Comparison of Pathogenicity of Various Candida albicans and C. stellatoidea Strains

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In order to clarify the pathogenicity and the pathogenic factors of various Candida species strains, three strains, NIH A-207 and J-1012 (serotype A), and NIH B-792 (serotype B) of Candida albicans and two strains, ATCC 20408 (karyotype II) and ATCC 36232 (karyotype I) of C. stellatoidea, a synonym for C. albicans, were tested for their lethality to mice, adherence to Hela cells, hydrophobicity, and cell growth under acidic conditions, pH 2.0—5.9. The pathogenicity for mice of all the strains was observed in the order NIH B-792, ATCC 36232, J-1012, NIH A-207, and ATCC 20408. The pathogenicity for mice by all the strains used was well correlated with adherence to the Hela cells, the hydrophobicity, and the cell growth under the acidic condition, pH 2.0. These results emphasize that these specific properties of the C. albicans and C. stellatoidea strains play an important role in the pathogenesis of candidosis.

Key words Candida albicans; Candida stellatoidea; pathogenicity; adherence; hydrophobicity; cell growth

Candida infections are a major cause of morbidity and mortality in compromised hosts. It is known that Candida virulence is a function of a multiplicity of factors working jointly to overcome the host defenses.1,2) Especially, studies of the pathogenic mechanisms of Candida infections have focused on dimorphism, protease production, cell growth, resistance to phagocytes, and adherence to host tissues. C. albicans is the most commonly isolated and the most medically important. The pathogenic C. albicans strains are well correlated with adherence to mucosal epithelial cells and hydrophobicity.3–6) The hydrophobic yeast cells are capable of abundantly binding to all tissues.7,8) During the course of Candida infection studies, we initially reported that several polysaccharide fractions showed a significant protective effect against the C. albicans infection in mice.9,10) Next, we studied the changes in the antigenicity (the structure) of the cell wall mannsans of several medically important Candida species cultured at several pH levels and temperatures.11–13) Recently, in order to investigate the pathogenic mechanisms of Candida infections, we reported the acid proteinase productivity by various Candida species strains14,15) and adhesion of the C. albicans NIH A-207 strain to Hela cells.16)

In this paper, we compared the lethality to mice, the adherence to Hela cells, hydrophobicity, and cell growth under acidic conditions of various C. albicans and C. stellatoidea strains to clarify the pathogenic elements.

MATERIALS AND METHODS

Organism and Growth Conditions The Candida albicans strains, NIH A-207 (Ca-A, serotype A), NIH B-792 (Ca-B, serotype B), and J-1012 (Ca-I, serotype A), were kindly supplied by Dr. A. Nishikawa, Meiji College of Pharmacy, Tokyo, Japan. The Candida stellatoidea ATCC 20408 (Cs-20408, karyotype II) and ATCC 36232 (Cs-36232, karyotype I) strains were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). The cultivation and preparation of the cells were conducted as previously described.12) Namely, each strain was cultivated in yeast extract-added Sabouraud liquid medium (YSLM) containing 0.5% (w/v) yeast extract, 1% (w/v) peptone, and 4% (w/v) D-glucose at 27 °C for 48 h on a rotary shaker (150 rpm). After harvesting the cells by centrifugation, the precipitated cells were washed three times with saline by centrifugation. The dilutions of the cell suspensions were made for the various experiments by counting the cells using a hemacytometer. The morphologies of the washed cells were photomicroscopically evaluated.

Infection Mice were intravenously (i.v.) injected with 1×10⁶ cells of the Candida strains. The survival of the mice was observed for up to 26 d.

Determination of Colony Forming Units (CFU) in Kidney Mice inoculated with 10⁶ cells of the Candida strains were sacrificed by cervical dislocation on day 26 after infection. Both kidneys from each surviving mouse were removed and then homogenized together in saline using a Teflon homogenizer. The number (CFU) of viable Candida cells in each kidney homogenate was determined by plating on a Sabouraud agar plate.10)

Adherence of Candida Cells to Hela Cells The adherence assay was conducted as previously described.16) Namely, a mixture of equal volumes (1.0 ml) of HeLa cells (10⁹/mL) and Candida cells (10⁷/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 µm pore size) (Nucleopore Corp., Pleasanton, CA, U.S.A.) and washed with 8 ml PBS with gentle agitation. The percentage (%) of the Candida cells attached to the HeLa cells was then determined. The average from triplicate samples was considered as the relative adhesion ability of the yeast cell population. Each experiment was repeated at least twice.

Hydrophobicity The hydrophobicity assay was conducted by reference to the previous description as follows.17) The polybead polystyrene microspheres (1.0 µm diameter, Polyscience Inc., PA, U.S.A.) were used. Binding of the beads to
the Candida cells was done as follows. A mixture of equal volumes (150 µl) of the Candida cells (2×10⁶/ml) and the beads (9×10⁸/ml) prepared by three-washing with PBS was mixed for 30 s using a vortex mixer. The percentage (%) of the positive Candida 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 Candida cell population. Each experiment was repeated at least twice.

**Determination of Growth Rate in Acidic Medium**

Growth rates of the Candida strains in the acidic medium at 27 °C were determined as previously described. Namely, Candida cells (1×10⁵) collected after a 48-h incubation in YSLM were transferred into fresh medium which had a non-adjusted pH of 5.9 and then adjusted to pH 2.0—4.0 with 6N HCl, and incubated for 72 h on a rotary shaker (150 rpm). At the incubation times of 24, 48, and 72 h, the number of cells was microscopically counted by diluting the cultures in saline.

**Statistical Analysis**

Comparisons between the two groups were analyzed by the Student’s t test. The significance was assessed at p<0.05.

**RESULTS**

**Virulence of Various C. albicans Strains to Mice**
This result was obtained as shown in Fig. 1. The increasing order of lethal activity of mice by the Candida strains was as follows: Cs-20408>Ca-A>Ca-J>Cs-36232>Ca-B. The surviving mice were sacrificed on day 26 after infection and their kidneys were examined for the pathological effects. The average number of CFUs recovered from the kidney homogenates is as follows: 1.92×10⁸, 3.52×10⁷, 1.00×10⁶, and 1.40×10⁴ for the Ca-A, Ca-J, Cs-36232, and Ca-B strains, respectively. The CFU counts showed a pattern very similar to the virulence of the Candida strains for mice.

**Adherence to Hela Cells**

As shown in Fig. 2, the Cs-20408 strain was the most adhesive strain to the Hela cells. Next, the Ca-A strain was relativey high. However, the Ca-B and Cs-36232 strains showed very low adherence activities.

**Hydrophobicity**

As shown in Fig. 3, the hydrophobici- ties of all the Candida cells were very similar to the adherence activities to the Hela cells. The Cs-20408 and Ca-A strains showed the highest and second highest hydrophobicity activities. However, the Ca-B and Cs-36232 strains showed relatively low activities.

**Growth Rates in Acidic Medium**

Figure 4 shows a comparison of the growth rates of various Candida strains in YSLM of different pHs, i.e., 5.9—2.0. We could not recognize any morphological changes in all examined the Candida cells in this study, and the cells showed the shapes of a typical budding yeast. Though the growth in the YSLM (pHs 2.0—5.9) increased in the pH order of 2.0, 3.0, 4.0, and 5.9 between all the Candida strains, the growth in the pH 2.0...
medium was recognized as a clear difference by the strains. It is obvious that the cells of the Ca-A, Ca-J, and Cs-20408 strains showed a good proliferation at pH 2.0, but the B-792 and Cs-36232 strains did not proliferate at pH 2.0.

DISCUSSION

It is known that the virulence of *C. albicans* and *C. stellatoidea* differs by the serotypes and the karyotypes. On the other hand, we have a report that no correlation is found between the virulence and the serotypes. In order to clarify this discrepancy, we first determined the survival rate of mice inoculated iv with various *C. albicans* and *C. stellatoidea* strains. As we expected, the *C. albicans* serotype A (Ca-A and Ca-J) and *C. stellatoidea* karyotype II (Cs-20408) expressed remarkable lethal activities, but not the *C. albicans* serotype B (Ca-B) and *C. stellatoidea* karyotype I (Cs-36232) (Fig. 1).

Next, we investigated several pathogenic factors, such as adherence to Hela cells, hydrophobicity, and cell growth under various pH conditions of the *Candida* strains. It is known that the pathogenic *C. albicans* strains are well correlated with the adherence to mucosal epithelial cells and the hydrophobicity. Our results also show that variations in the adherence to Hela cells and the hydrophobicity are directly related to the variation in the pathogenicity among both the *C. albicans* and the *C. stellatoidea* strains (Figs. 2, 3). This suggests that these factors are equally important for the pathogenicity of both *Candida* cells.

Several investigators have insisted the importance of the acid proteinase (AP) activity in *C. albicans* infections. However, no correlative variation in the pathogenicity and AP secretion was observed between the *Candida* strains as we already reported. It has been reported that the efficacy of the acidic pH tolerance test for *C. albicans* has been confirmed only for isolates of the yeast species most commonly encountered in clinical specimens. It is known that the Ca-A strain was able to clearly grow in acidic medium (pH 3—5), especially in pH 4.0, but was not observed at neutral media (pH 6, 7) by maintaining the pH of the media at fixed values throughout the cultivation. A recent study has demonstrated that *C. albicans* responds to the pH of the host niche and this response is critical for virulence. It has also been known that candidacidal activity of gamma interferon-activated peritoneal macrophages correlates well with induction of highly acidified phagolysosomes. Based on the pH 2.0 condition described in this report, we clearly found that only the strong virulence strains were able to grow in the strong acidic pH 2.0 YSLM medium (Fig. 4). Therefore, we can consider that the growing ability in the pH 2.0 medium of the strong virulence strains may be required to survive in the highly acidified phagolysosomes. Based on these results, we speculate that the growing ability in pH 2.0 medium is a good tool to evaluate the pathogenicity of the *C. albicans* strains in addition to the adherence activity to epithelial cells. Our present investigations provided evidence for a good correlation between the pathogenicity (including kidney colonization) and the adherence, the hydrophobicity, and the cell growth in the pH 2.0 medium of the *Candida* strains. An association of these parameters may be an important contribu-

Fig. 4. Cell Growth of Various *C. albicans* and *C. stellatoidea* Strains on Acidic Conditions

Growth rates of *Candida* cells in acidic medium were determined as described in Materials and Methods. (A) Ca-A, (B) Ca-B, (C) Ca-J, (D) Cs-20408, (E) Cs-36232. ○, pH 2.0; ●, pH 3.0; △, pH 4.0; ▲, pH 5.9.
tory factor to the pathogenicity of *C. albicans* and *C. stellatoidea*.

Based on the present study, we can state that the pathogenicity of the *C. albicans* serotype A and *C. stellatoidea* karyotype II strains was significantly higher than those of the serotype B and the karyotype I. This result may be explained as follows. We reported that the mannans of the *C. albicans* serotype A and *C. stellatoidea* karyotype II strains contained α-1,2 and β-1,2-linked oligomannosyl residues, 

\[ \text{Man}^\beta_1-2\text{Man}^\alpha_1- \text{Man}^\beta_1-2\text{Man}^\alpha_1- \text{Man}^\beta_1-2\text{Man}^\alpha_1- \text{Man}^\beta_1-2\text{Man}^\alpha_1-, \]

corresponding to the serum factor 6, and that the mannans of the *C. albicans* serotype B and *C. stellatoidea* karyotype I strains did not. Namely, the presence of the specific structures, the side chains containing the α-1,2 and β-1,2-linked oligomannosyl residues, in the mannans of the pathogenic *C. albicans* and *C. stellatoidea* strains may have a relation to the pathogenicity expression of their *Candida* strains. Based on these reasons, we are now investigating the structures and the functions of the β-1,2-mannosyltransferases of the virulence strains of various *Candida* species.

REFERENCES