Coccidioidal meningitis is a devastating complication of disseminated coccidioidomycosis. An animal model of this infection could enhance understanding of the pathogenesis of the disease and lead to improvements in therapy. A rabbit model of central nervous system infection simulating human disease was established using a blind cisternal tap technique to inoculate $4 \times 10^3$ to $1 \times 10^6$ arthroconidia of *Coccidioides immitis* into the cisterna magna. Systemic, neurologic, and histopathologic findings of meningitis were observed in all rabbits, but an inoculum of $2 \times 10^6$ arthroconidia produced a chronic illness in which meningeal endarteritis obliterans was consistently observed. Serial sampling of cerebrospinal fluid demonstrated an inflammatory response. Growth of *C. immitis* was demonstrated by quantitative fungal culture from brains and proximal spinal cords.

Coccidioidomycosis can be a symptomatic illness resulting from the inhalation of the arthroconidia of the dimorphic fungus, *Coccidioides immitis*, which resides in the soil of the southwestern desert region of the United States and in areas of Mexico and Central and South America.

Meningitis is the most severe complication of disseminated coccidioidal infection and generally is fatal within 2 years without treatment [1, 2]. When treatment is undertaken with intrathecal amphotericin B (the agent of choice before 1990), only 50%–60% of patients respond [3]. Oral azoles, the current preferred therapeutic agents for coccidioidal meningitis, control infection in upward of 80% of patients [4, 5]. However, cure is not obtainable with azoles due to their fungistatic nature at current recommended doses [6]. Furthermore, complications, including hydrocephalus, encephalitis, and vasculitis-associated stroke events, occur in 10%–40% of appropriately treated patients with a mortality rate of 70% [7]. Galgiani [8] estimated that 100–200 cases of coccidioidal meningitis occur annually in the United States in nonepidemic years [8].

An animal model simulating human coccidioidal meningoencephalitis and cerebrospinal vasculitis is essential for the evaluation of potentially useful antifungal and immunotherapeutic agents as they become available. Such a model enables the study of pathologic processes, including vasculitis, that cause the clinical complications commonly seen in humans and could improve our understanding of the immune response in this disease. A murine model of central nervous system coccidioidal infection has been reported in which direct intracerebral injections of *C. immitis* endospores were used to establish infection. However, it was not possible to sample cerebrospinal fluid (CSF) and no histopathology was reported [9]. Sorensen et al. [10] described a rabbit model of subacute coccidioidal meningitis in which arthroconidia were introduced into the cervical subarachnoid space using a catheter system. Sampling was accomplished using a blind cisternal tap procedure, which allowed them to document a progressive lymphocytosis as infection progressed; however, no histopathology studies were done.

Our previous efforts to develop a rabbit model of coccidioidal meningitis by intracarotid injection of endospores were unsuccessful (unpublished data). A surgically placed subcutaneous supracranial Ommaya reservoir system with an exit catheter positioned within the cervical subarachnoid space to allow for arthroconidia inoculation (inocula $2 \times 10^3$–$1 \times 10^6$) was partially successful, but significant surgical and postsurgical complications combined with prolonged recovery led us to abandon this technique [11].

More recently, we used a blind cisternal puncture technique to allow both inoculation of arthroconidia into the subarachnoid space and subsequent sampling of CSF from the rabbit. We report here the first animal model of coccidioidal meningoen-
Materials and Methods

Preparation of inocula. Arthroconidia of _C. immitis_ for infection were obtained by culturing strain Silveira (ATCC 28868) on slants of 2% glucose and 1% yeast extract (Difco, Detroit), with 2% agar for 4–6 weeks at 35°C. The arthroconidia were dislodged into sterile water, and the suspension was exposed to 3–4 bursts (2–3 s each) in a vortex mixer with glass beads to disarticulate the chains of arthroconidia.

Animal inoculation. New Zealand White male rabbits (Krauluck Farms, Turlock, CA) weighing 3–4 kg were sedated using ketamine (35 mg/kg), xylazine (3.5 mg/kg), and acepromazine (0.5 mg/kg) administered intramuscularly. Rabbits were shaved, sedated, and positioned in the left lateral decubitus position. With sterile technique within a class II biologic safety cabinet, a 25-gauge butterfly needle (1.905 cm with 30.48 cm tubing) was inserted into the cisterna magna. Free flow of CSF was identified within the proximal attached catheter. We removed 0.5–0.6 mL of CSF by gentle aspiration using a tuberculin syringe prior to inoculation. Arthroconidia inocula were administered in a 0.2–0.25 mL vol. A flush with 0.4–0.6 mL of sterile saline followed.

Immunosuppression. One day before infection, the day of infection, and for 3 consecutive days following infection, all rabbits received an intramuscular 2 mg/kg injection of either cortisone acetate (Merrick Sharpe & Dohme, West Point, PA) or hydrocortisone (Steris Laboratories, Florham Park, NJ).

Postinoculation monitoring. Rabbits were monitored twice daily for evidence of systemic, neurologic, or discomfort sequelae. Every 10–14 days, most rabbits were anesthetized with isoflurane anesthesia and blind cisternal taps were done. When CSF was obtained, it was used to determine protein and glucose concentrations, for fungal culture and cell counts, and in some instances for serologic testing.

When the rabbits met preestablished criteria (undue discomfort, paralysis, convulsions, stupor, or prolonged anorexia or dehydration) or reached the experimental completion date (usually at 4–7 weeks), they were sedated and blood was obtained for serologic testing from a marginal ear vein. Rabbits then were euthanized via intravenous (ear vein) injection of a concentrated pentobarbital solution (Euthasol; Delmaria Laboratories, Bristol, TN). Brains and proximal spinal cords were harvested and transected. One-half of each tissue specimen was placed into 10% buffered formalin for histopathologic study. The other half was placed on Mycosel agar. Plates were sealed and incubated at 35°C. After 48–72 h, the number of colony-forming units (cfu) was determined.

Results

Experiment 1. In this experiment, 3 rabbits were inoculated with 10^6 arthroconidia and 4 rabbits with 4 \times 10^4 arthroconidia. Those inoculated with 10^6 arthroconidia exhibited marked systemic (intermittent fever, anorexia, weight loss, agitation, lethargy) and neurologic (ataxia, head tilting) signs beginning 2–3 days after infection. Two rabbits developed seizures and a third exhibited paralysis of the right hind limb. All died or were euthanized within 8 days of infection. It was not possible to obtain CSF from these rabbits after infection, possibly due to intense inflammation within the meninges with resultant obscuration of the subarachnoid space. Histopathologic study demonstrated severe acute meningitis with mild-to-moderate encephalitis. No vasculitis was observed.

In the 4 rabbits given an inoculum of 4 \times 10^3 arthroconidia, systemic findings similar to those described above were observed 10–14 days after infection. All rabbits required oral nutritional supplementation and in 2 (rabbits 2 and 3), normalization of clinical status was observed. Neither of these 2 rabbits demonstrated neurologic abnormalities. The other 2 rabbits (rabbits 1 and 257) continued to manifest signs of illness for the full duration of the study. Both were ataxic beginning at the end of day 14 (rabbit 1) and on day 18 (rabbit 257). Rabbit 1 also appeared to have meningismus beginning on day 23. A flush with 0.4–0.6 mL of sterile saline followed.

Experiment 2. Our purpose in this experiment was to identify an inoculum of _C. immitis_ arthroconidia that would lead to chronic disease and a greater incidence of endarteritis than observed in experiment 1. Five rabbits were inoculated with 1.5 \times 10^5 arthroconidia and 4 were inoculated with 2 \times 10^4 arthroconidia. In the group given 1.5 \times 10^5 arthroconidia, severe systemic (anorexia, weight loss, agitation, lethargy, and intermittent fever) and neurologic (ataxia, head tilt) signs were observed in all rabbits beginning within the first week of infection. Two rabbits had seizures and 1 of these developed a right medial rectus palsy. All rabbits either succumbed to infection or were euthanized within 6–8 days following infection. CSF was obtained from only 1 rabbit due to the intense meningial inflammation. Histologically, all rabbits had an acute meningitis with a less prominent granulomatus component and varying degrees of extension of the infiltrate intraparenchymally (cerebrum). One rabbit had meningeal arteritis with an associated proximal cervical spinal cord infarct.

In the 4 rabbits given 2 \times 10^3 arthroconidia, a more attenuated illness pattern was observed. All rabbits developed lethargy, anorexia, weight loss, and intermittent fever by the end of the first week of infection. Ataxia and/or head tilting occurred in all rabbits within 2–3 weeks of infection. Paralysis...
Table 1. Cerebrospinal fluid (CSF), tissue culture, and serology data in two experiments in rabbits.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Arthroconidia inocula</th>
<th>Rabbit no.</th>
<th>Day of euthanasia</th>
<th>Tissue fungal Whiteculture (log_{10} cfu/g)</th>
<th>CSF data by week</th>
<th>CSF data by week</th>
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<tbody>
<tr>
<td>1</td>
<td>4 × 10^3</td>
<td>1</td>
<td>28</td>
<td>2.5 3.5 + IgG undiluted + IgG</td>
<td>2</td>
<td>4</td>
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<td></td>
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<td>2</td>
<td>28</td>
<td>2.8 3.5 ± IgG undiluted ± IgG</td>
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<td>0</td>
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<tr>
<td></td>
<td></td>
<td>3</td>
<td>28</td>
<td>2.4 2.7 + IgG 1:4 + IgG</td>
<td>0</td>
<td>2</td>
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<tr>
<td></td>
<td></td>
<td>257</td>
<td>28</td>
<td>3.6 3.6 + IgG 1:2 + IgG + IgM</td>
<td>2</td>
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<tr>
<td>10^4</td>
<td></td>
<td>651</td>
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<td>2</td>
<td>2 × 10^6</td>
<td>1185</td>
<td>22</td>
<td>3.1 4.6 + IgG 1:4 + IgG</td>
<td>ND</td>
<td>ND</td>
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<td></td>
<td></td>
<td>1186</td>
<td>47</td>
<td>2.1 3.9 + IgG 1:3 + IgG</td>
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<td>1187</td>
<td>11</td>
<td>4.0 4.7 + IgG undil + IgG</td>
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<td></td>
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<td>1188</td>
<td>47</td>
<td>2.5 3.8 + IgG 1:3 + IgG</td>
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<td>ND</td>
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<tr>
<td>1.5 × 10^5</td>
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<td>1189</td>
<td>8</td>
<td>4.2 5.3</td>
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<td>1193</td>
<td>7</td>
<td>3.9 4.8</td>
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</tr>
</tbody>
</table>

NOTE. +, positive; –, negative; ±, equivocal; ND, not done; undil, undiluted.
* Not done at week 0.
² Postinfection CSF serology was negative at day 10 in rabbits 1185 and 1187, at day 20 in rabbit 1188, and at day 31 in rabbit 1186.

was seen in 3 rabbits; this involved the left hind leg in 2 (rabbits 1185, 1186), and 1 rabbit (1188) developed paralysis of the left hind leg (day 12) and right front leg (day 43). Meningismus was observed in 3 rabbits: 1185 (day 18), 1186 (day 24), and 1188 (day 27). Two rabbits developed urinary incontinence. Two rabbits were euthanized early because of progressive illness (rabbits 1185, day 21; 1187, day 10). The remaining 2 rabbits were euthanized at the end of the 7-week experiment. We were able to repetitively sample CSF from 2 of the 4 rabbits, although we frequently observed blood-tinged CSF, possibly due to capillary vein fragility associated with intense inflammatory meningitis.

Histologically, all 4 rabbits had severe granulomatous meningitis with cerebritis and endarteritis within the meninges. Many multinucleated giant cells were observed. A hypothalamic infarct was observed in 1 rabbit. Cord edema was prominent in 2 rabbits (figure 1). Results of the quantitative brain and spinal cord cultures, serologic testing from serum and CSF, and CSF analysis are shown in table 1.

Discussion

We found the blind cisternal tap technique to be far less problematic than surgical implantation of a catheter reservoir system for both inoculation of C. immitis arthroconidia into the subarachnoid space of the rabbit and for serial sampling of CSF. With this technique, we produced infection resulting in both systemic and neurologic abnormalities simulating those observed in humans with complicated coccidioidal central nervous system disease. In addition, we were able to cause a consistent cerebrospinal meningitis, often with cerebritis. We found that an inoculum of 2 × 10^4 arthroconidia produced a subacute to chronic illness of 2–7 weeks duration with all rabbits demonstrating a chronic granulomatous cerebrospinal meningitis, often with cerebritis and consistent meningeal endarteritis. Also, infarction in 1 rabbit (hypothalamus) simulated the findings seen in complicated human disease [7].

We demonstrated an appropriate inflammatory reaction within the subarachnoid space, and serial analysis confirmed progressive pleocytosis, hypoglycorrhachia, and protein elevation reproducing findings associated with human coccidioidal infection [12]. Significant numbers of C. immitis were recovered on quantitative cultures from both brain and spinal cord. Slightly greater numbers of organisms were recovered from the proximal spinal cord than from the brain. Higher tissue cfu correlated positively with higher inoculum and disease severity. Few CSF cultures were positive for C. immitis. This finding is similar to experience in humans; C. immitis can be cultured from CSF in only 30% of persons with documented coccidioidal meningitis [12].

Low-titer serologic responses were documented for rabbits that received 4 × 10^3 arthroconidia. In general, higher titers were detected in rabbits inoculated with 2 × 10^6 arthroconidia. Rabbits inoculated with 10^3 and 1.5 × 10^5 arthroconidia appar-
Figure 1. A, Severe granulomatous meningitis in rabbit given \(2 \times 10^4\) arthroconidia of *Coccidioides immitis* 7 weeks before euthanasia. Subarachnoid space surrounding spinal cord is expanded and filled by exudate. Spinal cord is distorted and subpial areas of cord are vacuolated, indicating ischemia. Exudate extends focally into parenchyma (arrowheads). Hematoxylin-eosin stain, \(\times 14\). B, Numerous coccidioidal organisms surrounded by multinucleated giant cells in meningeal exudate. Periodic acid–Shiff (PAS) stain, \(\times 175\). C, Fibrin deposition (arrow) in wall of meningeal artery. Artery is surrounded by mononuclear cells. PAS stain, \(\times 278\). D, Same vessel as in C. Attenuation and disruption of internal elastic lamina, characteristic of endarteritis obliterans (arrowheads). Elastic Van Gieson stain, \(\times 278\). All views are of rabbit 1188.

### Acknowledgments

We thank Katharine Fletcher for expert secretarial assistance in the preparation of this manuscript. We appreciate the technical assistance of Martin G. Täuber, Jay Tureen, Charly Liu, and Lucian Chow, for instruction in the technique of performing the blind cisternal tap in the rabbit. We acknowledge David Hewitt and staff, Laboratory Division, Kaweah Delta Health Care District, for performing CSF protein and glucose determinations.

### References

Mannose-Binding Lectin Plasma Levels and Gene Polymorphisms in \textit{Plasmodium falciparum} Malaria

Adrian J. F. Luty, Jürgen F. J. Kun, and Peter G. Kremsner

The contribution of mannose-binding lectin (MBL) to protection from malaria was assessed by comparing plasma concentrations of MBL and the frequency of MBL gene polymorphisms in groups of Gabonese children participating in a prospective study of severe and mild malaria due to infection with \textit{Plasmodium falciparum}. At admission, a higher proportion of patients with severe malaria had a low level of MBL compared with subjects with mild malaria (0.35 vs. 0.19, \textit{P} = .02). Two mutations in codons 54 and 57 of the MBL gene were detected. They were present at higher frequency in those with severe malaria (0.45 vs. 0.31, \textit{P} = .04). These results suggest that deficient innate immune responses, in the form of low MBL levels, may be a risk factor for severe malaria in some young children who lack well-developed, clinically protective acquired immune responses.

More than 1 million children die every year from severe malaria in areas of sub-Saharan Africa where \textit{Plasmodium falciparum} is endemic [1]. In such areas, effective clinical immunity develops only slowly after birth, and the burden of risk of developing severe disease is thus carried by young children [2]. These observations have prompted the search for mechanisms of protection, and innate resistance or susceptibility to severe malaria has thus recently been linked to genetic factors influencing immune responsiveness [3, 4].

Mannose-binding lectin (MBL) is a member of the collectin family of proteins, which are constituents of the innate immune system [5]. MBL mediates its effect through carbohydrate recognition domains with multiple specificities for sugar residues on microbial surfaces, resulting in opsonization via collectin receptors and/or complement activation [6]. MBL is thought to be most effective at an early age, before effective acquired immune responses have developed, acting as an ante-antibody, and MBL deficiency has thus been linked to chronic susceptibility to infection in children [7]. Low plasma concentrations of MBL result primarily from mutations in the coding region of the MBL gene, causing structural alterations in the collagenous region of the molecule, rendering the protein nonfunctional due to inability to form stable oligomers [6].

We determined, by comparing the plasma levels of this protein and the MBL gene polymorphisms in individuals with either severe or mild malaria, whether MBL may influence the response to infection with \textit{P. falciparum} in young African children. In the study area, the majority of malaria cases requiring hospital care present with severe anemia, hyperparasitemia, or both. Both of these parameters can be considered to be markers of uninhibited parasite multiplication, which seems to be a prerequisite for a fatal outcome of malaria in this setting.

**Methods**

\textit{Study subjects.} The patients were from a matched-pair case-control study of severe malaria undertaken in Albert Schweitzer Hospital, Lambarene, Gabon.