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Endothelial Cell Proliferation Associated with Lesions of Murine Systemic Candidiasis

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Neovascularization is associated with tumor growth and some inflammatory diseases but has not been reported to be induced by infectious agents. In a mouse model of systemic *Candida albicans* infection, extensive endothelial cell proliferation was seen in the periphery of brain abscesses and in the areas of fungal pyelonephritis in the kidney. This finding is important for an understanding of the pathogenesis of fungal infections and may contribute to an analysis of the mechanisms of angiogenesis.

Angiogenesis, the formation of new capillaries in the fully developed vertebrate, can occur either physiologically, as in wound healing, or pathologically, as in neoplasms or inflammatory diseases. Angiogenesis has been shown to be mediated by factors present in a variety of normal tissues (6), and some cells of the immune system, such as T lymphocytes and macrophages, also show angiogenic activity (20).

Angiogenesis occurring in association with infection is uncommon. It has been reported as an unusual manifestation of *Rochalimaea* infection (18) and has been demonstrated in tissues from patients with human immunodeficiency virus type 1 (5) or human T-cell lymphotropic virus type III (3) infections by staining for factor VIII (von Willebrand's factor). However, the latter observations are thought to be related to the production of angiogenic factors by infected lymphoid cells (19). In other infectious diseases, the accumulation of immune or inflammatory cells at the site of a lesion has not been reported to result in neovascularization.

Systemic candidiasis in inbred mice closely resembles the human disease in that the lesions are similar in nature and distribution (15) and the brain is a prime focus of infection (16). After intravenous infection with the yeast *Candida albicans*, the organism colonizes the brain and other tissues by adherence to, and penetration through, the capillary endothelium (1) and establishes foci of infection. These are characterized by accumulations of leukocytes, especially granulocytes, together with yeast and hyphal forms of the organism, within the necrotic debris. To examine the characteristics of the lesions in detail, specific-pathogen-free female CBA/CaH mice, 6 to 8 weeks of age, were purchased from the Animal Resources Centre, Perth, Australia, and infected intravenously with 3×10^5 *C. albicans* blastoconidia (isolate 3630 from the Mycology Reference Laboratory at the Royal North Shore Hospital, Sydney, Australia). The brains and kidneys were removed on day 5 after infection and fixed in formalin, and random sections were taken at intervals across these organs. The sections were stained with hematoxylin and eosin and periodic acid-Schiff. Endothelial cells were identified by immunoperoxidase staining of paraffin sections, using a polyclonal rabbit antiserum (Dakopatts, Glostrup, Denmark) specific for factor VIII-related antigen (von Willebrand's factor) (4, 13, 17).

In the cerebral lesions, there was evidence of endothelial cell proliferation, seen as distinct vascular profiles at the periphery of abscesses (Fig. 1) or as aggregates of spindle-shaped cells when stained for factor VIII-related antigen by the immunoperoxidase technique (Fig. 2A). Sections developed with immunoperoxidase in the absence of the primary antibody were not stained. The structures were not seen in uninfected control mice or in mice challenged intravenously with an equivalent dose of the related yeast, *Saccharomyces cerevisiae*, which did not induce any detectable lesions (data not shown). This

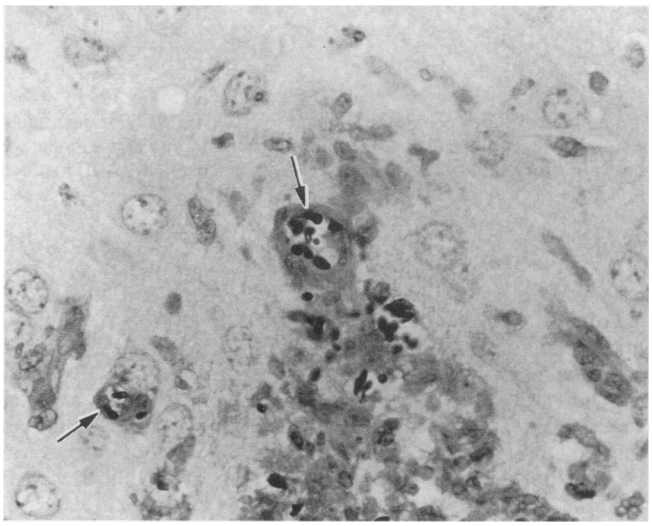

FIG. 1. Periphery of an abscess in the brain of an infected mouse examined 5 days after intravenous infection with 3×10^5 *C. albicans* blastoconidia. Capillary buds and small blood vessels (arrows), some of which contain the yeast forms of the organism, are lined with plump endothelial cells. Periodic acid-Schiff stain was used. Magnification, ×480.

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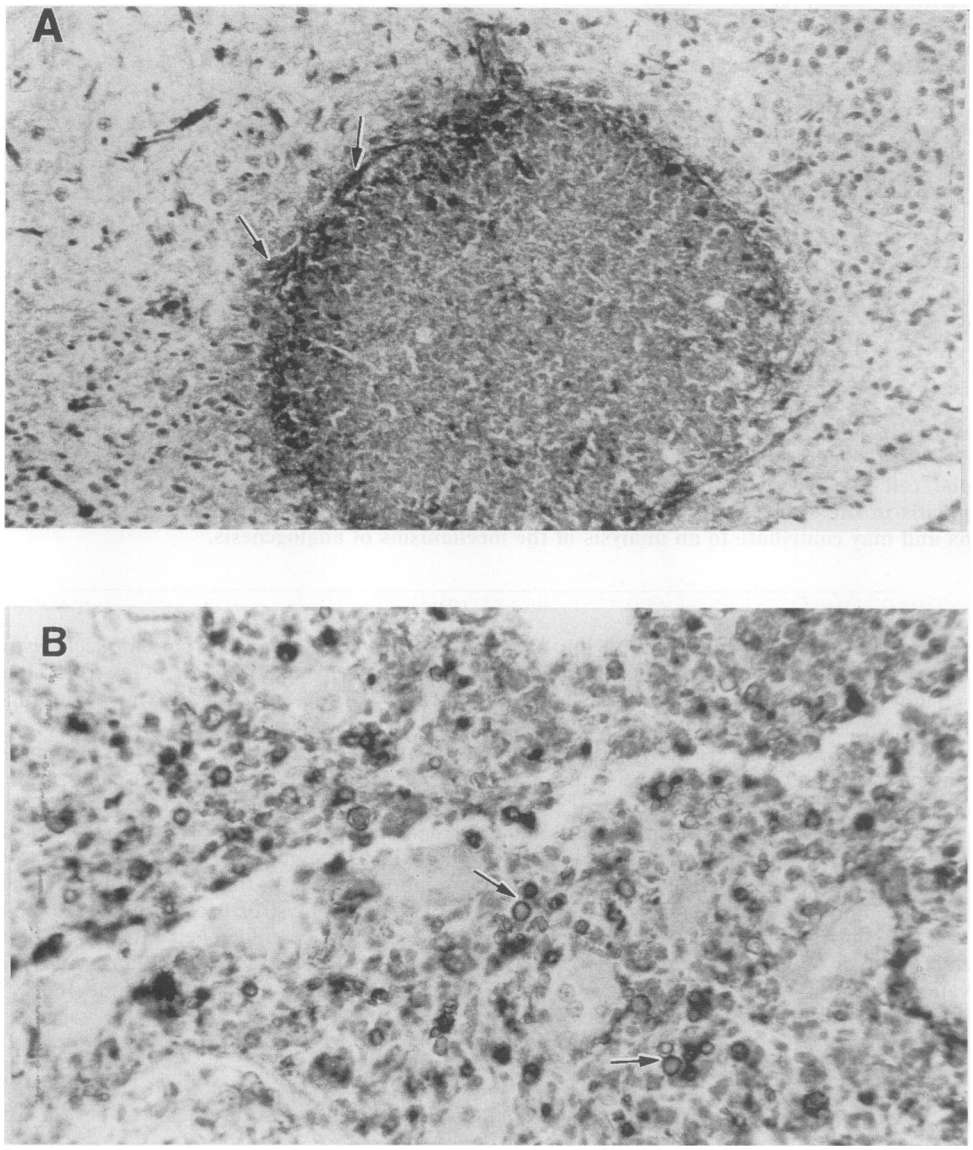

FIG. 2. (A) Periphery of an abscess in the brain of an infected mouse taken 5 days after intravenous infection and stained by an immunoperoxidase technique for factor VIII (von Willebrand's factor). Positively stained spindle-shaped cells are seen at the periphery of the lesion (arrows). Periodic acid-Schiff and immunoperoxidase stains were used. Magnification, ×120. (B) Section of kidney from a mouse taken 5 days after infection with *C. albicans* and stained for factor VIII by the immunoperoxidase technique. Many positively stained cells (arrows) are seen in a zone of pyelonephritis. Magnification, ×120.

reaction was not unique to the brain, as many endothelial cell aggregates were also demonstrated in the kidneys of infected mice in the areas of fungal pyelonephritis (Fig. 2B).

As angiogenic peptides have been extracted from "live yeast cell derivative" of *S. cerevisiae* (2), a concentrated *Candida* supernatant was tested for its ability to promote or maintain the growth of a mouse endothelial cell line. A *Candida* culture supernatant was prepared by inoculation of 500 ml of RPMI medium without serum with an aliquot of *C. albicans* blastoconidia. The culture was left to grow, with intermittent agitation, for 48 h, and then was centrifuged; the supernatant was recovered and concentrated in an Amicon pressure filtration apparatus, using a YM3 membrane. RPMI alone, similarly treated, was used as a negative control. The concentrates were tested for growth-promoting properties with a mouse brain endothelial cell line (MBE cells) developed in this department (4). Serial double dilutions of the concentrated supernatants were made in a 96-well tissue culture plate, and 2×10^4 MBE cells were added to each well. The assays were performed in duplicate in both 5 and 0.8% fetal calf serum. Cell proliferation was measured after 3 days of growth at 37°C in 5% CO_2, using tetrazolium dye reduction (14).

The concentrate showed no detectable activity when assayed on either MBE cells (data not shown) or BALB/c 3T3 cells, which are routinely used to assay the proliferative capacity of other growth factors (7). Although the stimulus for endothelial cell proliferation has not been identified, secretion of a growth-promoting factor by the organism itself has not been definitively excluded, because the MBE proliferation assay may not have been sufficiently sensitive, or the method of preparation

of the concentrate may not have preserved the activity. An alternative is that *Candida* spp. may stimulate macrophages or other lymphomyeloid cells to produce cytokines such as interleukin-8 (12), which can act on the endothelial cells to induce proliferation.

The pathogenesis of systemic *C. albicans* infection is not fully understood, but adherence of the yeast to the endothelium is undoubtedly crucial to the establishment of a focus of infection. There is evidence (9) that adherence of the yeast to endothelial cells is mediated by specific receptors, such as the α-subunit integrin analog that binds iC3b (8). The yeasts adhere preferentially to the surface of the endothelium at the junctions of adjacent cells (10) but bind much more avidly to exposed portions of the subepithelial extracellular matrix (11). A possible mechanism for tissue invasion by the organism is as follows: after initial adherence, the endothelial cells are induced to undergo proliferation. This involves a loosening of the tight junctions between the cells, exposing segments of the basement membrane, which provide a more favorable site for adherence. These segments are rapidly subjected to enzymatic digestion, and the individual cells migrate and divide, forming the basis of a microvascular network. The yeast could then either be drawn passively through the basement membrane or extend mycelial elements along these planes of weakness, thus establishing a focus of infection.

The mechanism of angiogenesis induced by *C. albicans* is of considerable interest, and further studies are being carried out to analyze the phenomenon more completely.

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