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Chromoblastomycosis

A Morphological Investigation of the Host-Parasite Interaction

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Summary. Chromoblastomycosis is a distinct cutaneous mycotic infection. Histologically it is characterized by the presence of fungus invariably in the yeast phase and by macrophages in large numbers. The morphology of fungus in both yeast and hyphal phases and its interaction with mononuclear phagocytes were examined using ultrastructural techniques. The major structural difference between the fungus in cutaneous tissue and that from a culture medium was a striking increase in cell wall thickness in the former. In the skin, the organisms were phagocytised by macrophages and enclosed in large membrane limited intracytoplasmic vacuoles. The thick, stratified, electron-dense fungal cell wall was observed in stages of alteration of varying severity. Most common was a granular modification of the outermost layers of the cell wall in contact with the vacuolar content. Fragmentation, splitting and rupture of this and deeper layers was also seen. Several ultrastructural features suggested that cell wall damage resulted from an active host cell digestion. The cell wall changes were in sharp contrast with the usual normal fungal cytoplasmic appearance. Only rare intracellular debris which we supposed to represent dead yeast cells were found. These findings suggested that the yeast responsible for chromoblastomycosis resisted fungicidal activities of cutaneous macrophages which possessed the ultrastructural features of stimulated phagocytes.

Key words: Chromoblastomycosis – Fungus ultrastructure – Yeast-macrophage interaction

Chromoblastomycosis is a chronic spreading mycosis of the cutaneous tissues with characteristic and well described clinical and histological features (Cespedes 1971; Carrion 1975; Binford and Dooley 1976). The pathogenic fungi involved belong to the *Dematiaceae*, a taxonomical group of pigmented fungi (Nielson 1974). Several dematiaceous species are responsible for

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morphologically similar skin lesions. Histologically similar pigmented fungi have also been implicated in a variety of mycotic infections including brain abscesses (Symmers 1960; Musella and Collins 1971), systemic infections (Itani 1970; Nishimoto et al. 1978) and subcutaneous cysts (Kempson and Sternberg 1963; Cains et al. 1977; Weedon and Ritchie 1979; Ziefer and Connor 1980). Following the suggestion of Zaias (1978), we restrict the term chromoblastomycosis to the classical cutaneous form and use chromomycosis as a generic, all-embracing title for the various lesions produced by the brown dematiaceous fungi. In patients with an altered immune response, chromoblastomycosis may produce a life threatening systemic chromomycosis with visceral metastases (Fukushiro et al. 1957; Tsai et al. 1966; Itani 1970; Bellefeuille et al. 1978).

The host-parasite relationship in chromoblastomycosis has not been extensively investigated. Antibodies have been detected in such patients (Gordon and Doory 1965; Buckley and Murray 1966) and the cell mediated immune response has been tested in two recent studies (Iwatsu et al. 1979; Kurita 1979). Also fungal form may be significant as chromoblastomycosis invariably involves fungi in the yeast phase, while both hyphae and yeast are found in other forms of chromomycosis. The aim of the present work is to 1) study by morphological means the host-parasite relationship and more especially the yeast-macrophage interaction in chromoblastomycosis and 2) compare the ultrastructural features of the fungus in both yeast and hyphal phases.

Material and Methods

Tissue for both light and electron microscopy was obtained from three African patients with chromoblastomycosis. The histological and ultrastructural studies in all 3 cases and the mycological investigations in one case were performed at the Centre International de Recherches Médicales de Franceville (C.I.R.M.F., Gabon, Africa). The mycological investigation from the two other cases were performed at the Department of Parasitology and Mycology, Centre Universitaire des Sciences de la Santé, Libreville (Gabon).

The specimens for light microscopy were fixed in formalin, embedded in paraffin, sectioned at 8 μm and stained with haematoxylin and eosin (H&E) and silver methenamine. Biopsies for electron microscopy were immediately fixed in 2.5% buffered glutaraldehyde; they were then post-fixed in osmium tetraoxyde and embedded in araldite. Ultrathin sections were stained with lead citrate and uranyl acetate and examined with a Siemens Elmiskop 102 transmission electron microscope (TEM).

Fungal species identification was made on a culture medium. Skin scrapings from one case were cultivated at room temperature on a Sabouraud agar medium containing chloramphenicol. After 2 weeks the emergent colonies were fixed on the medium with 2.5% buffered glutaraldehyde. The samples taken from the colonies were dehydrated through a graded series of ethanol, critical-point-dried with CO_2, sputter-coated with gold and viewed with an Etec Autoscan-scanning electron microscope (SEM).

Results

The 3 patients with recently diagnosed disease had received no treatment at the time of biopsy. In two cases large, late lesions, located on a foot and on a shoulder had a typical "mossy-like" appearance. In the third case, the verrucous lesion was early and located on the left index finger.

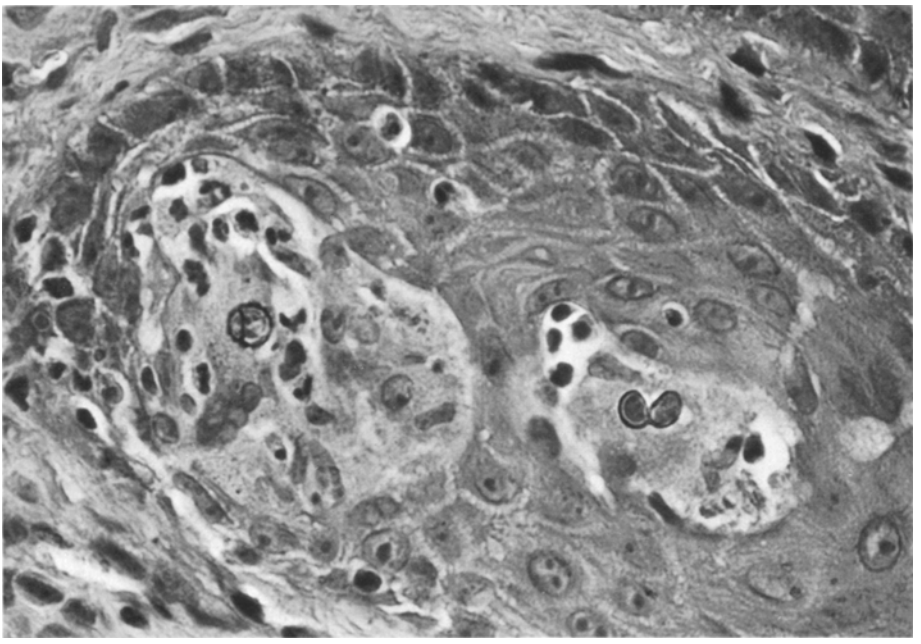

Fig. 1. Two intraepidermal microabscesses, both containing well identifiable intracellular thick-walled yeasts, septate at the left, in division at the right. H & E × 600

Morphology

Histology. Microscopic findings were similar in all three cases with only minor quantitative differences in epidermal reactive processes and in dermal yeast concentration. The dermis contained a dense inflammatory infiltrate dominated by macrophages and polymorphonuclears. Eosinophils were conspicuous; lymphocytes and plasma cells were also present. Macrophages were most numerous and were associated with multinucleated giant cells; true granulomas were not found. Polynuclears were usually most prominent in microabscesses. In one case abscesses were also found in epidermal rete ridges. The fungi consisted of single or multiple, thick walled and often septate brown yeast cells (Fig. 1). They were located in giant cells or in microabscesses. The diameters of the fungal cells ranged from 8 to 13 μm.

Ultrastructure. Transmission electron microscopy confirmed the presence of different types of inflammatory cells. Macrophages were associated with polynuclears, eosinophils, lymphocytes and plasmocytes. The macrophage population in the cutaneous lesions was composed of small to large mononuclear phagocytes and of multinucleated giant cells. The largest cells had one or several euchromatic nuclei, extensive cytoplasm with irregular contours (Fig. 2) producing interdigitated profiles with adjoining macrophages (Fig. 3). The abundant cytoplasm of the largest macrophages and giant

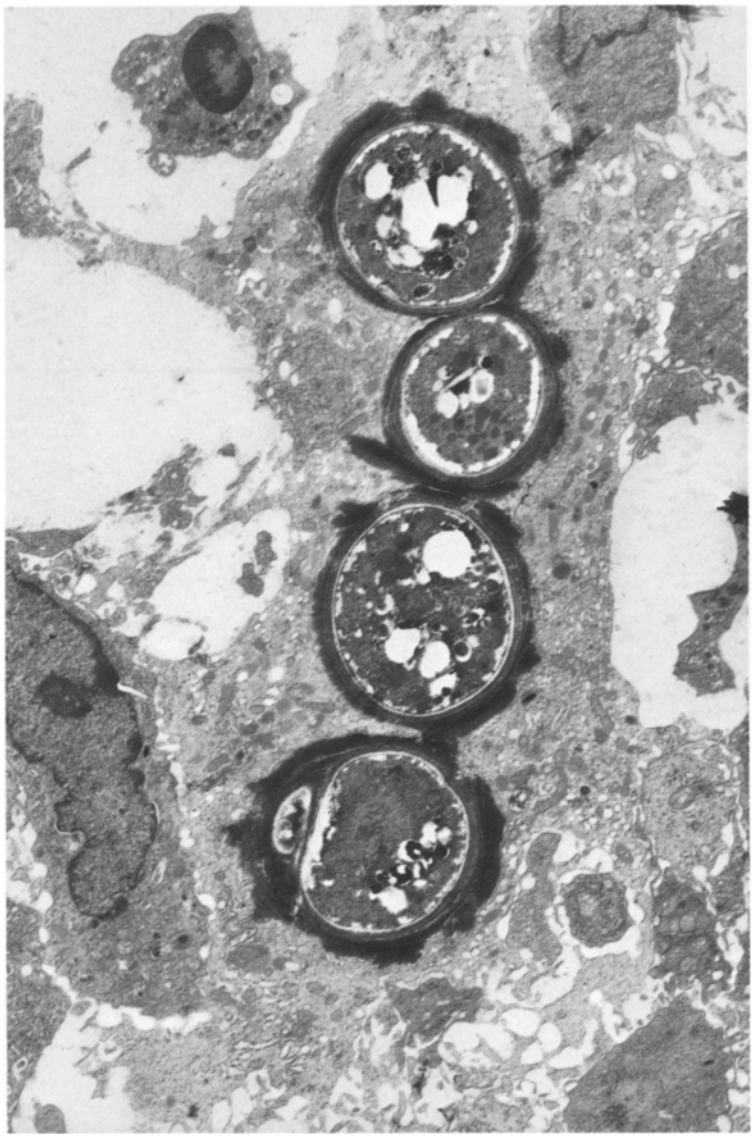

Fig. 2. T.E.M. micrograph. The central giant cell contains 4 yeasts in moniliform disposition without obvious degradative changes. The empty spaces in fungal cytoplasms are artefacts. (× 8,000)

cells contained numerous Golgi profiles, lysosomes, vesicles and mitochondria (Figs. 3–5). In each of the 3 ultrastructuraly investigated cases, some giant cells contained cytoplasmic intravacuolar inclusions such as cell debris or large, dense, lamellar bodies of unknown origin.

Fungal organisms were found in only one case. The yeast cells, single or multiple, in clusters or in moniliform disposition (Fig. 2), were located

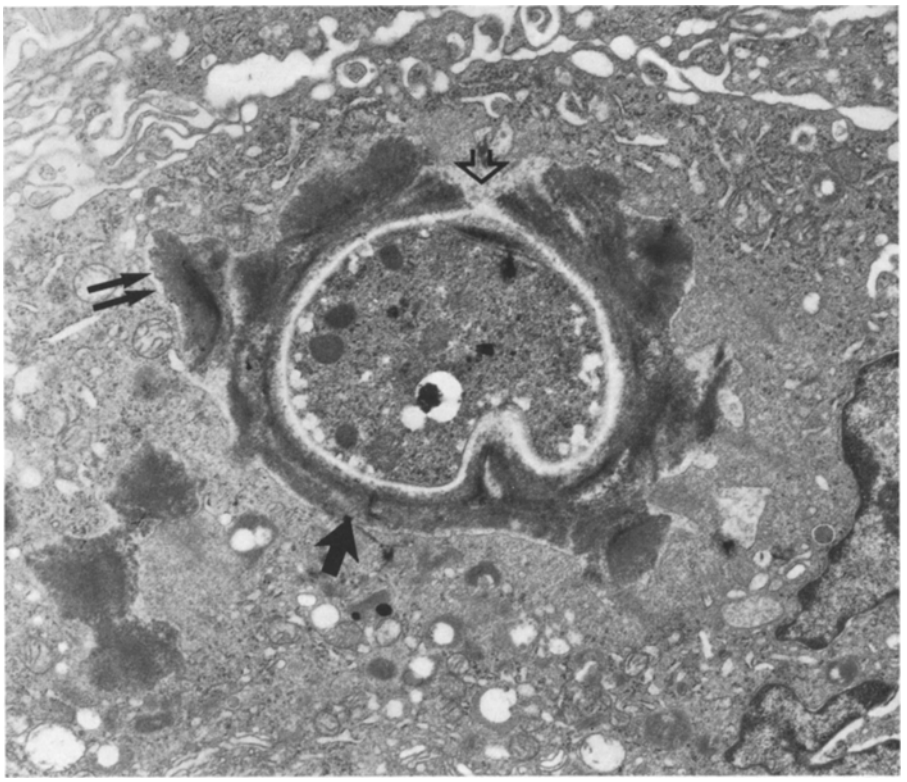

Fig. 3. T.E.M. micrograph. Multinucleated giant cell with a yeast enclosed in large membrane limited intracytoplasmic vacuole. The yeast cell wall (*arrow*) of irregular thickness has a lamellar appearance and is damaged with fragmentation (*double arrow*), rupture (*open arrow*) and splittings. Granular, cell-wall like material is present in separate cytoplasmic vacuoles (*lower left*). (×16,000)

in multinucleated giant cells; no extracellular fungus organism was found. Yeast cells were enclosed in a large intracytoplasmic vacuole (Fig. 3) and contact between the vacuolar membrane and yeast was continuous or occurred only focally. The large, membrane limited vacuole enclosing yeast cells contained additional small granules in highest concentration in the vicinity of and composed of the same electron dense material as the fungal cell wall. At the site of contact, apposition between the macrophage cytoplasm and the intravacuolar yeast was close (Fig. 4). The contours of the vacuolar membrane and the outermost cell wall layer were parallel. These adjacent structures were separated by a narrow slit (Fig. 4). The macrophage cytoplasm, directly adjacent to the fungal cell wall, contained numerous vesicles with dense granular bodies (Fig. 4). The fungal cells were round or crescent shaped. They were enveloped by a thick, dense, stratified cell wall (Fig. 2). The cell wall thickness varied within a single specimen and ranged from 0.2 μm to 1.5 μm (Fig. 3). The outermost layers of the cell wall were inhomogeneous and the innermost homogeneous (Fig. 4). The

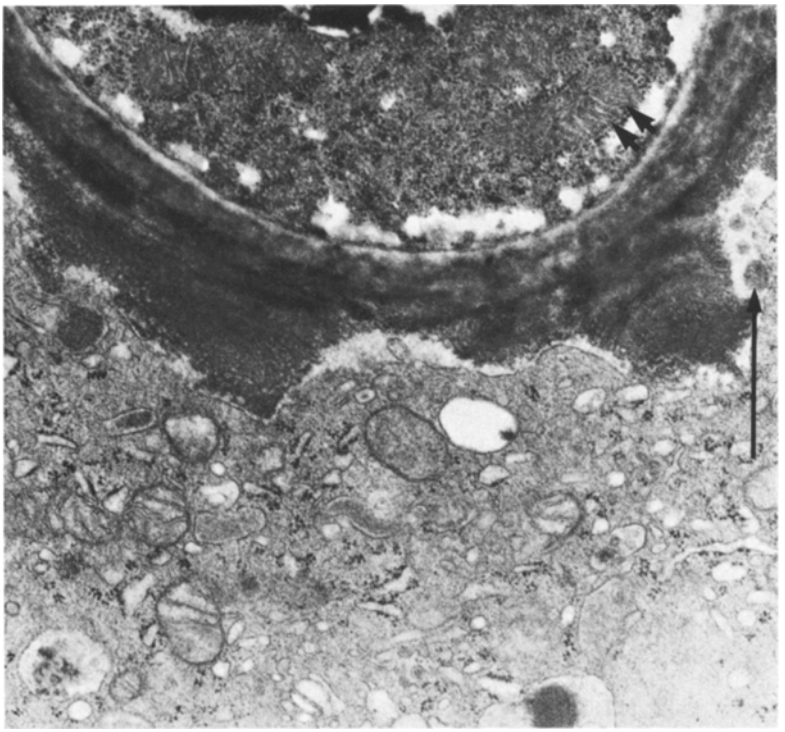

Fig. 4. T.E.M. micrograph. Detail from a phagocytised yeast. The membrane of the phagosome is separated from the cell wall surface by a narrow slit. The outermost cell wall layers have a granular appearance. Fine fragments of cell-wall like material were found in the widest parts of the slit (*arrow*). An electron-lucent thin layer separates the innermost homogeneous cell wall layers from the plasma membrane. The yeast cytoplasm contains mitochondria (*double arrow*) and fine dense granules (× 40,000)

cell wall surface was irregular; breaks, gaps and splitting were particularly frequent in the outermost layers at the site of contact with macrophage cytoplasm (Fig. 3). In these areas, granules, comparable in structure to the most superficial cell wall layers, were found in large numbers in the slit separating macrophage cytoplasm from the fungus (Fig. 4). In addition, transitional figures were found in some areas between the cell wall layer and these free granules. The cell wall was separated from the fungal plasma membrane by a continuous thin electron-lucent layer of 0.05 μm to 0.1 μm (Fig. 4). The plasma membrane was directly apposed on the cytoplasm or was separated from it by a rim of electron-lucent droplets without an identifiable membrane (Fig. 4). Such structures may correspond to lipid droplets, but features of degeneration or fixation artefacts could not be excluded. Apart from these droplets, the yeast cytoplasm was dense and numerous granules (probably glycogen storage granules) and mitochondria were identifiable (Fig. 4). The single nucleus was round, usually centrally located and less electron dense than the cytoplasm; it measured 1.5 μm to 2 μm in diameter. In rare structures which we supposed to represent degenerated

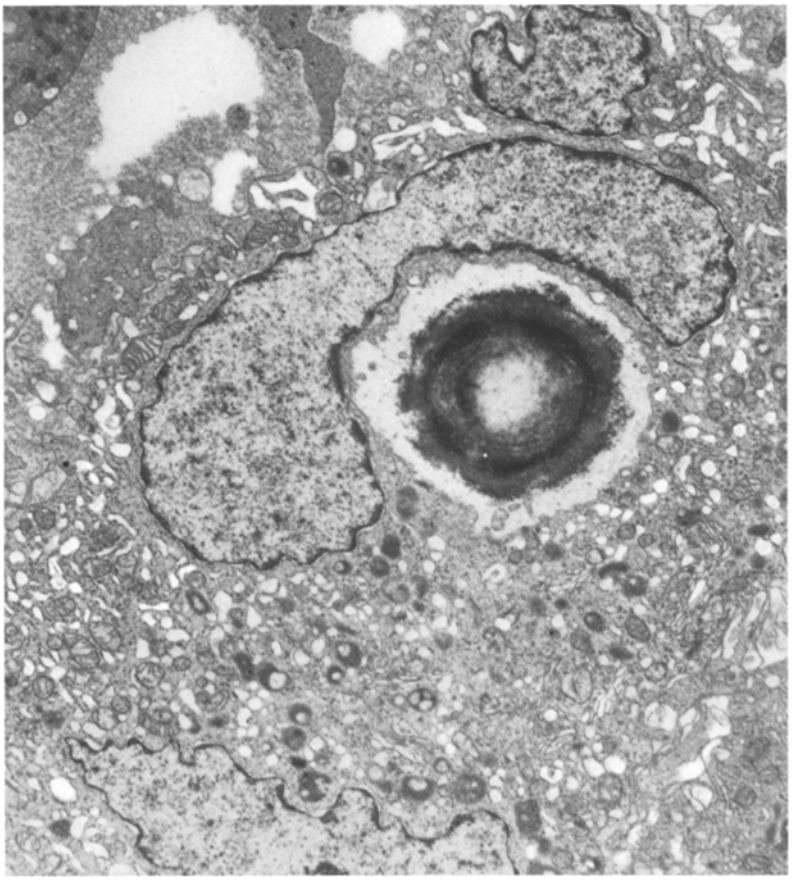

Fig. 5. T.E.M. micrograph. Multinucleated giant cell with a cytoplasm rich in vesicles, dense granules, Golgi complexes and containing a large intravacuolar, dense, lamellar bodie that we believed to be a residual dead yeast (× 12,000)

yeasts, the cell wall was still clearly identifiable, limiting an empty space without residual cytoplasmic components (Fig. 5).

Mycology

In each of the three cases, the cultured fungus was identified as *Fonsaceae pedrosoi.*

S.E.M. Careful examination of the samples processed for scanning electron microscopy revealed only the acrotheca type of sporulation (Fig. 6). The branched hyphae had a slightly rough surface, and measured 1.5 μm to 2 μm in diameter. The lateral and terminal conidiophores were masked by clusters of oval, smooth-walled conidia. Conidia had a maximal diameter of 2 μm and a lengh of 5 μm. The short tubercules between conidiophores and conidia were not distinguishable.

T.E.M. The septated hyphae had a cell wall with two distinct layers: a rough, thin, electron-dense outer layer and a broader, inner, electron-lucent one. The cytoplasm contained numerous

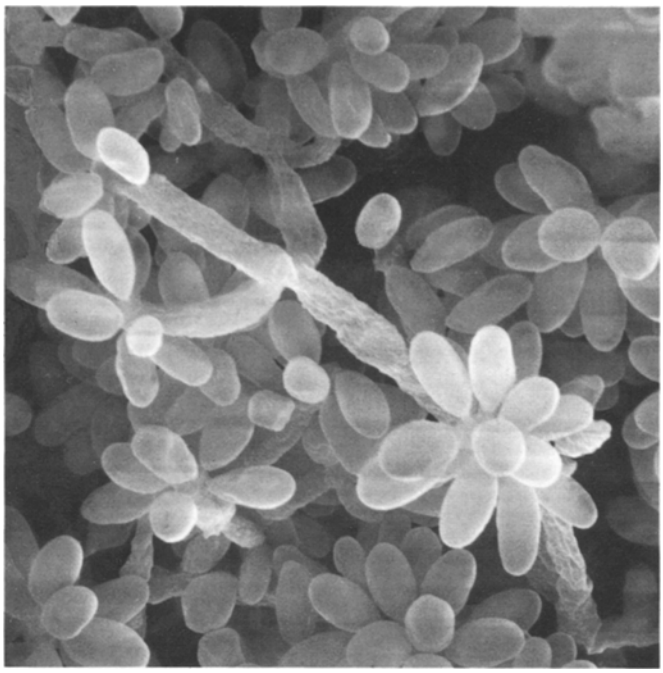

Fig. 6. S.E.M. micrograph. Acrotheca type of sporulation with smooth walled oval conidia, grouped in regular clusters. In diagonal, a branched, rough walled hyphae. × 3,000

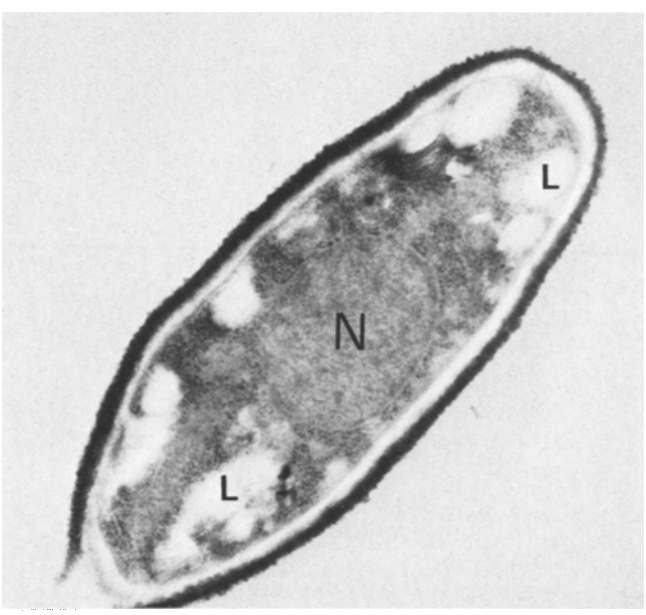

Fig. 7. T.E.M. micrograph shows a conidia obtained from culture; the attachment site on tubercule is on the lower left. The smooth cell wall is composed of electron-dense outer and an electron-lucent inner layer. The cytoplasm contained a central nucleus (*N*), lipid droplets (*L*) and dense granules. (× 25,000)

large lipid droplets. The conidia had a smooth thin cell wall composed of an electron-dense outer layer and an inner electron-lucent layer (Fig. 7). The whole cell wall measured 0.12 to 0.15 μm in thickness. The cytoplasm contained a regular oval shaped nucleus of 1 μm diameter, dense granules, mitochondria and lipid droplets (Fig. 7).

Discussion

The outstanding histological features of chromoblastomycosis are: 1) presence of a fungal organism, invariably in the yeast phase, 2) dermal inflammatory infiltration characterized by a predominance of polymorphonuclears and of macrophages associated with multinucleated giant cells.

Chromoblastomycosis is caused by at least 6 dematiaceous species: *Phialophora verrucosa, Fonsaceae pedrosoi, Fonsaceae compactum, Fonsaceae dermatitis, Cladosporium carrionni,* and *Cladosporium trichoides* (cf. Cespedes 1971; Nsanzumuhire et al. 1974). *F. pedrosoi* is the most commonly implicated of these fungi and was the species identified in the three cases of our study. Most of the dematiaceous fungal pathogens are natural saprophytes in the soil and in dead wood (Dixon et al. 1980) and are nearly universal. As a consequence, chromoblastomycosis has been reported from most areas of the world (Cespedes 1971). The dematiaceous fungi responsible for chromoblastomycosis are dimorphic: the yeast phase is present in cutaneous lesions and the hyphal phase develops on culture medium. The fact that only yeast cells are found in cutaneous chromoblastomycosis may be due to either an absence of factors necessary for a hyphal morphogenesis or a local inhibitory effect related to the cutaneous microenvironment including host defense mechanisms.

Host defense mechanisms in chromoblastomycosis have not been extensively investigated. Precipitating antibodies have been detected in several serological studies in patients with chromoblastomycosis (Gorden and Doory 1965; Buckley and Murray 1966), but the role of these humoral factors in the host defense mechanisms against dematiaceous fungi remains unclear. Granulocytes and macrophages are both implicated in host cellular defense mechanisms. According to experimental data with *Aspergillus fumigatus,* the effect of polymorphonuclears on fungal pathogens depends on fungal form. Lehrer and Jan (1970) demonstrated that polymorphonuclears were unable to kill ingested spores of this fungus. On the other hand, Diamond et al. (1978) reported that polymorphonuclears attached to, spread over and finally induced dramatic changes and probably death in hyphae. Other experimental studies with *Aspergillus* provided evidence that macrophages may damage fungi. The works of Merkow et al. (1971) and Sidransky et al. (1972) described interactions of phagocytic cells and spores in vitro. Chromoblastomycosis provided an opportunity to study the effect of macrophages on yeast in vivo. Although difference in structure of yeast cells must be interpreted with caution, the ultrastructural findings in the present study demonstrated that some phagocytised fungal organisms were damaged. Cell wall changes of variable severity and rare figures suggestive of dead yeast were found. A morphological continuum was found from yeast without evident changes to fungal cells with obvious cell wall damage. We

believed that these changes resulted from active host cell degradation processes because: 1) structural damage to the yeast cell wall was more marked on the outermost layers which were in direct contact with the phagocytic vacuolar content (Fig. 4), 2) the lysosome-like cytoplasmic bodies were in greatest number in macrophages containing phagocytised yeast (Figs. 3 and 5) and 3) fine or coarse fragments of cell wall-like material was found in vacuoles separated from but in the vicinity of phagocytised yeast (Fig. 3). These changes were in sharp contrast with the lack of obvious modification of the cytoplasm of almost all phagocytised yeast. Only rare empty yeast-like structures with recognizable residual cell wall were found (Fig. 5). While the specific processes leading to cell wall degradation and finally to yeast death are not ascertained, it is likely that lysosome fusion with and ensymes release into the phagosome is involved.

In recent years, it has become increasingly evident that macrophages require T-cells interaction to be activated (Cohn 1978). Such activation leads to morphological, biochemical and functional changes consistent with increased microbicidal and fungicidal activity of the macrophage (van Furth 1975).

The importance of stimulated macrophages i.e. of a well developed cell-mediated immune response in fungal infections has been proven by both experimental and clinical observations. Monga (1981) found that protection of mice against *Cryptococcus neoformans* was due to T cell-mediated activation of macrophages. Kimberlin et al. (1981) demonstrated that BCG treated macrophages were more efficient in *Histoplasma capsulatum* management than non-activated phagocytes. BCG is known to stimulate T lymphocytes which in turn activate macrophages (Mokyr and Mitchell 1975). Furthermore, it is well known that fungal infections have an increased incidence among patients given cytotoxic and immunosuppressive treatments. Also, patients with disseminated cryptococcosis, candidiasis, histoplasmosis and coccidioidomycosis are known or suspected to be deficient in cell-mediated immunity (Diamond and Bennett 1973; Catanzaro et al. 1974; Stobo et al. 1976).

In recent studies, a cell-mediated immune response was detected both in humans with chromoblastomycosis (Iwatsu et al. 1979) and in mice infected by *F. pedrosoi* (Kurita 1979). The present study provided indirect evidence for an activation of the cutaneous macrophages involved in yeast phagocytosis in a patient with chromoblastomycosis. On the other hand, the present study suggested also that, although macrophages were responsible for fungal cell wall damage, intracellular death of the yeast seemed to occur only rarely.

Further studies would be required to clarify the host-parasite relationships in chromoblastomycosis and to determine whether yeast persistance and multiplication in this disease is related to fungal form and/or to an inadequate host-cell-mediated immune response.

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