**Candida albicans** Endocarditis: Ultrastructural Studies of Vegetation Formation

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*Candida albicans* endocarditis was established in rabbits after transaortic catheterization. Within 30 to 90 min after infection, *C. albicans* was observed by scanning electron microscopy on the valve surface. The organisms were predominantly associated with host deposits of erythrocytes, phagocytes, platelets, and fibrinous-appearing material, which collectively appeared on the valve surface in response to trauma. Within 48 h after infection, vegetations composed of these same host components were observed on the heart valves, although *Candida* cells were not demonstrable on the valve or vegetation surface. When the vegetations were examined by transmission electron microscopy, *Candida* blastospores were only observed within phagocytic cells (predominantly monocytes) enmeshed within the vegetation matrix. Many of the intracellular organisms were undergoing degradation, as evidenced by a reduction in electron density of the cell wall. Other fungi had highly electron-dense cell walls and germ tubes. Phagocytic cells containing germinating *Candida* were highly vacuolated and were observed at various stages of cell lysis. After 7 days of infection, the vegetation contained a dense meshwork of fibrin and *Candida* pseudohyphae with 105 to 106 colony-forming units/g of vegetation. The mature vegetation was devoid of phagocytic cells and continued to grow until the death of the animal.

*Candida albicans*, as a normal commensal of humans, often becomes infectious in the compromised host with an underlying disease (7). Also, in postcardiac surgery patients, *C. albicans* and other fungi are now more commonly recognized as an important cause of infectious endocarditis (1, 11, 18). *Candida* endocarditis is particularly devastating, since the infrequency of positive blood cultures and the difficulty of interpreting serological data often postpone diagnosis until the disease has progressed (1, 18). Also, therapy with antifungal antibiotics is rarely successful.

Factors that increase the vulnerability of cardiac surgery patients to *Candida* endocarditis have been discussed previously (18) and have been summarized by Andriole et al. (1). However, the early events, including the factors that influence the initial attachment of *C. albicans* to the valve surface, its survival on the valve, and the development of a vegetation, have not been characterized.

Animal models of fungal endocarditis have been developed recently that simulate the disease in humans (2, 6, 17). The purpose of our investigation was to characterize some of the early events in the development of the disease with transmission and scanning electron microscopy and an animal model.

**MATERIALS AND METHODS**

*C. albicans*. The isolate of *C. albicans* used in this study was obtained from an infection of a valvular prosthesis and has been previously characterized (17).

Endocarditis model. The methods for the reproduction of endocarditis in rabbits have been previously described (6, 17). The aortic valve cusps of the rabbits were traumatized by passing a polyethylene catheter through the carotid artery across the aortic valve. Catheters were left in place for 1 h. The rabbits were then infected through the catheter with 5 × 108 colony-forming units of *C. albicans* blastospores per ml; the blastospores were obtained from a 48-h culture grown in Sabouraud glucose broth at 37°C. Catheterized rabbits were sacrificed at 30 and 90 min, 48 h, or 7 days after infection. Quantitation of *Candida* within the vegetation has been described previously (17).

Scanning electron microscopy. After rabbits were given an intravenous injection of pentobarbital, the thoracic cavity was opened, vessels leading to and from the heart were cross-clamped, and saline (0.9% wt/vol) was injected into the left ventricle until the aorta was cleared of blood. The heart was then removed and fixed in situ for 1 h with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.6). The tricuspid and aortic valves were removed and fixed for an additional 12 h in glutaraldehyde as described above.
The valve tissue was cut into small pieces and dehydrated in a graded series of ethyl alcohol (30 to 100%). Critical-point drying (Tousimis Corp., Rockville, Md.) was done with CO₂. The specimens were coated with gold (200 to 300 nm; Technics Instruments, Alexandria, Va.) and viewed in a scanning electron microscope (Autoscan; ETEC, Hayward, Calif.).

Transmission electron microscopy. After fixation in glutaraldehyde as described above, the tissue was washed three times in phosphate buffer, postfixed in 1% osmium tetroxide, and stained in 0.5% uranyl acetate. The tissue was then dehydrated in a graded series of ethyl alcohol, washed in propylene oxide, infiltrated with a propylene oxide-Maraglas (LADD Research Industries, Burlington, Vt.) (1:2) solution, and embedded in Maraglas (16). Ultrathin sections were stained with lead citrate or double stained with uranyl acetate and lead citrate and examined in a Philips EM 300 electron microscope. For light microscopy, 1-μm sections were stained in 0.5% azure A in 0.1% sodium borate (3). Other tissue was stained with silver methenamine for light microscopy.

RESULTS

*C. albicans* blastospores could be identified adhering to fibrin strands on the aortic valves of catheterized animals within 30 to 90 min after injection (Fig. 1) but not to the aortic valves of animals without precatheterization (Fig. 2). The initial vegetation consisted of fibrin, platelets, erythrocytes, and macrophages (phagocytes) adherent only to the area of endothelial trauma (Fig. 1 and 3).

Forty-eight hours after injection, macroscopic vegetations were present near the base of the valve (Fig. 4). Examination of the vegetation by scanning electron microscopy revealed a loose meshwork of fibrin strands with entrapped erythrocytes, but yeast cells could not be identified on the surface (Fig. 5).

Transmission electron microscopy of a 48-h vegetation revealed a dense peripheral layer of platelets and fibrin (Fig. 6). Mononuclear cells...
Fig. 3. Valve tissue 90 min after infection showing two cells that resemble phagocytes (P) in size. Also present are Candida (Ca) and platelets (Pt). Bar, 1.0 μm.

Fig. 4-16. Representations of animals infected for 48 h.
Fig. 4. Vegetation (foreground) seen at low magnification. Vegetations usually appeared close to the heart valve-wall junction. Normal valve tissue can be seen in the background. Bar, 50 μm.
FIG. 5. Vegetation surface at 48 h after infection showing fibrin (F) and erythrocytes (RBC). Yeast cells were not observed at 48 h on the vegetation surface. Bar, 1.0 μm.

FIG. 6. Transmission electron micrograph of fibrin (F) and plate (Pt) deposition at the periphery of a vegetation on a heart valve. Bar, 1.0 μm.
(macrophages) were observed throughout the vegetation enmeshed within the tight fibrin matrix (Fig. 7). Many of these macrophages contained phagocytized C. albicans cells (Fig. 8 and 9). The macrophages were usually isolated, but occasionally several cells, each containing yeast, could be found within the same microscopic field (Fig. 8). Extracellular (nonphagocytized) Candida were not observed within the vegetation 48 h after infection. As many as four to five fungal cells could be identified in a phagocytic cell (Fig. 9). Many Candida appeared to be extensively degraded, as determined by a reduction in the electron density of the cell wall (Fig. 10). Disruption of cytoplasmic structures was also observed. In other instances, macrophages containing the fungus were demonstrated at various stages of disintegration (Fig. 11 through 14). Many of the fungal cells identified in these phagocytes had produced germ tubes that could be observed breaking through the cell membrane of the phagocytic cell (Fig. 13); extensive growth of intracellular Candida occurred (Fig. 15) in some cases. Occasional polymorphonuclear leukocytes containing germ-tube-producing fungal cells could also be identified (Fig. 16).

Within 7 days, the mature vegetation consisted of a thick mesh of pseudohyphal forms of C. albicans containing high titers of the organism (10^6 to 10^8 colony-forming units/g). No host inflammatory cells were observed within the vegetation.

DISCUSSION

C. albicans is commonly found on the skin and mucous membranes of hospitalized patients, particularly those receiving antimicrobial agents. Surgical trauma or a number of different minor procedures, including tracheostomy or intravenous catheterization, readily provide for the entry of the organism into the bloodstream (18). Once within the bloodstream, the fungi are usually cleared rapidly by the reticuloendothelial system. However, when the endocardial surface of the cardiac valves is damaged by scarring or a foreign body, such as a prosthetic valve, localized intravascular infection may result. The use of an animal model of endocarditis has allowed us to study the initial events of colonization and development of Candida infection on a traumatized valve in vivo. Previous studies (6, 17) have documented the similarities of the an-

![Fig. 7. Mononuclear cell (monocyte, Mn) associated with fibrin (F) and platelet (Pt) deposits at the periphery of the vegetation. Bar, 1.0 μm.](http://iai.asm.org/ on January 18, 2018 by guest)
Fig. 8. Several phagocytic cells with intracellular yeasts (Ca) within the vegetation surrounded by erythrocytes. A neutrophil is also present. Bar, 3.0 μm.

Fig. 9. Monocyte (Mn) with several Candida (Ca) cells that differ in electron density. RBC, erythrocytes. Bar, 1.0 μm.
imal endocarditis model and human *C. albicans* endocarditis. The infection is highly reproducible, developing in all rabbits catheterized and infected with $10^6$ organisms. They develop agglutinating and precipitating antibody after 12 days of infection and survive for a mean of 26 days.

The sequence of events that resulted in endocarditis in our model began with the endocardial trauma. The host responded to the trauma by depositing platelets, erythrocytes, and fibrinous material on the valve surface. The fungus could be readily identified attached to these components and appeared to adhere preferentially to this developing vegetation; i.e., only rarely were yeast cells observed by scanning electron microscopy elsewhere on the valve surface within 90 min after infection. Cells resembling platelets (10) and phagocytes in size and histological characteristics were present on the traumatized valve surface early after infection (30 to 90 min). Durack and co-workers have also observed monocytes (macrophages) on the surface of a catheter-induced vegetation within 30 min after infection in a rabbit model of streptococcal endocarditis (3-5). The role of these phagocytic cells in this setting has not been established.

Although nonphagocytized *Candida* were observed on the valve early after infection, exhaustive sectioning of the vegetation at 48 h after infection failed to reveal any nonphagocytized *Candida* within the vegetation. Instead, the fungus was always observed within phagocytes, either appearing unaffected by the action of the phagocytes or at different stages of destruction, as evidenced by changes in the electron density of the fungal cell wall and cytoplasm. Most of the phagocytes within the developing vegetation were solitary. After the initial events, the further penetration and mobility of phagocytes within the vegetation appeared to be seriously limited by the presence of the tight vegetation matrix. Mackaness (12) has observed that the phago-

**Fig. 10.** Two intracellular Candida (Ca) within a monocyte. Note the differences in electron density of the cell wall and cytoplasm. Bar, 0.25 μm.
FIG. 11. Germ tube (Gt) formation by Candida within a phagocytic cell, probably a monocyte. Note the vacuolation (V) associated with the phagocyte. Bar, 1.0 μm.

FIG. 12. Phagocytic cell (with germinating yeast [Ca]) undergoing lysis. Bar, 0.5 μm.
Fig. 13. Phagocytic cell (with germinating yeast [Ca]) undergoing lysis. The germ tube appears to be emerging from the degraded phagocyte. Bar, 0.5 μm.

Fig. 14. Candida apparently free within the vegetation. The remains of a membrane partially around the yeast (arrows) can be seen in the insert at the upper left. Bar, 0.35 μm. Bar for insert, 0.15 μm.
cytic function (or mobility) of macrophages against staphylococci was reduced when both were held in a plasma clot.

Forty-eight hours after infection, intracellular Candida were either in the process of being destroyed by the phagocyte or had germinated, suggesting viability and growth of the fungus. This study clearly demonstrates microscopically that, in vivo, monocytes are capable of degrading *C. albicans*, in contrast to in vitro studies that have indicated that monocytes from nonimmunized animals failed to kill or even inhibit the intracellular growth of the fungus (14, 19). Our results are similar to those of Meister et al. (13), who reported studies of the granulomatous response in the liver of mice infected intravenously with *C. albicans*. They found that fungal cells were rapidly phagocytized by liver macrophages. Subsequently, Gram and periodic acid-Schiff stains and immunofluorescence reactions of fungal cells disappeared. Phagocytized cells exhibited a loss of cytoplasm as well as cell wall deformation and cell collapse. The authors suggest that these changes were associated with the degradation of cell wall mannan by macrophages. In the present study, we observed a change in the electron density of both the cell wall and the cytoplasm of some phagocytized *C. albicans*. The mechanisms by which normal monocytes (macrophages) degrade Candida species or other fungi have not been established (9). Nevertheless, in this study, the initial destruction of intracellular Candida was not adequate to tip the balance in favor of the host. Other intracellular Candida demonstrated no changes in electron density of the cell wall and were undergoing germ tube formation. Germination of the fungus was invariably associated with either disruption of the cell membrane or extensive vacuolation and lysis of the monocyte.

The fibrin-platelet-erythrocyte deposits that had continued to increase in size above the developing fungal cells now appeared to provide a protected environment devoid of further phagocytic cell infiltration, allowing unimpaired growth of the fungus. The importance of this “protection” is emphasized by a comparison of this model of endocarditis with a model of intramuscular *C. albicans* infection. Pearsall and Lagunoff (15) observed that, after injection of *C. albicans* into the thigh muscles of mice, eradication of the fungus from the site of infection was initially associated with an infiltration of

**Fig. 15.** Proliferation of *Candida* (Ca) within a monocyte. Some internal degradation of the monocyte is evident. Bar, 0.80 μm.

**Fig. 16.** Germ tube (Gt) formation within a neutrophil. RBC, Erythrocyte. Bar, 1.0 μm.
neutrophils. Within 3 weeks, an increase in mononuclear cells (macrophages and lymphocytes) paralleled the further decrease in the viable fungal organisms remaining in the lesion. In Candida endocarditis, this later granulomatous inflammatory response was absent from the vegetations, and the proliferation of the fungus, as determined by progressive enlargement of the vegetations, continued until death of the animal at 26 days (mean survival) (17). The factors that impair the influx of inflammatory cells once vegetation formation is established are unknown, but lack of a cellular response has also been observed in Aspergillus and various forms of bacterial endocarditis (2, 8).

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LITERATURE CITED