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Potentiation of Schistosome Granuloma Formation

By Lentinan—A T-Cell Adjuvant

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Lentinan is a fungal polysaccharide which acts as a T-cell adjuvant. When this glucan was administered to thymus-intact mice by intraperitoneal injection, conspicuously enlarged lung granulomas formed in response to either *Schistosoma mansoni* or *S japonicum* eggs or to antigen-coated polyacrylamide beads. Liver granulomas in cercaria-induced *S mansoni* infection were augmented up to eight-fold in volume. By contrast, nude mice showed a complete absence of hypersensitivity granulomas, regardless of whether they received lentinan. Lentinan-potentiated granulomas show a distinctive histopathologic picture characterized by abundant, large, pale-staining macrophages; reduced and redistributed eosinophil populations; and frequent, extensive central necrosis, uncommon in unpotentiated schistosome foci. They also differ in their distributions of egg antigen and of host immunoglobulins. Optimal lentinan effects followed a single 1-mg dose when given to sensitized mice on the day of intravenous challenge with *S mansoni* eggs rather than at the time of intraperitoneal sensitization or following challenge. This adjuvant appears to act on effector T cells or on macrophages interacting with T cells; its effect on macrophages in a latex bead foreign body granuloma was minimal. A number of other lentinan-associated systemic effects on parasite and host were noted and described, including reduced female schistosome egg output. (Am J Pathol 94:201–222, 1979)

GRANULOMATOUS TISSUE RESPONSES to infection contribute both to host defense and to host morbidity.[1] There is ample evidence that variant granuloma patterns in leprosy relate to host resistance and im-

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mune status[2]; yet, in most other infections, granuloma sizes or cell patterns are difficult to interpret.[3] During the chronic stage of murine schistosomiasis, when disease manifestations tend to decline, granuloma size is reduced, with lymphocyte responsiveness to soluble schistosome egg antigen (SEA) or mitogens.[4-8] Similar spontaneous declines in host responsiveness, varying in degree and selectivity, occur in patients with the chronic stages of filariasis,[9,10] trypanosomiasis,[11] syphilis,[12] and leprosy[13] and in some tuberculous individuals.[14,15] The roles of antigen–antibody complexes,[16] suppressor lymphocytes[17,18] or "adherent cells,"[19] or chemotaxis inactivators[20] in modulating granulomatous infections are under active scrutiny. Suppression of granulomas by lymphocytotoxic drugs and other procedures has also been well studied in experimental murine models of schistosomiasis.[21]

In contrast, little work has been done on their immunopotentiation, especially compared with the voluminous literature on the immunopotentiation of tumor and graft rejection.[22] Mycobacteria- or alum-containing adjuvants have long been known to elicit granulomas at their sites of deposition[23,24] but have not been reported to modify granulomas resulting from other antigens or materials. The systemic T-cell immunopotentiator levamisole showed little effect on schistosome granulomas[25]; parenteral lipopolysaccharide (LPS) induced hemorrhage, edema, and necrosis associated with mycobacterial granulomas.[26]

Since granulomatous hypersensitivity to *Schistosoma mansoni* eggs is thymic-dependent,[27-34] we have studied the effects on it of lentinan, a T-cell–oriented immunopotentiator.[35-37] *In vivo* administration of lentinan dramatically increased the size of schistosome granulomas and modified their morphology and cell composition. These cellular phenomena were clearly related to manipulation of the host T-cell reactivity, but their functional significance with respect to host resistance and morbidity remains to be clarified.

Materials and Methods

Lentinan

Lentinan is a $\beta(1,3)$ glucan, with molecular weight of approximately 1,000,000, purified from aqueous extracts of the edible Japanese mushroom *Lentinus edodes*.[38] A supply (lots No. 10063 and No. 731) was generously provided by Dr. Goro Chihara (National Cancer Center Research Institute, Tokyo). Lentinan aggregates were dispersed in sterile normal saline at a concentration of 1 mg/ml and were solubilized using a sonicator cell disruptor (Heat Systems–Ultrasonics, Plainview, NY). Mice received lentinan by intraperitoneal injection in doses of 250 μg, 500 μg, or 1 mg (Tables 1 through 8). Control mice were injected with an equivalent volume of sterile saline.

Animals

Male CBA J mice (Jackson Laboratory, Bar Harbor, Me), 6 to 8 weeks of age, were used for most experiments. Female Sch:Balb c BOM Cr (nu nu, nu nu) DF athymic nude mice and their corresponding heterozygotes (nu +), 5 to 6 weeks of age, were obtained from ARS Sprague-Dawley (Madison, Wis). Female outbred Spb:HA(ICR) mice from Spartan Research Animals (Haslett, Mich) were utilized as the egg source for the lung granuloma model.

Lung Granuloma Model (Tables 1, 2, and 6 through 8)

Schistosome eggs were isolated by a modification of the technique of Coker and von Lichtenberg [39] from the livers of egg source mice which had been infected by intraperitoneal injection 8 to 9 weeks previously with 125 cercariae of the Puerto Rican strain of *S mansoni* or 7 to 8 weeks previously with 30 to 35 cercariae of the Japanese strain of *S japonicum* (infected egg source mice supplied by the Museum of Zoology, University of Michigan, Ann Arbor, under contract with the U. S.–Japan Cooperative Medical Science Program, NIAID, NIH). Purified eggs were suspended in phosphate-buffered saline (PBS) at a concentration of 8000 eggs/ml, and 2000 eggs were injected into the mouse tail vein using a tuberculin syringe with a 12.5-mm, 26-gauge needle. Nonsensitized mice received only the primary intravenous egg injection. Sensitized mice received, in addition, an intraperitoneal injection of 2000 eggs at least 1 week prior to intravenous challenge.

For the bead experiments, styrene divinylbenzene (latex) beads 45.4 μ in diameter (Dow Diagnostics, Indianapolis) and Bio-Gel P-4 200 to 400 mesh polyacrylamide beads 37 to 73 μ in diameter (Bio-Rad Laboratories, Richmond, Calif) were utilized. Two hundred milligrams of the polyacrylamide beads were covalently linked to 20 mg of chromatographically pure bovine albumen (Cappel Laboratories, Cochranville, Pa) using EDAC (Bio-Rad), a carbodiimide reagent.[40,41] Two thousand beads suspended in 0.25 ml PBS were injected intravenously into each mouse.

Infections (Tables 3, 4, and 6)

To determine the effect of lentinan on liver granulomas, mice were infected percutaneously by the ring technique [42] with *S mansoni* cercariae (Puerto Rican strain) shed from infected *Biomphalaria glabrata* (supplied by the Museum of Zoology, University of Michigan, or Dr. Edward Michelson, Harvard School of Public Health).

Tissue Techniques

Mice utilized for the lung granuloma model were killed at the indicated times after intravenous challenge by cervical dislocation. The thoracic cavity was opened, and before removal the lungs were inflated with 1 ml of 10% phosphate-buffered formalin or Hollande's fluid. Necropsies were performed on infected mice 8 or 10 weeks after exposure, and all major organs were fixed in Hollande's fluid. Paraffin sections were cut at 4 μ and were stained with hematoxylin and eosin or by the Litt modification of the Dominici technique.[43] Lung and liver granulomas around single eggs and bead reactions were measured across two perpendicular diameters using an ocular micrometer, and the mean dimension was recorded.[44] Only eggs or beads showing a cellular response were measured. Significance probabilities for the difference in granuloma diameters between control and lentinan-treated groups were computed using the t statistic for two means.

Granulomas in frozen sections of control and lentinan-treated nu/+ mouse livers were stained by the indirect immunofluorescence technique for schistosome egg antigen (SEA) and mouse IgG as previously described.[34]

Tissue egg counts were made following digestion of small liver fragments in KOH.[45] Eggs were counted in liver sections following the matching of slides of control and

lentinan-treated mice having sections of approximately equal areas. Worm pairs were counted after the careful dissection of schistosomes from the mesenteric vessels and liver.

Results

In an initial pilot experiment, CBA/J mice were infected with the Puerto Rican strain of *S mansoni*. Half the mice were given three 250-μg doses of lentinan at 5-day intervals beginning at 4 weeks after exposure. Necropsies were performed on the treated and control mice at 6 weeks, and their liver granulomas were compared. Gross examination revealed that the granulomas in the lentinan-treated mice were larger and more opaque than those in controls and were irregular in shape. For more controlled and quantitative studies, the lung granuloma model was utilized.

Anamnestic Lung Granulomas

CBA/J mice were sensitized intraperitoneally with *S mansoni* eggs, followed in 7 days by intravenous egg challenge, producing synchronized hypersensitivity granulomas in their lungs (Table 1). Half the mice received 250-μg doses of lentinan intraperitoneally on Days −7, 0, and +4 in relation to egg challenge. Mice were killed 8 days after challenge, ie, at the time of peak granuloma size.[27] Control granulomas had a mean diameter of approximately 180 μ, with moderate variation in size. Lentinan-potentiated granulomas averaged almost double the diameter of con-

Table 1—Lentinan Potentiation of Pulmonary Granulomas Following the Intravenous Injection of *Schistosoma mansoni* Eggs Into CBA/J Mice

	No. of granulomas measured/ No. of mice	Mean lesion diameter ± SE (μ)	*P* value
Sensitized mice—eggs injected intraperitoneally on Day −7 then intravenously on Day 0			
Lungs taken on Day +8			
Control	432/8	179.8 ± 2.9	<0.001
Lentinan*	253/8	351.1 ± 10.4	
Unsensitized mice—eggs injected intravenously on Day 0			
Lungs taken on Day +8			
Control	124/6	164.7 ± 5.2	<0.001
Lentinan†	143/7	283.2 ± 12.2	
Lungs taken on Day +16			
Control	156/6	230.9 ± 8.3	<0.001
Lentinan†	147/7	319.4 ± 17.9	
Lungs taken on Day +16			
Control	70/6	187.7 ± 12.3	<0.001
Lentinan‡	90/6	294.4 ± 21.5	

* Lentinan dose = 250 μg (Days −7, 0, +4)
† Lentinan dose = 250 μg (Days −14, −7, 0, +4)
‡ Lentinan dose = 250 μg (Day 0)

trols, representing an approximate 8-fold increase in volume, and had much greater size variance. The control granulomas were typical of schistosome egg lung granulomas with sharp, rounded contours and the absence of central necrosis (Figure 1). These granulomas showed an abundant, evenly distributed eosinophil population and concentrically arranged, strongly staining macrophages and fibroblasts. In contrast, lentinan granulomas had irregular, scalloped borders showing thickened alveolar septums and spillage of alveolar cells and leukocytes into the peripheral air spaces (Figure 2). The predominant cells of the lentinan granulomas were pale-staining macrophages. These large macrophages (Figures 3 and 4) were remarkably uniform in appearance, differing both from the epithelioid macrophages and from actively phagocytic pigment-laden macrophages typical of control granulomas. Following formalin fixation, the lentinan macrophages appeared "foamy" or highly vacuolated; however, after Hollande's fixation these cells showed an even, well-preserved, lightly staining cytoplasmic matrix and contained no typical brown schistosome pigment. Eosinophils were proportionately reduced in numbers compared with controls and showed an irregular distribution within the granulomas. Many lentinan granulomas showed prominent circumoval necrosis but otherwise similar cellularity (Figure 5). The necrotic center of the lentinan granulomas contained cell debris principally originating from neutrophils and some eosinophils. Besides these distinctive egg reactions, there were others essentially similar to those of control mice, as well as some eggs with only minimal reaction.

Primary Lung Granulomas

In the next experiment (Table 1), mice received a single, primary intravenous injection of *S mansoni* eggs. Two hundred fifty micrograms of lentinan was injected on Days −14, −7, 0, and +4. Half of the lentinan and control animals were killed and processed on the eighth day after egg injection, when primary hypersensitivity granulomas are evident and are histologically typical but are less than peak size; the other half of the animals were killed on the 16th day, ie, at the time of peak primary granuloma size.[44] The effect of lentinan on primary schistosome egg granulomas was obvious by the eighth day and was further increased by the 16th day, paralleling the normal development of the primary granuloma. Histologically, lentinan effects were the same as described in the sensitized mice. At this point, we found that a single 250-μg lentinan injection on the day of intravenous egg challenge would produce the described effects although of a slightly lesser magnitude (Table 1).

Optimal Timing

When lentinan was administered to sensitized CBA/J mice in a single 1-mg dose (Table 2), the maximal potentiation of *S mansoni* egg granulomas occurred when lentinan was given on the day of intravenous egg challenge (Day 0). A much lesser effect was seen with treatment following challenge (Day +4), and a significant quantitative suppression followed a dose on the day of sensitization by intraperitoneal egg injection (Day −7), although the cytologic effects of lentinan were not totally abolished by either of these schedules.

S mansoni eggs incubated in a lentinan solution prior to intravenous injection gave no potentiated granulomatous response. Small lentinan aggregates, although relatively insoluble in saline at a neutral pH without sonication, when injected intravenously into CBA/J mice were quickly cleared by normal lung scavenging mechanisms and elicited no granulomatous response.

Hepatic Granulomas

To follow up our initial pilot experiment showing gross alteration of liver granulomas by lentinan, several groups of CBA/J mice were infected with *S mansoni* cercariae. Half of these mice were given lentinan weekly from the fourth week of infection, the week before significant egg deposition commences, until the week prior to necropsy. The lentinan doses were constant (250 μg) or were increased (from 250 μg to 1 mg) as the infections progressed. Significant potentiation of liver granulomas occurred in all groups of mice (Table 3). As expected, lentinan granulomas

Table 2—Effect of Lentinan on Pulmonary Granulomas 8 Days After Intravenous Injection of *Schistosoma mansoni* Eggs Into Sensitized CBA/J Mice As Determined By the Time of Administration of a Single 1-mg Dose*

Timing of Dose	No. of granulomas measured/ No. of mice	Mean lesion diameter ± SE (μ)	*P* value†
Day of sensitization			
Day −7	191/7	139.1 ± 5.7	<0.001
Day of challenge			
Day 0	253/7	290.8 ± 11.3	<0.001
After challenge			
Day +4	201/7	191.9 ± 6.9	NS
Control	216/6	192.9 ± 4.9	—

* Eggs injected intraperitoneally on Day −7 then intravenously on Day 0
† Compared with control
NS = no significant difference

Table 3—Lentinan Potentiation of Hepatic Granulomas in *Schistosoma mansoni*-Infected CBA/J Mice

	No. of granulomas measured/ No. of mice	Mean lesion diameter ± SE (μ)	*P* value
8-week infection, 30 to 35 cercariae			
Control	135/6	362.4 ± 12.8	<0.001
Lentinan*	113/6	518.9 ± 23.3	
10-week infection, 20 cercariae			
Control	176/9	344.2 ± 6.6	<0.001
Lentinan†	117/9	480.1 ± 20.4	
10-week infection, 20 cercariae			
Control	138/8	353.1 ± 9.4	<0.001
Lentinan‡	92/8	447.7 ± 20.8	

* Lentinan dose = 250 μg (Weeks 4 and 5), 500 μg (Week 6), 1 mg (Week 7)
† Lentinan dose = 250 μg (Weeks 4 and 5), 500 μg (Weeks 6 and 7), 1 mg (Weeks 8 and 9)
‡ Lentinan dose = 250 μg (Weeks 4–9)

were proportionally larger than those in the lung model, with some truly monstrous single egg granulomas exceeding 1 mm in diameter. The cellular alterations seen in the liver lentinan granulomas (Figure 6) were similar to those described for the lung reactions, with perhaps some increased eosinophil involvement in circumoval necrosis in the liver. Granulomas in the guts of these mice likewise showed lentinan potentiation and increased necrosis.

Granuloma Immunofluorescence

The distributions of schistosome egg antigen (SEA) and of host immunoglobulins in murine hepatic schistosome egg granulomas have previously been described.[34] Briefly, egg antigen is concentrated in the granuloma center within a distance of approximately one half the width of the egg from the egg shell, and immunoglobulins are distributed evenly throughout the granuloma except for a dramatic reduction in the area where SEA is concentrated adjacent to the egg shell. In lentinan granulomas, the area of SEA diffusion was considerably expanded, forming an intense fluffy bright green coating one to two times the width of the egg in thickness. Occasional lentinan macrophages in the vicinity of the egg showed an even, powdery, specific fluorescence throughout their cytoplasm. However, if a zone of circumoval necrosis surrounded the egg, the region outside the intensely staining SEA band was seen as a "black belt" essentially devoid of specific staining or autofluorescence.

Staining for host immunoglobulin was even throughout the lentinan granulomas, with the fluorescence more intense than in controls. Some lentinan macrophages close to the egg showed fine, even, cytoplasmic staining for IgG. Contrasted to controls, intense staining for host immunoglobulin was often seen immediately adjacent to the egg shell, corresponding to the area of SEA distribution. If a necrotic zone surrounded the egg, its outer aspect was devoid of specific staining and of most autofluorescence, while the cells encircling the necrotic area stained more intensely than those in the remainder of the granuloma.

General Effects on *S mansoni*-Infected Mice

In addition to the expected manifestations of murine schistosomiasis (hepatomegaly and splenomegaly), lentinan produced the following effects when control and lentinan-treated mouse groups infected with 20 cercariae were compared (Table 4): reduced body weight (21%), increased hepatomegaly (15 to 19%), increased splenomegaly (<200%), reduced egg output by the female schistosomes (39 to 49%), and increased granuloma necrosis (16 to 22%). Infected lentinan-treated mice showed moderate liver enlargement above that of uninfected lentinan-treated mice (Table 5); thus, their increased hepatomegaly, compared with infected control mice, was partly a reflection of reduced body weight. In addition to a 10 to 12% body weight reduction, lentinan treatment of uninfected CBA/J mice produced splenomegaly (<200%) (Table 5). Thus, the enormous splenomegaly (five to seven times normal) in lentinan-treated schistosome-infected mice, contrasted to untreated uninfected mice, was interpreted as an additive effect, resulting in part from the lentinan treatment and in part from the schistosome infection. On the other hand, depressed egg production in the lentinan-treated mice might actually have moderated esophageal varices in these animals compared with infected controls.

Nude Mice

We have previously shown that alymphoblastic nude mice respond to schistosome eggs injected into their lungs with little or no cellular reaction and that infected nudes produce greatly reduced hepatic egg reactions consisting primarily of monocytes and pigment-laden macrophages with a scattering of neutrophils.[34] The effect of lentinan in these T-cell–deficient animals and in immunocompetent controls heterozygous at the nude locus was therefore explored (Table 6). For the lung model, the mice were given 250 μg of lentinan on Days −4, −2, and 0 relative to intravenous egg injection and were killed on Day +16. The heterozygotes (Table 6)

Table 4—Hepatosplenic Manifestations of Schistosomiasis in Control and Lentinan-Treated CBA/J Mice 10 Weeks After Exposure to 20 Cercariae of *Schistosoma mansoni*

	No. of worm pairs	Worm pairs/ mouse	BW(g)	Liver wt (mg)	% BW	Spleen wt (mg)	% BW	Mice with varices
Control (9)	17	1.9	28.9 ± 0.2	2208 ± 104	7.7	252 ± 15	0.9	3/9
Lentinan (9)*	19	2.1	22.7 ± 0.5 (P <0.001)	2063 ± 78 (NS)	9.1	491 ± 18 (P <0.001)	2.2	0/9
Control (8)	13-14	1.6-1.8	27.6 ± 0.8	1920 ± 104	7.0	237 ± 14	0.9	3/8
Lentinan (8)†	13	1.6	21.8 ± 0.7 (P <0.001)	1736 ± 72 (NS)	8.0	318 ± 14 (P <0.001)	1.5	0/8

	Total egg count in liver	Eggs/g liver	Eggs/ worm pair	No. of liver sections counted/ No. of mice	Eggs	Necrotic remnants of eggs	Total egg count in sections
Control (9)	14,023 ± 2302	6105 ± 754	7424	39/9	714	4	718
Lentinan (9)*	9561 ± 1386	4754 ± 763	4529	39/9	442	61	503
Control (8)	8279 ± 1752	4118 ± 721	5095	30/8	474	6	480
Lentinan (8)†	4189 ± 883	2378 ± 497	2578	30/8	289	71	360

Values are expressed as mean ± SE. Number of mice/group is indicated in parentheses.
* Lentinan dose = 250 μg (Weeks 4 and 5), 500 μg (Weeks 6 and 7), 1 mg (Weeks 8 and 9)
† Lentinan dose = 250 μg (Weeks 4-9)
BW = body weight; NS = no significant difference

Table 5—Effects of Lentinan on Uninfected CBA/J Mice*

	BW (g)	Liver wt (mg)	% BW	Spleen wt (mg)	% BW
Untreated (9)	29.8 ± 0.7	1551 ± 42	5.2	95 ± 5	0.3
Lentinan (9)†	26.9 ± 0.8	1492 ± 54	5.6	216 ± 22	0.8
Lentinan (9)‡	26.1 ± 0.6	1356 ± 47	5.2	229 ± 14	0.9

Values are expressed as mean ± SE. Number of mice/group is indicated in parentheses.
* Mice age-matched to animals used in experiments detailed in Table 4.
† Lentinan dose = 250 μg (Weeks 4 and 5), 500 μg (Weeks 6 and 7), 1 mg (Weeks 8 and 9)
‡ Lentinan dose = 250 μg (Weeks 4-9)
BW = body weight

showed normal granuloma formation and potentiation (Figure 7) similar to CBA/J mice; nude mice showed a complete absence of hypersensitivity granulomas in their lungs, whether or not they had been treated with lentinan (Figure 8). The rare nude lung egg reactions that did occur consisted of a few nonepithelioid macrophages and were at most two cell diameters wide. Most eggs were found lodged in capillaries and showed no cell response at all, and for this reason nude lung reactions were not measured.

Half of the *S mansoni*-infected nudes and heterozygotes were given three weekly doses of lentinan, increasing from 500 μg to 1 mg beginning the fifth week after infection, with necropsy at 8 weeks (Table 6). Under

Table 6—Effect of Lentinan on Pulmonary Granulomas 16 Days After Intravenous Injection of *Schistosoma mansoni* Eggs Into Unsensitized Heterozygous (Nu/+) Mice and on Hepatic Egg Reactions in *S mansoni*-Infected Nude (Nu/Nu) and Heterozygous (Nu/+) Mice

	No. of granulomas measured/ No. of mice	Mean lesion diameter ± SE (μ)	*P* value
Lung granulomas, unsensitized mice—eggs injected intravenously on Day 0			
Nu/+ (control)	118/6	167.1 ± 7.2	<0.001
Nu/+ (lentinan)*	101/6	249.5 ± 12.4	
Liver egg reactions, 8-week infection, 30 to 35 cercariae			
Nu/Nu (control)	62/3	148.8 ± 4.7	
Nu/Nu (lentinan)†	95/4	160.9 ± 4.6	
Nu/+ (control)	108/6	320.1 ± 11.5	
Nu/+ (lentinan)†	135/6	477.7 ± 15.5	
Nu/Nu (control) vs Nu/Nu (lentinan)			NS
Nu/+ (control) vs Nu/+ (lentinan)			<0.001
Nu/Nu (control) vs Nu/+ (control)			<0.001
Nu/Nu (lentinan) vs Nu/+ (lentinan)			<0.001

* Lentinan dose = 250 μg (Days) −4, −2, 0)
† Lentinan dose = 500 μg (Weeks 5 and 6), 1 mg (Week 7)
NS = no significant difference

these conditions, heterozygotes produced typical granulomas and showed significant lentinan granuloma potentiation (Figure 9). Nudes treated or untreated with lentinan lacked hepatic hypersensitivity granulomas and produced egg reactions approximately one half the diameter of the control heterozygote granulomas, which were composed primarily of monocytes and nonepithelioid pigment-filled macrophages (Figure 10). Zonal hepatocellular alterations surrounding nude liver egg foci as previously described [34] were seen whether or not lentinan had been administered.

Artificial Bead Granulomas

Although lentinan had no obvious effect on macrophages in nude mouse egg reactions, we also studied its effect on inflammatory responses to latex beads in CBA/J mice. This foreign body response is known to be triggered by Hagemann factor, peaks within the first 2 days, then quickly regresses.[46] Lentinan produced a small but statistically significant increase in the size of this reaction (Table 7), which was marginal when compared with that noted in the earlier experiments involving hypersensitivity granulomas around schistosome eggs. No alterations were seen in the macrophages comprising these reactions.

To demonstrate that lentinan would potentiate hypersensitivity granulomas in general and not exclusively schistosome egg granulomas, the effect of lentinan on the pulmonary response to antigen-coated beads was next examined. CBA/J mice were sensitized by subcutaneous injection of purified bovine albumen in complete Freund's adjuvant followed in 16

Table 7—Effect of Lentinan on Foreign Body Granulomas Around Latex Beads and on Hypersensitivity Granulomas Around Antigen-Coated Polyacrylamide Beads Following Intravenous Injection of the Beads Into the Pulmonary Microvasculature of CBA/J Mice

	No. of granulomas measured/ No. of mice	Mean lesion diameter ± SE (μ)	*P* value
Styrene divinylbenzene (latex) beads injected intravenously on Day 0			
Lungs taken on Day +2			
Control	141/8	71.0 ± 1.3	<0.001
Lentinan*	151/9	82.6 ± 2.4	
Bovine albumen-coated polyacrylamide beads—200 μg bovine albumen in 0.25 ml complete Freund's adjuvant injected subcutaneously on Day −16; coated beads injected intravenously on Day 0			
Lungs taken on Day +6			
Control	119/6	87.5 ± 2.2	<0.001
Lentinan†	64/6	255.3 ± 11.2	

* Lentinan dose = 250 μg (Days −4, −2, 0)
† Lentinan dose = 1 mg (Day 0)

days by intravenous challenge with albumen-coated polyacrylamide beads. Half the mice received a single 1-mg lentinan injection at the time of challenge. On the sixth day after challenge, lentinan-treated mice showed a dramatic 3-fold increase in granuloma diameter compared with controls (Table 7). Control granulomas differed from foreign body reactions by having a significant eosinophil component; lentinan granulomas which formed around the antigen-coated beads consisted primarily of lentinan-modified, pale-staining macrophages.

S japonicum Granulomas

Since there is a growing perception that *S japonicum* egg granulomas differ substantially from *S mansoni* egg granulomas,[47-50] we investigated the effect of lentinan on them. CBA/J mice were injected intravenously with *S japonicum* eggs either following or without prior intraperitoneal egg sensitization. Half the animals were given 250 μg of lentinan on Days -7, 0, and $+4$ relative to intravenous egg challenge, and lungs were taken on Day $+7$. In both sensitized and unsensitized mice, a significant lentinan potentiation was realized (Table 8). The morphology of the *S japonicum* egg granulomas has been previously described [48,49] (Figure 11), and the lentinan granulomas (Figure 12) resembled those seen around *S mansoni* eggs with perhaps more central necrosis. The lentinan-potentiated *S japonicum* granulomas were smaller than the corresponding granulomas around *S mansoni* eggs (Tables 1 and 2) in proportion to the smaller mean size of the control *S japonicum* lung granulomas (Table 8). In contrast to previously reported studies,[48,49] an anamnestic response was seen both in control and in lentinan *S japonicum* granulomas following intraperitoneal egg sensitization (Table 8).

Table 8—Lentinan Potentiation of Pulmonary Granulomas 8 Days After Intravenous Injection of *Schistosoma japonicum* Eggs Into CBA/J Mice*

	No. of granulomas measured/ No. of mice	Mean lesion diameter ± SE (μ)	*P* value
Sensitized mice—eggs injected intraperitoneally on Day −7 then intravenously on Day 0			
Control	87/6	123.4 ± 5.1	<0.001
Lentinan	118/6	238.9 ± 17.9	
Unsensitized mice—eggs injected intravenously on Day 0			
Control	72/6	99.4 ± 3.2	<0.001
Lentinan	73/5	168.9 ± 12.8	
Sensitized (control) vs unsensitized (control)			<0.001
Sensitized (lentinan) vs unsensitized (lentinan)			<0.01

* Lentinan dose = 250 μg (Days -7, 0, $+4$)

Discussion

We are unaware of any prior reports on granuloma immunopotentiation by systemic agents. Our own pilot studies with BCG, *Corynebacterium parvum*, and *S typhosa* LPS have not thus far yielded any striking results, but these explorations are far from complete. Lentinan was found to potentiate granuloma formation in response to three particles of differing antigenic specificity: *S mansoni* eggs, *S japonicum* eggs, and polyacrylamide-bead–bound BSA; lentinan did not accelerate the attainment of *S mansoni* egg granuloma peak size (Table 1) as would have homologous sensitization [27]; rather it markedly altered the morphology of the granuloma cell components themselves. Thus, the observed granuloma potentiation is unlikely to result from antigenic cross-reaction but must represent a genuine adjuvant effect.

A single 250-μg lentinan dose on Day 0 of challenge was sufficient for near-maximal effect in the lung granuloma model (Table 1), and repeated weekly injections of the same dose worked well in full-scale schistosome infection (Table 3); the minimal effective dose remains to be determined.

As shown in Table 4, multiple lentinan doses did clearly inhibit schistosome egg production as well as body weight increase of treated vs untreated infected mice. Organ/body weight ratios were markedly affected in the spleen; they were less affected in the liver. In addition, lentinan minimally but significantly increased macrophage responses to nonantigenic latex particles in the lung (Table 7). No potent adjuvant lacks side effects, and several are known to stimulate spleen size or macrophage reactivity,[51] but these unexplained features are probably irrelevant to the main objectives of our study.

Our results (Tables 1 through 3 and 6 through 8) were entirely consistent with earlier indications that lentinan immunopotentiates via the T lymphocyte. Conversely, they also strengthen the evidence that granulomatous hypersensitivity to schistosome eggs [27-34] and to particle-bound protein antigen [52,53] is T-cell–dependent. Thus, lentinan had no perceptible effect on the impaired lung or liver egg foci of thymic-deficient mice, while heterozygote controls responded dramatically (Table 6). That lentinan has much less effect on the small foreign body foci around nonantigenic latex particles (Table 7) than on those around antigen-coated polyacrylamide beads in sensitized mice is also consistent with T-cell immunopotentiation. The effect of lentinan was optimal when its administration coincided with challenge rather than with sensitization (Table 2). In unsensitized mice, lentinan proved effective (Table 1) prior to the time when antibodies to SEA can normally be expected.[54,55] These findings suggest that lentinan's main target in our system may have been

the effector T lymphocyte but, without appropriate adoptive transfer studies, this cannot be considered proved. Earlier studies in which lentinan effect was measured in terms of carrier priming and of antibody titers has led to the conclusion that it acted on T-helper functions.[35,36] Our system did not clearly discriminate between T-cell helper effects and those exerted via lymphokines or other means. Alternatively, the primary target of lentinan in this system could have been macrophages interacting with T lymphocytes. The maximal potentiation coming after lentinan administration on the day of egg challenge and the altered macrophage morphology clearly point to lentinan activity being expressed on the efferent rather than the afferent side of the granulomatous response. Both *S mansoni* lung granulomas (Tables 1 and 2) and the smaller *S japonicum* granulomas (Table 8) were equally potentiated by lentinan, although it has been shown that the immune responses associated with granuloma formation around eggs of the two parasite species differ in several notable respects.[50]

The morphologic and immunocytochemical changes elicited in lentinan-modified granulomas were unexpected and visually striking. The formation of giant nodules, the prominent central necrosis, and the granulocyte degeneration in these granulomas were features that made these schistosome lesions appear more "tuberculoid" than normal. By contrast, the widened spread of SEA and immunoglobulins in these lesions and their numerous pale-staining (or vacuolated) macrophages were more reminiscent of lepromatous anergy; they also evoked the image of cultured macrophages *in vitro* whose endocytotic membrane fusion process has been impaired by lectins.[56] Thus, we can only speculate whether the augmented granulomatous cell response promoted by lentinan indicates an improved or an impaired capacity of the host cells to deal with schistosome egg antigens and metabolic products. Conceivably, immunopotentiation might have attracted more effector cells to deal with egg products but may also have rendered these effector cells excessively sensitive to specific schistosome antigen, thereby promoting early necrosis of eosinophils and foamy degeneration of macrophages. More detailed immunocytochemical studies at the light and electron microscopic levels might help to better focus on these questions. In addition, the effect of lentinan on the rate of schistosome egg destruction needs to be determined.

Although we have shown that lentinan is ineffective in the absence of an adequate host T-lymphocyte reservoir (Table 6), most instances of immunologic host unresponsiveness to infection are based on other more complex mechanisms.[16-20] It would therefore be of interest to ascertain

whether lentinan is capable of potentiating memory T cells in infected subjects in whom these cells are potentially available but have been functionally suppressed. That such rescue might be feasible has already been suggested by experiments involving the host rejection of Sarcoma 180 in rats [37]; the same principle might be applicable to granulomatous infections involving replicating bacteria or mycobacteria. Thus, under appropriate conditions, an effect deleterious to normal reactors might prove beneficial to immunosuppressed hosts by helping to normalize their deficient granulomatous sensitivity. This interesting possibility is open to experimental exploration.

Finally, the question is raised whether exogenous products other than lentinan, perhaps derived from intercurrent bacterial or parasitic infections, could act as potentiators of chronic granulomatous diseases such as schistosomiasis. In this regard, the diverse severity of schistosome pathology at otherwise comparable levels of infection intensity has often been discussed.[57] While immunosuppressive effects of malaria [58] and toxoplasmosis [59] on schistosome granulomas have already been demonstrated, the converse potentiating effect has thus far not been studied in either experimental or human infections, but it seems unlikely that lentinan's effect should be unique in that respect. Further study of this problem, both at the clinical and experimental levels, would therefore be of interest.

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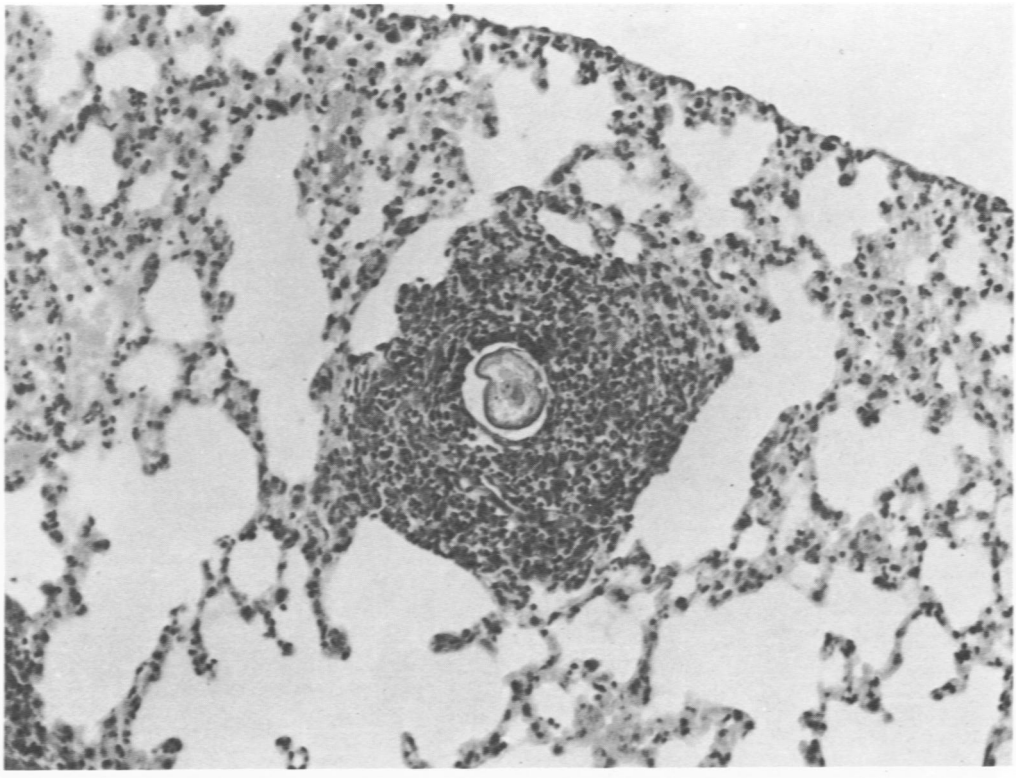

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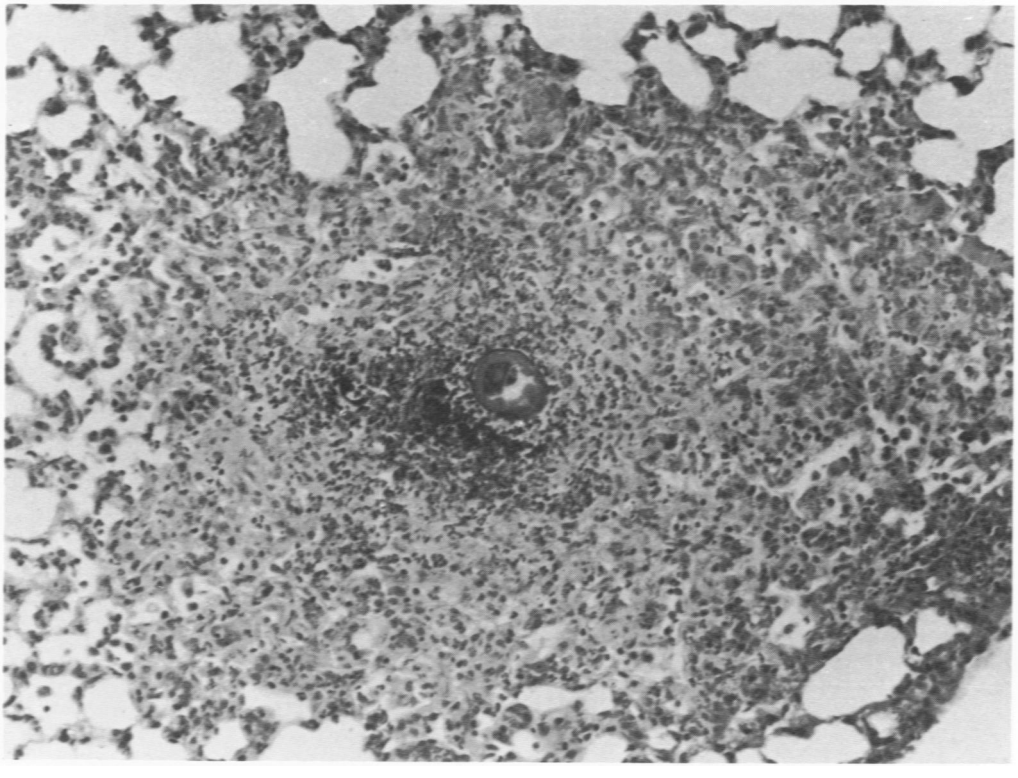

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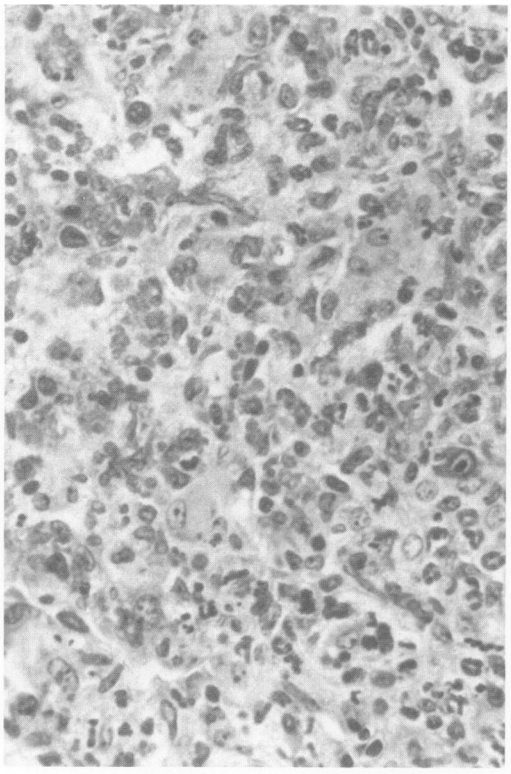
Figures 1 and 2—Lung granulomas 8 days after intravenous injection of *Schistosoma mansoni* eggs into sensitized CBA/J mice. **Figure 1**—Control granuloma. An evenly distributed eosinophil population is the most notable feature. **Figure 2**—Lentinan-potentiated granuloma, characterized by large, pale-staining macrophages. (Dominici, × 195)



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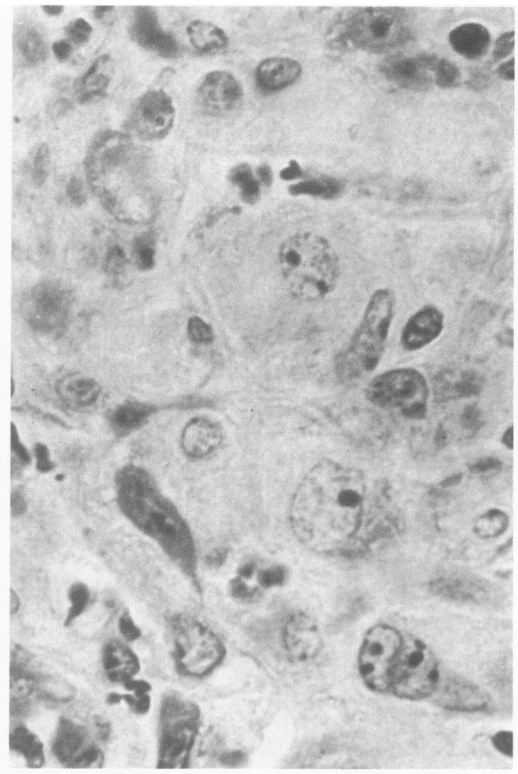

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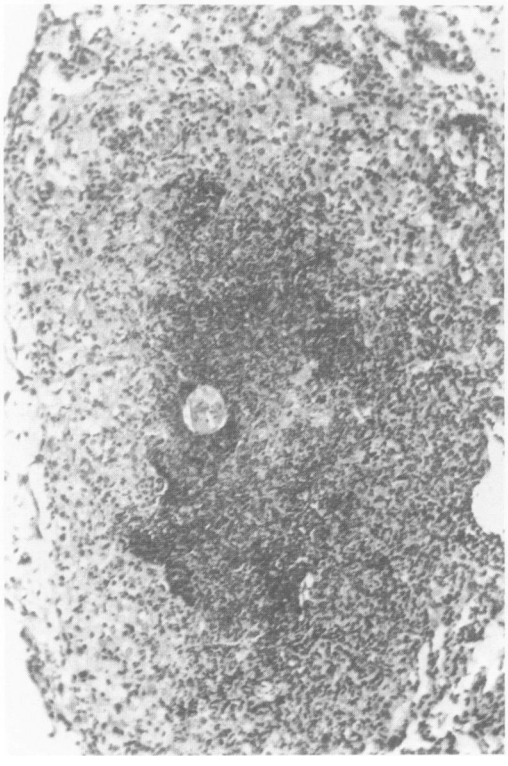

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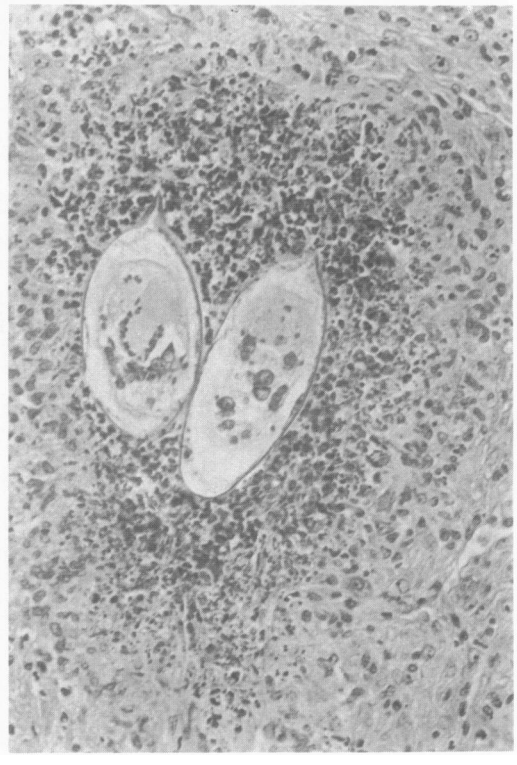

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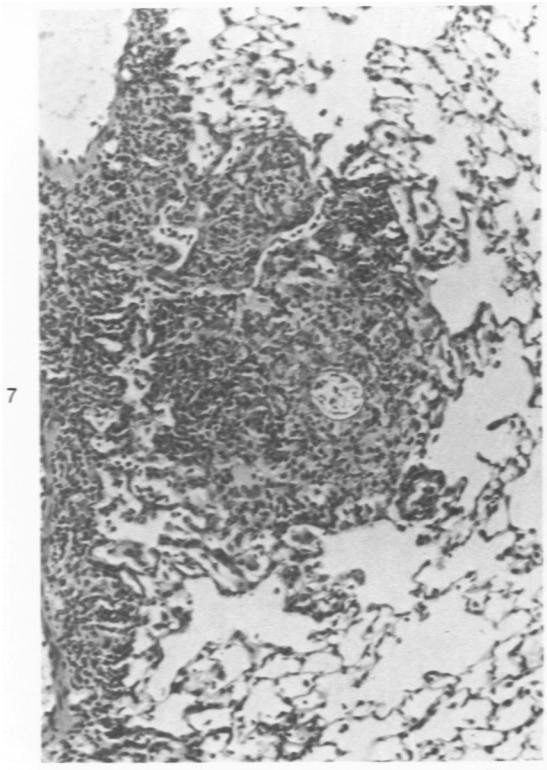
Figures 3 through 5—Lung granulomas formed after injection of *S mansoni* eggs into lentinan-treated CBA/J mice. **Figures 3 and 4**—Cellular details of potentiated lung granulomas 8 days after egg injection into sensitized mice. (Dominici, **3,** × 485; **4,** × 1210) **Figure 5**—Giant lentinan granuloma with prominent circumoval necrosis. Sensitized mouse, 8 days after egg injection. (H&E, × 120) **Figure 6**—Necrotic center of a lentinan-potentiated liver granuloma. CBA/J mouse. Eight-week *S mansoni* infection. (Dominici, × 300)



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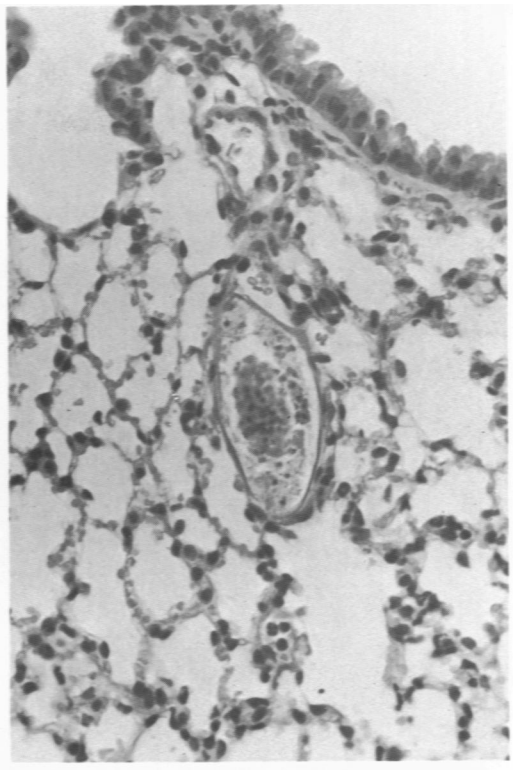

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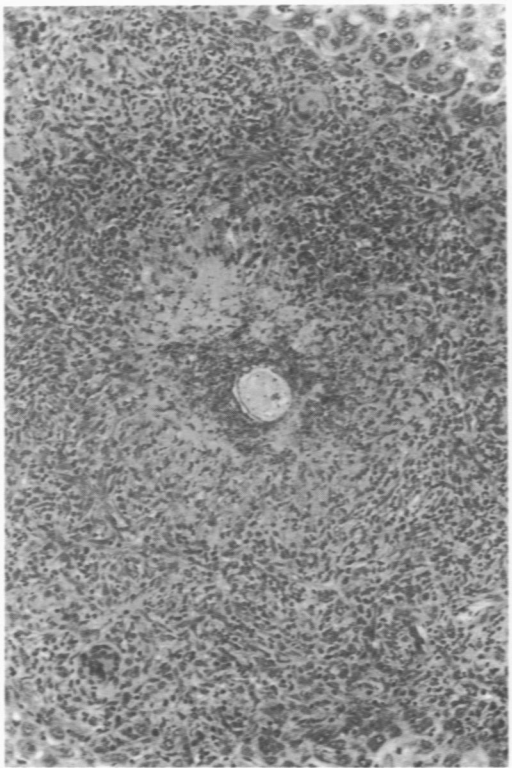

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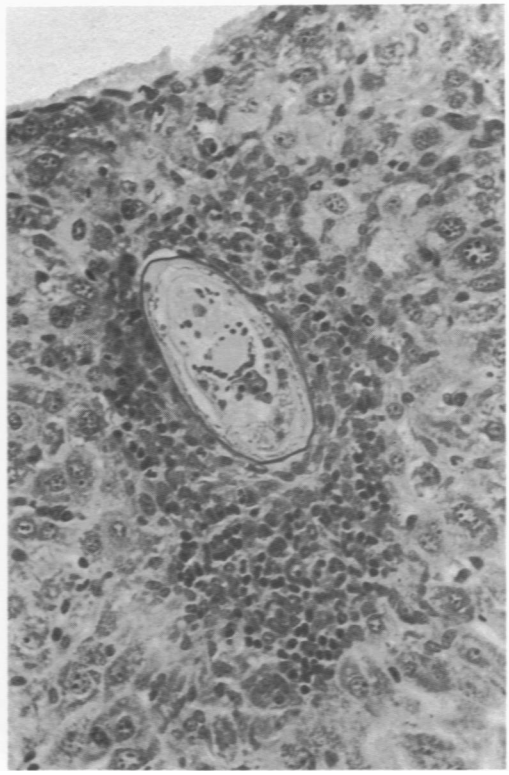

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Figures 7 through 10—Effects of lentinan treatment on lung and liver *S mansoni* egg reactions in nude and heterozygous mice. **Figures 7 and 8**—Lung responses 16 days after injection of eggs. **Figure 7**—Heterozygote. Lentinan-potentiated primary lung granuloma. **Figure 8**—Nude. Eggs injected into nude mice typically evoke little or no pulmonary response whether or not lentinan has been administered. (H&E, **7**, × 120; **8**, × 300) **Figures 9 and 10**—Liver granulomas. Eight-week infections. **Figure 9**—Heterozygote. Lentinan-potentiated hepatic hypersensitivity granuloma. **Figure 10**—Nude. *S mansoni* egg reaction characteristic of the nude mouse. Nude liver granulomas are greatly altered from the hypersensitivity granulomas seen in thymus-intact mice [24] and are composed mostly of monocytes and macrophages which show no perceptible cytologic alterations as a result of lentinan treatment. (Dominici, **9**, × 120; **10**, × 300)

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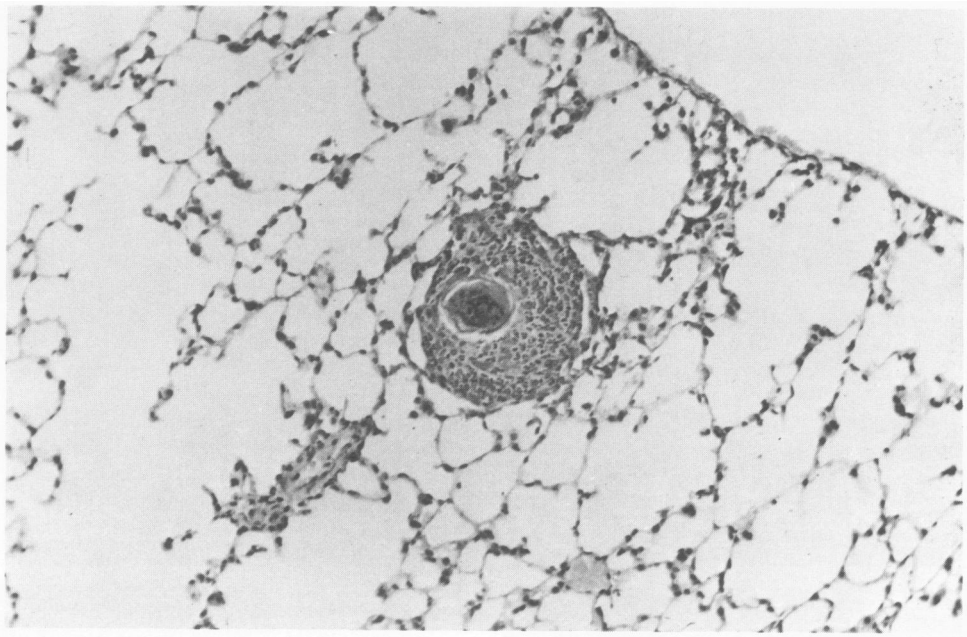

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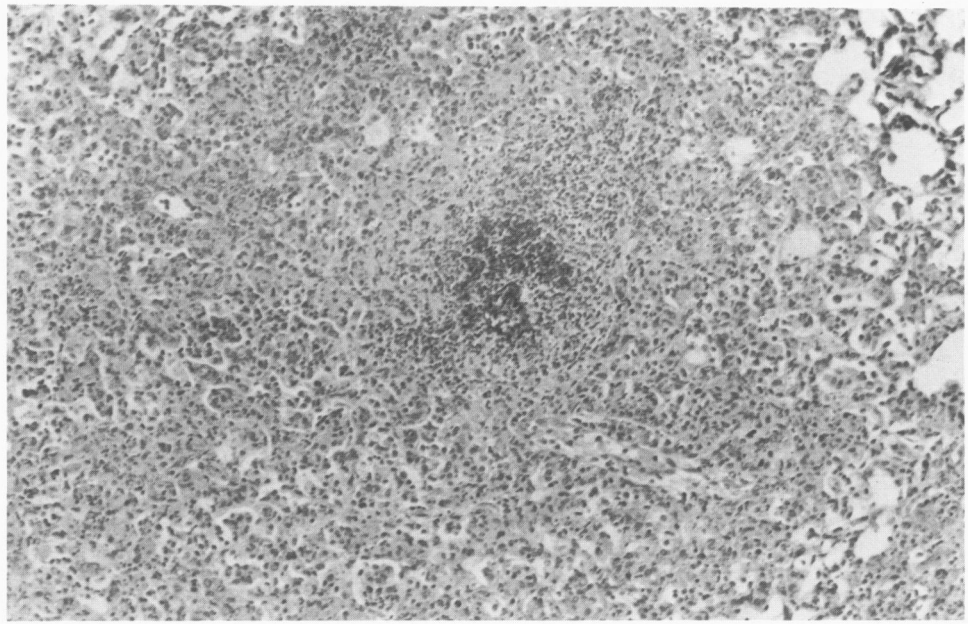

Figures 11 and 12—Lung granulomas 8 days after intravenous injection of *Schistosoma japonicum* eggs into sensitized CBA/J mice. **Figure 11**—Control granuloma. **Figure 12**—Lentinan-potentiated granuloma. (Dominici, × 155)