Effect of monoclonal antibodies directed against *Candida albicans* cell wall antigens on the adhesion of the fungus to polystyrene

Rosario San Millan,¹ Pilar A. Ezkurra,¹ Guillermo Quindós,¹ Raymond Robert,² Jean Marcel Senet² and Jose Pontón¹

Author for correspondence: Jose Pontón. Tel: +34 4 4647700 ext. 2746. Fax: +34 4 4648152.
e-mail: oipposaj@lg.ehu.es

¹ Departamento de
Inmunología,
Microbiología y
Parasitología, Facultad de
Medicina y Odontología,
Universidad del País Vasco,
Apartado 699, E-48080
Bilbao, Vizcaya, Spain

² Laboratoire
d'Immunologie-
Parasitologie, Section
Pharmacie, UER des
Sciences Médicales et
Pharmaceutiques,
16 bd Daviers, 49100
Angers, France

The adhesion of *Candida albicans* to polystyrene and the effect of three monoclonal antibodies (mAbs) reactive with *C. albicans* cell wall surface antigens on this process was assessed *in vitro* with several *C. albicans* strains. In the absence of mAbs, adhesion of *C. albicans* to polystyrene increased in parallel with germ-tube formation. However, the growth of the strains in the yeast phase at 25 °C or the use of an agerminative mutant inhibited adhesion to polystyrene. Serotype A and B strains showed similar kinetics of adhesion to polystyrene and no statistically significant differences in germination or adhesion were observed when strains from the two serotypes were compared. The three mAbs had different effects on both germination and adhesion of *C. albicans*. mAb 3D9 showed no influence on either germination or adhesion to polystyrene in two *C. albicans* strains. mAb B9E decreased both adhesion (45.6%) and filamentation (52.6%), and mAb 21E6 decreased filamentation (34.0%) but enhanced adhesion by 23.3%. This enhancement was also observed with the agerminative mutant and it was dose-dependent. It was not related to the binding capacity of the MAb to polystyrene nor to an increase in cell surface hydrophobicity of the antibody-treated cells. In conclusion, both growth phases of *C. albicans* can adhere to polystyrene, although the conditions for this process seem to be different in each phase. The two types of adhesion of *C. albicans* to polystyrene might have a role in the colonization of medical implants. The disparate effects shown by mAbs directed against cell wall mannoproteins of *C. albicans* on the adhesion of the fungus to polystyrene should be taken into consideration when designing strategies to block the adhesion of *C. albicans* to plastic materials with mAbs.

Keywords: *Candida albicans*, adhesion to polystyrene, filamentation, germ tubes, monoclonal antibodies

INTRODUCTION

Medical implants such as catheters, prosthetic cardiac valves, joint replacements and dental prosthesis have improved the health status of many patients. However, these devices can become colonized by different micro-organisms to form a biofilm and establish a reservoir for chronic inoculation and dissemination of microbial cells (Costerton *et al.*, 1987). Different studies have demonstrated the capacity of *Candida albicans* to adhere to plastic materials (Hawser & Douglas, 1994; Klotz *et al.*, 1985; McCourtie & Douglas, 1981; Minagi *et al.*, 1985) and factors such as the surface hydrophobicity, elec-

trostatic forces and germ tube formation seem to play a key role in the adhesion process (Kennedy *et al.*, 1989; Kimura & Pearsall, 1980; Klotz *et al.*, 1985; Rotrosen *et al.*, 1985). The yeast–mycelium transition, which may be associated with *C. albicans* pathogenicity (Cutler, 1991), results in a molecular reorganization of the cell wall that may facilitate the adhesion of the fungus to biomaterials by expressing a fibrillar layer associated with the outer part of the cell wall. In fact, we have identified four cell wall mannoproteins of > 200, 200, 68 and 60 kDa that remained attached to polystyrene when the *C. albicans* cells were removed (Tronchin *et al.*, 1988).

Attachment of *C. albicans* to medical implants may be a

critical step in the initiation of colonization and infection. Knowledge of the mechanisms for attachment of *C. albicans* to the materials of such devices could lead to the development of a novel class of antifungal agents whose mechanism of action would be to block adhesion of the fungus. In this paper we have studied the effect of different monoclonal antibodies (mAbs) 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 (Bristol, UK) was used in most experiments. In some experiments, *C. albicans* 1066 (Tronchin *et al.*, 1988) and *C. albicans* CA2, a germ-tube-deficient strain kindly supplied by Dr A. Cassone (Istituto Superiore di Sanita, Rome, Italy) were also used. The agerminative mutant is an echinocandin-resistant mutant of the parental strain 3153 (Bistoni *et al.*, 1986). To compare the adhesion and filamentation of serotype A and B strains, the strains *C. albicans* NCPF 3153 serotype A, *C. albicans* NCPF 3156 serotype B, and 28 clinical isolates (14 of each serotype) were used. The strains were maintained at 4 °C on slants containing 20 g glucose, 10 g yeast extract and 20 g agar l⁻¹. Yeast cells and germ tubes were grown in medium 199 (Sigma) as previously described (Pontón & Jones, 1986).

Serotype testing. *C. albicans* serotypes were distinguished on the basis of reactions of agglutination (serotype A) or non-agglutination (serotype B) with *Candida*-check antiserum 6 (Iatron) according to the manufacturer's instructions.

mAbs. The three mAbs, 21E6, 3D9 and B9E, were produced by standard methods. Briefly, BALB/c mice were immunized by subcutaneous injections of: (i) germ tube fibrinogen binding factor (3D9); (ii) germ tube cell wall components adhered to polystyrene Petri dishes (21E6); or (iii) a partially purified antigen of 260 kDa from a germ tube cell wall extract eluted from SDS-PAGE gels (B9E) (Pontón *et al.*, 1993). mAbs were purified from ascites fluid by affinity chromatography on an anti-mouse IgM (Sigma) coupled to an Affi-Gel 10 column (Bio-Rad). Purified antibodies were dialysed against PBS before use.

Adherence assay. The adhesion of *C. albicans* cells to polystyrene was studied by a previously described method (Tronchin *et al.*, 1988). Briefly, yeast cells in the stationary phase were inoculated in medium 199, pH 6.7, at a final concentration of 5×10^6 cells ml⁻¹ and incubated for 140 min at 37 °C in 35- or 120-mm-diameter tissue culture polystyrene Petri dishes (Greiner) containing, respectively, 0.65 or 50 ml of the yeast cell suspension. Germination was quantified by counting, every 20 min, both the total number of cells and the number of yeast cells bearing germ tubes, using a phase-contrast microscope with a graticule mounted in the focus of the ocular. For each Petri dish, 12 fields (each 0.64 mm²) were counted, and the percentage of germ tubes per field was calculated by the equation: % filamentation = (no. of yeast cells bearing germ tubes/total no. of cells) $\times$ 100. Petri dishes were then rinsed with distilled water to dislodge all the loosely adherent yeast cells. The fungal cells which adhered to the plastic surface were counted as described above. Results were expressed as a percentage of the whole cell population according to the equation: % adhesion = (no. of adhered cells/total no. of cells) $\times$ 100. All values quoted represent mean values derived from four independent assays.

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 min of 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. The Petri dishes were incubated at 24 °C for 40 min and the adhesion was quantified as described above. The controls consisted of cells incubated in Petri dishes for 40 min at both 37 °C and 25 °C.

Isolation of germ tube cell wall components adhered to polystyrene. After a 3 h incubation, the Petri dishes were washed with distilled water and the germ tubes were removed from the plastic surface with a cell lifter (Costar Europe). Plates were washed again with distilled water and the cell wall mannoproteins that remained adhered to the Petri dishes were solubilized with 0.1 M NaOH for 5 min at room temperature. After the treatment, the extracts were dialysed against distilled water for 48 h and lyophilized.

Effect of mAbs on germination and adherence. To determine the effect of the mAbs on adherence, the adhesion assay described above was slightly modified. Yeast cells were inoculated in medium 199 containing 154 µg mAbs ml⁻¹. Control media were supplemented with the same volume of sterile distilled water. Germination and adhesion were quantified as described above.

SDS-PAGE and Western blotting. SDS-PAGE was performed by the method of Laemmli (1970) in a minigel system (Bio-Rad). The total amount of protein loaded per lane was 1 µg for each extract. Electrophoresis was carried out in 10% (w/v) acrylamide at 200 V for 45 min. Standard molecular mass markers were from Bio-Rad. Subsequently, the gels were either stained with silver nitrate or were electrophoretically transferred to a nitrocellulose membrane (Bio-Rad) for 30 min at 60 V, 10 W and 5 mA cm⁻² using the Fast Blot System (Biometra). After the transfer, the nitrocellulose membranes were blocked in 10% (w/v) nonfat dry milk in Tris-buffered saline (TBS-Milk), washed in TBS and incubated with the mAbs (3D9 diluted 1:5, 21E6 diluted 1:5 and B9E diluted 1:10, all in TBS-Milk), washed, and incubated with peroxidase-labelled, affinity-purified goat anti-mouse IgM (Sigma). Immunoreactive bands were visualized after staining for 30 min with a substrate solution [0.05% (w/v) 4-chloro-1-naphthol (Sigma) and 0.015% (v/v) H₂O₂ in TBS]. In some experiments, the antigens present on the nitrocellulose membrane were treated with 50 mM NaIO₄ as described by Sundstrom & Kenny (1984) and incubated with MAb 21E6 as described above.

ELISA. The binding capacity of mAbs 21E6 and B9E to polystyrene was studied by ELISA. Briefly, wells of polystyrene plates (Greiner) were coated with 100 µl of the mAbs (150 or 75 µg ml⁻¹) suspended in medium 199 and incubated for 60 min at 37 °C. Plates were blocked by adding 200 µl PBS containing 10% nonfat dry milk and 0.05% Tween 20 (PBSBT) to each well for 1 h at 37 °C. They were then incubated with peroxidase-conjugated, goat anti-mouse IgM (Sigma) diluted 1:2000 in PBS-milk for 1 h at 37 °C. Plates were washed with PBS and 100 µl of a solution containing 0.05% *o*-phenyldiamine dihydrochloride (Sigma) and 40 µl 30% (v/v) H₂O₂ in 0.15 M phosphate/citrate buffer (pH 5.0) were added to each well. Plates were incubated in the dark at room temperature for 30 min. The reaction was stopped with 50 µl 1 M H₂SO₄ and A₄₉₀ was read with a Titertek Multiscan.

Cell surface hydrophobicity. To test if the pretreatment of *C. albicans* 3153 cells with mAbs 21E6 and B9E modified the cell surface hydrophobicity, we incubated the *C. albicans* cells at 37 °C for 40 min in the presence of mAbs 21E6 and B9E, and then the pretreated cells were incubated with latex beads as

proposed by Hazen & Hazen (1987). The experiments were performed in duplicate.

Statistics. Student's *t* test was used to assess the significance of differences between means in adhesion and filamentation of serotype A and B strains. Data were considered significant at $P < 0.05$. When using the mAbs, the percentage of bound cells and standard error was calculated for each Petri dish. The appropriate percentage and variance was then obtained considering all the independent assays. The hypothesis of two treatments being equal was tested ($\alpha = 0.05$) using the normal approximation to the distribution of the observed percentage. Results are presented as means $\pm$ SEM.

RESULTS

Adherence of *C. albicans* strains to polystyrene

The kinetics of adherence of several *C. albicans* strains to polystyrene was initially explored at two temperatures. All strains showed similar kinetics; as illustrated in Fig. 1 for *C. albicans* 3153 serotype A, adhesion at 25 °C was very low and it did not significantly change during the time studied. At 37 °C, however, there was an increase in the adhesion of the fungus in parallel with germ tube formation. Maximum levels of filamentation (96.6%) and adhesion (77.7%) were reached at 140 min. To assess if serotype A and B strains showed differences in filamentation and/or adhesion to polystyrene, 15 serotype A and 15 serotype B strains were incubated for 140 min in polystyrene Petri dishes. No statistically significant differences were observed between serotypes in either germination (serotype A $87.9 \pm 3.4\%$ versus serotype B $84.3 \pm 3.1\%$) or adhesion to polystyrene (serotype A $58.1 \pm 5.6\%$ versus serotype B $58.0 \pm 3.7\%$). The relationship between germ tube formation and adhesion to polystyrene observed in all the strains studied was confirmed using an agerminative mutant, CA2. As expected, no filamentation and consequently no adhesion to polystyrene was observed with this strain (data not shown).

Fig. 1. Kinetics of germination (□, ■) and adhesion (○, ●) to polystyrene of *C. albicans* 3153 (serotype A) incubated at 37 °C (■, ●) or 25 °C (□, ○). Data points represent the means of quadruplicate determinations $\pm$ SEM.

Fig. 2. Western blots of 10% slab gels loaded with NaOH extracts from fibrillar adhesins stained with silver nitrate (lane 1) and mAbs 21E6 (lanes 2 and 3), B9E (lane 4) and 3D9 (lane 5). In lane 3, antigens present on the nitrocellulose membrane were treated with sodium periodate before the incubation with the antibody. Positions of molecular mass standards are shown on the left.

In an attempt to separate germination from adhesion, the germ tubes were first induced by incubating cells in an Erlenmeyer flask for 40 min at 37 °C; the cells were then transferred to polystyrene Petri dishes and allowed to adhere for 40 min at 25 °C to avoid further germ tube growth. Pre-formed germ tubes adhered to polystyrene at a percentage higher than that shown by germ tubes induced directly in polystyrene Petri dishes ($19.68 \pm 2.04\%$ versus $10.4 \pm 1.3\%$). Differences in adhesion may be related to the different percentage filamentation observed in the two systems, since germ tubes induced in Erlenmeyer flasks with shaking showed a higher percentage filamentation ($47.75 \pm 2.57\%$, versus $18.7 \pm 1.69\%$ for germ tubes induced in polystyrene Petri dishes). However, yeast cells grown in Erlenmeyer flasks at 25 °C showed very low adhesion when transferred to polystyrene Petri dishes ($0.16 \pm 0.23\%$).

Reactivity of *C. albicans* adhesins with mAbs

We have previously shown that *C. albicans* germ tubes adhere to polystyrene through cell wall fibrillar adhesins that remain attached to polystyrene after the removal of adherent germ tubes (Tronchin *et al.*, 1988). SDS-PAGE analysis of those adhesins showed three components of ≥ 200 , 68 and 60 kDa after silver staining (Fig. 2). When the reactivity of these mannoproteins with different mAbs was studied, mAb 21E6 stained the antigens of 68 and

Fig. 3. Effect of incubation with mAbs 21E6, B9E and 3D9 on germination (□) and adhesion to polystyrene (■) of *C. albicans* 3153 (a) and *C. albicans* 1066 (b) incubated at 37 °C for 80 min. Results represent the means of triplicate determinations $\pm$ SEM. *Results were significantly different ($P < 0.001$) from control values without mAbs (by the normal approximation to the distribution of the observed percentage).

60 kDa, but it also reacted with the high molecular mass antigens after periodic acid oxidation of the manno-proteins. In this case, two antigens of > 200 and 200 kDa were observed. mAb B9E only reacted with the > 200 kDa antigen and mAb 3D9 did not show any reactivity with the fibrillar adhesins.

Influence of mAbs on adhesion

The effect of the mAbs on the adhesion of *C. albicans* strains 3153 and 1066 to polystyrene was initially studied after incubation at 37 °C for 80 min. When compared to the controls without mAbs, mAb B9E significantly reduced the adhesion of the strains to polystyrene (52.5% and 38.7%, respectively) (Fig. 3) and produced a statistically significant decrease in filamentation in both strains (50.3% and 55.0%). mAb 3D9 caused no statistically significant differences in either filamentation or adhesion in the two strains studied compared with controls. However, mAb 21E6 enhanced the adhesion of *C. albicans* 3153 and 1066 by 24.95% and 21.67%, respectively. The enhancement of adhesion observed with mAb 21E6 was largely independent of germ tube induction, since this mAb reduced the filamentation at all times studied (Fig. 4). The enhancement of adhesion was greater when the levels of filamentation were low. Thus, incubation of *C. albicans* 3153 cells at 37 °C for 40 min in the presence of mAb 21E6 enhanced the adhesion to

Fig. 4. Kinetics of germination (□) and adhesion to polystyrene (■) of *C. albicans* incubated at 37 °C for 40, 80 and 120 min in the presence (a) or absence (b) of mAb 21E6. Data points represent the means of triplicate determinations $\pm$ SEM. *Values were significantly different ($P < 0.001$) from control values without mAb 21E6 (by the normal approximation to the distribution of the observed percentage).

353.7% of the control level, whereas only a moderate increase in adhesion (26–38%) was observed at longer times of germ tube induction. The low correlation between adhesion to polystyrene and filamentation in *C. albicans* cells pretreated with mAb 21E6 was confirmed with the agerminative mutant, for which similar levels of adhesion (70.7 ± 2.7 , 77.9 ± 2.2 and 77.3 ± 2.5 %) were observed at 40, 80 and 120 min of incubation at 37 °C. This experiment clearly showed that, in the presence of mAb 21E6, yeast cells can also adhere to polystyrene. To study the effect of the dose of mAb 21E6 on the adherence of *C. albicans* 3153 to polystyrene, different dilutions of the mAb were tested. When the mAb was tested at a concentration of $154 \mu\text{g ml}^{-1}$, 7.9 ± 1.3 % filamentation and 63.1 ± 2.3 % adhesion were observed. Incubation of *C. albicans* with the mAb at a 10-fold lower concentration increased both filamentation (19.4 ± 2.1 %) and adhesion (77.8 ± 2.0 %). However, still lower concentrations of mAb 21E6 showed no effect on filamentation although there was a dose-dependent decrease in adhesion.

The effect of mAb 21E6 on the adhesion to polystyrene Petri dishes of yeast cells and germ tubes previously grown in Erlenmeyer flasks for 40 min was investigated. mAb 21E6 enhanced the adhesion of the pre-formed germ tubes to polystyrene Petri dishes by 382.8% compared with the control without mAb (75.35 ± 2.43 % versus 19.68 ± 2.04 %). Yeast cells grown in Erlenmeyer flasks at

25 °C and then incubated with the mAb 21E6 in polystyrene Petri dishes showed low levels of adhesion to polystyrene ($6.31 \pm 1.33\%$).

The different effects of mAbs B9E and 21E6 on adhesion of *C. albicans* to polystyrene were not related to the binding capacity of the mAbs to polystyrene since, when tested at the same concentration ($150 \mu\text{g ml}^{-1}$), mAbs B9E and 21E6 showed a similar binding to polystyrene ($A_{490} 0.110 \pm 0.005$ and 0.106 ± 0.005 , respectively).

Influence of incubation with mAbs on cell surface hydrophobicity

To assess if the enhancement of adherence to polystyrene observed in *C. albicans* cells incubated with mAb 21E6 was related to an increase in cell surface hydrophobicity we measured the cell surface hydrophobicity of *C. albicans* cells before or after incubation with mAbs 21E6 and B9E. Control cells without mAbs showed a cell surface hydrophobicity of $16.89 \pm 2.44\%$. Cells pretreated with mAb B9E were more hydrophobic than the controls ($27.42 \pm 2.87\%$). Conversely, pretreatment with mAb 21E6 rendered the cells more hydrophilic than the controls ($7.75 \pm 1.50\%$).

DISCUSSION

Among the factors shown to influence the adhesion of *C. albicans* to plastic, germ tube formation may be particularly important since adherent germ tubes have a fibrogranular layer that contains mannoproteins adhesive to polystyrene (Tronchin *et al.*, 1988). Hyphal growth also seems to be an important factor in biofilm formation and Hawser & Douglas (1994) have shown that a mutant unable to grow in the hyphal phase had a decreased capacity for biofilm formation on plastic catheters, whereas a related mutant unable to grow in the yeast phase gave values for biofilm formation similar to those of the wild-type. The results presented in this paper confirm a role for germ tube formation in the adhesion of *C. albicans* to polystyrene, since germ tubes, when induced, adhered to polystyrene and adhesion increased as the percentage of filamentation increased. In contrast, when the fungus was grown in the yeast form, either by growing strain 3153 at 25 °C or by using the agerminative mutant CA2 grown at 25 or 37 °C, there was no adhesion to polystyrene.

Factor 6, an epitope in the cell wall mannan responsible for serotype specificity, is another factor that has been implicated in adhesion of *C. albicans* to oral cells; serotype B or factor-6-deficient mutants have reduced adherence compared with serotype A strains (Miyakawa *et al.*, 1989, 1992). However, Kennedy *et al.* (1992) studied the adhesion to polystyrene of yeast cells from several serotype B isolates and observed that, in general, serotype B was more adhesive to plastic than serotype A. In our study, adhesion kinetics were very similar in both *C. albicans* serotype A and B strains. The similarities in adhesion to polystyrene observed between the two serotypes may be related to the participation of germ

tubes in adhesion to polystyrene, since both serotype A and B germ tubes express antigen 6 (Barturen *et al.*, 1995; Poulain *et al.*, 1983).

Adhesion of *C. albicans* germ tubes to polystyrene seems to be mediated by cell wall fibrillar adhesins of > 200, 200, 68 and 60 kDa (Tronchin *et al.*, 1988). The role of these adhesins in adhesion of *C. albicans* to polystyrene has been confirmed in this study, since only the mAbs reactive with some of these components modified the adhesion of *C. albicans* to polystyrene. Thus, mAb 3D9, which reacts with an epitope specifically expressed on the germ tube cell wall of *C. albicans* but not expressed on the fibrillar adhesins, showed no effect on adhesion of *C. albicans* to polystyrene. Conversely, mAb B9E, which reacts with the > 200 kDa component, and mAb 21E6, which reacts with components of 68 and 60 kDa, influenced the adhesion of *C. albicans* to polystyrene. Interestingly, mAb 21E6 was also able to label the high molecular mass components after periodic acid oxidation, suggesting that, in these antigens, the protein epitope reactive with the mAb is hidden by sugar residues. This situation seems to exist at the cell wall surface also, since, although the mAb does not label the cell wall surface of *C. albicans* when tested by indirect immunofluorescence (Pontón *et al.*, 1993), there was a strong labelling after periodic acid oxidation (P. A. Ezkurra and co-workers, unpublished).

Attempts to inhibit *C. albicans* adhesion to host cells or plastic surfaces have been made by various approaches, including antibodies, host proteins, *C. albicans* antigens and antifungals (Critchley & Douglas, 1987; Edgerton *et al.*, 1993; Epstein *et al.*, 1982; Vuddhakul *et al.*, 1988). Some mAbs have been used to study the role of cell wall epitopes in the adhesion of *C. albicans* to buccal epithelial cells. Bendel *et al.* (1993), using mAbs against the β_2 -integrin subunit α_M , inhibited the adhesion of *C. albicans* by 52.4–58.2%, and Miyakawa *et al.* (1989, 1992) showed a reduced adherence to buccal epithelial cells of *C. albicans* pretreated with a mAb against factor 6 (mAb-6). In this study, incubation of *C. albicans* with mAb B9E, which seems to react with an epitope of antigen 6 (Barturen *et al.*, 1995) expressed on the > 200 kDa component of the fibrillar adhesins of *C. albicans*, decreased the adhesion of the fungus to polystyrene. This effect seems to be independent of the strain, since the mAb inhibited adhesion to polystyrene of two *C. albicans* serotype A and B strains; it may be related to the reduction of germ tube formation by this mAb.

By contrast, incubation of *C. albicans* cells with mAb 21E6 enhanced the adhesion of *C. albicans* 3153 and 1066 strains to polystyrene. Interestingly, the effect was observed in both germ tubes and yeast cells. The effect of mAb 21E6 on the adhesion of yeast cells was very evident, since the agerminative mutant grown at 37 °C showed high adhesion levels. The effect of mAb 21E6 on the adhesion of germinating cells was greater when the percentage of germination of the cells was low (40 min). The reasons for this enhancement of adhesion mediated by mAb 21E6 are presently unknown but it does not seem to be related to an increase in cell surface hydrophobicity after incubation

with mAb 21E6, since pretreatment of *C. albicans* with mAb 21E6 rendered the cells more hydrophilic than those incubated with mAb B9E or the controls without antibody. Another possibility is a reorganization of the cell wall fibrillar adhesins induced by mAb 21E6, leading to an exposure of the adhesins on the cell surface. Interestingly, pretreatment of polystyrene with the cell wall fibrillar adhesins enhanced adhesion of the fungus (data not shown). The effect of mAb 21E6 on the adhesion of *C. albicans* to polystyrene implies that the mAb is able to interact with the cell wall surface. By using an ELISA, we have been able to demonstrate a low reactivity of mAb 21E6 with the cell wall surface of both the agerminative mutant and *C. albicans* 3153 germ tubes grown at 37 °C, a reactivity which is likely not to be observed by indirect immunofluorescence due to its lower sensitivity (P. A. Ezkurra and co-workers, unpublished).

In conclusion, both growth phases of *C. albicans* can adhere to polystyrene, although the conditions for this process seem to be different in each phase. Germ tubes can adhere to polystyrene in the absence of mAb 21E6 but the adhesion of the yeast cells was modulated by the mAb. The two methods of adhesion of *C. albicans* to polystyrene might have a role in the colonization of medical implants. In the absence of antibodies against the fibrillar adhesins, a situation which is likely to occur during the first stages of infection, the germ tube may use the fibrillar adhesins to adhere to polystyrene. The production of an antibody response against some cell wall antigens may decrease the adhesion of *C. albicans*, perhaps by reducing filamentation. However, the existence of antibodies like mAb 21E6, which react with the antigens of > 200, 200, 68 and 60 kDa present in the fibrillar adhesins, may help the yeast phase to adhere to polystyrene. The different effects shown by the mAbs studied on the adhesion of *C. albicans* to polystyrene should be taken into consideration when designing strategies to block the adhesion of *C. albicans* to plastic materials with mAbs. However, confirmation of these results with prosthesis or catheter materials must be performed before definitive conclusions can be drawn.

ACKNOWLEDGEMENTS

We thank L. Campos for photographic assistance and Dr A. Sanz for her help with the statistics. We also thank the reviewers of this manuscript for their useful suggestions. R. San Millan and P. A. Ezkurra were supported by grants from the Dirección General de Investigación Científica y Técnica del Ministerio de Educación y Ciencia. This work was financed by grant PM92-0108 from the Dirección General de Investigación Científica y Técnica, grant UPV 093.327-EC018/93 from the Universidad del País Vasco, and by Contract no. SCI 0361 (EDB) from the Directorate General for Science, Research Development, Commission of the European Communities.

REFERENCES

- Barturen, B., Bikandi, J., San Millan, R., Moragues, M. D., Regúlez, P., Quindós, G. & Pontón, J. (1995). Variability in expression of antigens responsible for serotype specificity in *Candida albicans*. *Microbiology* **141**, 1535–1543.
- 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.
- Bistoni, F., Vecchiarelli, A., Cenci, E., Pucetti, P., Marconi, P. & Cassone, A. (1986). Evidence for macrophage-mediated protection against lethal *Candida albicans* infection. *Infect Immun* **51**, 668–674.
- Costerton, J. W., Cheng, K. J., Geesey, G. G., Ladd, T. I., Nickel, J. C., Dasgupta, M. & Marrie, T. J. (1987). Bacterial biofilms in nature and disease. *Annu Rev Microbiol* **41**, 435–464.
- Critchley, I. A. & Douglas, L. J. (1987). Isolation and partial characterization of an adhesin from *Candida albicans*. *J Gen Microbiol* **133**, 629–636.
- Cutler, J. E. (1991). Putative virulence factors of *Candida albicans*. *Annu Rev Microbiol* **45**, 187–218.
- 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.
- 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 materials *in vitro*. *Infect Immun* **62**, 915–921.
- Hazen, K. L. & Hazen, B. W. (1987). A polystyrene microsphere assay for detecting cell surface hydrophobicity within *Candida albicans* populations. *J Microbiol Methods* **6**, 289–299.
- Kennedy, M. J., Rogers, A. L. & Yancey, R. J. (1989). Environmental alterations and phenotypic regulation of *Candida albicans* to plastic. *Infect Immun* **57**, 3876–3881.
- Kennedy, M. J., Johnson, A. M., Volz, P. A., Neely, A. N. & Yancey, R. J. (1992). Virulence and adhesive properties of serotype-A and serotype-B of *Candida albicans* isolated from paediatric burn patients. *J Med Microbiol* **36**, 428–436.
- Kimura, L. H. & Pearsall, N. N. (1980). Relationship between germination of *Candida albicans* and increased adherence to human buccal epithelial cells. *Infect Immun* **28**, 464–468.
- Klotz, S. A., Drutz, D. J. & Zajic, J. E. (1985). Factors governing adherence of *Candida* species to plastic surfaces. *Infect Immun* **50**, 97–101.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.
- 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.
- Minagi, S., Miyake, Y., Inagaki, K., Tsuru, H. & Suganaka, H. (1985). Hydrophobic interaction in *Candida albicans* and *Candida tropicalis* adherence to various denture base resin materials. *Infect Immun* **47**, 11–14.
- Miyakawa, Y., Kagaya, K., Kuribayashi, T., Suzuki, M. & Fukazawa, Y. (1989). Isolation and chemical and biological characterization of antigenic mutants of *Candida albicans* serotype A. *Yeast* **5**, S225–S229.
- 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.
- Pontón, J. & Jones, J. M. (1986). Analysis of cell wall extracts of

Candida albicans by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and western blot techniques. *Infect Immun* **53**, 565–572.

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.

Poulain, D., Tronchin, G., Vernes, A., Popeye, R. & Biguet, J. (1983). Antigenic variations of *Candida albicans* *in vivo* and *in vitro*—relationships between P antigens and serotypes. *Sabouraudia* **21**, 99–112.

Rotrosen, D., Edwards, J. E., Gibson, T. R., Moore, J. C., Cohen, A. H. & Green, I. (1985). Adherence of *Candida* to cultured vascular endothelial cells: mechanisms of attachment and endothelial cell penetration. *J Infect Dis* **152**, 1264–1274.

Sundstrom, P. M. & Kenny, G. E. (1984). Characterization of antigens specific to the surface of germ tubes of *Candida albicans* by immunofluorescence. *Infect Immun* **43**, 850–855.

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.

Vuddhakul, 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.

Received 26 October 1995; revised 27 February 1996; accepted 11 March 1996.