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Comparative ocular pathogenicity of Cryptococcus neoformans, Candida glabrata, and Aspergillus fumigatus in the rabbit.


In a previous study, 88% of rabbits with disseminated infection caused by Candida albicans developed ophthalmoscopically visible, hematogenous endophthalmitis (chorioretinitis) over a 2 week period. To determine the incidence of this ocular complication in disseminated infection caused by Cryptococcus neoformans, Candida glabrata, and Aspergillus fumigatus compared with that caused by C. albicans, the first three species of fungi were injected intravenously (between 107 and 109 organisms per animal) into 36 New Zealand white rabbits. No chorioretinal lesions were seen by indirect ophthalmoscopy over a 2 week period. C. glabrata and A. fumigatus were not cultured from chorioretinas despite positive cultures from brains and kidneys at 1 and 2 weeks. In contrast, C. neoformans was cultured from 12 of 18 chorioretinas (mean Log10 3.45 colony forming units/gm of tissue) as well as from the brains and kidneys. The less intense inflammatory cell response to C. neoformans compared with that to C. albicans seen on histopathologic examination most likely explains the nondetectability of the cryptococcal chorioretinitis by indirect ophthalmoscopy. These data suggest that C. glabrata, A. fumigatus, and possibly C. neoformans have less ocular pathogenicity than C. albicans in rabbits and correlate with the small number of documented human

Hematogenous Candida endophthalmitis (chorioretinitis) is a well-described manifestation of disseminated infection caused by Candida albicans. Hematogenous endophthalmitis also occurs in disseminated infection caused by other fungi, including Cryptococcus neoformans,\textsuperscript{3,4} Candida glabrata (formerly Torulopsis glabrata),\textsuperscript{4-8} and Aspergillus species.\textsuperscript{5,6} However, the frequency of this ocular complication is unknown and the clinical and microscopic features of the chorioretinal lesions caused by these three fungi have been characterized only partially. The purpose of this study was to (1) determine the frequency of chorioretinal involvement in disseminated infection in the rabbit caused by C. neoformans, C. glabrata, and Aspergillus fumigatus compared to the results of previous studies with C. albicans, and (2) determine whether these lesions, if clinically visible, could be differentiated by ophthalmoscopic examination from those of C. albicans and from each other. The rabbit was used for these studies because of its usefulness as a model to study hematogenous endophthalmitis caused by Candida species.\textsuperscript{2,3}

Materials and methods

Experimental animals. Thirty-six New Zealand white female rabbits, ages 8 to 10 weeks, were used.

Organisms. Three strains from each of the following species of fungi were studied: C. neoformans (3062, 4057, and 4877) and C. glabrata (2641, 3628, and 4678) were clinical isolates from Harbor-UCLA Medical Center. The three strains of A. fumigatus (ATCC 1028, ATCC 13073, and ATCC 14110) were obtained from the American Type Culture Collection (Rockville, Md.). Strains of C. neoformans and C. glabrata were grown in brain-heart infusion (BHI) broth (Difco, Detroit, Mich.) at 35°C for 4 to 6 days prior to injection. Strains of A. fumigatus were grown on BHI agar at 35°C for 7 days, and the spores were collected by washing the surface of the culture plate with sterile 0.9% sodium chloride.

Infection. Based on preliminary studies, inocula of $1 \times 10^4$, $1 \times 10^5$, and $1 \times 10^7$ organisms/rabbit were used for C. neoformans, C. glabrata, and A. fumigatus, respectively. In these pilot studies, lower inocula of the fungi produced no eye lesions at 7 days and higher inocula of A. fumigatus ($5 \times 10^6$ per rabbit) caused the death of three of four rabbits by 5 days. In the present study, each strain of fungus was counted with the hemacytometer and quantitative cultures performed prior to injection. Differences between the numbers of fungi obtained by hemacytometer and quantitative culture counts are shown in Table I. Each strain was suspended in 1 to 5 ml of sterile 0.9% sodium chloride and injected intravenously via the marginal ear vein into a group of four rabbits.

Clinical eye examinations. All surviving rabbits were examined twice weekly with an indirect ophthalmoscope (Frigi-Xonix, Shelton, Conn.) after the pupils had been dilated with 2 drops each of 10% phenylephrine hydrochloride (Neosynephrine; Winthrop Laboratories, New York, N. Y.) and 1% tropicamide (Mydriacil; Alcon Laboratories, Fort Worth, Tex.).

Organ cultures. Two rabbits from each group injected with C. neoformans (except one group in which three of four rabbits died within 4 days) and C. glabrata (except one group in which one rabbit died) were sacrificed at 1 and 2 weeks after injection. The five surviving rabbits out of 12 injected with A. fumigatus were sacrificed at 1 week. Quantitative cultures for fungi were performed on the chorioretinas, sections of brain, sections of both kidneys, and 1 ml of blood (via cardiac puncture) as described previously.\textsuperscript{7}

Eye sections for histopathology. In a separate experiment, two rabbits were injected with $1 \times 10^9$ organisms of C. neoformans strain 4877. One was sacrificed at 1 week, and the other, 2 weeks after injection. The eyes were removed and placed into 10% formalin prior to staining multiple chorioretinal sections with hematoxylin-eosin, periodic acid-Schiff (PAS), and Mayer's mucicarmine stains.

Results

C. neoformans. No chorioretinal lesions were seen by ophthalmoscopic examination during the 2-week period of observation despite the positive cultures in 12 of 18 chorioretinas (Table I). The average number of organisms in the chorioretinas (only those with positive cultures were tabulated) was Log$_{10}$ 3.45 (range 0.47 to 5.08) colony forming units (cfu)/gm of chorioretina. Strain 4057 was nearly nonpathogenic, since all organ cultures except for two chorioretinal cultures were negative. Strains 3062 and 4877 infected other organs; the average number of organisms in brain and kidney sections was Log$_{10}$ 3.66 (range 3.03 to 4.83) and 3.26 (range 2.33 to 4.50) cfu/gm, respectively.

Ocular histopathology. No lesions or organisms were seen in the eyes of the rabbit sacrificed at 1 week. In one eye obtained at 2 weeks, small lo-
calized collections of both polymorphonuclear and mononuclear leukocytes were present in several areas within the optic nerve. One modest collection was present within the optic nerve radiation, just superficial to the chorioretina (Fig. 1). Budding yeasts were seen within the cellular aggregation (Fig. 2).

*C. glabrata.* In rabbits injected with *C. glabrata,* no chorioretinal lesions were seen by ophthalmoscopic examination and no fungi were cultured from the choroid-retina despite positive cultures from brain and kidney sections (Table I). The average number of fungi present in brain and kidney sections in which the cultures were positive was Log$_{10}$ 1.02 (range 0.92 to 1.12) and 2.71 (range 0.80 to 4.61) cfu/gm of tissue, respectively.

To determine whether *C. glabrata* was present within the chorio-retina (either in the capillaries or in the tissues) at any time after injection, another experiment was done in which one rabbit was sacrificed 10 min after injection via the marginal ear vein of $1 \times 10^8$ spores of *A. fumigatus* strain ATCC 1028. Log$_{10}$ 2.46 and 2.95 cfu/gm of chorioretina were recovered from the right and left eyes, respectively.

*A. fumigatus.* Seven of 12 rabbits died within 6 days of injection. No chorioretinal lesions were seen by ophthalmoscopic examination, and cultures of chorioretinas of the five surviving rabbits sacrificed at 1 week were negative despite positive brain and kidney cultures (Table I). None of the rabbits that died within 6 days of injection had ophthalmoscopically visible eye lesions prior to death. The average numbers of fungi present in the brain and kidney sections that were culture positive were Log$_{10}$ 1.00 (range 0.45 to 1.55) and 2.32 (range 0.82 to 3.46) cfu/gm of tissue, respectively.

To determine if fungi were ever present in the chorioretina, a separate experiment was done in which one rabbit was sacrificed 10 min after injection via the marginal ear vein of $1 \times 10^7$ spores of *A. fumigatus* strain ATCC 1028. Log$_{10}$ 2.46 and 2.95 cfu/gm of chorioretina were present in the right and left eyes, respectively.

Discussion. No lesions of hematogenous endophthalmitis were seen by indirect ophthalmoscopy in rabbits injected with high numbers of *C. neoformans,* *C. glabrata,* and *A. fumigatus* during 2 weeks of observation. These findings differ from those of previously reported experiments with *C. albicans* in which 88% of rabbits had chorioretinal lesions that could be seen by ophthalmoscopy by 2 weeks after intravenous injection. These results occurred despite the use of higher inocula (between $10^4$ and $10^6$ cfu/rabbit) of *C. neoformans,* *C. glabrata,* and *A. fumigatus* than the inoculum used in previous studies with *C. albicans.*
Fig. 2. Budding yeasts (arrow) within the lesion depicted in Fig. 1. (Mayer's mucicarmine; bar = 10 μm.)

Table I. Organ culture results at 1 and 2 weeks (combined) after intravenous injection of fungi

<table>
<thead>
<tr>
<th>Species of fungi</th>
<th>Strain</th>
<th>Inoculum (cfu/rabbit)</th>
<th>Positive cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chorioretina</td>
</tr>
<tr>
<td>C. neoformans</td>
<td>3062A</td>
<td>6.7 x 10⁶</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td>4057</td>
<td>1.9 x 10⁷</td>
<td>2/8</td>
</tr>
<tr>
<td></td>
<td>4877</td>
<td>4.0 x 10⁶</td>
<td>8/8</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>2841</td>
<td>2.5 x 10⁶</td>
<td>0/8</td>
</tr>
<tr>
<td></td>
<td>3028</td>
<td>2.2 x 10⁷</td>
<td>0/8</td>
</tr>
<tr>
<td></td>
<td>4678</td>
<td>1.1 x 10⁹</td>
<td>0/6</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>ATCC 1028*</td>
<td>5 x 10⁶</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>ATCC 13073⁵</td>
<td>8.5 x 10⁷</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>ATCC 14110⁶</td>
<td>3 x 10⁶</td>
<td>0/4</td>
</tr>
</tbody>
</table>

*All rabbits sacrificed at 1 week.
*Positive only at 1 week.
*All rabbits died prior to time of sacrifice.

mechanisms accounting for the differences in ocular pathogenicity among these various species of fungi are as yet unknown. The germ tube-forming capability of C. albicans has been suggested as one possible factor for ocular pathogenicity.³

The lack of infection and development of clinically visible chorioretinal lesions in rabbits injected with C. glabrata and A. fumigatus may be attributable to either the inability of these fungi to adhere to the capillary endothelial cells of the chorioretina, or alternatively, if adherence did occur, the rapid clearance of the fungi by the rabbit's defense mechanism. The positive culture of the chorioretinas at 10 min after intravenous injection confirmed the early presence of C. glabrata and A. fumigatus in the eye. In contrast, C. neoformans infected the chorioretinas. The average number of C. neoformans per gram of chorioretina cultured...
was almost the same as that for C. albicans from eyes with ophthalmoscopically visible chorioretinal lesions. In contrast to rabbit chorioretina infected with C. albicans, which provokes an intense inflammatory response, only a modest degree of inflammation was present surrounding the cryptococcal organisms. This is a characteristic feature of cryptococcal infection in general and may explain the lack of ophthalmoscopically visible chorioretinal lesions.

The results of these experiments suggest that during disseminated infection in rabbits, C. albicans is more likely to produce ophthalmoscopically visible chorioretinal lesions than other fungi. If these observations can be extrapolated to humans and correlated with published clinical observations, they suggest that patients who are at risk of developing disseminated fungal infection and have ophthalmoscopically visible chorioretinal lesions during an illness of less than 2 weeks' duration are more likely to have disseminated candidiasis than disseminated infection caused by C. neoformans, C. glabrata, or A. fumigatus. Conversely, the eye may be an insensitive indicator of disseminated fungal infection caused by these latter three fungi.

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Key words: hematogenous endophthalmitis, Cryptococcus neoformans, Aspergillus fumigatus, Candida glabrata, ocular pathogenicity, chorioretina

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