of *C. albicans* 3153 to polystyrene to 61.7% of the control level ($P < 0.0001$). Adsorption of the saliva with germ tubes and yeast cells of *C. albicans* 3153 inhibited the adhesion of *C. albicans* to polystyrene to 24.4 and 30.9% of the control level, respectively. However, the salivary components eluted from the surface of the cell wall inhibited the adhesion to levels similar to that found with whole saliva (52.4 and 61.8% of the control level, respectively).

Inhibition of adhesion of *C. albicans* to polystyrene by mAbs

In one experiment, the capacity of three mAbs directed against epitopes of antigens expressed on the cell wall of *C. albicans* to inhibit the adhesion of *C. albicans* germ tubes to polystyrene was studied by the fluorometric method. Their reactivity against cell wall extracts from *C. albicans* was compared with that of the salivary IgA and is shown in Fig. 4. When compared to the control without mAbs, all mAbs studied significantly reduced the adhesion of *C. albicans* 3153 to polystyrene ($P < 0.0001$). The highest inhibition in the adhesion

Fig. 5. Inhibition of the adhesion to polystyrene of *C. albicans* 3153 incubated at 37 °C for 80 min by purified salivary slgA and mAbs B9E, C7 and N3B, individually or in combinations. Cells used in this experiment showed 31.3% filamentation. Results represent the mean $\pm$ SEM of four determinations.

(75.6%) was obtained with mAb N3B (Fig. 5). mAbs inhibited adhesion of *C. albicans* at levels equivalent to those for purified slgA. Combination of two or three mAbs produced an increase in the inhibitory effect obtained by combining the individual effects of each mAb. The best results were obtained when the three mAbs were combined. Similar results were obtained with a different strain of *C. albicans* (UPV-C2t, data not shown).

DISCUSSION

Adhesion of *C. albicans* to polystyrene in the presence of saliva or salivary components

Attachment of *C. albicans* to plastic materials such as those of dental prostheses, catheters and other medical devices seems to be a critical event in the initiation of colonization and infection. This process may be especially important in denture stomatitis where *C. albicans* can adhere to the acrylic to form a reservoir for chronic dissemination of fungal cells (Budtz-Jørgensen, 1974). A variety of methods have been devised for studying *in vitro* the adhesion of *C. albicans* to plastic materials and different types of cells, but all of them present methodological pitfalls including yeast factors such as the strain, and test conditions such as the quantification method (Kennedy, 1988). To minimize some of these methodological shortcomings, two methods have been used in the present study to determine the adhesion of *C. albicans* to polystyrene: a visual assay and a fluorometric assay. The excellent correlation shown by both assays confirmed the results obtained in our initial study and in subsequent experiments, each method was used depending on the characteristics of each experiment. The automatic method was rapid and convenient to carry out when a high number of tests was performed. The visual method, although more time-consuming, allowed for the estimation of the differential adhesion of yeast and mycelial cells, which

was an important feature in our model system of adhesion. By using the visual method, we have demonstrated that in our model system, adhesion of *C. albicans* to polystyrene is related to germ tube induction since adhesion increased in parallel with germination. These results are in agreement with previously published reports showing that the hyphae of *C. albicans* adhere more readily to plastic materials and buccal epithelial cells than yeast cells (Odds, 1988; San Millán *et al.*, 1996).

Although *C. albicans* can adhere directly to plastic materials (Tronchin *et al.*, 1988; Kennedy *et al.*, 1989; San Millán *et al.*, 1996), prostheses introduced into the oral cavity are rapidly coated by a pellicle containing selectively adsorbed salivary components, including high-molecular-mass mucin, amylase and sIgA (Edgerton & Levine, 1992) which can have opposite effects on the adhesion of *C. albicans*. Preincubation of acrylic strips with whole saliva has been shown to decrease adhesion of *C. albicans* to denture acrylic *in vitro* (Samaranayake *et al.*, 1980; McCourtie & Douglas, 1981). In contrast, coating denture acrylic with whole saliva as well as parotid and submandibular-sublingual saliva enhanced *Candida* adhesion (Vasilas *et al.*, 1992). The results presented in this study confirm both effects of saliva on the adhesion of *C. albicans* to plastic materials, since whole saliva decreased or enhanced the adhesion of *C. albicans* to polystyrene depending on the morphological phase of *C. albicans*. Whole saliva decreased adhesion of hyphae of *C. albicans* 3153 to polystyrene while the adhesion of yeast cells was enhanced. The enhancement of adhesion of blastospores to polystyrene promoted by whole saliva was confirmed with the mutant CA2, which was derived from *C. albicans* 3153. The adhesion of this mutant unable to form germ tubes was very low in the absence of whole saliva but its adhesion to polystyrene was significantly enhanced in the presence of whole saliva from different donors. Different components of the salivary pellicle may be responsible for the enhancement of adhesion observed in the yeast cells. Edgerton *et al.* (1993) have shown that mucin-containing human submandibular-sublingual saliva is capable of significant enhancement of adhesion of yeasts cells. However, other components, including antibodies, may also enhance the adhesion of yeast cells to polystyrene. In fact, we have described an mAb directed against the *C. albicans* adhesins involved in attachment to plastic materials which enhanced the adhesion of yeast cells to polystyrene (San Millán *et al.*, 1996).

Although other salivary components may also be involved, the decrease in adhesion of germ tubes to polystyrene seemed to be related to the presence in whole saliva of sIgA, since the inhibitory effect was not observed in sIgA-depleted saliva. Interestingly, Umazume *et al.* (1995) have shown that inhibition of adhesion of *C. albicans* to epithelial tumour cells caused by whole saliva was almost completely abolished by an anti-sIgA antibody, and saliva from patients receiving chemotherapy for oral carcinoma, which had reduced

concentrations of sIgA, showed reduced inhibition of *C. albicans* adhesion. The inhibitory effect mediated by sIgA is not related to an inhibition of germ tube induction, since similar levels of germination were observed in *C. albicans* cells in the absence or presence of sIgA. The effect is likely to be produced by blocking the adhesins present on the cell wall surface of the fungus, since adsorption of the saliva with yeast cells or germ tubes, a procedure which removed components bound to the fungal surface, reduced the inhibitory effect of whole saliva. Conversely, the salivary components eluted from the surface of the fungal cells showed an inhibition of adhesion similar to that observed with untreated whole saliva.

Inhibition of adhesion of *C. albicans* to polystyrene by mAbs

Knowledge of the mechanisms for attachment of *C. albicans* to plastic materials has allowed for the development of strategies to block the adhesion of the fungus. Different approaches have been tried to block the adhesion of *C. albicans* to plastic materials, including the use of sugars (Edgerton *et al.*, 1993), histatin 5 (Edgerton *et al.*, 1995) and saliva (Samaranayake *et al.*, 1980; McCourtie & Douglas, 1981; McCourtie *et al.*, 1986). Since the adhesion of *C. albicans* to plastic has been shown to be mediated by cell wall mannoproteins (Tronchin *et al.*, 1988) and salivary sIgA reacts with mannoproteins of *C. albicans* of different molecular masses (Pontón *et al.*, 1996), in this study we have tried to mimic the inhibitory effect on *C. albicans* adhesion mediated by sIgA with mAbs directed against epitopes expressed on cell wall surface antigens of *C. albicans* (Pontón *et al.*, 1993). All mAbs studied inhibited the adherence of *C. albicans* to polystyrene at levels equivalent to those for purified sIgA. Interestingly, the highest reduction in the adhesion was obtained with mAb N3B, an IgA which shows a reactivity with mannoproteins of *C. albicans* reminiscent of that shown by salivary sIgA. However, the results obtained with purified sIgA and mAbs are not directly comparable with those obtained with whole saliva since purified antibodies were used at a concentration 30 times higher than that of the sIgA in saliva. These results extend our previous observation that mAbs directed against epitopes expressed on *C. albicans* adhesins involved in attachment to polystyrene can inhibit adherence of *C. albicans* to this plastic (San Millán *et al.*, 1996). It is worth noting that the inhibitory effect can be enhanced by combining mAbs directed against different epitopes. It seems likely that mAbs mimic the inhibitory effect of saliva on the adherence of *C. albicans* to polystyrene through a different mechanism, since mAb B9E inhibited germination of *C. albicans* (San Millán *et al.*, 1996) while, as demonstrated in this study, sIgA did not. Historically, there has been controversy over the relative importance of antibody- and cell-mediated protection in candidiasis (Casadevall, 1995; Cassone *et al.*, 1997; Casadevall *et al.*, 1998). Data presented in this paper provide further support to the notion that

antibodies, and sIgA in particular, can modify the course of *C. albicans* infections associated with plastic devices by modulating the adhesion of the fungus to these materials.

In conclusion, we have demonstrated that whole saliva has a dual role in the adhesion of *C. albicans* to polystyrene, since it can enhance or inhibit the adhesion depending on the morphological phase of the fungus. The inhibitory effect shown by salivary sIgA can be mimicked by mAbs directed against epitopes of cell wall mannoproteins. If this effect is also observed in the adhesion of *C. albicans* to acrylic, the use of mAbs may open a new approach for inhibiting the adhesion of *C. albicans* to dental prostheses.

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REFERENCES

- Arendorf, T. M. & Walker, D. M. (1987). Denture stomatitis: a review. *J Oral Rehabil* **14**, 217–227.
- Bendel, C. M., Hostetter, M. K. & McClellan, M. (1993). Distinct mechanisms of epithelial adhesion for *Candida albicans* and *Candida tropicalis*. Identification of the participating ligands and development of inhibitory peptides. *J Clin Invest* **92**, 1840–1849.
- Budtz-Jørgensen, E. (1974). The significance of *Candida albicans* in denture stomatitis. *Scand J Dent Res* **82**, 151–190.
- Budtz-Jørgensen, E. (1990). *Candida*-associated denture stomatitis and angular cheilitis. In *Oral Candidosis*, pp. 156–183. Edited by L. P. Samaranayake & T. W. MacFarlane. London: Wright.
- Cannon, R. D., Nand, A. K. & Jenkinson, H. F. (1995). Adherence of *Candida albicans* to human salivary components adsorbed to hydroxylapatite. *Microbiology* **141**, 213–219.
- Casadevall, A. (1995). Antibody immunity and invasive fungal infections. *Infect Immun* **63**, 4211–4218.
- Casadevall, A., Cassone, A., Bistoni, F., Cutler, J. E., Magliani, W., Murphy, J. W., Polonelli, L. & Romani, L. (1998). Antibody and/or cell-mediated immunity, protective mechanisms in fungal disease: an ongoing dilemma or an unnecessary dispute? *Med Mycol* **36** (suppl. 1), 95–105.
- Cassone, A., Conti, S., De Bernardis, F. & Polonelli, L. (1997). Antibodies, killer toxins and antifungal immunoprotection: a lesson from nature? *Immunol Today* **18**, 164–169.
- Critchley, I. A. & Douglas, L. J. (1987). Isolation and partial characterization of an adhesin from *Candida albicans*. *J Gen Microbiol* **133**, 629–636.
- Delgado, W. & Aguirre, J. M. (1997). Las micosis orales en la era del sida. *Rev Iberoam Micol* **14**, 14–22.
- Edgerton, M. & Levine, M. J. (1992). Characterization of acquired denture pellicle from healthy and stomatitis patients. *J Prosthet Dent* **68**, 683–691.
- Edgerton, M., Scannapieco, F. A., Reddy, M. S. & Levine, M. J. (1993). Human submandibular-sublingual saliva promotes adhesion of *Candida albicans* to polymethylmethacrylate. *Infect Immun* **61**, 2644–2652.
- Edgerton, M., Raj, P. A. & Levine, M. J. (1995). Surface-modified poly(methyl methacrylate) enhances adsorption and retains anticandidal activities of salivary histatin 5. *J Biomed Mater Res* **29**, 1277–1286.
- Epstein, J. B., Kimura, L. H., Menard, T. W., Truelove, E. L. & Pearsall, N. N. (1982). Effects of specific antibodies on the interaction between the fungus *Candida albicans* and human oral mucosa. *Arch Oral Biol* **27**, 469–474.
- Hawser, S. P. & Douglas, L. J. (1994). Biofilm formation by *Candida* species on the surface of catheter material *in vitro*. *Infect Immun* **62**, 915–921.
- Kennedy, M. J. (1988). Adhesion and association mechanisms of *Candida albicans*. In *Current Topics in Medical Mycology*, vol. 2, pp. 73–169. Edited by M. R. McGinnins. New York: Springer.
- Kennedy, M. J., Rogers, A. L. & Yancey, R. J. (1989). Environmental alteration and phenotypic regulation of *Candida albicans* adhesion to plastic. *Infect Immun* **57**, 3876–3881.
- McCourtie, J. & Douglas, L. J. (1981). Relationship between cell surface composition of *Candida albicans* and adherence to acrylic after growth on different carbon sources. *Infect Immun* **32**, 1234–1241.
- McCourtie, J., MacFarlane, T. W. & Samaranayake, L. P. (1986). Effect of saliva and serum on the adherence of *Candida* species to chlorhexidine-treated denture acrylic. *J Med Microbiol* **21**, 209–213.
- MacFarlane, T. W. (1990). Ecology and epidemiology of *Candida*. In *Oral Candidosis*, pp. 21–46. Edited by L. P. Samaranayake & T. W. MacFarlane. London: Wright.
- Miyakawa, Y., Kuribayashi, T., Kagaya, K., Suzuki, M., Nakase, T. & Fukazawa, Y. (1992). Role of specific determinants in mannan of *Candida albicans* serotype-A in adherence to human buccal epithelial cells. *Infect Immun* **60**, 2493–2499.
- Odds, F. C. (1988). *Candida and Candidosis*. London: Baillière Tindall.
- Pontón, J., Marot-Leblond, A., Ezkurra, P., Barturen, B., Robert, R. & Senet, J. M. (1993). Characterization of *Candida albicans* cell wall antigens with monoclonal antibodies. *Infect Immun* **61**, 4842–4847.
- Pontón, J., Bikandi, J., Moragues, M. D., Arilla, M. C., Elósegui, R., Quindós, G., Fisicaro, P., Conti, S. & Polonelli, L. (1996). Reactivity of *Candida albicans* germ tubes with salivary secretory IgA. *J Dent Res* **75**, 1979–1985.
- Samaranayake, L. P., McCourtie, J. & MacFarlane, T. W. (1980). Factors affecting the *in vitro* adherence of *Candida albicans* to acrylic surfaces. *Arch Oral Biol* **25**, 603–609.
- San Millán, R., Ezkurra, P., Quindós, G., Robert, R., Senet, J. M. & Pontón, J. (1996). Effect of monoclonal antibodies directed against *Candida albicans* cell wall antigens on the adhesion of the fungus to polystyrene. *Microbiology* **142**, 2271–2277.
- Tronchin, G., Bouchara, J. F., Robert, R. & Senet, J. M. (1988). Adherence of *Candida albicans* germ tube to plastic: ultrastructural and molecular studies of fibrillar adhesins. *Infect Immun* **56**, 1987–1993.
- Umazume, M., Ueta, E. & Osaki, T. (1995). Reduced inhibition of *Candida albicans* adhesion by saliva from patients receiving oral cancer therapy. *J Clin Microbiol* **33**, 432–439.
- Vasilas, A., Molina, L., Hoffman, M. & Haidaris, C. G. (1992). The influence of morphological variation on *Candida albicans* adhesion to denture acrylic *in vitro*. *Arch Oral Biol* **37**, 613–622.

Vuddhhakul, V., McCormack, J. G., Seow, W. K., Smith, S. E. & Thong, Y. H. (1988). Inhibition of adherence of *Candida albicans* by conventional and experimental antifungal drugs. *J Antimicrob Chemother* **21**, 755–763.

Vudhichamnong, K., Walker, D. M. & Ryley, H. C. (1982). The effect of secretory immunoglobulin A on the in vitro adherence of

the yeast *Candida albicans* to human oral epithelial cells. *Arch Oral Biol* **27**, 617–621.

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