The pathogenesis of cerebral infection after Cryptococcus neoformans fungemia in outbred mice was investigated. Confocal microscopy and cultures on ficoll-hypaque gradient–separated blood cells were used to detect yeasts in the cytoplasms of monocytes. In semithin brain sections, poorly capsulated yeasts were seen in macrophages in the leptomeningeal space, in monocytes circulating in leptomeningeal capillaries, or in the endothelial cells themselves, strengthening the hypothesis that monocytes and endothelial cells play key roles in the pathogenesis of cryptococcal meningitis. Similar fungal loads and cellular reactions were seen in mice and in 1 patient with acquired immune deficiency syndrome (AIDS), all with acute cryptococcal meningoencephalitis, and in mice and in 1 patient with AIDS, all with cured cryptococcal infection. Immunostaining revealed both the presence of cryptococcal polysaccharide in various brain cells and antigenic variability both from yeast cell to yeast cell and over time. Thus, our data established the relevance and interest that this experimental model has for investigation of the pathogenesis of human cryptococcal meningitis.

Cryptococcus neoformans is an encapsulated yeast that causes severe meningitis and disseminated infections, mostly in patients with defective cellular immunity [1]. In France, 80% of the cases are observed in individuals infected with human immunodeficiency virus (HIV) [2], and, conversely, cryptococcosis is the most frequent mycosis of the central nervous system in patients with AIDS [3]. To investigate the pathogenesis of cryptococcal meningitis, some investigators have developed experimental models of local infection that have been obtained by direct inoculation of yeasts [4]. However, because brain invasion by C. neoformans is thought to follow fungemia, the latter is bypassed, thereby leading to potentially inaccurate pathophysiological conclusions. In mouse, we recently devised a cryptococcosis model mimicking the most common clinical situation—namely, disseminated meningoencephalitis. Using that model, we showed a direct correlation between the magnitude of fungemia and the degree of brain infection early after inoculation [5], and we demonstrated that the plasma cytokine profile in mice was similar to that in patients with AIDS who have disseminated cryptococcosis [6]. The aim of the present study was to investigate, in mice, the pathogenesis of the cerebral C. neoformans infection obtained by hematogenous yeast dissemination and to further assess the relevance of our experimental model.

Patients, Materials, and Methods

Human Cases of AIDS-Associated Cryptococcosis

Case 1. A 37-year-old man with no medical history of disease was hospitalized with acute meningitis. Results of a cerebral computed-tomography scan were normal. Cerebrospinal fluid (CSF) contained no cells, but staining with India ink revealed numerous encapsulated yeasts. Cultures of CSF, buffy coat, and urine grew C. neoformans later identified as serotype A. Results of serologic tests for HIV-1 were positive, and the CD4+ T cell count was 56/μL. The patient died suddenly 3 days after antifungal treatment had been started, and his brain was removed 20 h after death.

Case 2. A 44-year-old man who had been HIV seropositive since 1985 and who had disseminated nontuberculous mycobacterial infection in 1991 presented with cryptococcal meningitis in 1992. At that time, the CD4+ T cell count was 4/μL. Blood and urine cultures were negative for fungi. The isolate from the CSF was identified as C. neoformans serotype D. The clinical outcome was favorable after initial antifungal therapy, and fluconazole was prescribed as maintenance therapy. The patient later developed cytomegalovirus infection, digestive cryptosporidiosis, and diffuse cytomegalovirus infection, digestive cryptosporidiosis, and diffuse...
pulmonary aspergillosis, leading to death in November 1993. An autopsy was performed 20 h after death.

In both case 1 and case 2, gross examination of the brains was performed, after 1 month, in 10% buffered-formalin fixation, both by coronal sectioning of the cerebral hemispheres and by sectioning, perpendicular to their axes, of the brain stem and the cerebellum. Blocks from many regions of the cerebral hemispheres, the brain stem, and the cerebellum were embedded within paraffin and subsequently were studied as described below for murine brains.

Experimental Infections

Animals. Outbred male OF1 mice (mean body weight 22 g; Iffa Credo) were housed in our animal facilities and received food and water ad libitum.

Isolates. C. neoformans (strain NIH52D) serotype D and a clinical isolate (from case 1) serotype A were used. Yeasts were subcultured in yeast nitrogen base broth supplemented with 2% glucose (Difco Laboratories) for 18 h, on a rotary shaker, at 30°C and were washed in sterile PBS (pH 7.4) before use. For selected experiments, strain NIH52D was then labeled with fluorescein isothiocyanate (FITC), as described elsewhere [7]. FITC-labeled yeasts were washed extensively in PBS before use. In preliminary experiments, we determined that the labeling was stable for 7 days at 4°C and for ≥24 h at 37°C (data not shown).

The inoculum was prepared as has been reported elsewhere [5]. Mice were injected intravenously (iv) with (a) 2 × 10⁷ cryptococci (from both isolates), for the histopathological study, and (b) 2 × 10⁶ FITC-labeled or unlabeled cryptococci (strain NIH52D), for experiments designed to analyze the in vivo interaction with peripheral-blood mononuclear cells (PBMC).

The course of the infection (as represented by body weight, yeast counts in blood and brain, histopathological results, and immunohistochemistry of the brain) was monitored up to day 100 after inoculation (3–8 mice were killed at each time). After the mice had been killed, the brains were aseptically removed. The left hemispheres and the cerebellums were used for yeast counts, whereas, except for a small part of the frontal lobes, which was frozen at −80°C, the right hemispheres were fixed in 10% buffered formalin and were embedded within paraffin, for microscopy.

Fungemia. Blood from infected mice was obtained by cardiac puncture after they had been killed by chloroform inhalation. One milliliter of heparinized blood, diluted 1:5 in sterile saline, was layered onto 3 mL of ficoll-hypaque (J-prep; Vysis TechGen International) and was centrifuged at 350 × g for 30 min. Both the pellet and the PBMC layer were washed, and each was suspended in 200 μL of PBS. Each layer (pellet or PBMC) was divided into 2 equal aliquots. One was used for counts of colony-forming units, as described below, and the other was to prepare cytospin smears. Smears were fixed in 4% paraformaldehyde, were mounted with Mowiol 4.88 (Calbiochem), and were observed by means of a confocal microscope (Axiovert 100M, program LSM 510) when FITC-labeled cryptococci had been either inoculated or stained with May-Grunwald-Giemsa and were examined by light microscopy when unlabeled cryptococci had been injected.

Evaluation of the fungal load. After being removed, human or murine tissue samples were weighed and were homogenized in sterile distilled water. Tenfold dilutions of the homogenates were plated, in duplicate, on Sabouraud-chloramphenicol agar in petri dishes and incubated at 28°C for 48 h. Results are expressed as log10 colony-forming units per gram of organ. When the 1:10 dilution was confirmed to be sterile, the entire brain homogenate was plated on several Sabouraud dextrose-agar petri dishes. These brain samples were considered to be sterile if no colony-forming units were observed on any plate after 48 h of growth. Undiluted buoy coats or samples from the ficoll-hypaque gradients were cultured according to the same procedure [5].

Histopathology

Interactions between C. neoformans and the blood-leptomeningeal barrier. To analyze the interaction between C. neoformans and the blood-leptomeningeal barrier, the brain from a mouse with severe meningoencephalitis was aseptically removed after inoculation with NIH52D, was cut into 2-mm cubes fixed in 2.5% glutaraldehyde, was fixed in 1% osmium tetroxide, and was embedded in epoxy resin. Semithin sections were stained by toluidine blue and were examined by light microscopy.

Brain lesions. Brain hemispheres in mice infected with either NIH52D or the clinical isolate were processed to analyze the sequential histopathological changes and to compare them to those observed in human brains. Paraffin-embedded sections were stained with hematoxylin and eosin and with Alcian blue. Each sample was subjected to immunohistochemical analysis using an alkaline phosphatase-anti-alkaline phosphatase method, in most cases after microwave pretreatment. We used (i) a polyclonal antibody raised against the glial fibrillary acid protein (GFAP) (anti-GFAP; Dako) cross-reacting with several species, to identify astrocytes; (ii) a monoclonal anti-human major histocompatibility complex (MHC) class II (HLA-DR antibody, CR3/43 clone; Dako) and a polyclonal rabbit anti-mouse MHC class II antibody (kindly provided by Dr. Hugh Perry, Southampton, UK), to identify activated microglial cells and macrophages; (iii) a monoclonal anti-human macrophages (M3/84.6.34 clone; Pharmingen); and (iv) monoclonal anti–B lymphocytes (CD20, Ly M0755; Dako) and anti–T lymphocytes (CD3, M0452; Dako) antibodies, to characterize both the human and the murine lymphocyte subpopulations. CD4+ and CD8+ T cells were identified either on frozen sections of mouse brain, by means of a monoclonal rat anti-mouse antibody raised against CD4 (L3T4, RM-4.5; Pharmingen) and CD8a (Ly-2; 53-6.7; Pharmingen), and on paraffin-embedded sections of human brain, by means of monoclonal antibodies raised against CD4 (NCL CD4 1F6; Tebu Novoistra) and CD8 (CD8 144R; Dako). We also used a murine monoclonal IgG1 antibody (E1) reactive with cryptococcal capsular polysaccharide [8].

All sections were counterstained with hematoxylin and eosin. In control samples, the primary antibody was not included and positive material was simultaneously labeled. The intensities of the infiltrates (B- and T-cell populations, CD4+ T cells, CD8+ T cells, and macrophages), the gliosis, and E1 reactivity were semiquantitatively evaluated and were assigned an arbitrary score ranging from absent to intense. The slides were analyzed blindly by 2 observers (F.G. and F.C.), and the results were expressed as agreed by consensus.
Table 1. Ficoll-hypaque gradient separation of free (pellet) or peripheral-blood mononuclear cells (PBMC)–associated yeasts in blood samples (1 mL/mouse) drawn at various times after intravenous inoculation of Cryptococcus neoformans into outbred mice.

<table>
<thead>
<tr>
<th>Day(s) after inoculation</th>
<th>Mean ± SD yeasts* Free PBMC associated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>2</td>
<td>22 ± 7</td>
</tr>
<tr>
<td>3</td>
<td>13 ± 7</td>
</tr>
<tr>
<td>4</td>
<td>14 ± 6</td>
</tr>
<tr>
<td>5</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>10</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

*a In all instances, data are for 3 mice.

Results

Interactions between C. neoformans and Murine Host Cells In Vivo

We analyzed ficoll-hypaque gradient–separated blood samples from infected mice, at various times after inoculation with NIH52D, to study the interactions between yeasts and PBMC. Yeasts were recovered in the pellet and in the PBMC layer at all study times—that is, as early as 1 h (data not shown) after inoculation and on days 1, 2, 3, 4, 5, 10, and 20 after inoculation (table 1). Examination of smears stained with May-Grunwald-Giemsa revealed that the pellets were associated with free yeasts whereas the cell layer contained cryptococci associated with PBMC (data not shown). Both transmission-microscopy and confocal-microscopy examination of the smears of the PBMC layers obtained after inoculation of FITC-labeled cryptococci revealed that yeasts were internalized by monocytes (figure 1).

In the brain from a mouse with severe meningoencephalitis, poorly encapsulated yeasts were observed in mononuclear cells circulating in the lumen of leptomeningeal capillaries (figure 2). Others yeasts were seen to be phagocytosed by cells whose shape and structure strongly evoked those of endothelial cells. Of the numerous yeasts seen, usually the well-capsulated ones were free, whereas those poorly encapsulated and intracellular were mostly internalized within cells with vacuolated cytoplasm, a morphologic characteristic consistent with that of monocyte-derived macrophages. The latter cells sometimes appeared to be in close contact with the external capillary membranes.

Influence That the Isolate Has on the Course of Cerebral Infection in Mice

After iv inoculation with C. neoformans, the fungal burden increased, between days 3 and 8, in the brains of mice infected with either isolate (figure 3). In mice surviving inoculation with NIH52D, the mean number of colony-forming units decreased, and the mean body weight increased (data not shown) thereafter, until sterilization of both the blood and the brain in survivors. All 5 mice tested had no detectable colony-forming units in the blood at day 36, and 1 of the 5 mice tested had no detectable colony-forming units in the brain at day 48 after infection.

In a preliminary study, no significant histopathological changes were observed in the brains recovered 90 min–48 h after inoculation of the clinical isolate, despite the fact that colony-forming units were detectable at such times (colony-forming units per gram of brain, mean log ≥ 2.9). We thus chose to start the analysis on day 3 after inoculation. Follow-up was limited to the first 15 days for mice inoculated with the clinical isolate, since only 1 mouse survived until that time, whereas it continued until day 100 for mice inoculated with NIH52D.

In mice inoculated with either the clinical isolate or NIH52D, the only abnormality seen on day 3 was the presence of a few yeasts in the perivascular spaces, but there were no inflammatory infiltrates. On day 8, the changes consisted of leptomeningitis and microcysts filled with yeasts (figure 4). The inflammatory infiltrate around the cysts was moderate, composed primarily of CD4+ and CD8+ T lymphocytes, with rare B cells (table 2). A few reactive astrocytes and activated microglial cells also were seen. On day 15, in the brain of the only surviving mouse...
mouse infected with the clinical isolate, lesions were even more severe than those seen at day 8, with mild pericystic astrocytosis and microglial activation (figure 5). Inflammation was very severe, both in the leptomeninges and within the brain, and included numerous CD4<sup>+</sup> and CD8<sup>+</sup> T cells and macrophages and rare B lymphocytes. Marked gliosis and microglial activation also were present.

In mice recovering from challenge with NIH52D (i.e., from day 36 onward), brain changes consisted of granulomatous lesions—that is, scars composed primarily of macrophages. Although no recognizable yeasts were seen in them, they contained polysaccharide that was stained by Alcian blue. E1 also labeled polysaccharide around the scars and inside astrocytes, microglial cells, and leptomeningeal macrophages (figure 5). Gliosis was marked, but no lymphocytic reaction persisted.

**Brain Changes in Humans Are Similar to Those Observed in Mice**

As mentioned above, the patient whose isolate was used for the experimental study died of acute meningoencephalitis. Gross examination of the brain revealed thickened leptomeninges at the base of the brain, no cerebral atrophy, and no focal lesion. The fungal load in the brain was higher in the gray matter (colony-forming units per gram of brain, in log 6.7) than in the white matter (colony-forming units per gram of brain, in log 4.5). Numerous yeasts infiltrated the basal leptomeninges—and, to a lesser extent, the brain stem and the cerebellum—along the dilated Virchow-Robin spaces forming cysts (figure 4). A mild inflammatory infiltrate, composed primarily of CD8<sup>+</sup> T cells and macrophages, was seen (table 2). Immunolabeling also revealed slightly reactive astrocytosis and microglial activation around the cystic lesions (figure 4). There was no other opportunistic infection or focal lesion, and there was no evidence of HIV encephalitis.

In the second patient who did not have active cryptococcosis at the time of death, gross examination of the brain detected only a few small cavities (1–2 mm in diameter) in the basal ganglia. These cysts contained occasional yeasts that looked like ghosts (figure 5). The cytoplasm of rare macrophages was immunolabeled with E1. On the basis of either Alcian-blue staining or E1 reactivity, the antigen had not spread into the surrounding parenchyma. Inflammation was minimal, consisting of occasional CD8<sup>+</sup> T cells, without any CD4<sup>+</sup> cells. In contrast, microglial activation was intense, with reactive astrocytosis surrounding the cysts. There was no other opportunistic
Results are the means ± SD for 3–8 mice inoculated with NIH52D that were killed on various days after inoculation (mice inoculated with the clinical isolate did not survive beyond day 15). The asterisk (*) denotes that whole-brain homogenates were plated onto Sabouraud dextrose agar and were found to be negative.

infection or focal lesion, and there was no evidence of HIV encephalitis.

Antigenic Variability

Antigenic variability was evidenced by immunostaining with Mab E1. In vitro, E1 reacts strongly with the entire capsule of serotype A cells, whereas, in most cells within a given cell population, the reactivity pattern in the capsule of serotype D cells is weak and uneven [8]. Both in the brains of mice inoculated with the clinical isolate (serotype A) and in the brain of the patient from whom the isolate was recovered, most yeasts were recognized by E1, and the antibody reacted strongly with the antigen localized around the cysts and in the subpial regions (figure 4). However, the intensity of the reaction was variable from cell to cell, both in the patient’s brain (data not shown) and in murine brains (figure 6A). In mice inoculated with NIH52D (serotype D), very few yeasts were labeled with E1 early after inoculation (figure 6B), whereas later during the course of the infection the epitope recognized by E1 was sometimes strongly expressed (figure 6C and 6D).

Discussion

It is thought that *C. neoformans* penetrates into the brain after fungemia. However, no study had previously been performed to (a) assess the in vivo blood-brain interactions during disseminated cryptococcosis or (b) investigate the evolution of the infection and histopathological changes in the brain over time. Preliminary data obtained by the model used in the present study had suggested that yeasts circulate in contact with blood leukocytes [5]. In the present study, we have shown that

Figure 3. Evolution of fungal burden over time, in the brains of outbred mice killed after inoculation with either the clinical isolate (black bars) or NIH52D (white bars). Results are the means ± SD for 3–8 mice inoculated with NIH52D that were killed on various days after inoculation (mice inoculated with the clinical isolate did not survive beyond day 15). The asterisk (*) denotes that whole-brain homogenates were plated onto Sabouraud dextrose agar and were found to be negative.

Figure 4. Comparison of cerebral changes during acute cryptococcal infection in patient 1 (A and B) and in mice (C and D) infected with the clinical isolate and killed 8 days after inoculation. The cerebellar cortex shows the presence of a cyst containing numerous yeasts strongly expressing the epitope recognized by E1. Note that there is intense antigen labeling in the surrounding parenchyma and that there is almost no inflammation in either the human (A) or the murine (C) brains (magnification, ×200). The basal ganglia shows slight astrocytosis around a cyst filled with cryptococci, in both the human (B) and the murine (D) brains (immunolabeling with glial fibrillary acid protein; magnification, ×200).
Table 2. Histopathological findings in the brains of mice killed at various times after inoculation with $2 \times 10^5$ Cryptococcus neoformans (clinical isolate) and in a patient with AIDS whose isolate was used.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results in experimentally infected outbred mice killed on various days after inoculation</th>
<th>Results in patient with AIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3</td>
<td>Day 8</td>
</tr>
<tr>
<td></td>
<td>Sample 1</td>
<td>Sample 2</td>
</tr>
<tr>
<td>E1 reactivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B lymphocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T lymphocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microglia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glial fibrillary acid protein</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. –, Absent; ±, inconsistent; +, mild; ++, marked; ++++, severe.

Viable cryptococci can be found in the peripheral blood, either free or internalized in mononuclear cells, at various stages during the infection, thereby confirming previously reported results, obtained in vitro, that had suggested that C. neoformans can survive in vitro in human or animal monocytes or macrophages [9–11].

Microscopy of the leptomeninges of a mouse with severe meningoencephalitis showed cryptococci internalized both within mononuclear cells circulating within meningeal capillaries and within host cells touching the outer membrane of the capillaries and looking like the Hueco cells described by Feldmesser et al. [12]. Whether these latter cells were microglial cells that had phagocytosed free cryptococci in the meningeal space or were macrophages derived from C. neoformans–infected monocytes that had crossed the blood-brain barrier in a “Trojan horse” [13] remains to be determined. If the latter is
Figure 6. Variability in antigenic expression in vivo during Cryptococcus neoformans infection in outbred mice. Brain-tissue sections from mice killed either at day 3 after inoculation with the clinical isolate (A) or at day 8 (B), day 56 (C), or day 100 (D) after inoculation with NIH52D were immunostained with the monoclonal anti-polysaccharide antibody E1. Positive, negative, and weakly positive capsules can be seen early in the course of the infection in mice inoculated with either a serotype A (A) or a serotype D (B) strain; later, the epitope recognized by E1 (a minor epitope in vitro on serotype D) is strongly expressed both in yeasts and in brain tissues of mice infected with NIH52D (C and D).

the case, then the impaired anticryptococcal activity of monocytes from patients with AIDS [14–17] might partially explain their significantly higher frequency of meningeal dissemination, compared with that in seronegative individuals [2]. We also have observed, for the first time in vivo, yeasts phagocyted by endothelial cells of leptomeningeal capillaries, a finding that retrospectively validates the experimental models. Indeed, the role of endothelial cells has been studied, in vitro, with elegant demonstrations of cryptococci adhering to, phagocyted by, and damaging endothelial cells [18–20]. Even though the images were obtained at a late stage of the infection, our observations support the hypothesis that C. neoformans may co-opt monocytes and endothelial cells in order to cross the blood-brain barrier. The precise timing and molecular events that characterize the interactions between C. neoformans and these host cells remain to be determined.

To better investigate the relevance that the animal models of disseminated infection have for the study of cryptococcal meningitis, we studied brain-lesion pathogenesis over time, after iv inoculation of live cryptococci, and, on the basis of the outcome both in humans with AIDS and in mice, compared the brain lesions. Brain lesions consisting of accumulations of yeasts in leptomeningeal spaces and in dilated Virchow-Robin spaces with a little macrophagic reaction have been reported in patients with AIDS [21, 22] and in mice [23, 24]. In the present study, we have provided additional information on the pathogenesis of the lesions, showing that the early lesions in outbred mice with an intact immune system were comparable to those in a patient with AIDS who died from disseminated cryptococcosis. The only difference was the expected lack of detectable CD4+ T lymphocytes in this patient. Both in humans and in mice, inflammation in brain tissue was limited, a finding in agreement with both (a) our previous results showing the late production of low levels of tumor necrosis factor (TNF)–α and interleukin-6 in the brain of infected mice [6] and (b) the low concentrations of these cytokines in the CSF of patients with AIDS who have cryptococcal meningitis [25].

To further assess the relevance of our experimental model, we analyzed cerebral lesions in mice recovering from cryptococcosis; we found them comparable to those observed in the brain of a patient with AIDS who was cured of cryptococcal meningitis before dying. Scars composed of macrophages containing rare yeasts and/or capsular polysaccharide, surrounded by microglial and astrocytic reactions, were seen in mice and in this patient. This pattern was similar to the granulomatous reaction found in HIV-negative patients [22] and in rats resis-
tant challenge with *C. neoformans* [26]. We did not observe large areas of soluble polysaccharide in this patient’s brain, but the cytoplasms of various cells contained polysaccharide. Earlier studies have indeed documented both (a) the importance of brain and lung macrophages as reservoirs during infection in humans or animals [12, 26–28] and (b) the prolonged storage of the antigen in the liver and the spleen after injection of polysaccharide into nonimmune animals [29–31].

Finally, we have observed major strain-related differences in terms of survival, evolution of local fungal load, and intensity of the inflammatory response. In mice inoculated with NIH52D, intense inflammation coincided with a decline of the yeast load, whereas, in mice infected with the clinical isolate, the fungal burden continued to rise in the absence of macrophage reaction. This dichotomy confirms previous findings showing a relationship between granuloma formation and clearing of the organisms from rat lungs [26]. With other models of infection, it previously had been shown that different strains or even variants of the same strain could induce different local inflammatory responses [32–34], which could be related to differences in the major virulence factor—namely, the capsule presence (reviewed in [35]) or structure [36]. Although organ-related differences in capsule thickness have been found [37], in the present study we have shown that, for a given isolate, the expression of a specific capsular epitope can vary over time and from 1 yeast cell to another, not only in the brains of infected mice but also in the brains of humans, which could also influence the outcome [38]. In addition, differences in polysaccharide accumulation itself could influence the outcome, by modifying the occurrence of cerebral edema [27]. Lee et al. have suggested that widespread antigen dissemination in tissue is associated with rapidly proliferating organisms, probably because of ineffective inflammatory responses in infected humans [28]. Using knockout mice, Huffnagle et al. underlined the role that C-C chemokine receptor 5 plays in excessive capsule deposition and swelling in the brain [39] that Graybill et al. associate with early death in humans [40]. Whether additional differences in polysaccharide structure (serotypes) result in differences in the inflammatory response—and, consequently, in differences in the course of the disease—will be assessed in the ongoing prospective multicenter study by the French Cryptococcosis Study Group (Crypto A/D study) [41].

In conclusion, in the present study we demonstrated for the first time in vivo that live cryptococci can circulate in blood monocytes and that yeasts can be found inside endothelial cells during murine infection. These data strengthen the hypothesis that monocytes and endothelial cells play key roles and possibly cooperate in the pathogenesis of cryptococcal meningitis. We also have provided evidence that this experimental model of cerebral infection after fungemia indeed faithfully reproduces the major features of human disease and thus can be used to study various aspects of the pathophysiology of cryptococcal meningitis.

**Acknowledgments**

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