PHENOTYPIC SWITCHING AND ITS INFLUENCE ON EXPRESSION OF VIRULENCE FACTORS BY CANDIDA ALBICANS CAUSING CANDIDIASIS IN HUMAN IMMUNODEFICIENCY VIRUS-INFECTED PATIENTS

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Abstract

Purpose: The purpose of the present study was to determine the degree of expression of virulence factors such as adherence, cell surface hydrophobicity (CSH) and production of proteinase by different morphological forms of Candida albicans causing oral candidiasis in human immunodeficiency virus (HIV)-infected individuals. Methods: C. albicans 3153A and two strains isolated from oral thrush in HIV-infected individuals were induced to undergo phenotypic switching by exposure to UV light and the degree of expression of virulence factors by the different morphological forms was studied. Results: Three different morphological forms of C. albicans were obtained namely, star (S), wrinkled (W) and ring (R) types from the original smooth (O) variety. It was found that proteinase production was greatest with the W type followed by the R type and O type. The S type produced the least proteinase. Expression of cell surface hydrophobicity and adherence was greatest in the O type followed by the R and then the W type and finally the S type. Conclusions: The differential expression of virulence factors occurs with different phenotypic forms of C. albicans and this may provide a particular morphological type with a distinct advantage over other types in causing candidiasis.

Key words: C. albicans, phenotypic switching, virulence, candidiasis, human immunodeficiency virus

The transition of Candida spp. from a harmless commensal to an unrelenting pathogen is a fine line and one that is attributable to an extensive repertoire of virulence determinants selectively expressed under suitable predisposing conditions.1 Candida spp. colonizing the oral cavities of human immunodeficiency virus (HIV) infected individuals are subjected to selective pressure that may lead to the emergence of strains with altered genotypic/phenotypic characteristics and enhanced expression of virulence factors. Most strains of C. albicans are known to be capable of switching spontaneously, reversibly and at high frequencies between a number of general phenotypes distinguishable by colony morphology.2,3 Switching has been demonstrated to regulate a number of phenotypic characteristics involved in pathogenesis such as adhesion, expression of cell surface hydrophobicity (CSH) and secretion of proteinases.4,5

The aim of the present study was to investigate the link between high frequency phenotypic switching and pathogenicity of C. albicans. We compared the degree of expression of virulence factors such as secretion of proteinases, expression of CSH and the ability to adhere to human buccal epithelial cell (BEC) by various morphological forms of C. albicans isolated from oral thrush in HIV-infected individuals.

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After each assay, the colony phenotype was verified by plating 10 µl of the cell suspension on to a Lee’s medium agar plates and incubating the plates at 25°C for seven days.

Growth conditions

For estimation of proteinase production, cells from each of the different phenotype colonies were cultured and suspended in 1mL of sterile distilled water and counted with a hemocytometer. Erlenmeyer’s flasks containing 10mL Macdonald and Odds medium were inoculated with 10⁶ cells/ml and incubated at 25°C for seven days. The broth culture was centrifuged and the supernatant was used for the estimation of extracellular proteinase.

Assay of proteinase

The sample of culture supernatant (0.2mL) was mixed with 0.8 ml substrate (1% bovine serum albumin (BSA) in 0.025M sodium citrate buffer, pH 3.2) and incubated at 37°C for three hours. The reaction was halted by the addition of 2.0mL of 5% trichloroacetic acid (TCA) resulting in precipitation of BSA. The tubes were kept at 4°C overnight and centrifuged at 2000 rpm for 20 minutes. Proteolysis was determined by measuring the absorbance of the soluble peptides at 280 nm. For control, substrate was added to the culture supernatant and immediately treated with TCA. The absorbance of controls was subtracted from test samples to obtain values for enzyme activity. The experiments were repeated four times and mean (± SD) of the readings was determined.

Adherence assay

The adherence assay described by Kimura and Pearsall was used with minor modification. Buccal epithelial cells (BEC) were obtained from the buccal mucosa of a single healthy donor, on the day of the assay. BEC were washed thrice in PBS to remove unattached yeasts, collected by filtration, fixed by methanol on to a microscopic slide and stained by Gram stain. The number of adherent yeast cells on each of 100 epithelial cells was counted for each preparation. The experiments were repeated four times and mean (± SD) of the readings was determined.

Polystyrene microsphere assay for CSH

The CSH assay described by Hazen and Hazen was used with some minor modification. Blue dyed polystyrene microspheres (Sigma) having a diameter of 0.8 ±0.1 µM, were used in the study. A working solution containing approx. 9×10⁶ microspheres/ml in ice cold PBS was prepared from a stock of colloidal suspension of microspheres (10% solids). Equal volumes (200 µl) of microsphere suspensions and yeast cells (5×10⁶ cells/ml of PBS) were mixed, rapidly equilibrated to room temperature and vortexed for 30 sec. Cell surface hydrophobicity was determined as the percentage of yeast cells (from at least 100) with three or more attached microspheres, when viewed by bright field microscopy at 400x. The experiments were repeated four times and mean (± SD) of the readings was determined.

Statistical analysis

The expression of virulence factors by the different morphological forms of C. albicans were compared using the Kruskal Wallis Anova test.

Results

Induction of phenotype switching

Upon UV light treatment, the smooth (O) form of C. albicans demonstrated three variant phenotypes namely ring (R), wrinkled (W) and star type (S) (Fig. 1).

Proteinase activity of each phenotype, expressed as OD₂₈₀ varied dramatically among colony phenotypes (Table 1). While there was not much difference in the activities of all three strains tested, (2.42 by CA-O28 to 1.915 by C. albicans 3153A for smooth (O) phenotype, statistically not significant) there was significant difference (P<0.01) in proteinase activity between switch phenotypes of the same strain. Proteinase activity was highest with the ‘W’ type (2.847) of C. albicans CA-O28, followed closely by the ‘R’ type (2.645) and then ‘O’ type (2.424). The ‘S’ type was observed to produce least amounts of proteinase when compared to the parent ‘O’ type of C. albicans. This pattern of proteinase production was similar to all three strains of C. albicans tested.

Effect on adherence

The adhesion of ‘O’ type cells to buccal epithelium was significantly greater than adhesion of ‘R’ type, ‘W’ type or ‘S’ type (Table 2), but not significantly greater than the adhesion of ‘R’ type (Table 2). The adherence of smooth type (‘O’ type)
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of C. albicans strain CA - O28 to human buccal epithelial cells is shown in Fig. 2.

Effect on CSH

The Cell surface hydrophobicity of ‘O’ type cells was significantly greater than CSH of ‘R’ type, ‘W’ type or ‘S’ type, but not significantly greater than the CSH of ‘R’ type (Table 3). The adherence of polystyrene microspheres to smooth type of C. albicans CA – O68 is shown in Fig. 3.

Discussion

The present study indicates that the degree of expression of virulence factors such as CSH, adherence and proteinase does vary significantly among the different phenotypes of C. albicans. It was observed that protease production was greatest with the wrinkled (W) phenotype, followed by the ring (R) and smooth (O) types. The star (S) type was observed to produce the least protease. A previous study has shown that switching of laboratory strains of C. albicans plays an important role in regulating the transcription of certain secreted aspartyl proteinase genes SAP1 and SAP3.15 Therefore, the variation in secretion of proteases by the different morphological types observed might be due to the differential regulation of proteinase genes.

Variation was also observed in the ability of the different phenotypes of C. albicans to adhere to BEC and in their expression of CSH. The generalized hierarchy of adherence to BEC was as follows: smooth (O) > ring (R) > wrinkled (W) > star (S). The expression of CSH by the different phenotypes also exhibited a similar pattern. Changes in the adhesion and CSH due to phenotypic switching has been reported in earlier studies.4
Table 3: The expression of cell surface hydrophobicity of different morphological forms of *C. albicans*

<table>
<thead>
<tr>
<th>C. albicans strain</th>
<th>Percentage of yeast cells showing high cell surface hydrophobicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colony type</td>
</tr>
<tr>
<td>CA-O28</td>
<td>79 ± 11</td>
</tr>
<tr>
<td>CA-O68</td>
<td>71 ± 7.0</td>
</tr>
<tr>
<td>3153A</td>
<td>84 ± 9.0</td>
</tr>
</tbody>
</table>

All readings are the mean (± SD) of four independent assays. *P < 0.01, **P < 0.001 when compared to that of smooth.

* C. albicans is known to exhibit both yeast form and hyphal form in culture during phenotypic switching. It has been demonstrated that the percentage of yeast cells in a particular population has a significant effect on the adherence of *C. albicans*. Such phenomenon occurs more frequently with the smooth (O) type, which has been shown to adhere better to mucosal epithelia, probably due to formation of germ tubes rather than a population of *C. albicans* which has a greater percentage of hyphal forms (star type).

High frequency phenotypic switching has been shown to occur at an elevated level in *C. albicans* causing infection in HIV infected individuals. This might not only result in the emergence of variant phenotypes expressing different levels of virulence determinants, but also presumably, exhibiting very different combinations of virulence traits. Thus, a high level of spontaneous variability in such populations would provide them with the advantage of rapid adaptation and this might provide a particular morphological type with a distinct advantage over other types in causing candidiasis.

References


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