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Significance of modes of adherence in esophageal *Candida albicans*

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Abstract: Although esophageal candidiasis is the most common form of *Candida* infection in the gastrointestinal tract, little attention has been directed toward determining the mechanism of its infection. We have already clarified the existence of four modes of adherence of *Candida albicans* to the esophagus; attachment, subepithelial cell insertion, cavitation, and invasion. This study was undertaken to clarify the significance of each of these modes. Scanning electron microscopic observations were made of esophageal specimens from 8-week-old rabbits infected with *Candida albicans* IFO 1060. In this study, attachment and subepithelial cell insertion were found to be the most frequent modes of adherence. Cavitation occurred following subepithelial cell insertion, while invasion occurred following attachment and subepithelial cell insertion. These results suggest that attachment and subepithelial cell insertion play the most important role in the initial stage of adherence. The ratios of these modes for living yeast cells were similar to those for dead yeast cells and beads. This suggests that *Candida albicans* can gain a foothold on the esophageal epithelium solely by physical contact, after which colonization occurs.

Key Words: adherence, *candida albicans*, esophagus, cavitation, candidiasis

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Introduction

The mucosal surface of the gastrointestinal (GI) tract is an important portal of entry for *Candida albicans* into the body, leading to systemic candidiasis.[1] Although the esophagus is the most common site of *Candida* infection in the GI tract,[2] there has been little study of the mechanisms of adherence to the esophagus as the first step of Candida infection. Therefore, we decided to study the adherence of *C. albicans* to the squamous epithelium of the esophagus. We demonstrated using a scanning electron microscope (Hitachi S-570), that this adherence could be classified into four modes; attachment, subepithelial cell insertion, cavitation, and invasion, and we discuss these definitions.[3] Two of these modes have not been reported previously, and were suggested to be present in the clinical setting.[3] Since the significance of these modes was unclear, we carried out this study to clarify the significance of each mode.

Materials and methods

We used *C. albicans* IFO 1060 maintained on Sabouraud glucose agar at room temperature. Yeast form cells of *C. albicans* and cells with hyphae four or five times longer than the diameter of the yeast (in this study, we used the mycelial form) were prepared for experimental infections. Yeast form cells were cultured in Sabouraud glucose broth for 24 h at 37°C. Mycelial form cells were obtained by culturing the yeast form cells in arginine medium[3] for 48 h at 37°C.

Dead *C. albicans* cells and beads of comparable size (Immutex DRS-02, 4.72 μm in diameter; Japan Synthetic Rubber Co. Ltd., Japan) were also used. The dead *C. albicans* cells were produced by suspending *C. albicans* cells in 10% formalin for 48 h at room temperature. Both living and dead cells were washed three

times in the arginine medium. The cells and beads were suspended in the arginine medium at a concentration of 10^8 *C. albicans* cells/ml.

We used New Zealand white rabbits (n = 46; 8-week-old males, weighing 1.0–1.6 kg) because the fine structure of the surface of the squamous epithelium of the rabbit is very similar to that of human epithelium.[4–6] After the rabbits were fasted for 48 h, they were injected with pentobarbital sodium (200 mg/kg) and the esophagus of each rabbit was resected. The esophagus was then treated in a 5% solution of trypsin at room temperature for 75 min to produce mucosal damage. Suspensions (0.5 ml) of living yeast form cells of *C. albicans* were injected into the esophageal lumens of 22 rabbits, after which both ends of each esophagus were ligated. Equal suspensions of dead yeast form cells, beads, living mycelial form cells, and dead mycelial form cells were injected into the esophageal lumens of eight, six, six, and four rabbits, respectively, and both ends of each esophagus were ligated. Specimens were treated at 37°C for 1 h in the arginine medium[3] and then placed into the arginine medium again to remove extra *C. albicans* cells that had not adhered before fixation. The esophagi from four rabbits were treated by same method, minus trypsin treatment.

In 37 other rabbits, after a suspension of the living yeast form of *C. albicans* was injected into the lumen of the esophagus and both ends of the lumen were ligated, the ligations were removed after 1 h and the lumen of the resected esophagus was cut open. Each esophagus was then treated for another 3 h (13 rabbits) or 6 h (24 rabbits) in the arginine medium. The treated esophageal specimens were placed into the arginine medium once again to remove extra cells before fixation.

For observation with the scanning electron microscope, the esophagi were coated with gold-palladium after double fixation with 2.5% glutaraldehyde and 1% osmium tetroxide, dehydration, and critical point drying. Whole areas of the specimens were observed by scanning electron microscopy. Each *C. albicans* cell was classified according to its mode of adherence.

Results

All forms of *C. albicans* cells on the esophagi treated for 1 h maintained their fine structure. Exfoliating squamous cells were more frequently observed in the trypsin-treated specimens than in the non-trypsin treated specimens; however, the fine structure of the surface of the squamous cells was maintained. In the esophageal specimens initially injected with yeast form cells and treated for 4 h, the yeast form changed into mycelial form cells. In the specimens initially injected with yeast form cells and treated for 7 h, the hyphae grew longer than in those treated for 4 h. The cells on the esophageal specimens treated for 7 h were difficult to distinguish from other cells and difficult to classify into the four modes of adherence, because the hyphae had grown very long and were tangled. Therefore, we calculated the numbers of cells treated for 1 or 4 h, and beads, and classified the modes of adherence to squamous epithelial cells of the esophagus.

Ratio of each mode of adherence

Each *C. albicans* cell treated for 1 or 4 h, and beads, were classified according to their mode of adherence to the squamous epithelial cells of the esophagus. Table 1 shows the ratio of each mode and the calculated number of cells per specimen.

The most frequently observed modes of adherence for living yeast form cells were attachment and subepithelial cell insertion. Cavitation was very rare. Attachment, subepithelial cell insertion, and cavitation were also observed for dead yeast form cells, and

Table 1. Ratio of adherence modes of *Candida albicans* and beads

Form of *C. albicans*	Yeast form		Beads	Mycelial form		Yeast →Mycel.
Mode of adherence	Living 1 h	Dead 1 h	1 h	Living 1 h	Dead 1 h	Living 4 h
Attachment	72.87%	75.57%	89.15%	97.90%	99.36%	64.39%
Subepithelial cell insertion	27.10%	24.35%	10.85%	1.80%	0.60%	14.09%
Cavitation	0.03%	0.08%	0.00%	0.10%	0.04%	0.15%
Invasion	0.00%	0.00%	0.00%	0.20%	0.00%	21.37%
Total%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
Calculated *C. albicans* cells per specimen	3702 (n = 9)	1214 (n = 4)	106 (n = 2)	1003 (n = 2)	2294 (n = 3)	225 (n = 3)

Mycel, Mycelial form; n, number of specimens

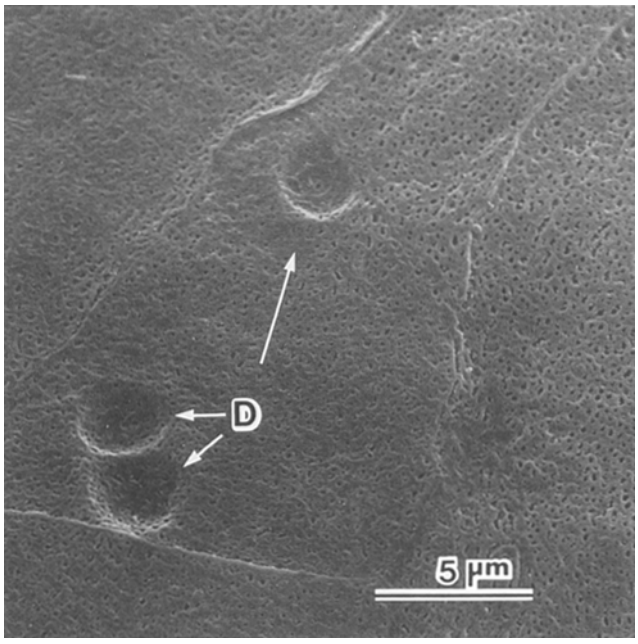

Fig. 1. Depressions (*D*) suspected to be imprints of cavitation by beads can be observed

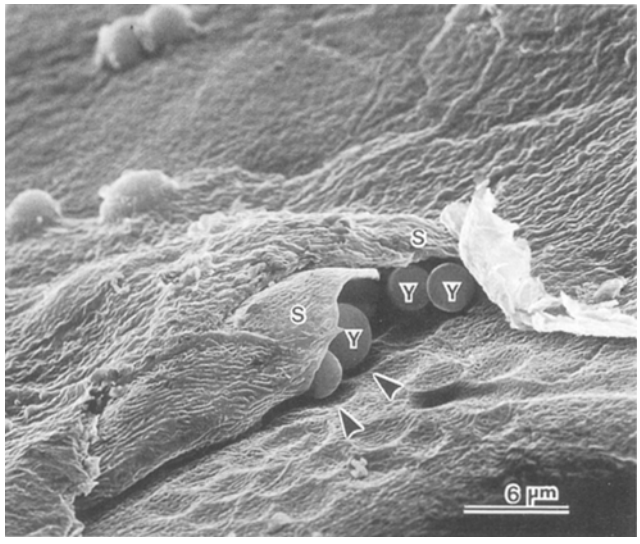

Fig. 2. *C. albicans* yeast form cells (*Y*) are located under the superficial epithelial cells (*S*) and embedded in the squamous cells with concave deformities (*arrowheads*)

the ratios of these modes were very similar to those for living yeast form cells. In the case of beads, attachment and subepithelial cell insertion were observed; the ratios of these modes were also similar to those of living yeast form cells. Cavitation was not observed, although depressions suspected to be imprints of cavitation were observed (Fig. 1).

Attachment was the most frequently observed mode of adherence for living mycelial form cells. Subepithelial cell insertion, cavitation, and invasion were very rare. Attachment, subepithelial cell insertion, and cavitation were also observed with dead mycelial form cells, but invasion was not. The ratios for the modes of dead *C. albicans* were very similar to those for living *C. albicans* cells.

In the esophageal specimens initially injected with yeast form cells of *C. albicans* and treated for 4 h, the cells changed into mycelial form. Subepithelial cell insertion and invasion were more frequently observed for these cells than for the mycelial cells initially injected into esophageal specimens, and attachment was less often observed.

In two non-trypsin treated specimens, the number of *C. albicans* cells classified as showing attachment and subepithelial cell insertion was 1091 (95.03%) and 57 (4.97%), respectively.

Cavitation

The fine structure of the epithelial surface was maintained in the depressions of cavitation, and cavitation

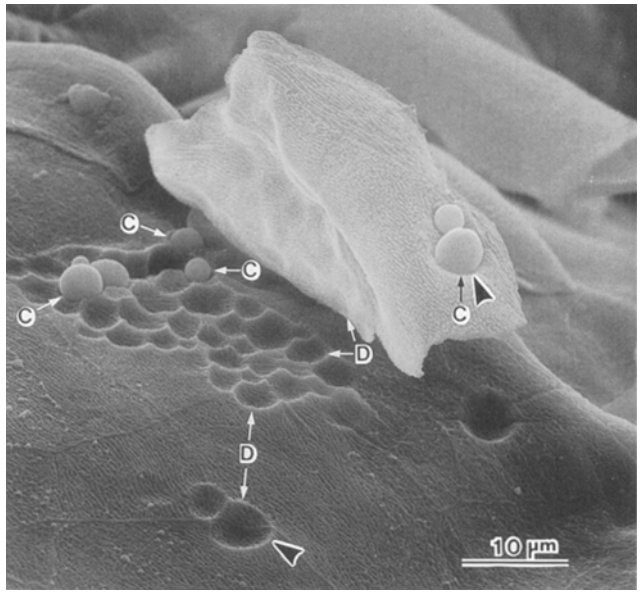

Fig. 3. Cavitations (*C*) and depressions (*D*) can be observed on both the back surface of exfoliating squamous epithelial cells and the surface of the remaining squamous cells. A few yeast cells are embedded in the back surface of exfoliating epithelial cells, and depressions are observed on the corresponding surface of the remaining squamous cells (*arrowheads*).

was observed even in dead fungi. In the case of beads, cavitation was not observed, but depressions that were suspected to be imprints of cavitation were noted.

Usually, subepithelial cell insertion and cavitation were observed independently; however, in only one

specimen, both modes were observed at the same place. *C. albicans* cells located under superficial epithelial cells (subepithelial cell insertion) were embedded in squamous cells located beneath the epithelial cells, with concave deformity of the surface (cavitation) (Fig. 2). In this specimen only, cavitations and depressions were noted on both the back surface of exfoliating squamous cells and the surface of the remaining squamous cells. A few yeast cells were embedded in the back surface of epithelial cells, and depressions were observed on the corresponding surface of the remaining squamous cells (Fig. 3).

Invasion

In the esophageal specimens initially injected with the yeast form of *C. albicans* and treated for 4 h, the yeast form cells changed into the mycelial form. Some mycelial elements of the yeast cells located on the surfaces of the squamous cells invaded the superficial squamous epithelial cells, while some other mycelial elements of yeast cells located under the superficial squamous epithelial cells penetrated these cells (Fig. 4). Invasion occurred through two modes of adherence; attachment and subepithelial cell insertion.

Discussion

The mucosal surface of the gastrointestinal (GI) tract is an important portal of entry for *C. albicans* into the body, this entry leading to systemic candidiasis.[1] However, the mechanisms responsible for the adherence and colonization of *C. albicans* on the GI mucosa and its entry through the mucosa have not yet been clarified.[7–11] Furthermore, little attention has been directed toward the study of *C. albicans* adherence to the squamous epithelium of the esophagus, despite the fact that esophageal candidiasis is the most frequent form of *Candida* infection in the GI tract. Therefore we decided to study the mode of adherence of *C. albicans* to rabbit esophagus as the first step in esophageal *Candida* infection. In our previous study,[3] four modes of adherence were noted; attachment, subepithelial cell insertion, and cavitation for both yeast and mycelial forms of *C. albicans*, and invasion for only the mycelial form.

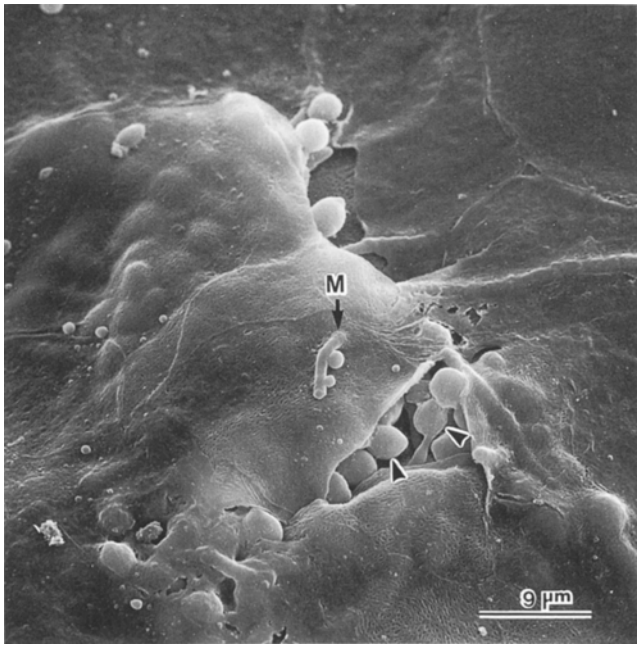

Fig. 4. A mycelial element (*M*) of *C. albicans* (*arrowheads*) located under a superficial squamous cell has penetrated that cell

In this study, we observed the first three modes of adherence, even on both dead forms of *C. albicans*. With beads, which have no activity, attachment and subepithelial cell insertion and a cavitation imprint were observed. The ratios of the modes for the living yeast form cells were similar to those for the dead cells and beads. The ratios of the modes for the living mycelial form cells were also similar to those of dead cells. These findings suggest that the mode of adherence essentially depends on physical contact. In non-trypsin treated specimens, the ratio of subepithelial cell insertion was lower than that in trypsin-treated specimens. The lower ratio may be related to the lower number of exfoliating squamous cells.

Subepithelial cell insertion in the esophagus and other areas of squamous epithelium, such as the skin, has not been reported previously. However, in our study, this type of adherence was usually observed in the yeast form cells of *C. albicans*.

Cavitation was very rare in the esophagus. This type of adherence has been reported in a study of the skin,[12] in which it was suggested that the cavitation was an active process affecting the corneocyte surface, since no cavitation was observed with killed blastoconidia or latex beads, neither of which have any activity. This cavitation did not appear to be an artifact. In our study, however, cavitation was observed with both living and dead *C. albicans* cells. With beads, we observed depressions that we suspected were cavitation imprints. In addition, the fine structure of the surfaces of squamous epithelial cells was maintained even with cavitation.[3] Thus, an active cavitation process affecting the esophageal squamous epithelial cell surface could not be confirmed. However, it was clarified that cavitation occurred following subepithelial cell insertion.

Invasion is a well known mode of adherence. In our study, invasion occurred following both attachment and subepithelial cell insertion.

The success of *Candida* infection depends on the organism gaining a foothold that prevents it being

washed away by food or water and/or by peristalsis of the esophagus at the initial stage. The results of this in vitro study suggest that *C. albicans* may gain a foothold in the esophagus as the first stage of *Candida* infection by the modes of attachment and/or by subepithelial cell insertion. During this first stage, cavitation may occur following subepithelial cell insertion. At the late stage of adherence, hyphal invasion may occur, and colonization may begin. However, at present, it cannot be concluded which mode of adherence, attachment or subepithelial cell insertion, is more successful in vivo.

It is well known that *Candida* infection occurs in the immunosuppressive state. However, the results of our study suggest that *C. albicans* was able to gain a foothold on the esophageal epithelium solely by physical contact. This finding indicates that the presence of an immunosuppressive state is not a prerequisite for the adherence of *C. albicans* to the esophagus. In fact, *Candida* infection has been reported in healthy individuals.[13] However, the mechanisms underlying infection in healthy individuals are unclear. Further in vivo investigation is required to confirm that, in the absence of immunosuppressive drugs, physical contact of *C. albicans* with the esophagus leads to the formation of candidial lesions.

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