Adhesion of *Candida albicans* to HeLa Cells: Studies Using Polystyrene Beads

Masayoshi MIYAUCHI,^a Philippe GIUMMELLY,^a Shin YAZAWA,^b and Yoshio OKAWA^{*,a}

^aDepartment of Infection and Host Defense, Tohoku Pharmaceutical University; 4–4–1 Komatsushima, Aoba-ku, Sendai, Miyagi 981–8558, Japan; and ^bDepartment of Legal Medicine, Gunma University School of Medicine; 3–39–22 Showa-machi, Maebashi, Gunma 371–8511, Japan.

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The adherence to HeLa cells by the yeast-type cells of the *Candida albicans* NIH A-207 strain cultivated for 2 d at 27 °C in the yeast extract-added Sabouraud liquid medium (YSLM) and the 500 mM galactose-added yeast nitrogen base medium (YNB-Gal) was compared. The yeast cells cultured in the YNB-Gal clearly increased the adherence to the HeLa cells compared to the YSLM. The adherence drastically decreased by pronase treatment of the yeast cells. Next, to define the sugar receptors of the yeast cells, lactosylceramide (LC)-, the H type 1 antigen (HA)-, Lewis^a antigen (Le^a)-, mannan- and BSA-coated polystyrene beads were used for the adherence to the yeast cells. Only the LC- and HA-beads were bound to the yeast cells. The adherence of the two beads to the yeast cells cultured in the YNB-Gal was higher than that to the yeast cells cultured in the YSLM. The yeast cell wall mannan-coated polystyrene beads did not adhere at all to the HeLa cells. Based on these results, it is evident that the protein parts involving the LC and HA receptors on the surface of the yeast cells correlate with the adherence to the HeLa cells.

Key words *Candida albicans*; HeLa cell; adherence; polystyrene bead; sugar receptor; mannan

The adherence to the host cells of *Candida albicans* is one of the important pathogenic factors during the initial stage of the infection. Many candidate adhesion molecules on the yeast cells have been reported. The protein parts^{1,2)} and the protein portion^{2,3)} of the mannoprotein of the yeast cells are more important than the carbohydrate moiety. On the other hand, several authors have shown that the cell-wall mannan parts^{4,5)} and chitin⁶⁾ are implicated to have some adhesive function to the host cells. It is also known that the glycosphingolipids⁷⁾ and the glycosides^{8,9)} could act as host receptors for *C. albicans* on the epithelial cells. Thus, the adhesion mechanisms of pathogenic yeasts are complex and the exact nature has not yet been completely solved. The elucidation of the adhesion mechanisms of pathogenic yeasts to hosts is important for understanding the pathogenic significance and to provide a means of preventions and the treatments of candidiasis. To assess the relevance to acid proteinase in candidiasis, we have already reported the production of the enzyme of several *Candida* species strains.^{10,11)}

In this study, we compared the adhesiveness to HeLa cells by *C. albicans* cultured in yeast extract-added Sabouraud liquid medium (YSLM) and yeast nitrogen base medium containing 500 mM galactose (YNB-Gal). Also, we assessed the adhesions using the polystyrene bead methods.

MATERIALS AND METHODS

Strain and Culture Conditions The *C. albicans* NIH A-207 strain (A-strain) was kindly supplied by Dr. A. Nishikawa, Meiji College of Pharmacy, Tokyo, Japan. The cultivation and preparation of the cells were conducted as previously described. The A strain was precultured in yeast extract-added Sabouraud liquid medium (YSLM) at 27 °C for 24 h on a rotary shaker (150 rpm).¹²⁾ The cells (2×10^7) in 100 to 200 μ l aliquots were then inoculated into 200 ml each of YSLM and of yeast nitrogen base medium containing 500 mM galactose (YNB-Gal)¹³⁾ in 500 ml Erlenmeyer flasks, and

the main cultivations were separately conducted at 27 °C for 2 d with shaking.

Preparation of Mannans The mannan fractions obtained from the A strain cells cultured at 27 °C for 2 d in YSLM (pH 5.9)¹²⁾ and in YSLM (pH 2.0)¹⁴⁾ were designated mannans A-5.9-M and A-2.0-M, respectively.

Culture of HeLa Cells HeLa cells, derived from a human cervical carcinoma, were donated by the Research Institute for Tuberculosis and Cancer, Tohoku University, Sendai, Japan. The cells were incubated in RPMI 1640 medium containing 10% fetal bovine serum at 37 °C under 5% CO₂ in air.

Adherence Assay of Yeast Cells to HeLa Cells The adherence assay was conducted with a slight modification as previously described.¹⁵⁾ The HeLa cells were gently detached from the monolayer in tissue culture flasks by a cell scraper (Falcon Co., Ltd.) and washed twice with phosphate buffered saline (PBS). The A-strain cells were prepared for the adherence experiments by washing twice with PBS. Both cell concentrations were determined by direct counts using a hemacytometer. A mixture of equal volumes (1.0 ml) of HeLa cells (10^5 /ml) and *C. albicans* cells (10^7 /ml) was incubated on a rotator at 37 °C for 30 min. Three tubes were used for each experiment. After incubation, the HeLa cells were collected on polycarbonate filters (8 μ m pore size) (Nucleopore Corp., Pleasanton, CA, U.S.A.) and washed with 8 ml PBS under gentle agitation. The percentage (%) of the A-strain cells attached to the HeLa cells was determined. The average from triplicate samples was considered as the relative adherence ability of the yeast cell population. Each experiment was repeated at least twice.

Pronase Treatment of Yeast Cells The A-strain cells (10^7 /ml) were treated with 100 μ g/ml of pronase-P (Kaken Pharmaceutical Co., Ltd., Tokyo, Japan) in PBS for 20 h at 37 °C. The pronase-treated yeast cells were washed twice with PBS and the adherence ability was measured by the method described above.

* To whom correspondence should be addressed. e-mail: okawa@tohoku-pharm.ac.jp

Binding Assay of Lactosylceramide- and H Antigen-Coated Beads to Yeast Cells The preparation of the lactosylceramide-coated beads was performed by reference to the previous descriptions^{7,16)} as follows. Briefly, 100 μ g of lactosylceramide (LC, Sigma Co., Ltd.) was dissolved in 100 μ l of a lipid solvent containing 400 μ g each of cholesterol and phosphatidylcholine in 10 ml of methanol. The 9×10^8 polybead polystyrene microspheres (1.0 μ m diameter, Polysciences, Inc., PA, U.S.A.) were added to the lactosylceramide solution and coated for 3 h at room temperature. The solvents were removed using an aspirator. The beads were then washed three times with PBS, blocked for 2 h at room temperature with 1% bovine serum albumin (BSA)-PBS, and then washed three times with PBS and finally suspended in 1.0 ml of PBS. BSA-blocked cholesterol- and phosphatidylcholine-coated beads and BSA-blocked new beads were used as the controls. Binding of the beads to the A-strain cells was done as follows. A mixture of equal volumes (150 μ l) of the A-strain cells (2×10^6 /ml) and the beads (9×10^8 /ml) was mixed for 30 s by a Vortex Mixer. The percentage (%) of the positive A-strain cells was determined on the basis of the number of cells from at least 100 cells with three or more attached beads. Binding of the other beads to the A-strain cells was also conducted with a similar manner using the H type 1 antigen (HA)-, Lewis^a (Le^a)-, and BSA-coated beads.¹⁷⁾

Binding Assay of Mannan-Coated Beads to HeLa Cells The preparation of the mannan-coated polystyrene beads was conducted by reference to the previous description¹⁸⁾ as follows. An appropriate concentration of 9×10^8 polybead polystyrene microspheres (1.0 μ m diameter) was coated with 1.0 ml each of two types of mannan solutions (A-5.9-M and A-2.0-M, 100 μ g/ml) dissolved in 50 mM sodium carbonate buffer, pH 9.6. The beads were incubated overnight at 4 °C, and then washed three times with PBS containing 0.05% (v/v) Tween 20 (PBST). The mannan-coated beads and new beads (as the control) were blocked for 2 h at room temperature with 1% BSA-PBST, then the beads were washed three times with PBST and twice with PBS and finally suspended in 1.0 ml of PBS. Binding of the beads to the HeLa cells was done as follows. A mixture of equal volumes (150 μ l) of HeLa cells (10^5 /ml) and beads (9×10^8 /ml) was mixed for 30 s by a Vortex Mixer. The percentage (%) of the positive HeLa cells was determined on the basis of the number of cells from at least 100 cells with three or more attached beads. The average from triplicate samples was considered the relative adherence ability of the A-strain cell population. Each experiment was repeated at least twice.

Statistical Analysis Values are shown as means $\pm$ S.D. Comparisons between two groups were analyzed by the Student's *t* test. The significance was assessed at $p < 0.05$.

RESULTS AND DISCUSSION

Adherence to HeLa Cells of A-Strain Cells Cultured with Two Media and Effect of Treatments of the Yeast Cells with Pronase The host cell recognition to *C. albicans* is extremely complex. Calderone and Braun¹⁹⁾ categorized these systems by considering the components of each ligand-receptor. The importance of the cell surface hydrophobic proteins has been reported by Hazen *et al.*^{1,20,21)} and Lopez-Ribot *et al.*²⁾ Douglas *et al.*^{3,9,22)} also reported that

Fig. 1. Comparison of Adherence to HeLa Cells of A-Strain Cells Cultured in Two Media

The adherence to HeLa cells of A-strain cells cultured in two media (A: YSLM and B: YNB-Gal) were measured as described in Materials and Methods. Data represent the means $\pm$ S.D. ($n=3$). * $p < 0.001$ vs. A: YSLM.

during the course of the adhesion studies of *C. albicans* cells to epithelial cells, the yeast cells cultured with the YNB-Gal produces an extracellular polymeric material which contains a mannoprotein adhesin. They suggested that the protein portion of the mannoprotein adhesin is more important than the carbohydrate moiety in mediating yeast attachment to the buccal epithelial cells. Therefore, we first conducted the adherence to epithelial tumor cells, HeLa cells, of the A-strain cells cultivated in YSLM and YNB-Gal (Fig. 1). The yeast cells obtained by the cultivations in the YNB-Gal clearly increased (28 to 45%) the adhesion to the HeLa cells, in contrast to those obtained by the standard cultivation in YSLM to the buccal epithelial cells as mentioned by Critchley and Douglas.²³⁾ In our separate experiments, almost all the adhesion activity (about 70–80%) of the two types of yeast cells cultured with the two media disappeared following treatment with pronase P for 20 h (data not shown). These results show that the protein parts on the surface of both A-strain cells correlate with the adherence to the HeLa cells. We already separately reported the adhesion-enhancing substances of A-207 cells cultured with YNB-Gal.²⁴⁾

Binding to Various Sugar Coated-Polystyrene Beads of A-Strain Cells Cultured with Two Media Next, we conducted the binding study using polystyrene beads. The glycosphingolipids and sugars are known as adhesion receptors for yeasts.^{7,8)} Therefore, binding of the polystyrene beads to the A-strain cells cultured with two media (YSLM and YNB-Gal) was tested with various kinds of polystyrene beads coated with lactosylceramide (LC, Gal β 1-4Glc β 1-1Cer), the H type 1 antigen (HA, Fuc α 1-2Gal β 1-3-GlcNAc-Gal-GalNAc-), Lewis^a antigen (Le^a, Gal β 1-3-(Fuc α 1-4)-GlcNAc β 1-Gal-GalNAc-), A-5.9-M, and BSA (control) (Fig. 2). Only the LC- and HA-beads (the receptor activity) in addition to the non-coated beads (the positive control indicating the non-specific hydrophobicity)¹⁶⁾ were bound to both of the A-strain cells. The binding of the three beads to the A-strain cells cultured in the YNB-Gal was higher than that to the yeast cells cultured in the YSLM. These results well correlated with the adherence to the HeLa cells of both A-strain cells cultured with two media (Fig. 1). Brassart *et al.*⁸⁾ reported that the Lewis antigens failed to act as an adhesion inhibitor as the Lewis antigens are not structural components of the epithelial cells. It seems that this is the reason why the Lewis antigens have no binding activity to the HeLa cells.

Fig. 2. Binding of Various Sugar-Coated Polystyrene Beads on A-Strain Cells Cultured in Two Media

Binding of the sugar-coated polystyrene beads to the A-strain cells cultured in two media (A: YSLM and B: YNB-Gal) as described in Materials and Methods. The lactosylceramide (LC)-, the H type 1 antigen (HA)-, Lewis^a (Le^a)-, A-5.9-M (M)-, and BSA (control)-coated polystyrene beads were used. The non-coated polystyrene beads were used as the positive control. Data represent the means $\pm$ S.D. ($n=3$). * $p<0.05$ vs. LC and None groups of A: YSLM. ** $p<0.01$ vs. HA group of A: YSLM.

Fig. 3. Binding of Mannan-Coated Polystyrene Beads to HeLa Cells

Binding of the mannans to the HeLa cells was conducted as described in Materials and Methods. The polystyrene beads coated with two types of mannans (A-5.9-M and A-2.0-M) and BSA (control) were used. The non-coated polystyrene beads were used as the positive control. Data represent the means $\pm$ S.D. ($n=3$).

Our results using the beads were the same as the previous descriptions.^{7,8)}

Binding to HeLa Cells by Polystyrene Beads Coated with Two Types of Mannans Evidence has also been reported for the mannose-mediated adherence of *C. albicans* to several tissue cells.^{4,5,25,26)} To define the participation of the yeast surface mannans (mannoproteins) on the adherence to HeLa cells, the beads coated with two types of mannans (A-5.9-M¹²⁾ and A-2.0-M¹⁴⁾ were used (Fig. 3). It was found that the two mannan-coated beads did not adhere at all to the HeLa cells, similar to the beads coated with the control BSA, though the non-coated polystyrene beads adhered to the cells. This result shows that the mannans, in spite of the presence or the absence of the β -1,2-linked mannopyranose units,^{12,14)} did not adhere to the HeLa cells. Tojo *et al.*²⁷⁾

showed that the hydrophobic peptide moiety existing in mannans (mannoproteins) participated in the adhesion onto the surface of plastic microtiter plates. Namely, we can mention that the polysaccharide parts of the *C. albicans* surface mannan-coated beads did not act as adhesives to the HeLa cells. The present study using polystyrene beads showed that the adherence to the HeLa cells of *C. albicans* is achieved by the protein parts involving the glycosphingolipid (LC) and the sugar (HA) receptors, while no participation by the polysaccharide parts of the mannans was found.

Further studies of the adhesion mechanisms of *Candida* cells to various host cells may be attributed to the elucidation of the pathogenicity and to the development of the diagnosis procedures and the medical treatments of candidiasis.

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