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Variable Efficacy of Passive Antibody Administration against Diverse *Cryptococcus neoformans* Strains

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The efficacy of monoclonal antibody (MAb 2H1) against diverse strains of *Cryptococcus neoformans* was studied in a murine model of intravenous infection. For six of eight strains, administration of MAb prior to infection prolonged survival of mice. For two strains, 371 and SB4A, administration of MAb prior to infection did not prolong survival in multiple experiments with inocula ranging from 10^2 to 10^6 yeast cells per mouse. Mice infected with strains 371 and SB4A had fewer CFU than non-MAb-treated controls, but the CFU reduction was not sufficient to affect survival. Serum glucuronoxylomannan (GXM) levels varied for the different *C. neoformans* strains. For mice that did not receive MAb 2H1, there was a positive correlation between lung fungal burden and serum GXM levels. MAb 2H1-treated mice had significantly reduced serum GXM levels. The results indicate that the efficacy of MAb 2H1 administration in prolonging survival and/or reducing organ CFU varies with the *C. neoformans* strain.

The importance of antibody immunity against a pathogen is usually established by correlating the presence of serum antibodies with protection and/or demonstrating protection with passive antibody administration. In recent years, numerous studies from three independent groups have shown that administration of monoclonal antibodies (MAbs) to the *Cryptococcus neoformans* polysaccharide capsule can modify the course of infection in mice (12–14, 35–42, 46, 56). However, there are also studies arguing against an important role for antibody immunity. B-cell-deficient mice were not more susceptible to infection (23, 33), mice with high serum antibody titers were not protected (19), and administration of polyclonal sera prolonged survival in some studies (1, 15, 20) but not in others (31, 44). Some of these seemingly contradictory observations may be explained by the findings that antibody efficacy is dependent on antibody quantity (13), isotype (38, 46, 47, 56), and specificity (36, 38). Polyclonal sera may or may not be effective depending on the quantity and composition of the specific antibody present.

Another consideration in antibody efficacy studies is the *C. neoformans* strain used in experimental infection. *C. neoformans* strains differ in virulence (22, 54), tissue tropism (21), antigenic composition (50), polysaccharide structure (8), resistance to phagocytosis (48), and complement activation (55) and manifest extensive genetic diversity (3). Given these strain differences, we hypothesized that antibody efficacy may also depend on the strain. In this study, we examined the ability of the immunoglobulin G1 (IgG1) MAb 2H1 to modify the course of infection for diverse strains of *C. neoformans*. MAb modified the course of infection for all strains tested, but its efficacy varied with the *C. neoformans* strain.

MATERIALS AND METHODS

C. neoformans. Strains 24065 (serotype B) and 24067 (serotype D) were obtained from the American Type Culture Collection (ATCC). Strain 371 (serotype A) was obtained from John Bennett (Bethesda, Md.). Strains J9A, J11A, J22A, SB4A, and SB6A are recent clinical isolates (7, 51, 52) which are genetically distinct and were isolated from patients who relapsed despite antifungal therapy (7, 52). CN1 is a laboratory strain of uncertain origin. All strains produced melanin-like pigments on L-dopa agar (27). Strains were maintained on Sabouraud dextrose agar slants (Difco Laboratories, Detroit, Mich.) at 4°C and grown in Sabouraud dextrose broth (Difco Laboratories) at 37°C overnight. Strain characteristics are summarized in Table 1. MAb 2H1 has been shown to bind the capsular polysaccharide and promote phagocytosis for each of these strains (11) (unpublished data for strain CN1).

Antigens and MAbs. Glucuronoxylomannan (GXM) was isolated by cetyltrimethylammonium bromide precipitation (10). For strains J22A and 24065, *C. neoformans* capsular polysaccharide was prepared by ethanol precipitation (6). GXM-binding MAbs 2H1 (IgG1) and 2D10γ1 (IgG1) have been described previously (4, 41). MAb-containing ascitic fluid was obtained by paracentesis of pristane-primed BALB/c mice given intraperitoneal (i.p.) injections of hybridomas. MAb concentrations were determined by enzyme-linked immunosorbent assay (ELISA) relative to isotype-matched standards (53).

Animal experiments. A/JCr mice were obtained from the National Cancer Institute (Frederick, Md.) and Jackson Laboratories (Bar Harbor, Maine). MAb was administered i.p. as ascites. Control groups were given i.p. injections of sterile 0.02 M phosphate-buffered saline, pH 7.2 (PBS). Previous studies have shown no difference between the use of PBS and ascites containing irrelevant antibody as the intervention for the control group (37, 38). *C. neoformans* cells were washed three times, suspended in PBS, and counted in a hemocytometer, and their cell viability was determined in Sabouraud dextrose agar (Difco). Intravenous (i.v.) infection was done via the lateral tail vein (40, 41, 57). Mouse deaths were recorded every 12 h in the first 2 to 3 weeks of each experiment and once per day thereafter.

Organ fungal burden was determined as described previously (41). Briefly, mice were bled from the orbital sinus, killed by cervical dislocation, and weighed, and the organs were harvested. Lungs were removed, inspected to ascertain that all lobes were present, and weighed. The skull was sectioned with a razor blade along the sagittal plane, brain tissue was removed and weighed, and the cranial vaults were inspected to ensure that they were empty. The entire lung or brain was then homogenized in sterile PBS, and dilutions of the homogenate were spread on Sabouraud dextrose agar. Colonies were counted after incubating plates for at least 2.5 days at room temperature. A potential concern in organ CFU studies involving MAb-treated mice is the theoretical possibility that CFU are reduced because of yeast cell agglutination by residual serum MAb 2H1 during tissue homogenization. The CFU reductions in MAb 2H1-treated mice were not artifacts of agglutination because (i) visual inspection of agar surface with a microscope revealed primarily single yeast forms without evidence of agglutination or clumping after spreading and (ii) the concentration of MAb in the homogenate calculated from serum GXM-binding IgG1 levels, organ weights, and a homogenization volume of 10 ml was at least 10 times lower than the agglutination threshold (11) (in most experiments, the homogenates were further diluted by a factor of 10^1 to 10^3 and vortexed before being plated on agar).

Experimental design. A/JCr mice were used because they are very susceptible to *C. neoformans* infection (45). Inocula and MAb doses were based on prior experience (38, 40, 41). The timing of MAb administration (i.e., 4 h before

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TABLE 1. Summary of strain characteristics

Strain	Origin	GCB agar[a]	Agglutination endpoint[b]	L-Dopa agar[c]	% Sterol[d]	Serotype[e]
371[f]	Cookos bird nest, Thailand	−	31.2	+	NA[g]	A
J9A	CSF,[h] AIDS patient, NYC[i]	−	15.6	+	1.1	D
J11A	CSF, lymphoma patient, NYC	−	7.8	+	2.9	A
J22A	CSF, AIDS patient, NYC	−	7.8	+	4.6	D
SB4A	CSF, AIDS patient, Stony Brook, N.Y.	−	15.6	+	1.6	AD
SB6A	CSF, AIDS patient, Stony Brook, N.Y.	−	7.8	+	0.31	AD
24065	ATCC	+	23.4	+	NA	B
24067	ATCC	−	1.9	+	NA	D
CN1	Uncertain	−	NA	+	NA	NA

[a] Glycine-canavanine-bromothymol blue agar (28) distinguishes between *C. neoformans* var. *neoformans* (serotypes A, D, and AD) and *C. neoformans* var. *gattii* (serotypes B and C).
[b] For agglutination endpoint, see reference 11.
[c] All strains produced black melanin-like pigments in agar containing L-dopa.
[d] For cell membrane sterol data, see reference 16.
[e] Serotype of strains 371, 24065, and 24067 as per ATCC description. Serotype of strains J9A, J11A, J22A, SB4A, and SB6A was determined by nuclear magnetic resonance and serologic analysis (9).
[f] 371 isolate obtained from John Bennett (Bethesda, Md.).
[g] NA, not available.
[h] CSF, cerebrospinal fluid.
[i] NYC, New York City.

infection) was based on the pharmacokinetics of i.p. injection, which indicate significant levels in serum by this time (see below). Day 4 was chosen for CFU determination because (i) CFU differences between MAb-treated and control groups occur by this time (14); (ii) it is early in infection, which avoids attrition from early deaths; and (iii) the overwhelming majority of mice lack endogenous serum antibody at day 4 (6). Lung and brain were chosen for CFU study because they are heavily infected in i.v. infection (46). A sample size of 10 per group was chosen to avoid problems of small sample size given experimental variation (41).

Serum GXM level and anti-GXM titer. Serum for GXM detection was digested with proteinase K and boiled, and GXM was measured by capture ELISA as described previously (5, 40, 41). For all strains except J22A and 24065, GXM levels were determined relative to GXM standards made by dissolving GXM in water. For strains J22A and 24065, serum polysaccharide levels were determined relative to ethanol-precipitated capsular polysaccharide, which is 80 to 90% GXM (10).

Pharmacokinetics of i.p. MAb administration were studied by administering 1 mg of MAb 2H1 to A/J mice and monitoring the serum titer by ELISA. Blood was collected from the orbital sinus before and 4, 16, 24, 48, 72, and 96 h after MAb administration. Titers in serum were determined by an ELISA on polystyrene plates coated with a 1-μg/ml solution of strain 24067 GXM in 10 mM Tris-HCl–150 mM NaCl–1 mM NaN_3 and developed with goat anti-mouse IgG1 alkaline phosphatase-conjugated antibody (Fisher Biotech, Orangeburg, N.Y.) as described previously (5). The titer was defined as the dilution which gave an A_{405} of twice the background (6). A similar ELISA was used to measure the level of GXM-binding antibody at the time of CFU determination except that antibody concentrations were obtained relative to MAb 2H1 standard curves.

Data analysis. Statistical analysis was done by or with the advice of C. J. Chang, a statistician at our institution. Survival and CFU data were analyzed by parametric and nonparametric methods with the SAS software package (SAS Institute, Cary, N.C.) or the True Epistat Version 3.1 (Epistat Services, Richardson, Tex.) program. Survival data were analyzed by log rank and Wilcoxon tests which yielded comparable *P* values. CFU were analyzed by Wilcoxon and Kruskal-Wallis tests which yielded comparable *P* values. Student's *t* test was used to compare organ weights and serum GXM and antibody levels by the Primer of Statistics: The Program (McGraw-Hill Co., New York, N.Y.) and Sigmastat (Jandel Scientific, San Rafael, Calif.). Regression analysis and analysis of variance were done with SigmaStat. Numbers shown in the test and tables are averages ± standard deviations.

RESULTS

Pharmacokinetics of i.p.-administered MAb 2H1. Administration of 1.0 mg of MAb 2H1 into the peritoneal cavity of A/J mice resulted in high titers in serum of GXM-binding antibody 4 h after injection (inverse titers of 1,000 to 10,000). Using a plasma volume of 1.5 ml per mouse and the concentration of MAb required to produce the measured serum titers, we estimated that >40% of the MAb 2H1 dose was in serum 4 h after i.p. injection.

Survival studies. The ability of MAb 2H1 to prolong survival of mice lethally infected by i.v. inoculation was studied for eight *C. neoformans* var. *neoformans* strains (24067, SB4A, SB4A, CN1, J11A, J9A, J22A, and 371) and one *C. neoformans* var. *gattii* strain (24065). For six strains, 24067, SB4A, CN1, J11A, J9A, and J22A, administration of MAb 2H1 4 h before infection resulted in prolongation of survival (Table 2). For strain SB6A, the average survival of MAb 2H1-treated mice was significantly longer than that of controls ($P < 0.05$) in two of three experiments (Table 2). For two strains, 371 and SB4A, administration of MAb 2H1 did not prolong survival in multiple experiments (six for strain 371; nine for strain SB4A) with infecting inocula ranging from 10^2 to 10^6 yeast cells per mouse (Fig. 1). At inocula of 10^5 to 10^6 cells per mouse, mice died in 8 to 20 days with little variation in average survival (Fig. 1). Reducing the inocula prolonged survival and significantly increased variation in the average survival of both MAb 2H1-treated and control groups (Fig. 1). Administration of MAb 2H1 24 h before i.v. infection with 5×10^4 yeast cells (to maximize serum MAb levels) did not prolong survival, with mice in the MAb 2H1 and control groups surviving 17.7 ± 2.9 days and 17.7 ± 2.31 days, respectively ($P = 0.8191$). For *C. neoformans* var. *gattii* 24065, survival studies were not feasible because infected mice did not die. For example, there were no deaths after 22 days with an inoculum of 10^6 yeast cells per mouse in 20 mice. An attempt to increase virulence by passaging strain 24065 in mice was not successful since 9 of 10 mice infected with a colony recovered from lung tissue were still alive after 1 year.

Brain and lung CFU. The effect of MAb administration on organ CFU was studied with strains 371, SB4A, SB6A, J22A, and 24065. For all strains, administration of MAb 2H1 reduced brain and/or lung CFU, but the effect depended on the inoculum (Tables 3 and 4). For strain 371, no reduction in brain or lung CFU was observed with MAb 2H1 treatment with i.v. infection with an inoculum of 2×10^5 yeast cells. When the inoculum was reduced to 5×10^3 or 5×10^2 cells, reduction in brain CFU but not lung CFU was observed in the MAb 2H1-treated group (Table 3). For strain J22A, reductions in brain and lung CFU were observed in the MAb 2H1-treated group for mice infected with 2×10^5 yeast cells (Table 3). For *C. neoformans* var. *gattii* 24065, a reduction in lung CFU was noted in the MAb 2H1-treated group for mice infected with

TABLE 2. Summary of survival data for strains 24067, SB6A, CN1, J9A, J11A, and J22A

Strain	Expt	Inoculum[a] (10^6) (%)	MAb administration (h)	Avg survival (days)		% Increase in survival	P^b
				PBS	2H1		
24067	1	2.5 (136)	−4	1.0 ± 0.2	5.1 ± 2.7	400	≤0.001
SB6A	1	2.0 (59)	−4	8.4 ± 2.1	8.8 ± 1.4	5	0.6987
	2	2.0 (70)	−5	7.1 ± 0.7	8.4 ± 1.2	17	0.0036
	3	2.5 (66)	−4	5.0 ± 3.2	9.6 ± 1.4	92	0.0028
CN1	1	2.0 (340)	−4	2.1 ± 0.2	2.5 ± 0	19	0.0078
	2	2.0 (66)	−4	1.6 ± 0.2	3.6 ± 0.7	125	<0.0001
J9A	1	2.0 (88)	−4	2.9 ± 0.9	9.7 ± 1.0	234	<0.0001
J11A	1	1.0 (20)	−4	7.5 ± 1.2	9.3 ± 0.3	17	0.0002
J22A	1	2.0 (60)	−4	5.0 ± 1.2	17.2 ± 2.5	244	0.0001

[a] Inoculum determined by hemocytometer counts. Number in parentheses is the percentage of viable cells in the inoculum as determined by CFU on Sabouraud dextrose agar.

[b] *P* values were calculated for the comparison of mean survival values of the control and MAb 2H1-treated groups by parametric (log rank) and nonparametric (Wilcoxon) tests, and the results were similar. The table shows *P* values obtained with the Wilcoxon statistic.

2×10^6 yeast cells (Table 3; note that despite the high inoculum of 2×10^6 cells, the lung tissue CFU with strain 24065 were fewer than for other strains consistent with reduced virulence). For strain SB4A, reduction in lung but not brain CFU was observed in the MAb 2H1-treated group with an inoculum of 2×10^5 yeast cells (Table 4). Decreasing the SB4A inoculum to 2×10^4 cells resulted in reduced lung and brain CFU in MAb 2H1-treated mice relative to controls (Table 4). For strain SB6A, a reduction in brain CFU but not lung CFU was observed in MAb 2H1-treated mice infected with an inoculum of 2×10^5 cells (Table 4). MAb 2H1-treated mice infected with 2×10^4 cells of strain SB6A had reduced lung and brain CFU relative to controls (Table 4).

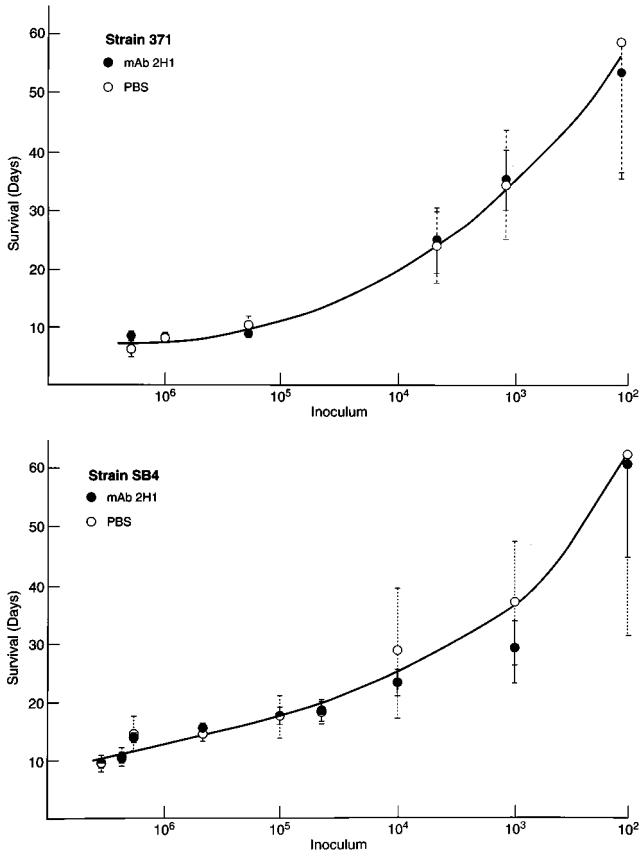

FIG. 1. Plot of inoculum versus survival for 16 MAb 2H1 protection experiments using strains 371 and SB4A. For each experiment, there were 10 mice in each of the PBS and MAb 2H1-treated groups. Points denote the mean survival time, and bars denote the standard deviation of the mean. For 13 of the 16 experiments shown, the *P* value was >0.05. For the 371 experiments using inocula of 2×10^6 and 2×10^5 cells, the calculated *P* values were 0.0009 and 0.0024, respectively. For one SB4A experiment (inoculum of 10^3 cells), the calculated *P* value was 0.0372. The significance of occasional *P* values of <0.05 given multiple experiments showing no significant difference between groups is uncertain.

Organ weights. Previously, we noted that MAb 2H1-treated mice had lower brain and lung weights than control mice infected with strain 24067 i.v. at day 7 of infection (39, 41). Histopathological studies have correlated increased organ weights in strain 24067 infection with more severe infection and inflammation (41). For the strains studied here, differences in lung and brain weight for the MAb and control groups were smaller than those measured for strain 24067 in previous studies. Significant differences ($P < 0.05$) were noted between organ weights in MAb 2H1-treated and control groups for some experiments with strains SB4A, SB6A, and 371 (Tables 3 and 4). In each case, the organs from MAb 2H1-treated mice weighed less than those of controls. The results were unchanged if brain and lung weights were normalized for body weight (data not shown).

Serum GXM. Serum GXM antigen levels were studied for strains 371, SB4A, SB4B, J22A, and 24065 in the presence and absence of MAb 2H1. Serum GXM was detectable in the serum of mice infected with each of these strains. Comparison of GXM levels in control mice revealed differences in GXM concentration depending on the strain. For example, in mice infected with 2×10^5 organisms, serum GXM levels at day 4 were 6.5 ± 5.14, 2.42 ± 1.28, and 23.5 ± 6.6 μg/ml for strains 371, SB6A, and J22A, respectively (Tables 3 and 4). Plots of serum GXM versus lung CFU for individual mice infected with strains 371, SB6A, and J22A revealed positive correlations for each strain, with correlation coefficients of 0.528, 0.501, and 0.810, respectively (Fig. 2). In all experiments, MAb 2H1-treated mice had significantly reduced serum GXM levels compared with control mice (Tables 3 and 4). For strain 371, a reduction in serum GXM levels for MAb 2H1-treated mice was observed in experiment 1 despite no reduction in lung or brain CFU (Table 4).

Serum anti-GXM IgG1 levels. GXM-binding IgG1 levels at day 4 of infection were measured for mice infected with 2×10^5 yeast cells of strains 371, SB4A, SB6A, and J22A which had received 1.0 mg of MAb 2H1 (Table 5). There were significant differences in levels in serum of IgG1 to GXM with the highest

TABLE 3. Summary of CFU data for strains 371, J22A, and 24065

Strain	Expt	Inoculum[a] (%)	Group	Body wt (g)[b]	Lung wt (g)[b]	Lung $\log_{10}$ CFU[c]	Brain wt (g)[b]	Brain $\log_{10}$ CFU[c]	Serum GXM level (μg/ml)[b]
371	1	2×10^5 (81)	PBS	18.4 ± 0.92	0.15 ± 0.06	5.26 ± 0.56	0.34 ± 0.04	6.07 ± 0.18	6.85 ± 5.14
			2H1	17.7 ± 0.90	0.15 ± 0.01	5.07 ± 0.49	0.35 ± 0.04	5.90 ± 0.45	<1.0
			P	0.0899	0.6287	0.9688	0.4808	0.1981	0.0021
	2	5×10^3 (NA)	PBS	18.6 ± 1.51	0.14 ± 0.01	4.27 ± 0.34	0.36 ± 0.17	4.91 ± 0.27	0.11 ± 0.09
			2H1	18.4 ± 0.86	0.14 ± 0.01	4.22 ± 0.15	0.33 ± 0.03	4.45 ± 0.39	<0.04
			P	0.6536	0.7007	0.1857	0.0143	0.0233	0.0315
	3	5×10^2 (92)	PBS	21.3 ± 1.28	0.14 ± 0.02	—[d]	0.34 ± 0.02	2.61 ± 0.68	ND[e]
			2H1	21.4 ± 2.3	0.15 ± 0.02		0.34 ± 0.04	2.11 ± 0.26	ND
			P	0.908	0.499		0.241	0.124 (0.043)[f]	ND
J22A	1	2×10^5 (40)	PBS	19.7 ± 1.28	0.15 ± 0.01	5.80 ± 0.15	0.34 ± 0.03	5.17 ± 0.30	23.5 ± 6.6
			2H1	19.0 ± 1.02	0.15 ± 0.02	4.89 ± 0.41	0.33 ± 0.02	4.66 ± 0.30	0.20 ± 0.31
			P	0.2086	0.5001	0.0002	0.7088	0.0024	≤0.0001
24065	1	2×10^6 (73)	PBS	ND	ND	2.94 ± 0.28	ND	ND	10.8 ± 4.8
			2H1	ND	ND	2.21 ± 0.44	ND	ND	<1.0
			P	ND	ND	0.0007	ND	ND	≤0.0001

[a] Inoculum determined by hemocytometer counts. Number in parentheses is the percentage of viable cells in the inoculum as determined by CFU on Sabouraud dextrose agar. NA, not available. MAb 2H1 was administered 4 h before infection except for experiment 3 with strain 371 when it was given 24 h before infection. There were 10 mice in each group.
[b] *P* value calculated by Student's *t* test. Values are averages and standard deviations.
[c] *P* value calculated by Kruskal-Wallis test. Values are averages and standard deviations.
[d] At this inoculum, the lungs were not heavily infected with this strain. Our CFU assay had a level of detection of $>10^2$ CFU per organ. Of the 10 mice in the PBS and MAb 2H1 groups, 5 and 7, respectively, had no colonies in the lungs.
[e] ND, not determined.
[f] *P* value in parentheses is that obtained if Student's *t* test is used.

and lowest levels occurring in mice infected with strains J22A and SB4A, respectively.

DISCUSSION

Previous studies of MAb 2H1 efficacy against *C. neoformans* were limited to strain 24067 (35–41, 57) with the exception of a single experiment using the clinical isolate GH (38). Strain 24067 was originally chosen for study because all the MAbs in our collection were reactive with its capsular GXM, permitting comparisons of antibody efficacy. Furthermore, strain 24067 was a reference ATCC serotype D strain which had been used in several immunological studies (24–26, 30) and was well characterized. In this study, we tested the ability of MAb 2H1 to modify the course of murine i.v. infection for eight additional strains. MAb reacted with the capsular polysaccharide of all strains (11). Administration of MAb 2H1 prior to i.v. infection modified some aspects of the course of infection for all strains, but antibody efficacy varied greatly depending on the strain.

MAb 2H1 prolonged survival relative to controls for strains 24067, SB6A, J9A, J11A, J22A, and CN1 but not strains 371 and SB4A. For all strains studied (SB4A, SB6A, J22A, 371, and 24067), MAb 2H1 administration reduced brain and/or lung CFU in MAb-treated mice relative to those of controls. However, the inoculum at which MAb 2H1-mediated lung

TABLE 4. Summary of CFU data for strains SB4A and SB6A

Strain	Expt	Inoculum[a] (%)	Group	Body wt (g)[b]	Lung wt (g)[b]	Lung CFU[c] ($\log_{10}$)	Brain wt (g)[b]	Brain CFU[c] ($\log_{10}$)	Serum GXM (μg/ml)[b]
SB4A	1	2×10^5 (91)	PBS	18.1 ± 2.12	0.15 ± 0.01	5.22 ± 0.24	0.33 ± 0.04	3.31 ± 2.85	3.1 ± 2.8
			2H1	14.9 ± 1.32	0.14 ± 0.01	2.08 ± 2.69	0.30 ± 0.05	2.59 ± 2.73	<0.01
			P	0.0007	0.1498	0.0149	0.1475	0.1739	≤0.0001
	2	2×10^4 (NA)	PBS	17.9 ± 1.35	0.15 ± 0.01	4.40 ± 0.10	0.32 ± 0.03	3.56 ± 0.95	0.26 ± 0.25
			2H1	17.3 ± 1.01	0.12 ± 0.02	3.54 ± 0.50	0.26 ± 0.05	3.20 ± 0.42	0.03 ± 0.02
			P	0.3324	0.0009	0.0002	0.006	0.0342	0.011
SB6A	1	2×10^5 (80)	PBS	16.1 ± 1.72	0.14 ± 0.03	5.13 ± 0.27	0.33 ± 0.04	4.70 ± 0.19	2.42 ± 1.28
			2H1	15.8 ± 2.10	0.14 ± 0.02	5.01 ± 0.22	0.32 ± 0.04	4.28 ± 0.44	<1.0
			P	0.7303	0.4546	0.5180	0.8692	0.0471	0.002
	2	2×10^4 (83)	PBS	18.7 ± 1.23	0.16 ± 0.01	3.89 ± 0.18	0.35 ± 0.02	4.30 ± 0.13	0.31 ± 0.42
			2H1	19.3 ± 1.20	0.14 ± 0.02	3.19 ± 0.30	0.34 ± 0.02	3.96 ± 0.26	0.04 ± 0.01
			2D10γ1	18.2 ± 0.89	0.14 ± 0.02	3.05 ± 0.45	0.34 ± 0.01	3.36 ± 0.47	ND[d]
			P[e]	0.2840	0.0156	0.0002	0.1435	0.0003	0.0624
			P[f]	0.3424	0.0189	0.0002	0.1020	0.0002	ND

[a] Inoculum determined by hemocytometer counts. Number in parentheses is the percentage of viable cells in the inoculum as determined by CFU on Sabouraud dextrose agar. NA, not available. MAb 2H1 was administered 4 h before infection in all experiments. There were 10 mice in each group.
[b] *P* value calculated by Student's *t* test. Values are averages and standard deviations.
[c] *P* value calculated by Kruskal-Wallis test. Values are averages and standard deviations.
[d] ND, not determined.
[e] *P* for comparison of PBS and 2H1 groups.
[f] *P* for comparison of PBS and 2D10γ1 groups.

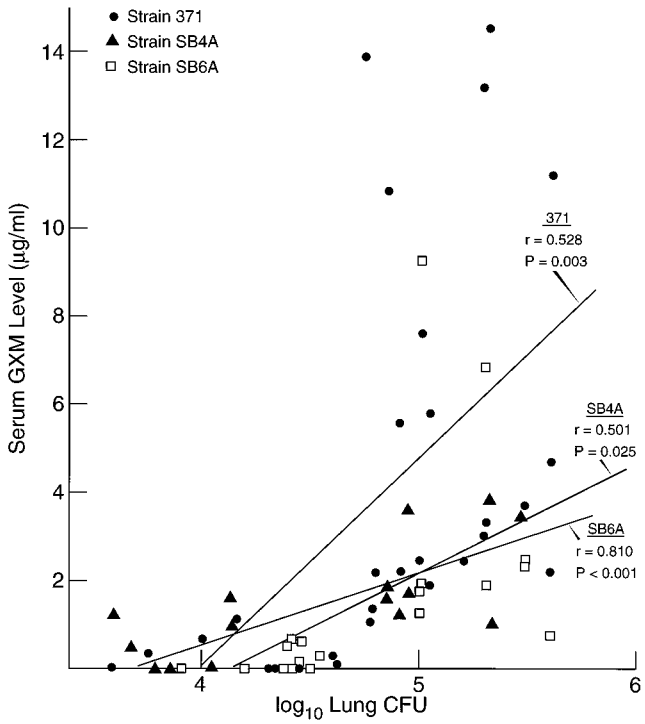

FIG. 2. Plot of serum GXM level versus $\log_{10}$ CFU for individual mice infected with 10^5 cells of either strain 371, SB4A, or SB6A at day 4 of infection. Each point corresponds to an individual mouse. All mice had serum GXM levels equal to or greater than 0.1 μg/ml, but many points appear as 0 because of the scale of the *y* axis. For each strain, *r* is the correlation coefficient calculated by linear regression analysis and *P* is the probability that serum GXM levels are correlated with lung CFU.

and/or brain CFU reductions were observed differed with the strain. At inocula of 2×10^5 yeast cells, reductions in CFU were observed in MAb 2H1-treated mice infected with strains J22A (lung and brain), SB4A (lung only), and SB6A (brain only) but not strain 371. At inocula of 2×10^4 cells, reductions in brain and lung CFU were observed in MAb 2H1-treated mice infected with strains SB4A and SB6A. For strain 371, a reduction in brain CFU, but not lung CFU, was observed for MAb 2H1-treated mice when the inoculum was reduced to 2×10^3 yeast cells per mouse. Reducing the strain 371 inoculum further to 2×10^2 cells did not increase the magnitude of the MAb 2H1 effect because at this very low inoculum there was significant scatter in the CFU data. MAb 2H1 administration resulted in a significant reduction in lung CFU for mice infected with 2×10^6 yeast cells of *C. neoformans* var. *gattii* 24065 (brain was not studied).

MAb 2H1 administration did not prolong the survival of mice infected with strains 371 and SB4A despite reducing organ CFU. The plot of survival time versus infecting inoculum for strains 371 and SB4A suggests a potential explanation for this result (Fig. 1). For both strains 371 and SB4A, it appears that a 10-fold reduction in the inoculum is necessary to provide a significant difference in survival. Assuming (i) that antibody efficacy mimics reductions of the inoculum by enhanced fungal clearance (see below) and (ii) that lung and brain CFU correlate with total fungal burden, our results suggest that MAb 2H1 could not increase survival for mice infected with strains 371 and SB4A because the magnitude of the CFU reductions was too small. For strains 371 and SB4A, it is possible that MAb-mediated prolongation in survival could be demonstrated at a very low inoculum (i.e., $<10^2$ cells per mouse). However, demonstration of statistically significant differences in experiments using very low inocula is likely to be difficult because not all mice are infected at low inocula and the larger variation in average survival would necessitate much larger sample sizes. In contrast to the results with strains 371 and SB4A, previous studies with strain 24067 have consistently demonstrated 10- to 1,000-fold organ CFU reductions in MAb-treated mice (37, 39–41). Sanford et al. (46) also described reduction in organ CFU without prolongation of survival for mice treated with another IgG1 MAb (46). Thus, the ability of a MAb to prolong survival in this murine model may depend on the magnitude of the organ CFU reduction. Organ CFU appear to be a more sensitive parameter for studying passive antibody efficacy in murine i.v. infection than survival.

Previous studies of passive antibody protection have shown reductions in lung but not brain CFU (41, 46). The inability of MAb to reduce brain CFU in i.v. infection has been interpreted as a reflection of poor penetration into the central nervous system or consumption by peripheral infection (41, 46). In this study, reductions in brain CFU were observed in MAb 2H1-treated mice infected with strains 371, SB4A, SB6A, and J22A at the lower inocula. Thus, MAb 2H1 can enhance clearance of the organism in the brain and/or reduce dissemination to the brain. Nevertheless, all mice studied had significant tissue infection despite high levels in serum of GXM-binding antibody. For example, mice given MAb 2H1 had IgG1 GXM-binding antibody levels of 100 to 300 μg/ml (Table 5), which is 10 to 50 times higher than the concentration required for opsonization of these strains in vitro (11). This suggests that the inability of MAb 2H1-treated mice to clear infection in this model is not due to lack of serum opsonins. However, serum and tissue opsonin concentrations may not be equivalent. *C. neoformans* collections in infected tissues are often surrounded by shed GXM antigen (17, 41), which may prevent antibody from reaching yeast cells once tissue infection is established.

There was a correlation between lung fungal burden and serum GXM levels at day 4 of infection in control mice, suggesting that serum GXM levels reflect CFU burden. There were differences between strains in serum GXM levels, suggesting differences in GXM production and/or clearance. Administration of MAb 2H1 reduced serum GXM levels for all strains. In some experiments (i.e., experiment 1 for strain 371), MAb 2H1-treated mice had little or no GXM in serum despite no significant reduction in lung or brain CFU, suggesting that antibody can reduce serum GXM levels independently of its effects on organ CFU. In rats given GXM followed by MAb 2H1, GXM is rapidly cleared by the liver and spleen (18).

TABLE 5. Levels of IgG1 to GXM in serum at day 4 of infection

Group[a]	Serum IgG1 to GXM (μg/ml)	$P < 0.05$ vs SB4A group?[b]
SB4A	157 ± 84	
371	200 ± 43	No
SB6A	337 ± 122	Yes
J22A	303 ± 116	Yes

[a] Groups correspond to SB4A experiment 1 in Table 4, 371 experiment 2 in Table 3, SB6A experiment 1 in Table 4, and J22A experiment 1 in Table 3. Serum was obtained at the time that the mice were killed for CFU determination. Values are averages and standard deviations.

[b] *P* values were calculated by Student's *t* test after a one-way analysis of variance revealed statistically significant differences between groups (<0.001) and pairwise multiple comparisons (Student-Newman-Keuls method) revealed significant differences for the comparison of SB4A versus SB6A and SB4A versus J22A but not SB4A versus 371. Analysis of variance and multiple comparisons were done with the SigmaStat program.

There were significant differences in serum GXM-binding IgG1 levels in mice infected with equal inocula of strains 371, J22A, SB4A, and SB6A. Serum GXM-binding IgG1 levels for strains 371 and SB4A were lower than those for strains J22A and SB6A (Table 5), suggesting that the half-life of MAb 2H1 in infected mice can differ depending on the *C. neoformans* strain.

The exact mechanism by which antibody modifies the course of *C. neoformans* infection in mice is uncertain. MAb 2H1 has been shown to enhance *C. neoformans* phagocytosis by macrophages and microglia (57), promote *C. neoformans* killing by murine J774.16 macrophages (42), increase nitric oxide production by J774.16 cells (34), and reduce serum and tissue GXM concentrations (41). Antibody can enhance NK cell activity against *C. neoformans* (32). The relative contribution of these mechanisms to prolongation in survival and reduction in tissue CFU is unknown. Similarly, the mechanism by which some strains are more resistant to MAb 2H1 is unknown and is the subject of ongoing studies. Because of the relatively small number of strains studied here, we cannot correlate antibody efficacy with strain phenotypes such as serotype classification. Strain-specific factors such as protease production (2), complement activation (55), and resistance to intracellular killing could contribute to variable MAb 2H1 efficacy. The relative ineffectiveness of MAb 2H1 against strain 371 is interesting given that MAb 2H1 was generated from mice immunized with a conjugate vaccine of strain 371 GXM linked to tetanus toxoid (4). Strain 371 has the highest agglutination endpoint of all the strains, suggesting fewer MAb 2H1 binding sites per capsule (11). It is noteworthy that another murine IgG1 MAb (3E5) has prolonged survival of A/J mice given lethal inocula of a mouse-passaged isolate of strain 371 (56). MAbs 2H1 and 3E5 share the same idiotype and variable region structure but differ in several amino acid residues (35). The differences in the efficacy of MAbs 3E5 and 2H1 against strain 371 may reflect subtle differences in MAb specificity or affinity or differences in the mouse-passaged 371 isolate (36).

Strain type and inoculum size appear to be important variables in studies of antibody efficacy in addition to antibody quantity (13), isotype (38, 46, 49), specificity (36), and animal model (20). Differences in strain susceptibility to antibody immunity could have contributed to the contradictory observations that antibody prolonged survival in some studies (13, 15, 20) but not others (19, 31, 46). The relevance of our findings to ongoing efforts to develop antibodies for prevention and therapy of human infection is unclear. The murine i.v. infection model is an artificial system in which a large inoculum is administered via a nonphysiological infection route. Significant differences between murine and human immune systems (i.e., nitric oxide [43], defensins [29], etc.) indicate caution in extrapolating results of murine studies to humans. Nevertheless, our findings suggest that variation in the efficacy of antibody-based vaccines or passive antibody reagents may occur depending on the strain. The results illustrate the complexity of antibody immunity against *C. neoformans* and suggest that studies of all aspects of *C. neoformans* immunity should include multiple strains.

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