# CYTOHISTOLOGIC AND ULTRASTRUCTURAL FEATURES OF THE EARLY STAGES OF INTERACTION BETWEEN Candida CELLS AND EPITHELIOCYTES

UDC 616.992.282-07:
616-018.7-076.5

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KEY WORDS: candidiasis, pathogenesis, ultrastructure, adhesion.

There is an extensive literature on the pathomorphology of the various forms of candidiasis [1, 4-6, 11, 14]. Meanwhile the study of the earliest stages of interaction between Candida cells and the host cells, namely adhesion to the surface of epitheliocytes (EC), which determines colonization of the mucous membranes and the subsequent mycotic process, began only in recent years [2, 8], and has been devoted mainly to analysis of the adhesive properties of Candida cells (CC).

The aim of this investigation was to study cytohistologic and ultrastructural features of the early stages of interaction between CC and EC of mucous membranes.

## EXPERIMENTAL METHOD

In experiments in vitro, EC obtained from the oral cavity (154 observations) or vagina (43 observations) of human subjects and CBA mice (62 animals) were incubated with CC (Candida albicans, strain 2565) under standard conditions, and the number of CC adherent to one EC was determined. Details of the method were described previously [3]. In experiments in vivo, CBA mice (132 animals) and Wistar rats (40 animals) were infected by the intravaginal or peroral route with C. albicans cells (infecting dose $10^6$-$10^8$ cells per animal). Material for cytologic, histologic, and electron-microscopic investigation was obtained from 30 min to 24 h after infection. Preparations for cytologic investigation were stained by Gram's method, and the mucous membrane of the oral cavity and vagina was studied histologically: sections of the organs were stained by the PAS reaction and with hematoxylin. In some cases film preparations of the epithelium were obtained [9]. Material for electron microscopy was fixed in Karnovsky's aldehyde mixture, postfixed in $OsO_4$, and embedded in Araldite. Ultrathin sections were studied in the JEM-100-CX-II electron microscope. For scanning electron microscopy the fixed material was dehydrated, dried in vacuo, sprayed with gold, and examined in the JEM-ASID-4D scanning attachment.

## EXPERIMENTAL RESULTS

General characteristics of adhesion of CC to EC are described in [3]. A study of the distribution of adherent CC on histologic and cytologic preparations showed that the EC population within a tissue is heterogeneous for its adhesive properties. In experiments in vitro, absolute values of adhesion varied depending on the conditions; with a ratio of CC (C. albicans): human EC = 100:1 and with a duration of incubation of 1 h, an EC population (15-20% of the total number of cells) with high adhesive properties (4.5 times higher than the mean values for the remaining EC) is present. This population, estimated by a special parameter, namely the adhesion index [3, 12], is particularly important pathogenetically, for it is in regions of such EC that invasion by CC is most probable. Within the limits of the same EC and the layer of epithelial tissue, a different type of inequality of CC distribution is found: blastospores are distributed mainly around the periphery of EC, as may be seen by examination of the cytologic smears (Fig. 1a), histologic film preparations (Fig. 1b), and

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Smol'yannikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 12, pp. 752-754, December, 1987. Original article submitted July 30, 1986.

 0007-4888/87/0012-1766$12.50 

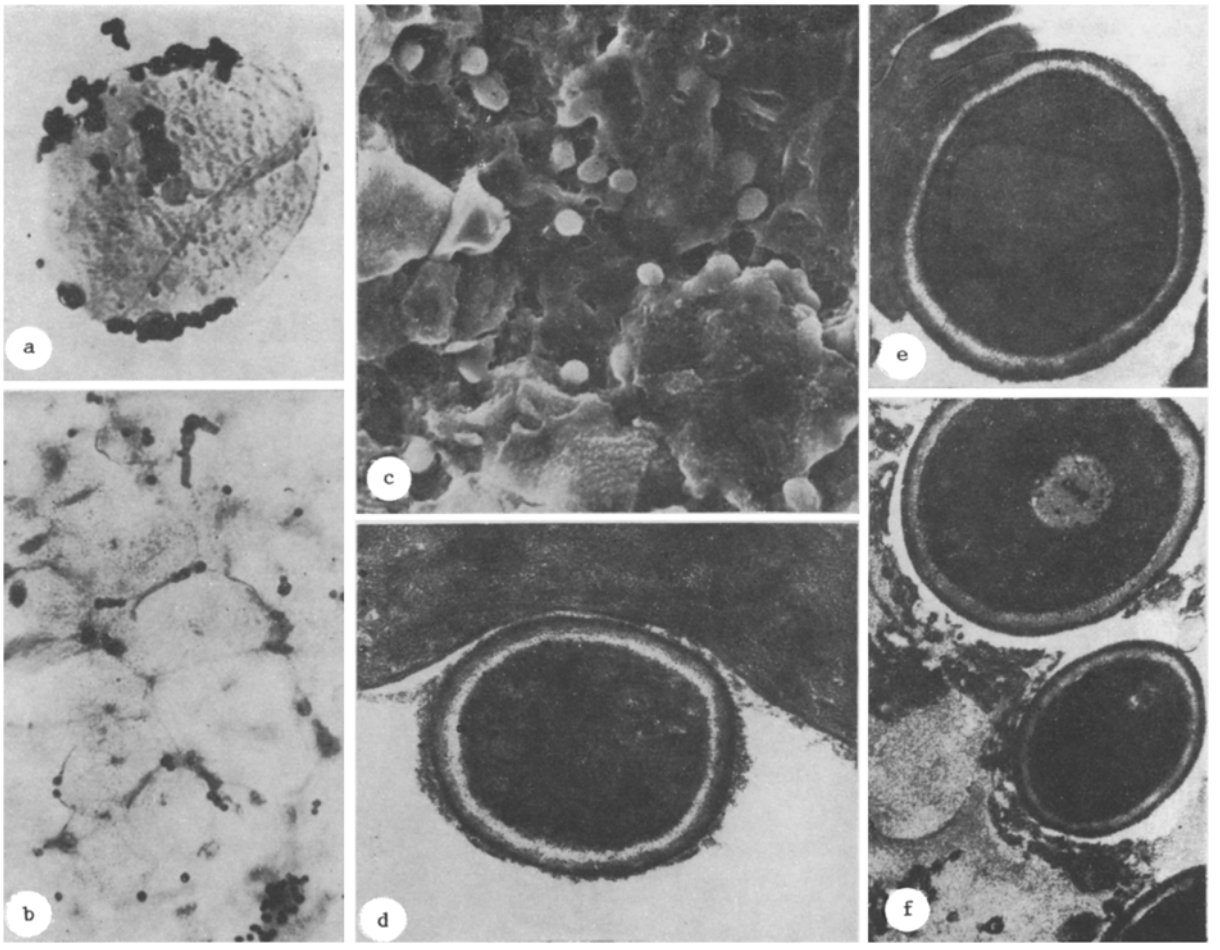


Fig. 1. Adhesive interactions between Candida cells and epitheliocytes. a) Adhesion of blastospores of C. albicans to EC of human oral mucosa in vitro. Gram's stain. 600 ×; b) film preparation of mouse vaginal epithelium 4 h after infection. Fungal cells located mainly around periphery of EC and in region of intercellular boundaries. Staining: PAS reaction and hematoxylin. 160 ×; c) electron micrograph obtained under scanning conditions. Adherent blastospores arranged along boundaries of epitheliocytes on surface of mouse vagina. 3000 ×; d) initial phase of adhesion of a blastospore to surface of epithelium with the formation of "bridges" of finely dispersed fibrillary material. 28,000 ×; e) firm attachment of blastospore in late stages of adhesion to EC. Fibrillary-granular layer in zone of contact is indistinguishable. 28,000 ×; f) formation of zone of translucency in cytoplasm of epithelial cell in region of contact with Candida blastospores. 18,000 ×.

electron micrographs obtained on the scanning electron microscope (Fig. 1c). To verify this observation, the ratio of CC adherent to the peripheral zone of EC (width 7.2 μ – the mean diameter of a blastospore) and to the remainder of EC, was calculated. For EC from the human oral cavity the ratio of these values was 1.9 ± 0.02 (mean for 20 subjects), and if calculated per area of zones (density of distribution of CC) it was 2.9 ± 0.02. For EC of the mouse vagina (20 animals) these ratios were 2.3 ± 0.04 and 3.1 ± 0.03, respectively. The predominantly peripheral and intercellular distribution of CC on adhesion to the epithelium must be taken into account when the invasion process is analyzed, for during it many of the blastospores penetrate into the depth of the epithelial cells [13].

Ultrastructural analysis of the adhesion process revealed finely dispersed fibrillary material on the surface of CC, forming "bridges" with EC (Fig. 1d). Stronger attachment of CC to EC also was discovered with disappearance of the outer fringe layer of the fungal wall (Fig. 1e). These observations agree with those of others [10] who regard such pictures as a reflection of two alternative versions of adhesive interactions, although it seems likely that these versions correspond to successive stages of the same process.

During analysis of the cytologic material, the formation of cytoplasmic pockets (Fig. 1a), which were described previously [7], was observed in EC at the site of the adherent blastospores. On electron microscopy, this version of interaction corresponded to the formation of a zone of translucency in the cytoplasm of EC around the periphery of CC (Fig. 1f). The appearance of pockets as a result of lysis and deformation of the peripheral parts of EC evidently reflects interactions that are not yet adhesive, but the cytopathic effect of the fungus, combined with signs of invasion of the epithelium or preceding them.

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