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Lung Weight Parallels Disease Severity in Experimental Coccidioidomycosis[1]

M. HUPPERT,* S. H. SUN, I. GLEASON-JORDAN, AND K. R. VUKOVICH

Veterans Administration Hospital, Long Beach, California 90822, and University of California, Irvine, California 92664*

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Evidence provided by histopathological study of lesions is a valuable adjunct for evaluating chemotherapeutic efficacy in experimental animal models. In addition, this should be correlated with a measure of disease severity in the same animal. The latter could be obtained by homogenization of infected organs and quantitative enumeration of viable cells of the etiological agent, but this would preclude histopathological studies in the same animal. Progression of disease in pulmonary infection is associated with replacement of air space by fluid, cells, and cellular debris. Therefore, an increase in lung weight should reflect severity of disease. Results with the murine model of coccidioidomycosis demonstrate that increasing lung weight parallels the increasing census of fungus cells in the lungs of both treated and nontreated infected mice. This was supported with evidence obtained from microscopic studies of lesions indicating that specific chemotherapy limited spread of the infection and inhibited multiplication of the fungus in the lung. Therefore, lung weight can be used as a measure of disease severity in the murine model of coccidioidomycosis.

In vitro studies of the antifungal activity of combined drugs have demonstrated that polyenes can act synergistically with certain other antibiotics (8, 9, 14, 15). A similar effect occurred in vivo in the experimental murine model of coccidioidomycosis when the animals were treated with amphotericin B and tetracycline (7). In the latter report, antibiotic therapy was limited to a short period (5 to 9 days postinfection) so that drug effects could be measured before detectable immunity, which should occur at approximately 30 days postinfection (10-12). Although the results showed that combined drug therapy produced statistically significant increases in survival time and total survivors, it was apparent also that mortality rates increased after cessation of therapy and that the anticipated immunity response was not prominent through 40 days postinfection. One possible explanation for these results is that the antibiotics, either singly or in combination, interfered with the complete development of an effective immune response.

An important consideration for such studies would be to learn what is occurring in these lesions and to relate this to the severity of the disease in the same animal. The latter can be measured as the number of viable fungus cells in the tissue, but homogenization and quantitative culture of the tissue would obviate study of lesion histopathology in the same animal. Since coccidioidomycosis is initiated as a pulmonary infection, we considered that evolution of the disease in the lungs involved progressive replacement of air space with fluid, cells, and cellular debris. Therefore, increasing severity of the disease should be accompanied by an increase in the weight of the lung. The following experiments were designed to test this hypothesis. The results show that changes in lung weight do serve as an objective measurement of the severity of pulmonary disease.

MATERIALS AND METHODS

Animals. Male mice (dba strain) were purchased from Jackson Laboratory, Bar Harbor, Me. On arrival, mice were weighed, distributed into cages at random, and quarantined for at least 2 weeks before use in experiments. Weighings were repeated at weekly intervals. Food and water were available ad libitum at all times.

Chemicals. Amphotericin B (Fungizone intravenous, amphotericin B for injection, U.S.P.) was obtained from E.R. Squibb and Sons. The antibiotic was diluted for use with sterile 5% (wt/vol) aqueous glucose solution.

Culture and infection inoculum. A single strain of *Coccidioides immitis* (C-34) was used. Cultures were grown at room temperature for 1 to 2 months on a medium containing 1% glucose, 0.5% yeast extract, and 1.5% agar (4). Arthrospores were harvested with minimal amounts of sterile saline by the spinning-bar technique described earlier (6). Visual

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arthrospore counts were made with a hemocytometer, the suspensions were adjusted to contain the desired number of arthrospores per milliliter, and counts of viable cells were obtained by quantitative dilution and plating.

Design of experiments. The protocol for the murine model of coccidioidomycosis was similar to that reported earlier (7). Mice, weighing within ±2 standard deviations (SD) of the average for the entire population (25.7 ± 2.5 g), were anesthetized lightly (sodium pentobarbital, 54 mg/kg) and were infected intranasally by inhalation of a measured drop (0.02 ml) of the spore suspension. Control mice (sham infection) received 0.02 ml of sterile saline or heat-killed arthrospores (autoclaved). The infected mice were distributed at random into numbered cages (five per cage), and the cages were assigned to experimental groups according to a table of random numbers.

Four conditions were used to test the hypothesis that lung weight paralleled the severity of infection. One group of mice was infected with 1,000 arthrospores (average viable count = 970), and a second group was infected with 200 spores (average viable count = 226). Some of the animals within each group were injected intraperitoneally once daily with 0.01 mg of amphotericin B in 0.5 ml (0.4 mg/kg) for 5 days beginning on day 5 postinfection. The remaining infected mice within each group were treated similarly with the 5% glucose solution instead of amphotericin B.

As animals died or were sacrificed at intervals, lungs were removed for weighing and for culturing or histopathology. The lungs were obtained after quickly removing the heart and lung as a package and dissecting away the heart. Representative lungs were placed in neutral buffered formalin, sections were cut at 5-μm thickness, and serial sections were stained by the following methods: hematoxylin-eosin, Gomori methenamine silver nitrate, Gridley, Masson trichrom, and reticulum stains. The selection of lungs for histopathology was based on lung weights approximating the average for the group at each time interval. The lung weights of organs used for histopathology were not included in Tables 3, 4, and 5. Lungs from all other infected mice were weighed and homogenized with tissue grinders, and colony counts were obtained by quantitative dilutions and serial plating on Mycosel agar (Baltimore Biological Laboratories).

Statistical evaluation of differences in lung weights among various groups was done by the Student *t* test (Monroe computer program 3011N). Correlation of lung weight with count of viable fungus cells from homogenized lungs was performed by linear regression analysis with a computer (Monroe computer program 4022N)

RESULTS

Since the hypothesis postulated that lungs from infected animals would increase in weight during the course of the infection, it was necessary to sacrifice sham-infected mice at intervals to obtain lung weight determinations from non-infected animals as a reference point. These are presented in Table 1, representing combined data from several experiments. Several points should be noted. The average weights and measures of variability were almost constant throughout the 40-day period. Nevertheless there were differences, particularly on days 30 and 40, and therefore the weight of the lung from each animal was converted to a lung weight index (LWI) by dividing the weight of the lung by the average lung weight for sham-infected mice at the same time period. This method was used earlier by Youmans and Youmans (19) and served two purposes. The LWI measured the individual lung weights as units of average weight for the sample control population at a specified postinfection time, and therefore the LWI at different time intervals could be compared in terms of equivalent units. This becomes obvious in Table 1, where the average LWI for sham-infected mice at each time interval equals unity. Second, since variability for each average is both low and quite uniform, one can estimate the significance of any LWI for infected animals in the following manner. Since 1.96 times ± SD defines the limits which include 95% of the population, one would expect that only 5% of a normal population would lie outside these limits; i.e., 2.5% would be greater and 2.5% would be smaller. Furthermore, since our hypothesis predicts that the LWI for infected animals will be greater than that for sham-infected animals, we are concerned only with the higher limit. Therefore, we could state that an LWI greater than the mean ($\bar{X}$) plus 1.96 SD would be expected by chance only 2.5 times among 100 normal animals. Similarly, the 99% confidence limit would be the $\bar{X}$ + 2.58 SD, and an LWI

TABLE 1. *Average weight of lungs and average lung weight index (LWI) for mice receiving 0.02 ml of saline intranasally (noninfected controls)*

Days postinfection	No. of mice	Lung wt (mg)[a]			LWI[b]		
		$\bar{X}$	±SD	±SE	$\bar{X}$	±SD	±SE
0	21	163	18	4	1.01	0.10	0.02
5	15	170	27	7	1.00	0.16	0.04
7	18	162	25	6	1.00	0.15	0.03
9	19	180	26	6	1.00	0.15	0.03
12	8	170	20	7	1.00	0.12	0.04
14	13	168	18	5	1.00	0.11	0.03
16	15	167	23	6	1.00	0.14	0.04
20	8	159	14	5	1.00	0.09	0.03
30	15	190	27	7	1.00	0.15	0.04
40	17	215	18	4	1.00	0.08	0.02
All	149	176	27	2	1.00	0.11	0.01

[a] SD, Standard deviation; SE, standard error of the mean = SD $\sqrt{n}$; $\bar{X}$, mean.

[b] LWI = lung weight/mean lung weight.

greater than this would be expected by chance only 0.5 times among 100 normal animals. Therefore, for all of the sham-infected mice, the upper limit of LWI for the $\bar{X}$ + 1.96 SD = 1.22 and for the $\bar{X}$ + 2.58 SD = 1.28.

The effect that tissue response to killed spores as inert particles might contribute to increasing lung weights had to be considered. None was found (Table 2). Although we believe that there must be some tissue response to these killed spores, apparently the number of spores is too small in this model for any increase in weight to be detectable.

Since most of the infected mice dying during the experimental period did so at night, the question had to be considered whether there was a significant increase in lung weight during the postmortem period before necropsy the following morning. Twenty infected mice were sacrificed (nembutal euthanasia) on postinfection day 12. Ten were necropsied immediately, and the remaining 10 mice remained in their cages until the following morning, 16 h after death. The results (Table 3) demonstrated that there were no significant differences among these two groups with respect to lung weights or viable count from homogenized total lung. It was also obvious that the LWI for each animal was significant with respect to sham-infected mice (see Table 1). It was concluded, therefore, that there was no significant increase in lung weight or number of viable fungi attributable to a postmortem delay of as long as 16 h before necropsy could be performed.

The principal question asked in this study was whether the lung weights of infected animals would parallel the severity of disease. The viable count ($\log_{10}$) from homogenized entire lung was chosen as the measure of disease severity, and this was compared with the weight of the lung (as the LWI) before homogenization (Fig. 1). Each mark in these figures represents one animal of a group, some of which had been sacrificed and the remainder had died during a 40-day postinfection period. The correlation coefficients (r) were very high (Table 4): 1/ for each of the four conditions (A, B, C, D) whether the sacrificed and dying animals were considered separately or together; 2/ when mice were combined regardless of treatment (A + B, C + D); and 3/ when all mice were combined, including different doses of arthrospores for infection, treated or not treated, and sacrificed or dying (A + B + C + D). Even though the mice that died tended to higher viable counts and greater LWI than those that were sacrificed, it is apparent that the strong correlation of viable count with LWI was maintained for both groups. We concluded, therefore, that lung weight (expressed as the LWI) was a valid measure of disease severity in this murine model of coccidioidomycosis.

Since the LWI could be considered a measure of the severity of the disease, and since the strong correlation existed regardless of whether the disease was treated or not, we considered the question whether the LWI could be used as the measure by which the efficacy of antifungal therapy could be evaluated. The identical LWI values (LWI = 1) for infected and noninfected mice on day 0 (Fig. 2) indicated that inhalation of a suspension of as many as 1,000 arthrospores was no different than inhalation of saline with respect to lung weights, and therefore the infected and noninfected animals could be considered as drawn from the same population with respect to lung weight on day 0. It was apparent that the curve for infected mice

TABLE 2. *Average LWI for mice receiving 0.02 ml of the heat-killed arthrospore suspension*

Days postinfection	No. of mice	LWI[a]		
		$\bar{X}$	±SD	±SE
0	5	1.00	0.07	0.03
5	5	1.00	0.13	0.06
7	5	1.00	0.07	0.03
9	5	1.00	0.16	0.07
14	5	1.00	0.09	0.04
16	5	1.00	0.06	0.03
20	5	1.00	0.09	0.04

[a] See footnotes *a* and *b*, Table 1.

TABLE 3. *Viable count (total lung) and lung weight index (LWI) for 20 mice sacrificed on postinfection day 12, with 10 mice necropsied on day 12 and 10 mice necropsied on day 12 + 1*

Viable count ($\log_{10}$)		LWI	
Day 12	Day 12 + 1	Day 12	Day 12 + 1
6.83	6.84	2.04	2.82
8.15	7.15	3.39	3.32
8.23	7.04	3.80	3.29
8.00	8.28	2.59	3.96
8.11	8.15	3.16	3.43
8.11	8.23	3.14	3.57
8.87	7.00	4.65	3.09
6.86	8.20	2.51	3.52
8.04	8.18	3.12	3.43
8.20	6.92	3.75	3.05
$\bar{X}$[a] 7.94	7.60	3.22	3.35
SD ±0.63	±0.65	±0.75	±0.32
SE ±0.20	±0.21	±0.24	±0.10
t[b] = 1.20; $P > 0.20$		$t = 0.52$; $P > 0.20$	

[a] $\bar{X}$, Average; SD, standard deviation; SE, standard error.

[b] t, Student t test; P, probability.

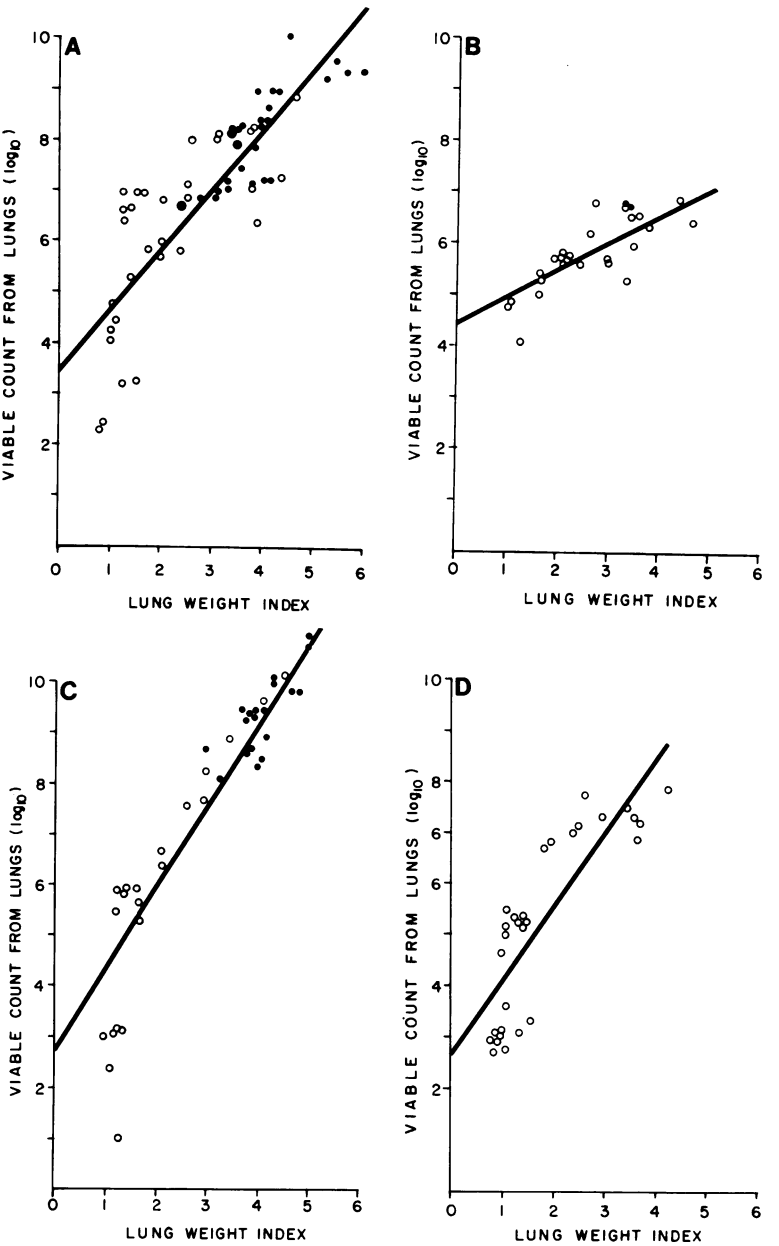

FIG. 1. *Correlations of viable count from homogenized lung (log_{10}) with lung weight index. (A) Infection with 1,000 arthrospores, treated with glucose solution intraperitoneally (i.p.); (B) infection with 1,000 arthrospores, treated with 0.4 mg of amphotericin B per kg i.p.; (C) infection with 200 arthrospores, treated with glucose solution i.p.; (D) infection with 200 arthrospores, treated with 0.4 mg of amphotericin B per kg. Symbols: (○) Necropsy after sacrifice; (●) necropsy after death. Linear regression curve computed for all animals.*

treated with amphotericin B differed from that for infected mice treated with placebo, and that both differed from the constant LWI for both groups of noninfected animals. During the critical period from day 5 (initiation of therapy) to day 16 (one week post-therapy), LWI values for amphotericin B-treated mice differed significantly from those for placebo-treated mice. This was apparent by day 7 for animals infected with 200 arthrospores ($P < 0.01$) but was delayed

TABLE 4. *Correlation of lung weight index with viable count (log_{10}) from homogenized entire lungs of infected mice*

Group	Infection (no. of arthrospores)	Treatment (0.5 ml, i.p.)	Necropsy after:	No. of mice	Linear regression[a]			Significance	
					r	*m*	*b*	*t*	*P*
A	1,000	Glucose solution	Sacrifice	35	0.85	1.34	3.03	7.59	<0.001
			Death	28	0.76	0.89	4.57	5.94	<0.001
			Combined	63	0.83	1.18	3.45	12.21	<0.001
B	1,000	Amphotericin B[b]	Sacrifice	25	0.77	0.50	4.47	5.98	<0.001
			Death	2					
			Combined	27	0.77	0.52	4.44	6.22	<0.001
A + B	1,000	Glucose or amphotericin B	Sacrifice	60	0.72	0.92	3.72	7.44	<0.001
			Death	30	0.77	0.95	4.29	6.34	<0.001
			Combined	90	0.81	1.06	3.54	12.17	<0.001
C	200	Glucose solution	Sacrifice	21	0.86	1.86	2.30	6.49	<0.001
			Death	19	0.80	1.20	4.48	5.41	<0.001
			Combined	40	0.92	1.65	2.70	13.31	<0.001
D	200	Amphotericin B	Sacrifice	30	0.83	1.44	2.60	7.93	<0.001
			Death	0					
			Combined	30	0.83	1.44	2.60	7.93	<0.001
C + D	200	Glucose or amphotericin B	Sacrifice	51	0.84	1.64	2.44	10.43	<0.001
			Death	19	0.80	1.20	4.48	5.41	<0.001
			Combined	70	0.92	1.69	2.39	18.38	<0.001
(A+B + C+D)	1,000 or 200	Glucose or amphotericin B	Sacrifice	111	0.77	1.21	3.11	12.07	<0.001
			Death	49	0.71	1.10	4.13	6.94	<0.001
			Combined	160	0.85	1.34	2.94	19.69	<0.001

[a] *r*, Correlation coefficient; *m*, slope; *b*, intercept in the regression equation.
[b] Amphotericin B treatment = 0.4 mg/kg, single daily dose, postinfection days 5 to 9.

until day 9 for mice infected with 1,000 arthrospores (day 7, $P > 0.10$; day 9, $P < 0.005$), probably reflecting the much heavier infection load before antifungal therapy was measurably effective. After day 16 there were relatively few survivors among placebo-treated infected mice, and both the range of LWI values and the recovery of viable fungi from homogenized lungs varied considerably (Table 5). In contrast, among amphotericin B-treated mice, although the average LWI values remained relatively constant during the second postinfection week, there was a significant increase in these values from day 16 to day 20 ($P < 0.025$ for mice infected with 1,000 arthrospores and $P < 0.01$ for mice infected with 200 arthrospores). This pattern of containment of infection by specific antifungal therapy during the second postinfection week followed by increasing severity of disease during succeeding periods was very similar to that reported in earlier studies for cumulative mortality rates (7). It seems probable, therefore, that the average LWI could be used for evaluating the efficacy of antifungal therapy, especially during the 9- to 16-day postinfection period.

The histology of lesions in the lungs supported the above evidence that lung weight measured severity of disease. At 5 days postinfection (Fig. 3A), lung sections from placebo-treated mice showed an intense patchy bronchopneumonia with focal abscesses composed of polymorphonuclear (PMN) leucocytes. There was associated hemorrhage and edema. During succeeding time intervals, the pathology progressed to an extensive bronchopneumonia with focal parenchymal necroses, abscess formation, hemorrhage, and edema. Eventually this became an extensive, confluent, necrotizing bronchopneumonia, with the host cellular response consisting primarily of PMN leucocytes and approximately 15% mononuclear (MN) cells (Fig. 4A). The mixed PMN and MN response was present also in alveoli adjacent to lesions. No identifiable giant cells were evident. Mature endosporulating spherules were present at postinfection day 5, and some of these spherules had ruptured and dispersed endospores into air sacs and bronchi (Fig. 3B). Therefore, the spherule-endospore cycle was well established by day 5, and it became progressively more numerous and widespread with time (Fig. 4B). This was comparable to the census of viable fungus cells.

The host cellular response in lung tissue from mice treated with amphotericin B did not differ from that in the placebo-treated group until after the first postinfection week. The effect of antibiotic treatment was apparent by day 10 (Fig. 5). Only a few small necrotic foci were evident, and the alveolar wall integrity was well maintained. The PMN and MN cell re-

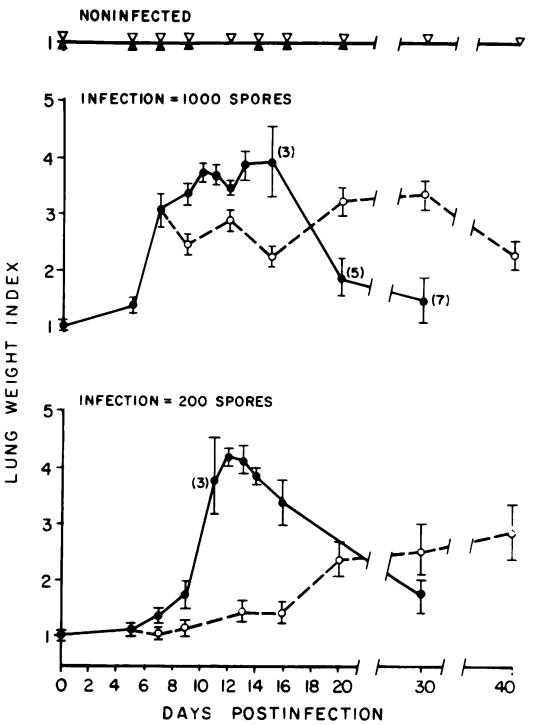

FIG. 2. *Changes with time in average lung weight index. Symbols: (△) noninfected controls, saline intranasally (i.n.) (10 mice per indicated day; standard error range, ±0.02 to ±0.04); (▲) noninfected controls, heat-killed arthrospores i.n. (5 mice per indicated day; standard error range, ±0.03 to ±0.07); (●) treated with glucose solution i.p.; (○) treated with 0.4 mg of amphotericin B per kg i.p.; (I) standard error of the mean. For mice infected with 1,000 arthrospores, each point is the average of 10 to 15 mice except where indicated in parentheses. For mice infected with 200 arthrospores, each point is the average of 5 to 7 mice except where indicated in parentheses.*

sponses were 50% greater and less diffuse than that seen in tissues from the placebo-treated mice at a comparable time. Spherules were numerous but mostly immature, with only a few endosporulating spherules identified (compare Fig. 4B and 5B). By day 15, a definite granulomatous bronchopneumonia was evident in the treated animals. Most of the lesions were miliary in size, and few foci of necroses were present. New collagen deposits were focally prominent throughout the infected areas of parenchyma. A granulomatous pleuritis with focal necrosis was also evident. Accumulations of plasma cells and lymphocytes were present about granulomata, and a few giant cells were seen in alveoli at the periphery of granulomata containing spherules. The spherules were still predominantly immature, but had spread widely and were present in alveoli and bronchi adjacent to lesions. By day 20 (Fig. 6), the granulomata had become confluent and fully developed and involved the pleura. Focal necroses and abscesses were present within these lesions, indicating continuing activity. A plasma cell and lymphocyte cell response was present in small numbers, particularly about lobular septa and blood vessels. An MN response was evident in the necrotic granulomata and in adjacent air sacs, but giant cells were inconspicuous. The lung parenchyma surrounding the lesions revealed moderate congestion and edema. Immature spherules, about 36 per high dry field in areas of greatest concentration, had disseminated from purulent lesions into adjacent alveoli and bronchi, but to a lesser extent than at 15 days postinfection. These were accompanied by a fresh PMN response, indicating a spreading infection. Additional changes through day 40 were not notable except for the following. Spherules, still predominantly immature, were markedly reduced in number, i.e., to about 20% of the number found on day 20. Granulomata in one of the two animals sacrificed on day 40 were without obvious necrosis, although the lung weight was high (LWI = 2.69), indicating that the infection had been as severe as in other mice. In both mice, collagen production was pronounced in old lesions but occasional new lesions were still present, as evidenced by clusters of PMN cells in alveoli immediately adjacent to granulomata and by young granulomata with primarily epithelioid response and no collagen production.

TABLE 5. *Lung weight index (LWI) values for placebo-treated infected mice sacrificed on postinfection days 20 and 30*

Infection (arthrospores)	Sacrificed on postinfection day: 20 LWI	20 Viable count (log_{10})	30 LWI	30 Viable count (log_{10})
1,000	0.82	0	0.82	2.30
	0.89	0	0.84	2.41
	1.10	4.45	1.01	4.23
	2.35	5.81	1.15	0
	4.18	7.21	1.32	0
			1.41	0
200	ND[a]	ND	3.89	6.38
			1.11	0
			1.23	1.00
			1.62	5.63
			2.92	7.65
			2.97	8.23

[a] ND, Not done.

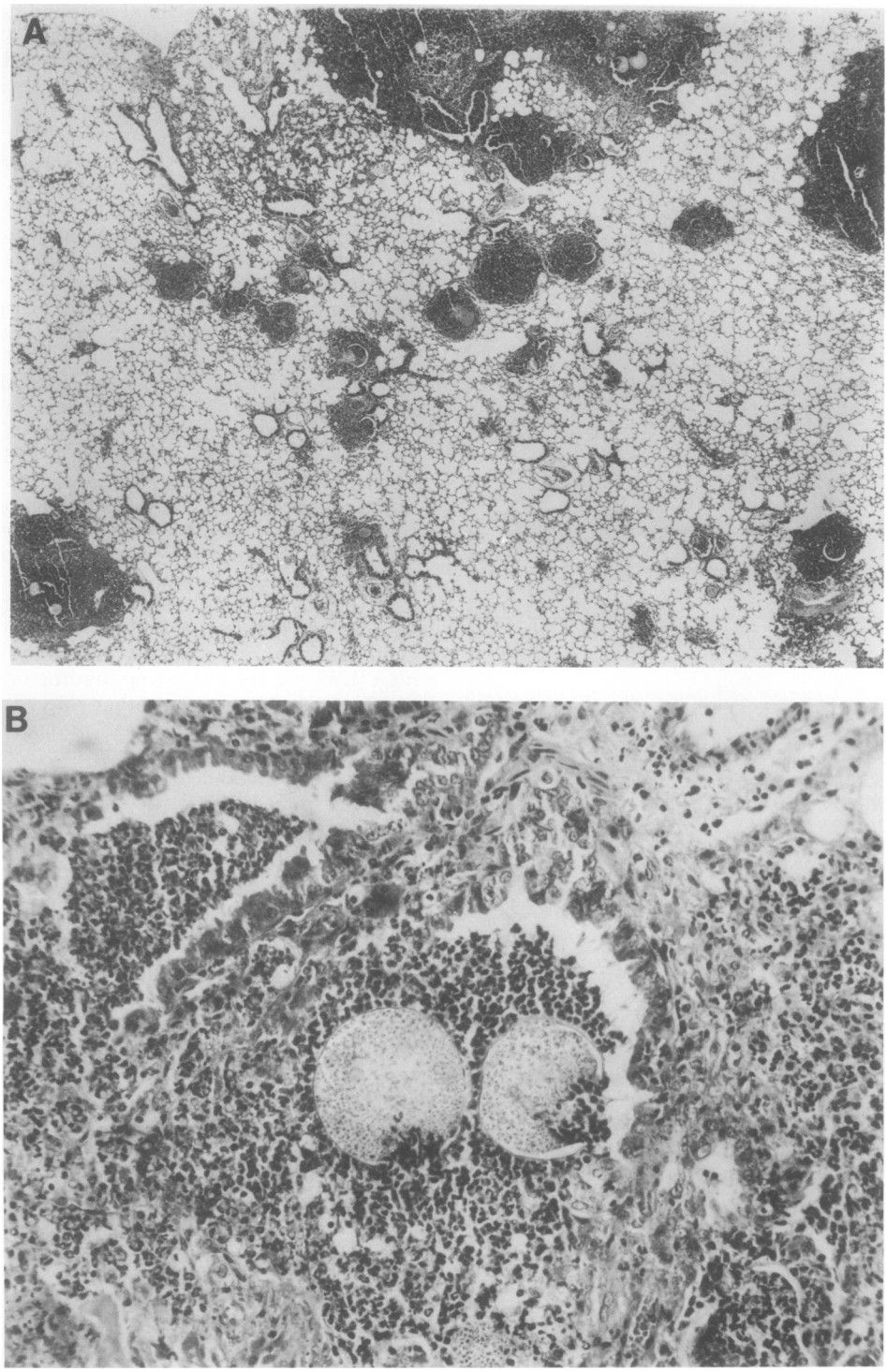

FIG. 3. *Tissue section from lung of placebo-treated infected mouse at 5 days postinfection showing patchy bronchopneumonia and mature endosporulating spherules in air sacs and bronchi. (A) ×40 magnification; (B) ×200 magnification.*

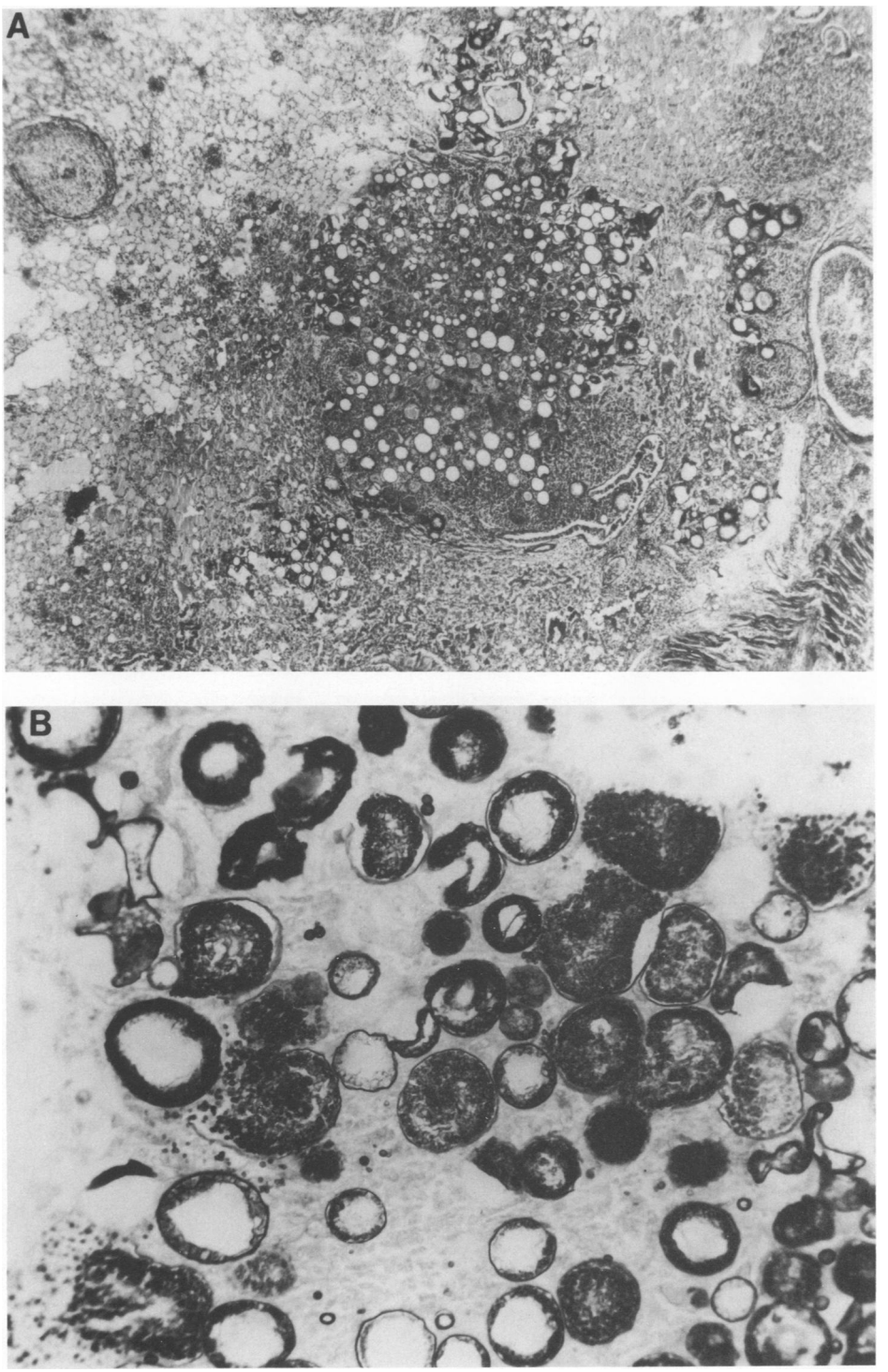

FIG. 4. *Tissue section from lung of placebo-treated infected mouse at 10 days postinfection showing confluent bronchopneumonia and numerous endosporulating spherules. (A) ×40 magnification; (B) ×200 magnification.*

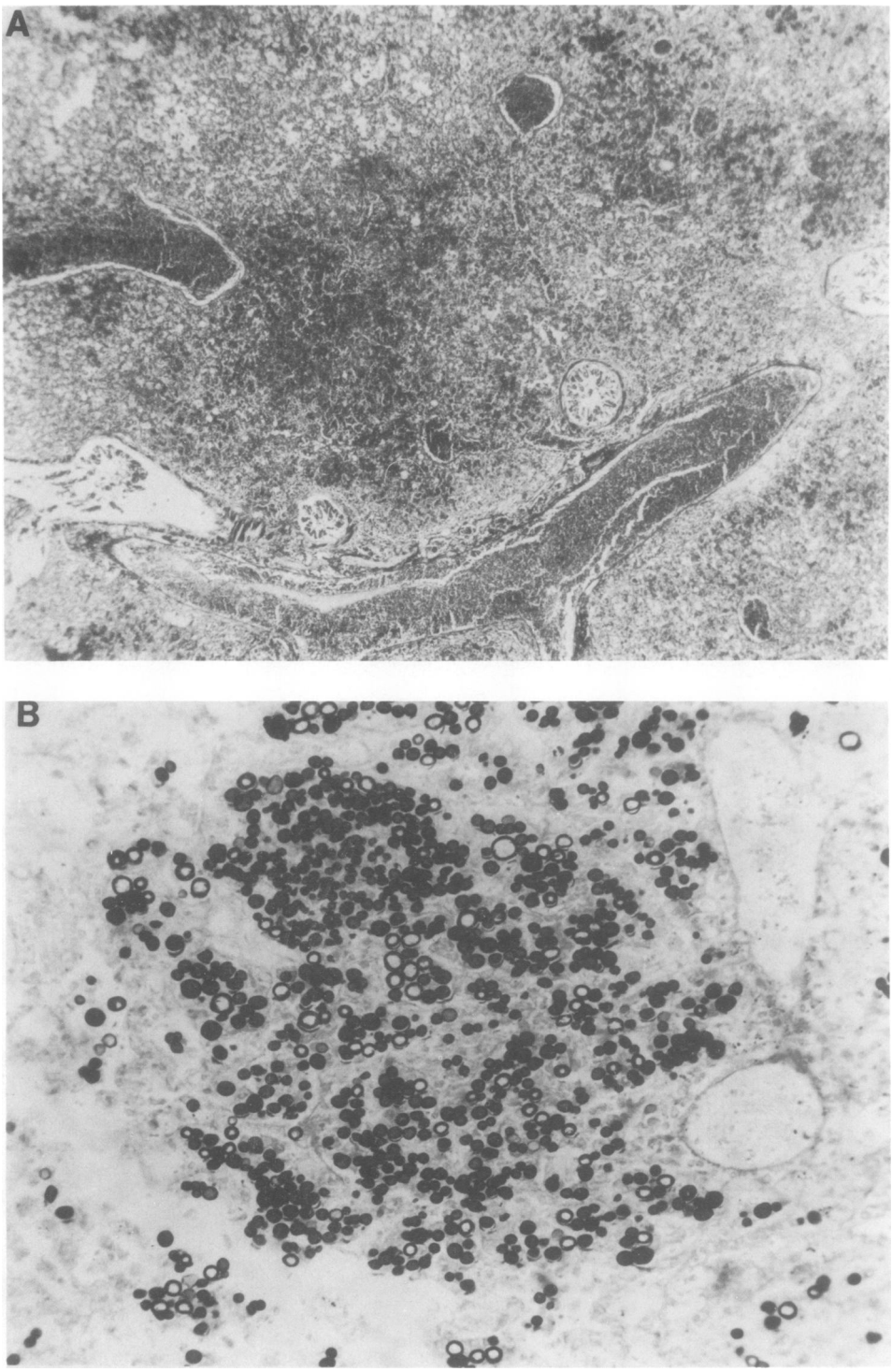

FIG. 5. *Tissue section from lung of amphotericin B-treated infected mouse at 10 days postinfection showing necrotic foci, confluent granulomata, and immature spherules: (A) ×40 magnification; (B) ×200 magnification.*

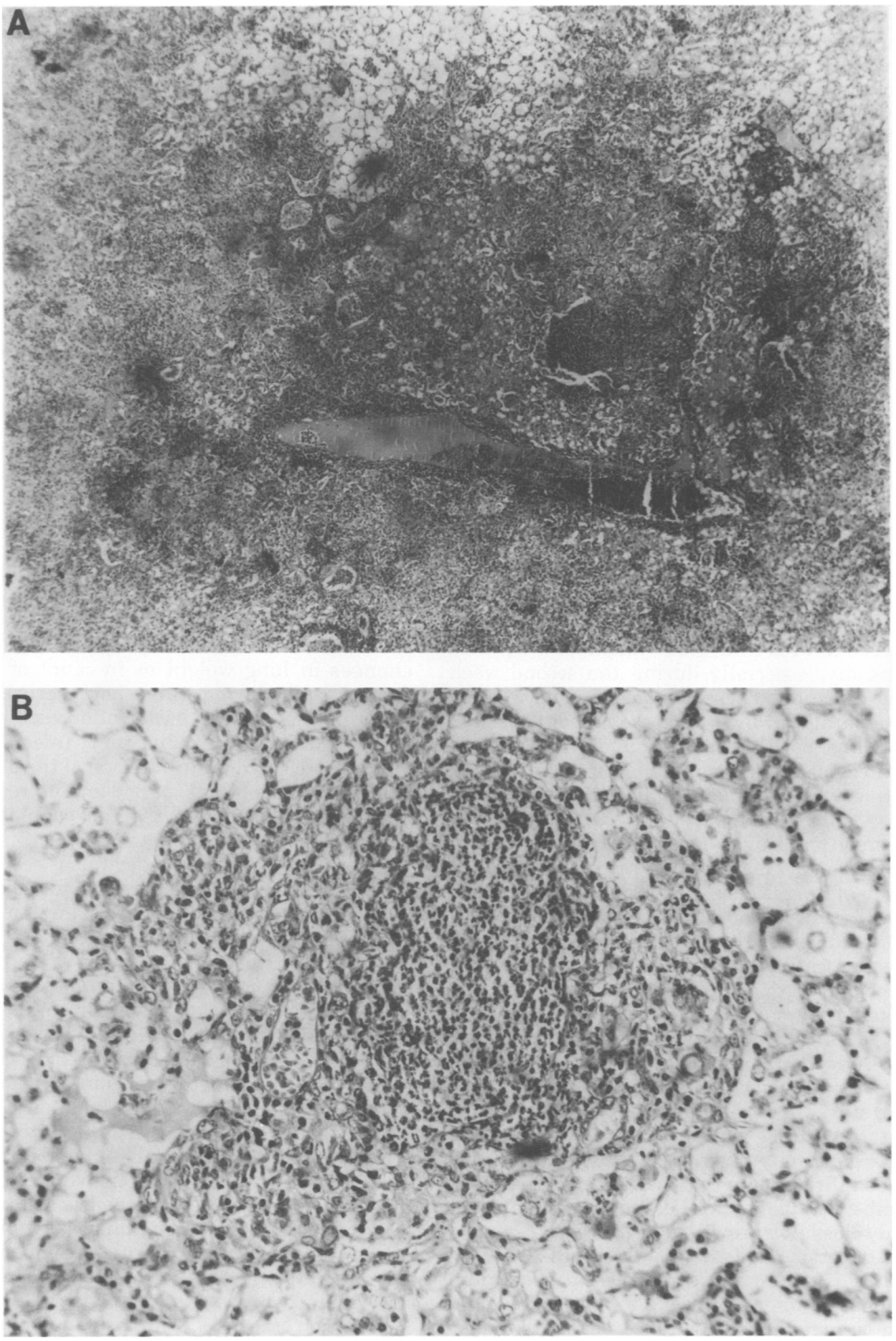

FIG. 6. *Tissue section from lung of amphotericin B-treated infected mouse at 20 days postinfection showing confluent granulomas, focal necroses, and abscesses with satellite lesions. (A) ×40 magnification; (B) ×200 magnification.*

Since the number of spherules was reduced markedly, some of the fresh PMN response in alveoli may have been induced by a nonspecific pneumonia secondary to the granulomatous process. The MN response was still moderate within and at the periphery of granulomata. The giant-cell response was still inconspicuous and in air sacs adjacent to granulomata containing an occasional spherule. Plasma cells and lymphocytes were present within granulomata and around bronchi, septa, and blood vessels. It was apparent, therefore, that lung air space had been replaced with solid lesions.

DISCUSSION

The principal question posed for this study was whether increasing lung weights would reflect severity of disease. The results demonstrated that total lung weight did increase with pulmonary infection, and there was evidence that the lung weights could be used as an index of disease severity. The weight of lungs from noninfected animals, expressed as the LWI, remained consistent throughout the experimental period, whereas the weight of lungs from placebo-treated infected animals increased markedly, especially during the second week after infection. These changes in lung weights were paralleled by an increasing population of fungus cells within the lung and by progressive pathological changes in lung parenchyma, resulting in replacement of air space with fluid, cells, and cellular debris. Treatment with an effective antifungal agent interrupted the rising curve for lung weights, restricted multiplication of fungus cells, and reduced lung pathology. It was apparent, therefore, that the weight of the lungs in this experimental pulmonary infection correlated with the number of viable pathogenic organisms in the lung and was reflected in damage to lung tissue. The question has to be considered whether allergic (hypersensitivity) inflammation contributed to the increase in lung weights, particularly during the later stages of the disease. This would be important since lung weight increase caused by a hypersensitivity response would not necessarily be reflected in a comparable increase in the number of viable fungus cells. The present study does not provide a definitive answer to this question, and the question will be investigated further. Nevertheless, maximum changes of lung weight and fungus cell population occurred in less than 2 weeks after infection. One would expect that specific hypersensitivity responses would be minimal during this period, although they might contribute significantly to lung weight at a later time. In support of this is the observation by Levine et al. (10), who performed footpad tests for delayed hypersensitivity among mice sensitized by subcutaneous infection with *C. immitis* and reported, "Cellular infiltrates were minimal in mice on the 18th day of infection but were pronounced on the 33rd day."

Since lungs were obtained from infected placebo-treated mice after death and from infected amphotericin B-treated mice after sacrifice, one must consider whether significant changes occurred during the period from death to necropsy (less than 15 h). This question was considered in the experiment reported in Table 3, in which one-half of the sacrificed mice were not necropsied until the following day. There were no significant differences among these two groups either in viable count from the lungs or in LWI. Furthermore, the high correlation coefficients for LWI values compared with fungus cell population of the lung indicated that if any fungus multiplication had occurred between death and necropsy, it was either consistent for all animals or not great enough to be significant. Therefore, we believe that the several hours' delay from death to necropsy among mice dying from infection did not result in significant changes in lung weight or in count of viable fungus cells during this short period. This might not be true for bacterial or viral experimental models of pulmonary infection, in which more rapid multiplication of the etiological agent during this period might result in significant increases in the cell population at necropsy.

The progressive pathological changes occurring in the lungs were similar to those described by other investigators for human (summarized in reference 3) and animal infections (1, 2, 5, 13, 16, 17). Initially there was an acute bronchopneumonia, with an intense PMN response and abscess formation. In animals not receiving specific chemotherapy, the spherule-endospore cycle was established early and became widespread, with dissemination into air sacs, bronchi, and probably lymphatic channels, although we did not see the latter in the present study. Ultimately these nontreated animals succumbed to an extensive necrotizing bronchopneumonia. The most significant difference among the treated animals was inhibition of the maturation of spherules (compare Fig. 4B and 5B) and, as a consequence, marked limitation of spread of the infection. This led to localization of lesions, with formation of granulomas and deposition of collagen. Nevertheless, the presence of PMN cells within granulomata and in foci at the periphery of lesions indicated that the infection was still both active and

spreading. The MN and giant-cell response was somewhat less than anticipated, and the possibility that this reflected an immunosuppressive effect by amphotericin B is to be explored in further studies. This possibility is supported in the report by Thomas et al., who found that amphotericin B increased mortality among mice infected with *Listeria monocytogenes* when the antibiotic was injected 24 h after infection (18).

The use of an index (LWI) to indicate the increase in weight of diseased lungs relative to that of lungs from noninfected animals is similar to the granulomatous index reported by Youmans and Youmans (19). They found a marked increase in lung weights of mice after intravenous injection of large numbers (1 to 5 mg) of either living or heat-killed *Mycobacterium tuberculosis* $H_{37}Ra$, and the response was proportional to the number of cells injected intravenously. A significant increase in granulomatous index was found in 1 day after the 5-mg dose and in 4 days after the 1-mg dose. The tissue response in the lungs was primarily granulomatous. In our animal model, however, a granulomatous response with deposition of collagen was not definite until postinfection day 15, and earlier lesions were primarily suppurative. For this reason we chose the term "lung weight index" as more descriptive and less restrictive than "granulomatous index." The reason for the different tissue responses in these two animal models probably lies in the experimental methods used. In the coccidioidomycosis model, we used comparatively small doses of viable fungus cells and infection via the respiratory route. In the tuberculosis model, large doses of viable cells were used (up to 2×10^8 viable particles [20]), and infection was by intravenous injection. In addition to these two factors, there may be a fundamental difference in the host response to these very different microorganisms.

A potential advantage to be gained from lung weight determination would be the use of this measurement for a quicker evaluation of animal models of pulmonary mycoses. For example, in our murine model of experimental coccidioidomycosis, highly significant differences were evident by days 9 through 16 when lung weights from placebo-treated infected mice were compared with those from amphotericin B-treated infected animals. New antifungal agents could be screened for potential efficacy by testing them at a maximum tolerated dose, sacrificing survivors at day 10 (or day 16), weighing lungs of dying and sacrificed mice, and evaluating the average LWI from treated and nontreated animals for statistical significance. If the difference proved significant, further study of the new drug would be justified. This approach would be more rapid as a screening test than determinations of 50% survival times or dose required to protect 50% of the animals, although the latter two determinations would be required for more precise evaluation of therapeutic efficacy. The same approach could be used for studies of immune responses.

In the past, we have used infection with 1,000 arthrospores for our murine model of coccidioidomycosis. The results illustrated in Fig. 2 indicate that infection with 200 arthrospores would produce a more sensitive model for detecting the efficacy of antifungal therapy. This will be investigated in future studies.

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