Pathogenesis of Pulmonary Cryptococcus neoformans Infection in the Rat

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The pathogenesis of Cryptococcus neoformans pulmonary infection in the rat was studied after intratracheal inoculation. Lungs were examined at various times following infection for histopathology in conjunction with macrophage markers, proliferating cell nuclear antigen (PCNA), and capsular glucuronoxylomannan (GXM) antigen. Serum GXM, immunoglobulin M (IgM) and IgG titers and organ fungal burden were compared with pathological findings. C. neoformans organisms were in the lung parenchyma 2 h postinoculation, and GXM antigen was present in surrounding tissues shortly thereafter. Extrapulmonary dissemination occurred early in infection. Two phases of host cellular inflammatory response were discernible: early local macrophage recruitment at 2 to 4 days followed by granulomatous inflammation, which reached maximum intensity 14 days after infection. The granulomatous phase was preceded by lymphocyte influx with macrophage proliferation and maturation into epithelioid histocytes; this was paralleled by a shift of yeasts from extracellular to intracellular spaces. Tissue IgG deposits, serum IgG to GXM, and localization of tissue GXM immunoreactivity to epithelioid cells were noted at 2 to 4 weeks. A 10-fold decrease in lung fungal burden occurred 25 days postinfection and was associated with resolving granulomas, fewer proliferating cells, and decreased tissue GXM. The present study demonstrates that (i) C. neoformans penetrates the lung parenchyma shortly after infection; (ii) immunocompetent rats control pulmonary cryptococcosis efficiently, with minimal extrapulmonary dissemination and low levels of serum GXM; and (iii) macrophage activation is likely to play a crucial role in limiting C. neoformans infection in the rat lung.

The lungs are the presumed entry site of Cryptococcus neoformans infection in humans. Local pulmonary defense mechanisms are believed to play a pivotal role in the control of infection and the prevention of extrapulmonary dissemination. In immunocompetent hosts, pulmonary infection has minimal symptomatology (10). In contrast, extrapulmonary dissemination with meningoencephalitis is associated with immunosuppressed states including AIDS, lymphoproliferative disorders, organ transplants, and corticosteroid therapy (10). In the immunocompetent host, histopathologic findings of pulmonary cryptococcosis consist primarily of well-defined granulomas and suggest the importance of cellular immunity in controlling the infection (24). Animal models provide further support for this hypothesis. In mice, CD4 and CD8 T cells have been shown to be important for clearance of C. neoformans pulmonary infection (17–19, 23).

Among laboratory animals, cryptococcosis has been most extensively studied in mice, and significant work has been done with steroid-treated rabbits (29). Rats have been used sporadically to study C. neoformans infection (12, 16) and test antifungal drugs (28). In 1935, Benham demonstrated systemic infection after intraperitoneal inoculation of C. neoformans in rats (1). In 1947, Kliger attempted to elicit antibodies to C. neoformans capsular polysaccharide in rats but found no agglutinins (20). Rat pulmonary cryptococcosis has been studied by Gadebusch and Gikas (14) and Graybill et al. (16), who noted that immunocompetent rats have effective mechanisms for clearing and preventing the dissemination of pulmonary infection. C. neoformans can be a pathogen for rats, since pulmonary infection has been described in a wild rat (30). There is a need for better animal models of cryptococcal infection. Rats have certain advantages relative to mice by virtue of their larger size. Comparative studies of C. neoformans infections in different animals can provide new insights into the pathogenesis of cryptococcosis. (The findings of this study were presented at the 94th General Meeting of the American Society for Microbiology, Las Vegas, Nev., 23 to 27 May 1994.)

MATERIALS AND METHODS

C. neoformans. Serotype D strain 24067 was obtained from the American Type Culture Collection (Rockville, Md.). Strain 24067 was used because of prior experience with it in murine infection (27, 29). Serotype D strains constitute ~7% of North American isolates, and this serotype is the most common isolate in some European countries (22). Strain 24067 was maintained in Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.). Rats were infected with cells grown in Sabouraud dextrose broth (Difco) at 30°C for 3 days, washed three times with 0.2 M phosphate-buffered saline (PBS), pH 7.2, and counted in a hemocytometer.

Intratracheal (i.t.) and intraperitoneal (i.p.) infection. Male Fischer rats weighing approximately 200 g were obtained from the National Cancer Institute (Frederick, Md.). Rats were anesthetized with methoxyflurane (Pitman-Moore, Mundelein, Ill.) and placed on an incline wood board. By using an otoscope (Welch Allyn, Skaneateles Falls, N.Y.) with a slot carved into the speculum, the vocal cords were visualized and a 0.3-ml suspension of 1.7 × 107 C. neoformans organisms in PBS was placed in the trachea distal to the vocal cords with a curved,
blunt-tip 23-gauge spinal needle. Following inoculation, 0.3 ml of air was injected at the same site to aid in the dispersion of organisms. The rats tolerated the procedure well. I.p. infection was done by injecting \(1.8 \times 10^7\) yeast cells in PBS into the peritoneum.

**Organ fungal burden.** Rats were killed by prolonged exposure to methoxyflurane. Organs were removed, weighed in an analytical balance, and homogenized by mechanical grinding in PBS, and dilutions of the tissue homogenate were plated in Sabouraud dextrose agar for CFU determination. Colonies were counted after 48 h of incubation at 30°C.

**Serum GXM levels.** Serum glucuronoxylomannan (GXM) levels were measured by latex agglutination and enzyme-linked immunosorbent assay (ELISA). Latex agglutination was done with the CALAS kit (Meridian, Cincinnati, Ohio) according to the manufacturer’s instructions. The ELISA for GXM has been described and used to study GXM levels in mice (6, 26, 27). Sera were digested with proteinase K and heat denatured prior to latex agglutination and ELISA as described previously (26, 27). Antigen levels were determined relative to GXM standards made by cetyltrimethylammonium bromide (Aldrich Chemical Co., Milwaukee, Wis.) precipitation (9). Serum antibody to GXM was measured by a double-sandwich ELISA. This ELISA was necessary because rat serum contained antibodies reactive with the usual polystyrene plate blocking agents bovine serum albumin (BSA) and casein, which made measurements of titer on GXM plates difficult. Briefly, anti-rat antibody (either immunoglobulin G [IgG] or IgM) was absorbed on 96-well polystyrene plates (Corning, Corning, N.Y.), plates were blocked for nonspecific binding with 1% BSA in PBS, and serial dilutions of serum were incubated in each well. GXM was added to each well at a concentration of 1 \(\mu\)g/ml; this was followed by incubation with a mouse monoclonal antibody (Mab) to GXM. Alkaline-phosphatase conjugated goat anti-mouse isotype-specific reagents (Southern Biotechnology, Indianapolis, Ind.) were applied, and then p-nitrophenyl substrate was added. A positive titer was defined as a dilution giving an \(A_{405}\) of greater than two times the background. The opsonizing activity of rat sera was tested on J774 cells exactly as described previously (26).

Rat sera were heated at 56°C for 30 min to inactivate complement. Sera from each of four rats obtained on days 21 and 25 of infection were pooled to provide an adequate volume, and the opsonizing activity was compared with that of sera from four noninfected rats. The phagocytic index was the number of attached plus ingested cryptococci divided by the number of macrophages per field (eight observations per measurement, approximately 100 macrophages counted per observation).

**Histopathology.** For each rat studied, the right upper lobe (RUL) of the lung was preserved in 10% buffered formalin for histopathologic studies and the remainder of the lung was used for CFU determination. The RUL comprised ~10 to 15% of the weight of the lung. By reserving the RUL for pathologic examination, we were able to correlate histopathologic events with fungal burden for individual rats. Tissue sections, 4 \(\mu\)m thick, in paraffin were stained with hematoxylin and eosin, periodic acid-Schiff, and modified methenamine silver stains for fungi.

**GSA lectin histochemistry.** Tissue macrophages were visualized by lectin histochemistry as described previously (27). Briefly, deparaffinized tissue sections were treated with 0.3% H\(_2\)O\(_2\) for 30 min and incubated with peroxidase-conjugated *Grifonia simplicifolia* agglutinin (GSA) B\(_2\) isoelectin (Sigma, St. Louis, Mo.) at 10 \(\mu\)g/ml in cation buffer (PBS containing CaCl\(_2\), MgCl\(_2\), and MnCl\(_2\) at a concentration of 0.1 mM each) for 16 h at 4°C, and dianminobenzidine was added as a chromogen.

**GXM immunohistochemistry.** Tissue GXM was determined as described previously (27). Briefly, deparaffinized tissue samples were treated with 0.3% H\(_2\)O\(_2\) for 30 min and incubated with 10% goat serum in PBS for 1 h, and primary GXM-binding MAb 3E5 was applied at a concentration of 2.6 \(\mu\)g/ml. Peroxidase-conjugated goat anti-mouse IgG3 (Fischer Scientific, Orangeburg, N.Y.) was diluted 1:250 in PBS and applied, and the mixture was incubated for 1 h at room temperature. Color was developed with dianminobenzidine.

**PCNA.** Proliferating cells were identified by staining tissues for proliferating cell nuclear antigen (PCNA), a marker for replicating cells. Briefly, lung tissues were reacted with anti-PCNA antibody (mouse IgG2a; DAKO, Carpinteria, Calif.) at 1:50 in 10% goat serum for 1 h and then incubated with peroxidase-conjugated goat-anti-mouse IgG2a (Southern Biotechnology) at 1:250 for 2 h at room temperature, and color was developed with dianaminobenzidine.

**Tissue IgG deposits.** IgG in the lung was identified by immunostaining. Deparaffinized sections were incubated with alkaline phosphatase-conjugated goat-anti-rat IgG (Southern Biotechnology) at 1:250 in 10% goat serum in PBS for 16 h at 4°C, and color was developed with nitroblue tetrazolium in 5-bromo-4-chloro-3-indolyl phosphate buffer containing levamizole to block endogenous alkaline phosphatase activity. For GXM and PCNA immunohistochemistry, incubation with isotype-matched irrelevant antibodies served as negative controls.

**Statistical analysis.** \(t\) tests were performed using Primer of Biostatistics software (McGraw-Hill, Inc., New York, N.Y.).

**RESULTS**

Three experiments were performed. The first experiment was done to determine the reliability of i.t. inoculation of *C. neoformans* in inducing pulmonary infection. i.t. inoculation of a *C. neoformans* suspension successfully produced pulmonary infection in 93% (13 of 14) rats studied. Subsequent experiments have produced even higher success rates. In all rats, the infection involved the right and left lungs as determined by CFU. Infected rats did not appear ill, and none manifested signs of respiratory distress. The second experiment used 18 rats to study the course of infection in the first 5 days. The third experiment used 15 rats to study infection at days 7, 14, and 25.

**Organ fungal burden.** Figures 1A and B show lung fungal burden at various times after infection. There was a transient decrease in average lung log\(_{10}\) CFU/g from 7.15 ± 0.16 to 6.71 ± 0.11 (\(P = 0.017\)) in the first day of infection relative to the fungal burden 2 h after inoculation. Subsequently, average lung CFU per gram remained relatively constant until day 14 of infection, after which there was a marked decrease. At day 25, the average lung log\(_{10}\) CFU/g (5.17 ± 0.11) was ~1.6 logs lower than at day 7 (6.79 ± 0.48; \(P < 0.001\)). The reduction in lung CFU per gram was temporally associated with increased lung weights, which paralleled increased inflammation (see below). Low-level extrapulmonary dissemination (~4 log units less than the pulmonary burden) occurred in four of six rats by day 7 and resulted in kidney, spleen, and brain involvement. Two rats had kidney involvement with an average log\(_{10}\) CFU/g of 2.1 ± 0.8, two rats had spleen involvement with an average log\(_{10}\) CFU/g of 2.0 ± 0.4, and one rat had brain involvement with a log\(_{10}\) CFU/g of 2.5. Blood cultures were negative for *C. neoformans* at 7, 14, and 25 days.

**Serum GXM levels.** Despite extensive pulmonary infection, serum GXM levels were low. Ten days after infection all serum
GXM determinations by latex antigen agglutination were <1:2 with the exception of one. By day 25 all rats tested (five of five) had low positive latex endpoints (>1:4). GXM capture ELISA confirmed that the vast majority of rats had antigen levels <1 µg/ml. The low level of serum GXM in pulmonary infection was surprising and led us to study GXM levels in systemic infection. Three rats given 1.8 × 10⁷ C. neoformans organisms i.p. had disseminated infection, with average lung, brain, spleen, and kidney log₁₀ CFU of 8.4, 6.5, 6.6, and 6.0, respectively, and serum GXM levels >1,000 µg/ml 2 weeks postinfection. Detection of high levels of GXM by latex agglutination and capture ELISA in i.p. infection indicate that these assays work well with rat sera.

Serum antibody response to GXM in infection. Three of six rats had an IgM titer to GXM of >1:243 detected on day 7. However the IgM response was transient and, no significant IgM was detected subsequently. C. neoformans infection elicited IgG to GXM in most rats, such that at day 7 five of six rats had a greater than threefold increase in IgG titer. In contrast to IgM, serum IgG persisted and there was an upward trend in IgG titer, with three of five rats having ninefold increases in IgG to GXM at day 25 relative to day 2. Sera from infected rats were more opsonic than sera from noninfected rats. Average phagocytic indexes for sera from uninfected and infected rats were 0.03 ± 0.04 and 0.13 ± 0.08, respectively (P < 0.01).

Histopathology of pulmonary infection. Histopathological findings are summarized in Table 1. At 2 h postinoculation, several foci of C. neoformans deposition were apparent. C. neoformans organisms were localized to the alveolar and bronchiolar air spaces as well as to tissue parenchyma (Fig. 2a). Parenchymal organisms were predominantly extravascular and not associated with tissue necrosis or inflammation. A few macrophages with intracellular C. neoformans were seen in occasional bronchial lumens (not shown). The organisms showed little variation in size and shape (Fig. 2a). Since the C. neoformans doubling time in vitro is ~2.5 h, the homogeneity of yeast forms is consistent with origin from the infecting inoculum. GXM immunoreactivity was localized to the capsule (Fig. 2a), with minimal diffusion to surrounding tissues. GSA lectin staining revealed macrophages in alveolar and interstitial spaces, with a predilection for subpleural spaces (Fig. 2i). Similar macrophage distribution was noted in control rats. At 12 h postinoculation, immunohistochemical staining revealed diffusion of GXM into surrounding tissues as well as adjacent alveolar epithelial surfaces (Fig. 2b). There was little change in the number and morphology of organisms or in host inflammatory reaction, but tissue penetration by C. neoformans was greater. Similarly, 24 h postinoculation there was little tissue inflammatory response or appreciable increase in the number of C. neoformans organisms. However, several bronchi demonstrated increased secretions containing C. neoformans organisms and desquamated epithelial cells, consistent with upward clearance of fungi from alveoli. At 48 h, GXM immunoreactivity and yeast forms were detected in the peri-bronchial lymphoid tissue (not shown). There was a steady increase in the bronchial secretion in association with peri-bronchial inflammation. Also noted at this time was variation in the size of C. neoformans cells, with emergence of small forms indicative of replication in vivo (not shown). At 72 h, GSA lectin histochemistry revealed recruitment of local macrophages to foci of C. neoformans, with GSA⁺ cells containing several ingested yeast forms (Fig. 2j). In one rat, early perivascular lymphocyte cuffing was noted. At 120 h, perivascular lymphocyte cuffing was more pronounced (Fig. 2e). Adjacent parenchymal lung tissue showed a polymorphous inflammatory infiltrate composed of lymphocytes, macrophages, neutrophils, and rare eosinophils. Diffuse, widespread tissue immunoreactivity for GXM persisted, and C. neoformans was found predominantly extracellularly (Fig. 2f).

At 7 days, early granuloma formation was noted; changes at

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**Table 1. Summary of histopathological findings**

<table>
<thead>
<tr>
<th>Time</th>
<th>C. neoformans Location⁺</th>
<th>GXM⁺</th>
<th>Macrophage recruitment⁺</th>
<th>Lymphocyte infiltration⁺</th>
<th>Granuloma formation⁺</th>
</tr>
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<tbody>
<tr>
<td>2 h</td>
<td>I &lt; E</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12 h</td>
<td>I &lt; E</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>24 h</td>
<td>I &lt; E</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>48 h</td>
<td>I &lt; E</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>72 h</td>
<td>I &lt; E</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>96 h</td>
<td>I &lt; E</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
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<tr>
<td>120 h</td>
<td>I &lt; E</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
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<tr>
<td>7 days</td>
<td>I &gt; E</td>
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<td>+++</td>
<td>++</td>
<td>++</td>
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<tr>
<td>14 days</td>
<td>I &gt; E</td>
<td>NA</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>25 days</td>
<td>I &gt; E</td>
<td>NA</td>
<td>++</td>
<td>+/−</td>
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</tbody>
</table>

⁺I, intracellular; E, extracellular.

⁺GXM immunoreactivity was determined by immunohistochemistry of lung sections, using an MAb to C. neoformans GXM.

⁺Mononuclear macrophage infiltration was examined by GSA lectin histochemistry excluding epithelioid histiocytes in granulomas. NA, not applicable.
14 days were qualitatively similar to those occurring at 7 days, but granuloma formation was the predominant finding, involving 50 to 70% of lung areas, with loss of air spaces. Granulomas were characterized by sheets of epithelioid cells (Fig. 2g), occurring predominantly in the area of terminal bronchioles (Fig. 2h) and often forming nodular protrusions into bronchial and bronchial lumens. Lymphocytes were no longer limited to perivascular space but infiltrated through parenchyma at the periphery of granulomas. Occasional neutrophils, eosinophils, and plasma cells were mixed with lymphohistiocytes. Numerous mitotic figures were present in macrophages (not shown). PCNA immunocytochemistry demonstrated that up to 30% of the cells in inflammatory areas were proliferating (Fig. 2i and j). PCNA^+ cells were identified as macrophages (Fig. 2i and j), lymphocytes (Fig. 2l) (both in lesional and in peribronchial lymphoid tissue), and damaged bronchial epithelium (not shown). Control rats (n = 3) had few or no PCNA^+ cells. Coinciding with granuloma formation was a shift of yeast cells from extracellular to intracellular spaces (Fig. 2f), which occurred mostly in epithelioid histiocytes (Fig. 2g). Many yeast cells were sickle shaped, suggestive of dead organisms. Epithelioid histiocytes expressed very low levels of diffuse cytoplasmic staining for GSA lectin (Fig. 2k). Several foci of confluent granulomas showed central necrosis with PAS^+ debris and karyorrhectic figures. In contrast to the diffuse tissue staining noted earlier in infection, GXM immunoreactivity was localized to granulomas within epithelioid histiocytes (Fig. 2c). The peribronchial lymphoid tissue showed hyperplasia, granulomatous inflammation, and C. neoformans penetration into the parenchyma of the lung. Immunocytochemistry for tissue IgG revealed staining of serum in vascular spaces (in both control and infected rats) and diffuse staining of perivascular inflammatory cells, including macrophages. Clusters of cells with dot-like discrete paranuclear staining (plasma cells) were detected in infected rats, consistent with local immunoglobulin production (not shown).

At 25 days, granulomatous inflammation subsided considerably but many vessels showed persistent lymphocyte cuffing and many histiocytes were large foamy macrophages exhibiting GXM immunoreactivity (Fig. 2d). PCNA^+ cells were much less frequent than at 14 days, consistent with resolution of the inflammatory process (Fig. 2n). The histologic pattern at 25 days was consistent with a resolving inflammatory process.

DISCUSSION

i.t. inoculation of C. neoformans reliably produced pulmonary infection in rats without the need for surgical techniques to expose the trachea. Our model differs from those described earlier (14, 16) in that it involves no disruption of tissue planes or damage to pulmonary structures. i.t. inoculation results in rapid deposition of C. neoformans at the alveoli. At 2 h postinfection C. neoformans organisms were seen within the parenchyma of the lung, indicative of rapid penetration at the level of alveoli. In vitro, C. neoformans has been shown to be internalized by primary rat lung cultures (25) and alveolar macrophages (2), even in the absence of opsonins. Despite initial deposition of yeasts in the alveoli, organisms and inflammation were subsequently localized to the peribronchial regions such that at 7 days the histologic picture was that of a bronchial pneumonia. This observation contrasts with primary alveolar infection in steroid-suppressed rats (14). In immunocompetent rats efficient alveolar clearance could result from alveolar macrophages and/or upward mucociliary transport.

Extrapulmonary dissemination occurred early in the course of infection. However, the extrapulmonary burden was low despite extensive pulmonary involvement. Containment and reduction of C. neoformans infection in the lungs appear to depend largely on an effective cellular immune response. The course of pulmonary infection in the rat is similar to that described for mice (17). The rat immune response reduced the C. neoformans burden in the lung by day 25 of infection. Reduction in lung CFU was temporally correlated with granuloma formation following lymphocyte and macrophage infiltration of the lung parenchyma. Granuloma formation was accompanied by tremendous cellular proliferation by day 14 of infection. The sequence of cellular changes suggests that effective limitation of infection requires expansion and activation of macrophages. Lymphocyte recruitment occurred 4 days after infection, and an appreciable increase in macrophage number was not apparent before this time. Macrophage expansion would have increased the number of phagocytic cells, and the influx of lymphocytes may have activated macrophage function.

The signals responsible for the lymphocyte influx and macrophage expansion and activation in the rat are unknown. Early in infection there were isolated pulmonary macrophages
was in sharp contrast to the high serum GXM levels present diffusely through infected tissues, and yeast cells known to be affected by a variety of stimuli in Mycobacterium tuberculosis by monocytic cells leads to rapid secretion of granulocyte-macrophage colony-stimulating factor (8). Direct evidence for cytokine involvement awaits studies using immunohistochemical or molecular probes.

The rat antibody response to GXM in i.t. C. neoformans infection included IgM and IgG. IgM was transient and measurable only at day 7 for only 50% of the rats. For most rats an increase in IgG was noted with time. When interpreting the serum titers it is important to note that the ELISA immobilized serum immunoglobulin and assayed its capacity to bind GXM. This ELISA circumvents the problem of rat antibody cross-reacting with the ELISA blocking proteins and has the theoretical advantage that it could detect antibody in antigen-antibody complexes. However, since specific antibody to GXM composes only a small fraction of the total antibody pool, the titers measured are relatively low and not comparable to those obtained with direct GXM binding ELISAs (5, 7). Similar ELISAs have been used to measure IgA in C. neoformans-infected mice (5). The source of the serum IgG was not determined, but some may have originated from the pulmonary plasma cells noted by immunohistochemistry.

Reduction in lung CFU was accompanied by a shift in the ratio of extracellular to intracellular C. neoformans organisms. In the absence of opsonins, phagocytosis of C. neoformans is inefficient (3). The shift to intracellular predominance suggests effective opsonization and may have been associated with local antibody production. For some rats there was a reduction in lung CFU without an appreciable increase in serum IgG titer, suggesting phagocytosis without opsonins (2) or alternative opsonins such as complement proteins (3, 4) or IgA.

Immunohistochemistry revealed GXM release into tissues by C. neoformans 12 h after infection. GXM immunoreactivity was present diffusely through infected tissues, and yeast cells stained less distinctly. Capsular polysaccharide synthesis is known to be affected by a variety of stimuli in vitro including CO2 tension (15), osmolality (11), and sugar concentration (11). Release of GXM into lung tissues may contribute to successful establishment of pulmonary infection, since capsular polysaccharide can inhibit phagocytosis (21). Serum GXM levels were low despite extensive pulmonary deposits of GXM. The low serum GXM level accompanying pulmonary infection was in sharp contrast to the high serum GXM levels in rats i.p. infected. GXM shed into pulmonary tissues either does not efficiently disseminate to the serum compartment or is removed from serum. In mice MAB administration reduces GXM levels, and serum IgG could have contributed to low serum GXM levels (26). Human primary pulmonary cryptococcosis has been reported to be serum antigen negative (31). The rat observations are consistent with this finding.

C. neoformans pulmonary infection in rats is a dynamically evolving process on the part of the host and the pathogen. The rat responds to pulmonary infection with a complex array of effector cells including macrophages, neutrophils, lymphocytes, and occasional eosinophils, which effectively reduce fungal burden. Despite massive infection the rat pulmonary defenses are effective at minimizing dissemination and controlling infection. The rat i.t. infection model may have some unique advantages as an animal system for the study of C. neoformans pathogenesis. In contrast to rabbits, rats can be infected without the need of immunosuppression. Because rats are approximately 10 times the size of mice, it is possible to do certain experimental procedures such as direct tracheal visualization, bronchoalveolar lavage, and repeated cerebrospinal fluid sampling that are difficult if not presently impossible in mice. However rats are more expensive than mice to obtain and maintain, and cost is a significant factor in the design of large studies. The rat provides new insights into C. neoformans pathogenesis, and this animal system is fertile ground for the dissection of the host-pathogen interaction.

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