Ultrastructure of Human Cutaneous Candidosis

CHRISTIAN SCHERWITZ, M.D.
Abteilung Dermatologie I der Universität, Tübingen, Germany

Human skin biopsies were taken from patients with candidosis of the groin, axillary and submammary areas. The majority of the fungal cells were situated inside epithelial cells. The fungi invaded the entire stratum corneum. They were often found in parakeratotic epithelial cells. They could not be detected in noncornified cells of the malpighian layer. Mycelial forms predominated by far. They apparently invade the epidermis actively. Blastospores were found less often and they mostly were situated between or in superficial cells of the horny layer. Pseudomycelia and germ tubes were rarely observed. Remarkable was the frequent finding of lomasomas in structures were rarely demonstrable in vitro. They probably represent structures that occur in damaged fungal cells as a result of defense mechanisms of the host. The fungal elements inside the epithelial cells were often surrounded by electron-transparent areas. These areas possibly resulted from keratolytic activities of the fungus. Characteristic manifestations of candidosis of the human skin were parakeratosis, spongiosis, and intracorneal and subcorneal micro-abscesses. However, fungal elements failed to occur in the center of these abscesses, possibly because the process of phagocytosis, killing, and lysis of the fungi had been completed.

There are many reports of light microscopic investigations on human and animal candidosis concerning skin, mucous membranes, and systemic involvement [1-10]. It was only in 1968 that Montes and Wilborn published an ultrastructural study on the host-parasite relationship in oral candidosis [11]. This was in fact the first description of Candida (C.) albicans in human tissue by means of electron microscopy. We believe that additional basic knowledge of the host-fungus relationship in human candidosis at the electron microscopic level is needed for a better understanding of pathogenetic principles and for therapeutic reasons. The present study deals with our ultrastructural investigations in human candidosis of the glabrous skin.

MATERIALS AND METHODS

Patients
Skin biopsies were obtained from 9 patients suffering from intertrigino us candidosis involving the axillae, groin, and intermammary folds. Local anesthesia (1% mepivacaine) was applied subcutaneously in a ring around the lesion to avoid mechanical alteration of the tissue. Four patients had diabetes mellitus and 1 had diabetes and pancreatic carcinoma. The remaining 4 patients were young male adults suffering from candida balanoposthitis. In each case there was a history of candida vaginitis in their partners. Scrapings from the cutaneous lesions showed the characteristic mixture of yeast and mycelial phase organisms in all 9 patients. Candida albicans was identified by chlamydo­spore production on chlamydospor test medium [12], by germ tube production in human serum [13] and by carbohydrate fermentation and carbohydrate assimilation tests [14].

Electron Microscopy
Skin biopsies were suspended in 0.1 M phosphate buffer solution containing 3% sucrose (pH 7.2), cut in cubes of 1 mm³. Skin scrapings were fixed directly. Four different fixation solutions were used to obtain satisfactory results.

Fixation (F): The samples were fixed at 4°C for 4 hr in Karnovsky's fixative [15]. After an overnight rinse in 0.1 M cacodylate buffer the samples were postfixed with 2% osmium tetroxide in cacodylate buffer for 3 hr, dehydrated in graded ethanols, blockstained in saturated uranylaceatate in 100% ethanol for 30 min at room temperature. The blocks were dehydrated in ethanol and 1.2 epoxipropane and embedded in ERL 4206 [16] in a low vacuum at 60°C. Thin sections were cut with a diamond knife, stained with lead citrate [17] and examined with a Philips EM 301 or a Zeiss EM 10 electron microscope with an accelerating voltage of 80 kv.

RESULTS

Site of the Parasite in Tissue
The fungi were located in the epidermis within the region of the stratum corneum (Fig 1). Only fungi lying on the surface or between very superficial horny cells of the stratum corneum were in an extracellular position like the concomitant bacterial flora often seen. Sometimes we found "ghost cells" extracellularly. These are dead C. albicans cells. Only the rigid cell wall and a few threadlike and vesicular inner structures were preserved. Such ghost cells were never seen in deeper epidermal layers. The clear majority of C. albicans cells was found intra­cellularly in the cells of the stratum corneum. This was true not only for blastospores but also for hyphae. Occasionally C. albicans cells were found in nucleated cells of the epidermal layers (Fig 2A).

Yeast (Y) and Mycelial (M) Form
In the parasitic stage we observed different tissue forms of C. albicans. Blastospores in different sprouting stages were seen especially in superficial horny cell layers, less often at the border to the rete malpighii (Fig 2B). Pseudomyecia, i.e., elongated blastospores were rarely found (Fig 2A). In all layers of the stratum corneum we encountered mycelia, i.e., threadlike, filamentous forms of C. albicans (Fig 2C). Rarely, we found germ tubes (Fig 2D) which are precursors of mycelia [20]. Occasionally septa formed in the mycelia (Fig 3).

Morphology of Candida albicans in vivo
The cell wall structure of in vivo blastospores was similar to the in vitro findings [20-22]. The outer granular layer described
Mitochondria were without an exception of the cristae type and of various configurations extending in one case to the length of 2.5 μm. The membranous structures of the mitochondria and the endoplasmic reticulum were most distinct in lithium permanganate samples. The endoplasmic reticulum consisted of irregularly distributed threadlike, round to oval vesicular structures in the cytoplasm, depending on the sectional plane (Fig 5). In one cell there was a continuous transition of threads of the endoplasmic reticulum into the mitochondrial membrane.

Free ribosomes were preserved in osmium tetroxide fixed cells in the form of small round electron dense particles distributed over the cytoplasm (Fig 2B, 3). Spherosomes or lipid granules were osmiophilic round structures without apparent membranous bordering (Fig 9).

Vacuoles of different sizes were regularly seen in *C. albicans* cells in *vitro*. The vacuole was often situated in the vicinity of the nucleus. It appeared as an electron transparent, membrane-bound area.

The cytoplasmic ground substance consisted of a finely granular material of varying electron lucency.

**Host-parasite Relationship in the Epidermis**

In superficial layers of the stratum corneum some *C. albicans* cells, mostly in the Y-form, were encountered in the extracellular spaces. The great majority of the fungal cells was inside epithelial cells. Sometimes the process of invasion of the horny cells by the fungal elements was observed. The horny cells apparently offered resistance to the fungi about to invade the cells. As a consequence they were deformed and squeezed together under the mechanical pressure. This was conspicuously demonstrable in horizontal sections through the stratum corneum (Fig 6).

The epithelial cells involved often showed a clear perifungal zone of varying size. These areas surrounding the fungi were either completely structureless and electron transparent (Fig 2C) or microfilamentous spines extended perpendicularly to the yeast cell surface through the perifugal area (Fig 4A). Adjacent to this area, the tonofilaments were often closely packed together, obviously resulting from their mechanical displacement and compression.

The reaction of the skin to the infection by *C. albicans* was manifested as acute to subacute dermatitis (Fig 1). There was an edema in the stratum papillare of the corium. It contained a mixed-cell infiltrate of lymphocytes and neutrophils. The intercellular spaces of the rete malpighii were widened, some of the intercellular bridges were ruptured. The plasmalemma of the basal keratinocytes showed many fingerlike microvilli protruding into the intercellular spaces. Microabscesses formed when the immigration of neutrophils increased (Fig 1). The microabscesses were situated between the stratum intermediate and the stratum corneum, or in the lower stratum corneum. There was parakeratosis in some parts, the horny cells contained clearly discernible nuclei. The content of the microabscesses was composed almost exclusively of polymorphonuclear leukocytes, recognizable by their lobated nuclei and numerous granules representing lysosomes. We failed to find any fungal elements in the region of the microabscesses.

**DISCUSSION**

The present electron microscopic investigations contribute to extending our knowledge of the morphology, site and behavior of *C. albicans* in human candidosis of the glabrous skin. Our findings show that *C. albicans* only occurs rarely in the intercellular spaces of the outer layers of the stratum corneum. The fungus is found rather regularly inside the horny cells. Filamentous forms of the parasite predominate by far. Blastospores are rarely seen. The fungi tend to invade the deep layers of the stratum corneum and sporadically reach the stratum intermediate, in which the maturing processes preceding the keratinization take place. There is a close relationship between *C.*
**FIG 3.** Mycelium of *Candida albicans* with septum inside epithelial cell. Plasmalemma, mitochondria (M), ribosomes (R), lipid granules (L) are seen. *F 2. (× 51,000). Scale line represents 0.5 μm.*

*albicans* cells and epidermal cells with preserved nuclei. Ray and Wuepper showed in experimental cutaneous candidosis in rodents that fungal filaments invaded the malpighian layer of the epidermis and came close to the epidermodermal junction [9]. However, it is not known which factors inhibit the invasion of the malpighian layer in human candidosis. Inoculation of *C. albicans* cells into the serum-rich surface of stripped human skin under occlusion greatly enhanced the infection [10]. There was thus no indication of a possible inhibitory effect by previously described anti-candidal-factors [23-25]. It is not known whether epithelial cells play a part in defense mechanisms against fungal infections. The increased epidermal proliferation and parakeratosis as a consequence of the inflammatory process may be regarded as a defense mechanism against the invasion of *C. albicans*. Positive delayed hypersensitivity reactions in experimental cutaneous candidosis act to increase the rate of basal cell turnover. However, the effect appears to be nonspecific, since other forms of inflammation are also capable of causing increased epidermal proliferation [26].

**Morphology of the Parasite in vivo**

The fungal cells did not show any essential difference compared with the *in vitro* findings. The plasmalemma showed multiple invaginations. Those structures were described in young and older cells of *C. albicans* and in dermatophytes *in vitro* [18,27-29].

We observed lomasomas in our *in vivo* material very frequently, whereas they were rarely seen *in vitro*. Neither the

**FIG 4.** Cross-sectioned mycelia of *Candida albicans* inside horny cells. A, Note perifungal clear zone and microfilaments (arrow) arranged perpendicularly to the fungal cell wall surface. *F 3. (× 51,000). Scale line represents 0.5 μm.* B, Group of 3 lomasomas (arrow) lying between plasmalemma and cell wall. Note deep invagination of the plasmalemma (double arrow). *F 3. (× 37,500). Scale line represents 0.5 μm.*
Host-parasite Relationship in the Epidermis

The great majority of the fungal elements were found inside epithelial cells. According to the investigations of Müller, Takamiya, and Jaeger the extracellular coating material observed in Fig 5 and 6 may be interpreted as an antigen-antibody precipitate [34]. The microfilaments arranged perpendicularly to the cell wall surface in cross-sectioned mycelia (Fig 4A, B) correspond possibly to a radial pattern of glycoprotein mediating adhesion to the host cell as described by Costerton, Geesey, and Cheng in bacteria [35].

The process of penetration through epithelial membranes was frequently seen. The invasion of C. albicans cells into the stratum corneum also involved the desmosomal disks between the horny cells. Enlarged intercellular spaces in the immediate vicinity of the infected epithelial cells indicated loss of cohesion of the epithelial structure following alteration of the desmosomal disks. Probably the loosening of the tight epithelial cohesion allows following fungal elements to invade the stratum corneum easier and faster. The question is: how does C. albicans invade the horny cells of the human skin? In our opinion, it is unlikely that essentially mechanical effects are decisive for the spreading of the Candida infection. We believe that the perifungal clear zone in involved epithelial cells indicates keratinolytic activity of C. albicans cells. Kapica and Blank showed that C. albicans is able to grow in vitro on keratin as its only nitrogen source [36]. Phospholipase activity was found in C. albicans [37] and thought to play a part in the invasion of host tissues in lesions of candidosis by damaging host cell membranes and allowing the hyphal tip to enter the cytoplasm [38,39]. Strain-specific, proteolytic activity of C. albicans was reported [40]. The pathogenetic significance of this with regard to human cutaneous candidosis is still unknown.

Clear perifungal zones were not reported in candidosis of mucous membranes [41,42]. Only Montes and Wilborn [11] found that tonofibrils were absent in the immediate vicinity of the fungus within an epithelial cell in oral candidosis. However, electronlucent areas surrounding the microorganisms were observed in tinea versicolor and erythrasma raising the question of keratinolytic activity [43,44]. In some epithelial cells invaded by fungal elements there were absolutely no hints as to any destructive activity of the parasite, damage or reaction of the host cell. The fungi behaved like inert corpuscles. Surprisingly, we failed to find any fungi in the area of the subcorneal or intracorneal micro-abscesses. In vitro C. albicans cells are easily phagocytized by polymorphonuclear leukocytes [21,45]. Therefore in vivo we expected to find fungi intracellularly in the phagolysosomes of neutrophils or extracellularly in the area of the micro-abscesses. Maibach and Kligman also stressed by means of light microscopic observations that the fungus does not reside in pustules. They state that after the inflammation develops the organism disappears rapidly [6]. One has to assume that the great majority of neutrophils had terminated the process of phagocytosis, killing and lysis of the fungal elements in the abscess area.

REFERENCES


