INFECTION AND IMMUNITY, Sept. 1995, p. 3448–3453
0019-9567/95/$04.00+0
Copyright © 1995, American Society for Microbiology
Vol. 63, No. 9

Tissue Localization of *Cryptococcus neoformans* Glucuronoxylomannan in the Presence and Absence of Specific Antibody

DAVID L. GOLDMAN,[1] SUNHEE C. LEE,[2] AND ARTURO CASADEVALL[3,4]*

Departments of Pediatrics,[1] Pathology,[2] Medicine (Infectious Diseases),[3] and Microbiology & Immunology[4] of the Albert Einstein College of Medicine, Bronx, New York 10461

Received 11 April 1995/Returned for modification 26 May 1995/Accepted 7 June 1995

During infection, *Cryptococcus neoformans* capsular glucuronoxylomannan (GXM) is released into tissues, where it may be associated with a variety of deleterious immunological effects. Relatively little is known about the organ distribution and cellular localization of GXM antigen. Intravenous administration of GXM to rats resulted in persistent serum levels which declined with a half-life of 14.3 h in the first 74 h and 3 h thereafter, coincident with the appearance of serum antibodies to GXM. GXM was sequestered primarily in spleen tissue, with localization to marginal zone and follicular cells. Administration of the murine immunoglobulin G1 monoclonal antibody (MAb) 2H1 resulted in >99% reduction in serum GXM level within 3 h. MAb 2H1 administration resulted in liver GXM deposition, with cellular localization primarily to Kupffer cells. GXM was also found in the spleens of MAb 2H1-treated rats, with localization to the marginal zones and follicles. Endotracheal administration of GXM resulted in low serum levels, with lung tissue having the highest GXM organ levels, localized primarily to alveolar macrophages. The results indicate that (i) intravenous administration to rats produced persistent serum GXM levels with a half-life similar to that found in mice and rabbits; (ii) endotracheal administration of GXM resulted in low serum levels; (iii) in the absence of specific antibody, GXM organ deposition occurs primarily in the spleen and is localized primarily to marginal zone macrophages; (iv) in the presence of specific immunoglobulin G1 antibody, GXM organ deposition occurs primarily in the liver and is localized primarily to Kupffer cells; and (vi) reticuloendothelial cells sequester GXM in the presence and absence of specific antibody.

Cryptococcus neoformans is an encapsulated yeast which produces meningoencephalitis in immunocompromised individuals. *C. neoformans* polysaccharide (CNPS) consists of at least two components: glucuronoxylomannan (GXM) and galactoxylomannan, with GXM being the major constituent (9). Nonencapsulated strains have markedly reduced virulence, suggesting that CNPS is important for virulence (5, 24). CNPS is antiphagocytic and may function in virulence by interfering with fungal clearance by host effector cells (20, 22). CNPS may also contribute to virulence through direct effects on the immune system (9). For example, CNPS can inhibit phagocytosis (20), antigen presentation (10), leukocyte migration (12, 14), lymphocyte proliferation (26), and specific antibody responses (21, 33, 39, 40) and induce suppressor responses (27, 32). Administration of small amounts of CNPS prior to infection can reduce survival in mice (2). Patients with cryptococcal infections are tolerant to CNPS (16, 17). Capsular polysaccharide can enhance human immunodeficiency virus replication in vitro (35, 36). These studies provide a large body of direct, indirect, and circumstantial evidence indicating an important role for CNPS in the pathogenesis of cryptococcal infection.

In cryptococcal infection, CNPS is released into tissues, and its detection in serum and cerebrospinal fluid serves as an important diagnostic marker (3). AIDS patients with *C. neoformans* infections often have high levels of CNPS in serum and cerebrospinal fluid. Increasing CNPS titer during therapy for cryptococcal meningitis has been associated with poor prognosis (11). In some patients, polysaccharide persists in body fluids after sterilization of body fluids with antifungal therapy (37). Despite the importance of CNPS in the diagnosis and pathogenesis of cryptococcal disease, relatively little is known regarding its organ distribution and metabolism in vivo. In 1965, Bennett and Hasenclever showed that CNPS administered into the cerebrospinal fluid of rabbits was rapidly cleared from the cerebrospinal fluid but persisted in serum (2). Kozel et al. used immunofluorescence to demonstrate that polysaccharide antigen persisted in kidney tubular epithelial cells (21). Muchmore et al. found that mice given 1 mg of CNPS had detectable serum levels of CNPS for 63 days, with the highest and most persistent tissue concentrations of CNPS in the liver and spleen (28). Kappe and Muller found that administration of 20 μg of CNPS to rabbits resulted in persistent serum levels with a half-life ($t_{1/2}$) of 24 h (19). Mukherjee et al. demonstrated that passive administration of antibody in a murine model of cryptococcosis reduced serum polysaccharide, but the fate of polysaccharide antigen in the presence of antibody was not studied (30).

In this paper, we report studies of GXM clearance in rats and focus on the question of tissue distribution of GXM antigen in the presence and absence of specific antibody. The results indicate marked differences in serum elimination, tissue distribution, and cellular localization of GXM in the presence and absence of specific antibody.

MATERIALS AND METHODS

Animals. Male Fischer rats were obtained from Taconic Farms (Germantown, N.Y.). Rats weighing approximately 200 g were used for the intravenous (i.v.) experiments. Rats weighing 350 to 400 g were used for the endotracheal (e.t.) experiment to facilitate intubation.

***C. neoformans* and antigen preparation.** Strain 24067 (serotype D) was obtained from the American Type Culture Collection (Rockville, Md.). This strain was studied because it had been used in prior studies of rat infection (15) and

* Corresponding author. Mailing address: Department of Medicine, 1300 Morris Park Ave., Bronx, NY 10461. Phone: (718) 430-4260.

passive antibody efficacy (29–31). CNPS was prepared from the culture supernatant by alcohol precipitation (30, 34) followed by repeated extractions with a 1:1 chloroform-butanol solution to remove protein components (13). The amount of GXM in total CNPS preparations was determined by capture enzyme-linked immunosorbent assay (ELISA) (7) relative to a GXM standard made by dissolving a known amount of GXM in water. Rats were injected with CNPS. However, since we measured GXM in the CNPS and in the serum by capture ELISA, subsequent discussion will refer to the material as GXM.

Antibody. Monoclonal antibody (MAb) 2H1 is a murine immunoglobulin G1 (IgG1) with specificity for GXM (6). MAb 2H1 ascites fluid was generated by injecting 2H1 hybridomas into peritoneal cavities of pristane-primed BALB/c mice. MAb 2H1 was purified from ascites fluid by protein G affinity chromatography (Pierce, Rockford, Ill.). Antibody concentrations were determined by ELISA relative to isotype-matched standards of known concentration (41).

i.v. GXM injections. Experiment 1 was designed to study the pharmacokinetics of GXM after i.v. administration and determine the effect of passive antibody administration on serum GXM levels and organ localization. Twelve rats (3 per group) were injected with 200 μg of GXM via a lateral tail vein and 4 h later given either 300, 30, 3, or 0 μg of MAb 2H1 in phosphate-buffered saline (PBS) via penile vein injection. Blood was collected from the tail vein at various times after administration of GXM. Two days after i.v. administration of GXM, rats given 300 μg of MAb 2H1 were killed with sodium pentobarbital (Anpro Pharmaceutical, Arcadia, Calif.), and their lungs, livers, spleens, brains, and kidneys were removed, weighed, and placed in formalin. Urine was obtained by bladder puncture. This procedure was repeated in rats which received no antibody 6 days following i.v. administration of GXM. Organs were harvested at different days from groups treated with MAb 2H1 and no antibody because the serum elimination of GXM is very different in the presence of specific antibody (see below).

Experiment 2 was designed to study the effect of MAb 2H1 on tissue distribution at early time points. Six rats (three per group) were injected with 200 μg of GXM in a lateral tail vein and bled 1 h later. MAb 2H1 (300 or 0 μg) was administered 2 h after GXM injection, and rats were sacrificed 3 h later. Spleens, livers, and kidneys were removed and placed in formalin. Urine and blood samples were also obtained at this time.

e.t. GXM administration. Four rats were anesthetized with methoxyflurane (Pitman-Moore, Mundelein, Ill.) and inoculated e.t. with 200 μg of GXM under direct visualization, using an otoscope (Welch Allyn, Skaneateles Falls, N.Y.) and a modified spinal needle as described previously (15). Rats were sacrificed 5 days after e.t. inoculation; organs were removed and placed in formalin.

Determination of GXM in organ tissues. Approximately 0.2 g of organ tissue was placed in 2 ml of PBS and homogenized with an Ultraturex T25 (Janke and Kunkel, Staufen, Germany). Proteinase K (1 mg/ml; Boehringer Mannheim, Mannheim, Germany) was added, and the suspension was incubated at 37°C overnight. Samples were then heated to 100°C for 15 min and tested by ELISA (see below).

Measurement of GXM in organ tissue. Serum, urine, and organ levels of GXM were determined by a MAb capture ELISA as described previously (15, 31). Body fluids and organ tissue samples from rats which did not receive any GXM were used as controls. Since some rats had high serum GXM levels at the time of determination of organ GXM levels, organ levels were corrected by multiplying the amount of plasma per organ (99 μl/g for liver, 86 μl/g for spleen, and 92 μl/g for kidney) by respective serum GXM levels and subtracting this value from the original GXM level (1). Correction for serum GXM content did not affect relative amounts of organ GXM.

Measurement of rat serum antibody to GXM. In rats receiving no MAb 2H1, the level of serum anti-GXM antibody was determined by ELISA (30). Briefly, 1 μg of GXM per ml was absorbed on 96-well polystyrene plates (Corning Glass Works, Corning, N.Y.). Plates were blocked with 1% bovine serum albumin and serial dilutions of serum were added to each well. Alkaline phosphatase-labeled anti-rat IgG or IgM (Southern Biotechnology, Birmingham, Ala.) was added, and the presence of antibody was detected by colorimetric reaction with *p*-nitrophenyl substrate. A positive titer was defined as the dilution giving an A_{405} of greater than two times the background signal (8).

Tissue immunohistochemistry. Localization of GXM in tissue was done by immunohistochemical techniques as described previously (15). Organs were fixed in formalin, embedded in paraffin, and sectioned into 4-μm slices. Tissue sections were deparaffinized, treated with proteinase K (10 μg/ml), and incubated with the IgG3 anti-GXM MAb 3E5 as described previously (15). Peroxidase-conjugated goat anti-mouse IgG (Fischer Scientific, Orangeburg, N.Y.) was used as a secondary antibody, and color was developed with diaminobenzidine. Sections were counterstained with hematoxylin or periodic acid-Schiff reagent. To enhance the contrast in spleen sections, an alkaline phosphatase-labeled secondary antibody (Southern Biotechnology) was used in place of the peroxidase-conjugated antibody. Color was developed with Sigma Fast Red TR/Naphthol AS-MX (Sigma, St. Louis, Mo.), which contains 1 mM levamiosole to block endogenous alkaline phosphatase activity. Two rats which did not receive GXM were used as negative controls.

Statistics and serum $t_{1/2}$. *P* values were calculated with a *t* test by using Primer of Biostatistics software (McGraw-Hill, Inc., New York, N.Y.). Some *P* values were calculated by a *t* test with the Satterthwaite method for unequal variances by using True Epistat version 3.1 (Epistat Services, Richardson, Tex.). Serum GXM $t_{1/2}$ was calculated from clearance curves by nonlinear regression by using a pharmacokinetics software package (PCNONLIN; SCI Software, Lexington, Ky.).

RESULTS

Pharmacokinetics of GXM. i.v. administration of 200 μg of GXM to rats weighing 197 ± 10 g resulted in an average serum GXM concentration of 46.9 ± 10.3 μg/ml 2 h after injection (Fig. 1). The kinetics of GXM elimination differed with time. In the first 74 h of GXM administration, the $t_{1/2}$ of GXM was approximately 14.3 ± 1.8 h. After 74 h, serum elimination was more rapid with a $t_{1/2}$ of 3.4 h and correlated with the appearance of serum antibody to GXM (see below). To determine the effect of specific anti-GXM antibody on serum GXM levels, rats were given MAb 2H1 i.v. 4 h after the injection of GXM (Fig. 1). Rats receiving 30 or 300 μg of MAb 2H1 had significantly lower serum GXM levels than rats receiving no MAb within 3 h of MAb administration. The reduction in serum GXM levels correlated with the amount of MAb 2H1 given. No reduction in serum GXM was observed for rats receiving 3 μg of MAb 2H1. GXM was not detected in the urine (<0.050 μg/ml) of antibody-treated and -untreated rats 5 h following the administration of GXM and remained undetectable at 2 and 6 days, respectively.

Organ GXM levels. In rats receiving i.v. GXM alone, the GXM concentrations in the spleen and liver were 2.98 ± 2.08 and 0.41 ± 0.58 μg/g, respectively, 5 h after GXM injection (Fig. 2). No GXM (<0.08 μg/g) was detected in the kidneys of these animals. Six days after the administration of GXM, the spleen GXM concentration in rats not receiving antibody was 1.00 ± 0.27 μg/g, with no detectable GXM in the brain, lungs, liver, or kidneys. In rats receiving 300 μg of MAb 2H1, the corrected average organ GXM concentrations in the spleen, liver, and kidneys at 5 h were 3.59 ± 0.34 μg/g ($P = 0.65$), 3.91 ± 0.07 μg/g ($P < 0.001$), and 0.18 ± 0.02 μg/g, respectively. After 2 days, the average organ GXM concentrations for the rats receiving antibody were 1.15 ± 0.15 and 0.78 ± 0.14 μg/g in the spleen and liver, respectively. No GXM was detected in the lungs, kidneys, or brain.

Rat antibody response to GXM. The rapid decline in serum GXM levels observed at the latter time points for rats not given MAb 2H1 (Fig. 1) suggested the possibility that rats were producing endogenous antibody to GXM. Antibody to GXM was measured in three rats not receiving MAb 2H1. Two of three rats had an IgM titer of 1:9 by day 4. All three rats developed an IgG anti-GXM titer of 1:27 by day 5 (Fig. 3).

Immunohistochemistry. Immunohistochemical studies of rats sacrificed 5 h after i.v. injection of GXM alone revealed strong GXM immunoreactivity in scattered cells of the outer marginal zone of the spleen (Fig. 4A). The morphology of the immunoreactive cells indicated that they are marginal zone macrophages. Light GXM staining was also noted in spleen follicles and vascular spaces of the kidneys and liver, with occasional staining of the sinusoidal spaces. Six days after the administration of GXM, serum GXM levels were undetectable and immunohistochemistry was performed. These studies revealed increased GXM immunoreactivity in the follicles compared with the 5 h time point (Fig. 4B). Some follicles contained GXM immunoreactivity in close proximity to large cells, representing early germinal center formation. GXM was not detected in the livers, brains, and kidneys of these rats.

In rats receiving GXM and 300 μg of 2H1, there was a large increase in GXM immunoreactivity within the liver. Five hours following the administration of GXM, immunoreactivity was localized to the cells lining the sinusoidal spaces as well as the sinusoidal space itself. Many of these GXM immunoreactive

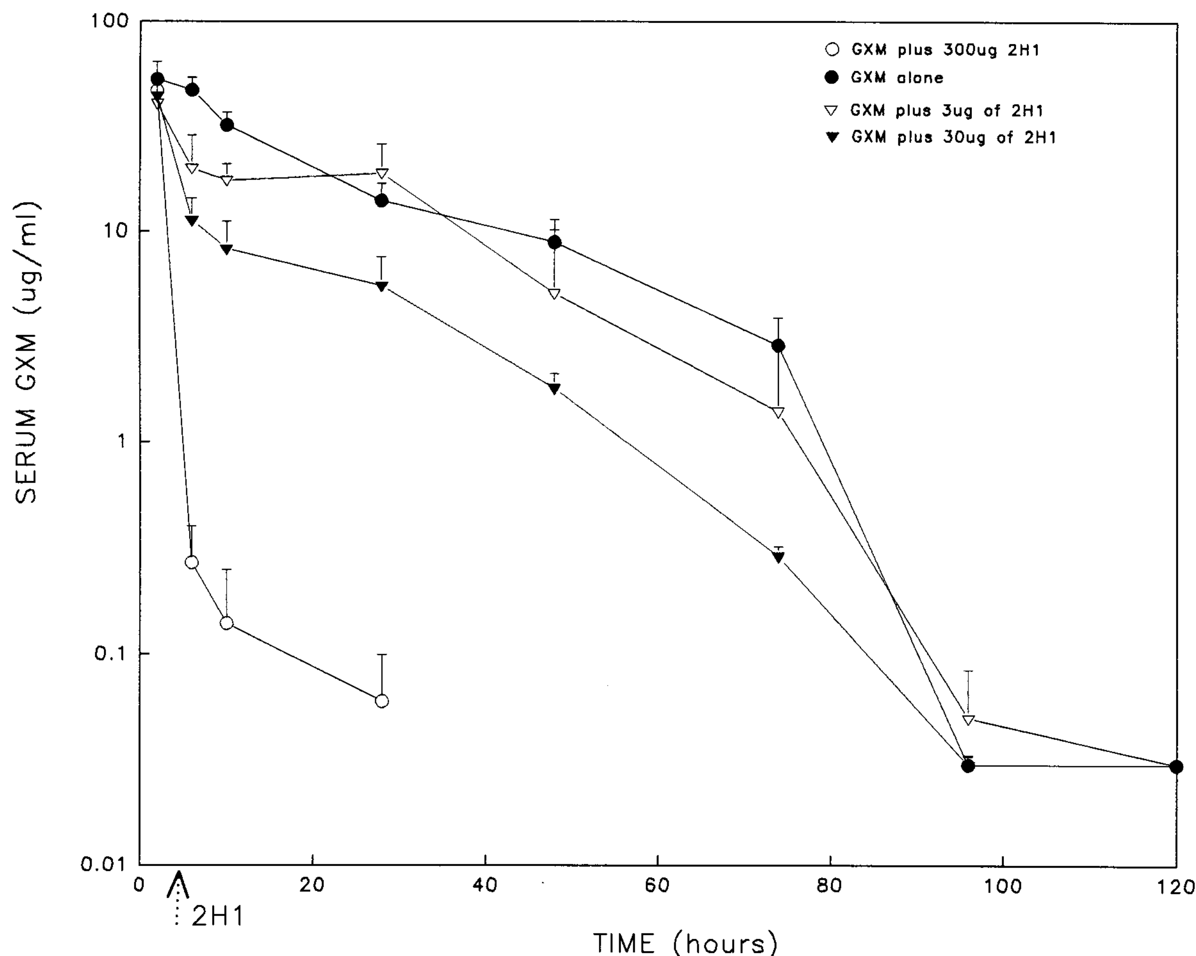


FIG. 1. Average serum GXM levels in the presence and absence of specific antibody. Rats were given 200 μg of GXM followed by 300, 30, 3, or 0 μg of MAb 2H1 i.v. Error bars indicate standard deviations of the means ($n = 3$). Arrow indicates time of 2H1 administration.

cells were stellate in appearance and periodic acid-Schiff positive, features consistent with those of activated Kupffer cells. GXM-immunoreactive macrophages and polymorphonuclear cells were also noted within the sinusoidal spaces. There was no hepatocyte staining. MAb-treated rats also demonstrated GXM immunoreactivity in cells of the marginal zone and follicles of the spleen in a pattern similar to that described for rats which received GXM alone.

Two days after the administration of GXM and MAb 2H1, immunohistochemistry revealed persistent GXM immunoreactivity in the liver and spleen. For rats receiving MAb 2H1, tissue immunohistochemistry was done at 2 days because this was the time when serum GXM was no longer detectable. The distribution of GXM immunoreactivity in the liver at 2 days was similar to that at 5 h (see above), except for an apparent decrease in GXM immunoreactivity within the sinusoidal space itself as well as a decrease in the number polymorphonuclear leukocytes within the sinusoidal spaces. Study of the spleens of these animals revealed GXM immunoreactivity in the marginal zones and increased follicular staining relative to that at 5 h. There was no immunoreactivity detected in the brains, lungs, or kidneys of MAb 2H1-treated rats.

e.t. administration of GXM. Our earlier study of rat *C. neoformans* pulmonary infection found low levels of serum GXM despite significant lung tissue infection. To study whether GXM penetrated into the serum from the airways, rats were given GXM e.t., and serum GXM levels were determined at various times afterward. Serum GXM levels in all four rats given GXM e.t. were low (<0.050 μg/ml) at 3, 6, 10, 24, 48, 72, 96, and 120 h following e.t. administration. Two rats had isolated serum concentrations of less than 0.2 μg/ml (48 and 120 h), and one rat had an isolated serum GXM concentration of 0.74 μg/ml (72 h). GXM was detected in lung homogenates by capture ELISA for all rats, with an average concentration of 1.08 ± 0.53 μg/g. In none of the rats was GXM detected in the brain homogenate. In three of four rats, no GXM was detected in liver or spleen homogenate. In one rat (the rat with the serum GXM level of 0.74 μg/ml), GXM was detected in the liver (0.25 μg/g) and spleen (1.32 μg/g) homogenates. In two of the four rats, GXM was detected in the kidney homogenate, with an average GXM concentration of 0.21 ± 0.04 μg/g. Lung immunohistochemistry at day 5 revealed localized GXM to large foamy macrophages within the alveolar spaces and septa. Spleen immunohistochemistry localized GXM to the marginal zone in the one rat in which the spleen homogenate was positive for GXM. GXM immunoreactivity was not detected in the kidneys, livers, or brains of these rats.

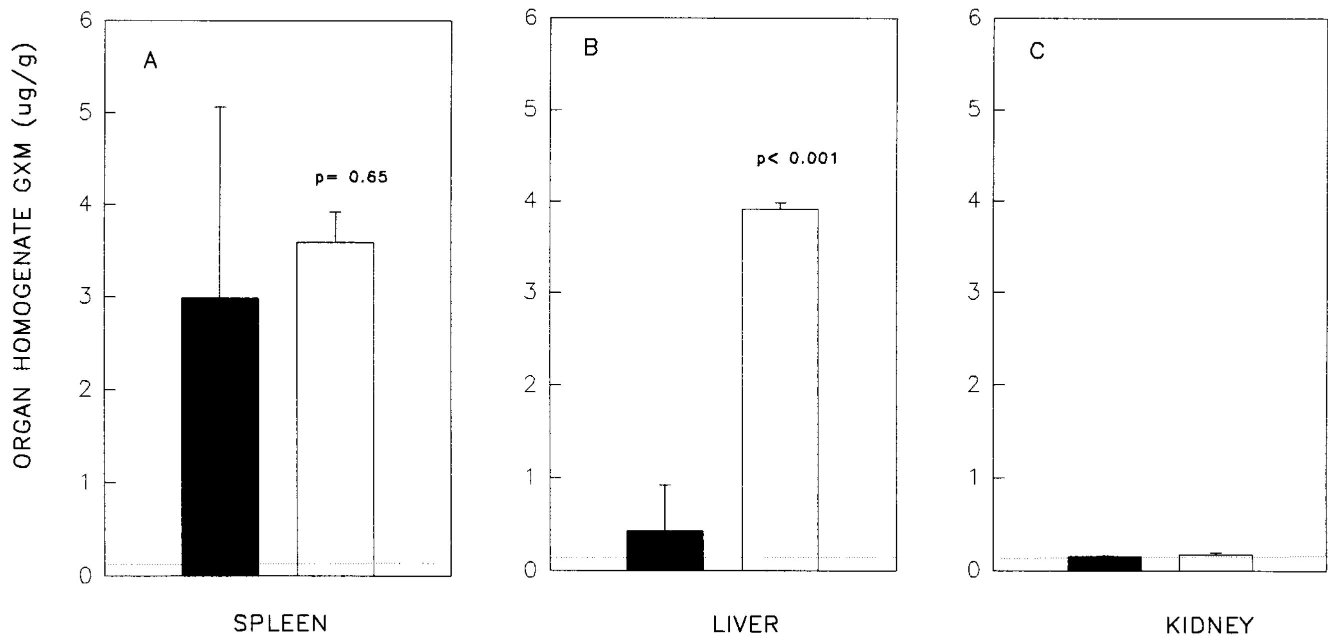


FIG. 2. GXM concentrations in spleens, livers, and kidneys of MAb-treated (open bars) and non-MAb-treated (black bars) rats 5 h after administration of GXM. Dotted lines represent the lower limits of GXM detection in our assay (0.15 μg/g). Error bars indicate standard deviation of the mean ($n = 3$). P values were calculated by a t test. P values calculated by a t test with Satterthwaite's method for unequal variances were comparable, being 0.6926 and 0.0067, respectively, for the spleen and liver comparisons: P values for kidney homogenates were not calculated because the GXM concentration was below the limit of detection in the non-MAb-treated rats.

DISCUSSION

Injection of GXM into the tail veins of rats resulted in sustained serum levels. i.v. administration of 200 μg of GXM into rats weighing approximately 200 g resulted in serum levels of 46.9 ± 10.3 μg/ml at 2 h. The calculated volume of distribution of GXM was 4 ± 2 ml. This volume is very similar to the calculated plasma volume for a 200-g rat of 5.5 ml, assuming a blood volume of 64 ml/kg and a hematocrit of 43% (25). The equivalence of the volume of distribution and the plasma volume indicate that shortly after injection, GXM is found primarily in the serum compartment. There were two different phases of GXM elimination. The initial phase lasts until about 74 h and has a calculated $t_{1/2}$ of 14 h. This $t_{1/2}$ value is similar to that the reported cryptococcal polysaccharide $t_{1/2}$ in mice (28) and rabbits (19). After 74 h, there is a rapid decrease in serum GXM which coincides with the appearance of endogenous serum antibody to GXM and is likely to result from antibody-mediated clearance of GXM (see below).

Organ GXM localization was studied by two techniques: GXM organ levels measured by capture ELISA and immunohistochemistry. Each technique provided complementary information. Organ levels provide information of GXM amount per organ, whereas immunohistochemistry provides information on cellular localization. In the absence of exogenously administered antibody, GXM antigen localized to the spleen. This is consistent with the findings of Muchmore et al. (28), who reported that administration of cryptococcal polysaccharide localized to the spleen of mice and persisted there for over 70 days. Immunohistochemical analysis of spleen tissue revealed that GXM initially (within 5 h) localized primarily to macrophages in the outer marginal zone, with light staining of the follicles. This observation is consistent with studies that have demonstrated a preferential uptake of other T-cell-independent type 2 (TI-2) antigens by marginal zone macrophages and may be the result of specialized carbohydrate receptors on the marginal zone macrophage (23).

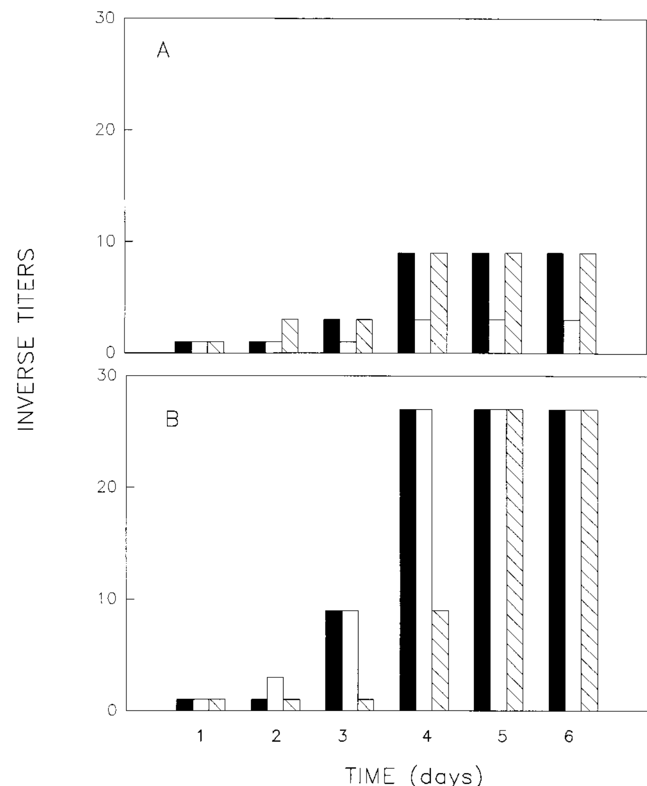


FIG. 3. (A) Inverse IgM titers to GXM for three rats following the administration of 200 μg of GXM in the absence of exogenous antibody as determined by ELISA. (B) Inverse IgG titers to GXM for three rats following the administration of 200 μg of GXM in the absence of exogenous antibody as determined by ELISA.

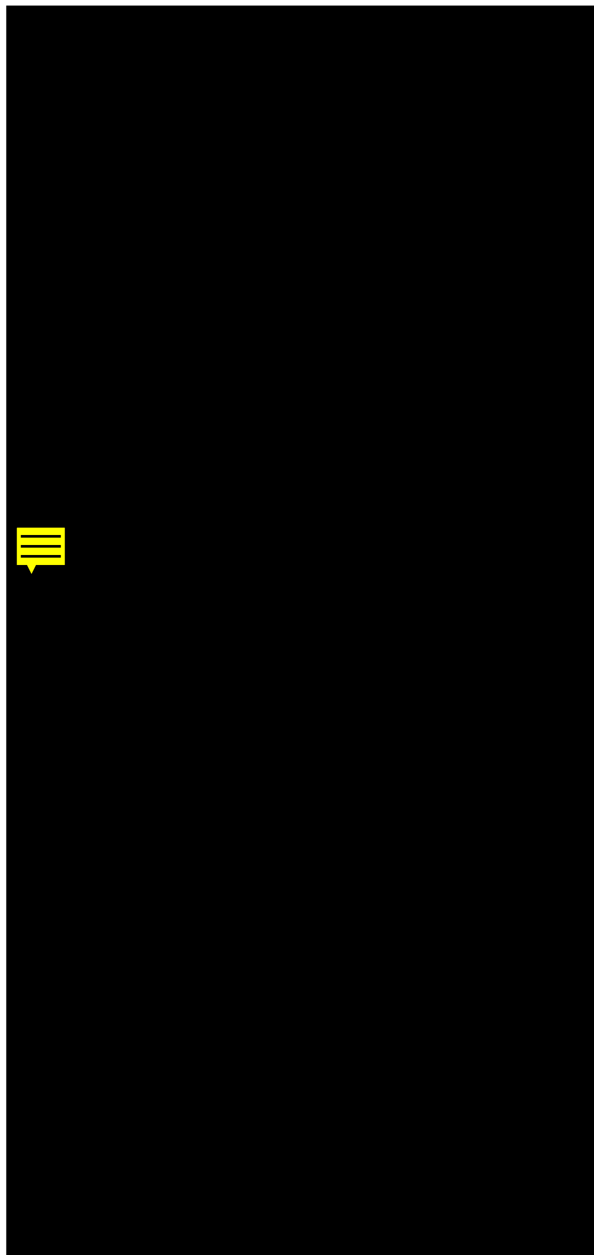


FIG. 4. (A) Immunohistochemistry for GXM in rat spleen 5 h after the administration of GXM reveals immunoreactivity in the outer marginal zone and inside the follicle. Magnification, ×240. The secondary antibody was alkaline phosphatase goat anti-mouse IgG antibody. mz, marginal zone: f, a follicle identified at lower power. (B) Immunohistochemistry for GXM in rat spleen 6 days after administration of GXM reveals persistent immunoreactivity in the marginal zone and increased follicular localization. Arrow points to an early germinal center. Magnification, ×240. (C) Immunohistochemistry for GXM in liver in MAb 2H1-treated rat reveals immunoreactivity within Kupffer cells. The secondary antibody was peroxidase-conjugated goat anti-mouse IgG antibody. Magnification, ×600.

Six days after GXM administration, there was a marked increase of GXM immunoreactivity in the follicles. Although follicular localization is not usually associated with TI-2 antigens, complement-dependent localization to the follicle has been reported for trinitrophenyl-Ficoll, another TI-2 antigen (18). In addition, follicular localization of antigen-antibody complexes is a well-described phenomenon (42) which may account for the presence of GXM immunoreactivity within follicles, since most rats developed an antibody response to GXM. The development of germinal centers in proximity to cells exhibiting GXM immunoreactivity is atypical for TI-2 antigens but has been reported following the administration of dextran to mice (43). The follicular pattern of GXM immunoreactivity may contribute to the relatively brisk anti-GXM antibody response demonstrated by rats receiving GXM alone.

Administration of GXM binding MAb 2H1 resulted in a rapid reduction of serum GXM levels. Rapid elimination of serum GXM also occurred in rats not given MAb after 72 h and was temporally correlated with the appearance of rat serum antibody to GXM. This result is consistent with the findings observed after MAb 2H1 administration. Previous studies had shown that administration of MAb 2H1, but not an irrelevant isotype-matched IgG1 control, reduced serum GXM in murine i.v. infection (31). However, it was unclear if this effect was the result of antibody-mediated reduction in *C. neoformans* infection or a direct effect of antibody on GXM (30, 31). The results presented here indicated that MAb 2H1 can reduce GXM directly. The magnitude of the antibody-mediated reduction in serum GXM was dependent on MAb 2H1 dose. No reduction was observed with the small dose of 3 μg of MAb 2H1. MAb 2H1 reduction in serum GXM is likely to result from the formation of antigen-antibody complexes and their subsequent removal from serum by reticuloendothelial cells. Administration of MAb 2H1 to rats with high GXM levels was well tolerated, and the rats did not manifest signs of distress or illness. Organ GXM deposition was different in MAb 2H1-treated rats. At 5 h, the amounts of GXM per gram of spleen and liver tissue in MAb 2H1-treated rats were equivalent. Since the liver weighs approximately 13 times more than the spleen, the amount of GXM in the liver was much greater than that in the spleen. These findings are in agreement with those of Russell et al., who demonstrated a preferential localization of radiolabeled pneumococcal polysaccharide to the livers of mice treated with either an IgG3-, an IgA-, or an IgM-specific MAb (38). Thus, organ tissue localization of GXM antigen can be altered by specific antibody. For spleen and liver, tissue deposition is much greater in the spleen than in liver in the absence of antibody and less in the spleen than in the liver in the presence of antibody.

e.t. administration of GXM resulted in low levels of serum GXM levels comparable to those measured during e.t. infection (15). At 120 h after e.t. administration, the organ levels of GXM remained greatest in the lung. In the lung, GXM was localized primarily to pulmonary macrophages, which may reflect opsonin-independent phagocytosis by rat macrophages (4).

The ultimate fate of cryptococcal GXM antigen in infection is unknown. No mammalian enzymes are known to digest GXM. Persistence of GXM antigen in spleen and liver cells for prolonged periods of time (weeks to months) has been documented in mice. In the absence of antibody, GXM appears to accumulate in the spleens of rats and may also persist there for long periods of time. Relatively little polysaccharide antigen was found in the urine, arguing against significant kidney elimination in the short term. However, urinary excretion may be able to eliminate significant amounts of GXM over the long term. Persistence in the spleen may contribute to the unresponsiveness of cured patients to rechallenge with polysaccharide antigen.

In summary, clearance of GXM occurs primarily by cells of the reticuloendothelial system. In the absence of specific antibody, GXM is sequestered primarily by spleen macrophages. In the presence of specific antibody, GXM is sequestered pri-

marily by hepatic Kupffer cells. Differences in organ localization may affect immune responses to GXM and *C. neoformans* and suggest another mechanism by which the presence of antibody immunity to GXM may modify the course of infection.

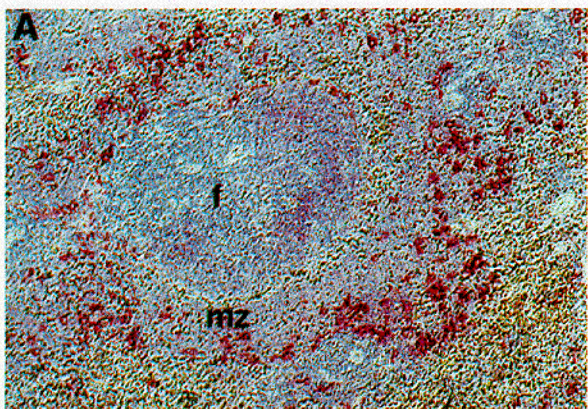
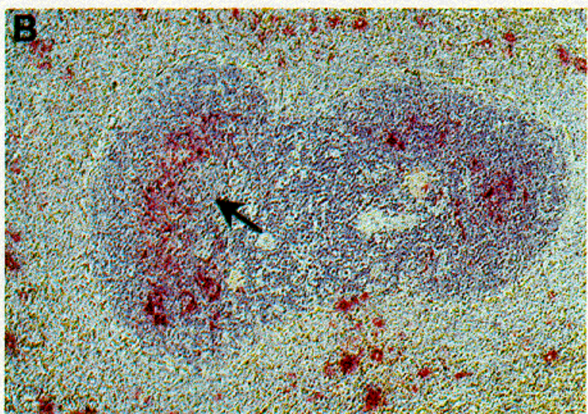
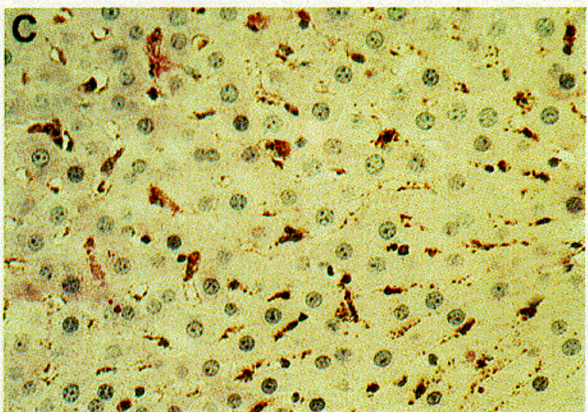
ACKNOWLEDGMENTS

A.C. is supported by a James S. McDonnell Scholar Award and NIH grants AI33774 and AI13342. S.C.L. is supported by NIMH grant MH 47667.

We thank Lionel Zuckier for help in computing the serum $t_{1/2}$ values. We thank Dennis W. Dickson and Meng-Liang Zhao for the help with immunocytochemistry and helpful discussions. We thank Jorge Bermudez for processing of tissues for histopathological analysis.

REFERENCES

1. **Altman, P. L.** 1961. Blood and other body fluids. Federation of American Societies for Experimental Biology, Washington, D.C.
2. **Bennett, J. E., and H. F. Hasenclever.** 1965. *Cryptococcus neoformans* polysaccharide: studies of serologic properties and role in infection. J. Immunol. **94:**916–920.
3. **Bloomfield, N., M. A. Gordon, and D. F. Elmendorf, Jr.** 1963. Detection of *Cryptococcus neoformans* antigen in body fluids by latex particle agglutination. Proc. Soc. Exp. Biol. Med. **114:**64–67.
4. **Bolanos, B., and T. G. Mitchell.** 1989. Phagocytosis and killing of *Cryptococcus neoformans* by rat alveolar macrophages in the absence of serum. J. Leukocyte Biol. **48:**521–528.
5. **Bulmer, G. S., M. D. Sans, and C. M. Gunn.** 1967. *Cryptococcus neoformans*. I. Nonencapsulated mutants. J. Bacteriol. **94:**1475–1479.
6. **Casadevall, A., J. Mukherjee, S. J. N. Devi, R. Schneerson, J. B. Robbins, and M. D. Scharff.** 1992. Antibodies elicited by a *Cryptococcus neoformans* glucuronoxylomannan-tetanus toxoid conjugate vaccine have the same specificity as those elicited in infection. J. Infect. Dis. **65:**1086–1093.
7. **Casadevall, A., J. Mukherjee, and M. D. Scharff.** 1992. Monoclonal antibody ELISAs for cryptococcal polysaccharide. J. Immunol. Methods **154:**27–35.
8. **Casadevall, A., and M. D. Scharff.** 1991. The mouse antibody response to infection with *Cryptococcus neoformans*: V_H and V_L usage in polysaccharide binding antibodies. J. Exp. Med. **174:**151–160.
9. **Cherniak, R., and J. B. Sundstrom.** 1994. Polysaccharide antigens of the capsule of *Cryptococcus neoformans*. Infect. Immun. **62:**1507–1512.
10. **Collins, H. L., and G. J. Bancroft.** 1991. Encapsulation of *Cryptococcus neoformans* impairs antigen-specific T-cell responses. Infect. Immun. **59:** 3883–3888.
11. **Diamond, R. D., and J. E. Bennett.** 1974. Prognostic factors in cryptococcal meningitis. Ann. Intern. Med. **80:**176–181.
12. **Dong, Z. M., and J. W. Murphy.** 1995. Intravascular cryptococcal culture filtrate (CneF) and its major component, glucuronoxylomannan, are potent inhibitors of leukocyte accumulation. Infect. Immun. **63:**770–778.
13. **Dromer, F., J. Salamero, A. Contrepois, C. Carbon, and P. Yeni.** 1987. Production, characterization, and antibody specificity of a mouse monoclonal antibody reactive with *Cryptococcus neoformans* capsular polysaccharide. Infect. Immun. **55:**742–748.
14. **Drouhet, E., and G. Segretain.** 1951. Inhibition de la migration leucocytaire in vitro par un polyoside capsulaire de *Torulopsis* (*Cryptococcus*) *neoformans*. Ann. Inst. Pasteur **81:**674.
15. **Goldman, D., S. C. Lee, and A. Casadevall.** 1994. Pathogenesis of pulmonary *Cryptococcus neoformans* infection in the rat. Infect. Immun. **62:**4755–4761.
16. **Henderson, D. K., J. E. Bennett, and M. A. Huber.** 1982. Long-lasting, specific immunologic unresponsiveness associated with cryptococcal meningitis. J. Clin. Invest. **69:**1185–1190.
17. **Henderson, D. K., V. L. Kan, and J. E. Bennett.** 1986. Tolerance to cryptococcal polysaccharide in cured cryptococcosis patients: failure of antibody secretion *in vitro*. Clin. Exp. Immunol. **65:**639–646.
18. **Humphrey, J. H.** 1981. Tolerogenic or immunogenic activity of hapten-conjugated polysaccharides correlated with cellular localization. Eur. J. Immunol. **11:**212–220.
19. **Kappe, R., and J. Muller.** 1991. Rapid clearance of *Candida albicans* mannan antigen by liver and spleen in contrast to prolonged circulation of *Cryptococcus neoformans* antigens. J. Clin. Microbiol. **29:**1665–1669.
20. **Kozel, T. R., and E. Gotschlich.** 1982. The capsule of *Cryptococcus neoformans* passively inhibits phagocytosis of the yeast by macrophages. J. Immunol. **129:**1675–1680.
21. **Kozel, T. R., W. F. Gulley, and J. Cazin, Jr.** 1977. Immune response to *Cryptococcus neoformans* soluble polysaccharide: immunological unresponsiveness. Infect. Immun. **18:**701–707.
22. **Kozel, T. R., G. S. T. Pfrommer, A. S. Guerlain, B. A. Highison, and G. J. Highison.** 1988. Role of the capsule in phagocytosis of *Cryptococcus neoformans*. Rev. Infect. Dis. **10:**S436–S439.
23. **Kraal, G., H. Ter Hart, C. Meelhuizen, G. Venneker, and E. Classen.** 1989. Marginal zone macrophages and their role in the immune response against T-independent type 2 antigens: modulation of the cells with specific antibody. Eur. J. Immunol. **19:**675–680.
24. **Kwon-Chung, K. J., and J. C. Rhodes.** 1986. Encapsulation and melanin formation as indicators of virulence in *Cryptococcus neoformans*. Infect. Immun. **51:**218–223.
25. **Lee, H. B., and M. D. Blaufox.** 1995. Blood volume in the rat. J. Nucl. Med. **25:**72–76.
26. **Mody, C. H., and R. M. Syme.** 1993. Effect of polysaccharide capsule and methods of preparation on human lymphocyte proliferation in response to *Cryptococcus neoformans*. Infect. Immun. **61:**464–469.
27. **Mosley, R. L., J. W. Murphy, and R. A. Cox.** 1986. Immunoadsorption of *Cryptococcus*-specific suppressor T-cell factors. Infect. Immun. **51:**844–850.
28. **Muchmore, H. G., E. Nan Scott, F. G. Felton, and R. A. Fromtling.** 1982. Cryptococcal capsular polysaccharide clearance in nonimmune mice. Mycopathologia **78:**41–45.
29. **Mukherjee, J., M. D. Scharff, and A. Casadevall.** 1992. Protective murine monoclonal antibodies to *Cryptococcus neoformans*. Infect. Immun. **60:**4534–4541.
30. **Mukherjee, J., L. Zuckier, M. D. Scharff, and A. Casadevall.** 1994. Therapeutic efficacy of monoclonal antibodies to *Cryptococcus neoformans* glucuronoxylomannan alone and in combination with amphotericin B. Antimicrob. Agents Chemother. **38:**580–587.
31. **Mukherjee, S., S. Lee, J. Mukherjee, M. D. Scharff, and A. Casadevall.** 1994. Monoclonal antibodies to *Cryptococcus neoformans* capsular polysaccharide modify the course of intravenous infection in mice. Infect. Immun. **62:**1079–1088.
32. **Murphy, J. W., and R. A. Cox.** 1988. Induction of antigen-specific suppression by circulating *Cryptococcus neoformans* antigen. Clin. Exp. Immunol. **73:**174–180.
33. **Murphy, J. W., and G. C. Cozad.** 1972. Immunological unresponsiveness induced by cryptococcal polysaccharide assayed by the hemolytic plaque technique. Infect. Immun. **5:**896–901.
34. **Neill, J. M., C. G. Castillo, R. H. Smith, and C. E. Kapros.** 1949. Capsular reactions and soluble antigens of Torula histolytica and Sporotrichum schenckii. J. Exp. Med. **89:**93–106.
35. **Pettoello-Mantovani, M., A. Casadevall, T. R. Kollman, A. Rubinstein, and H. Goldstein.** 1992. Enhancement of HIV-1 infection by the capsular polysaccharide of *Cryptococcus neoformans*. Lancet **339:**21–23.
36. **Pettoello-Mantovani, M., A. Casadevall, P. Smarnworawong, and H. Goldstein.** 1994. HIV-1 infectivity is increased in vitro by the presence of the capsular polysaccharide of *Cryptococcus neoformans* and *Haemophilus influenzae*. AIDS Res. Hum. Retroviruses **10:**1079–1087.
37. **Powderly, W. G.** 1992. Therapy for cryptococcal meningitis in patients with AIDS. Clin. Infect. Dis. **14:**S54–S59.
38. **Russell, M. W., T. A. Brown, L. J. Clafin, K. Schroer, and J. Mestecky.** 1983. Immunoglobulin A-mediated hepatobiliary transport constitutes a natural pathway for disposing of bacterial antigens. Infect. Immun. **42:**1041–1048.
39. **Sundstrom, J. B., and R. Cherniak.** 1992. A glucuronoxylomannan of *Cryptococcus neoformans* serotype A is a type 2 T-cell-independent antigen. Infect. Immun. **60:**4080–4087.
40. **Sundstrom, J. B., and R. Cherniak.** 1993. T-cell-dependent and T-cell-independent mechanisms of tolerance to glucuronoxylomannan of *Cryptococcus neoformans* serotype A. Infect. Immun. **61:**1340–1345.
41. **Tijssen, P.** 1985. The immobilization of immunoreactants on solid phases, p. 297–328. *In* R. H. Burdon and P. H. van Knippernberg (ed.), Practice and theory of enzyme immunoassays. Elsevier, Amsterdam.
42. **van Roojin, N., G. Kraal, and C. D. Dijkstra.** 1989. Cytological basis of immune functions of the spleen. Prog. Histochem. Cytochem. **19:**1–67.
43. **Wang, D., S. M. Well, A. M. Stall, and E. A. Kabat.** 1994. Reaction of germinal centers in the T-cell-independent response to the bacterial polysaccharide alpha(1-6)dextran. Proc. Natl. Acad. Sci. USA **91:**2502–2506.


A
f
mz

B

C