The Inhibition of Granuloma Formation Around Schistosoma Mansoni Eggs

III. Heterologous Antilymphocyte Serum

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GRANULOMA FORMATION around schistosome eggs appears to play a major role in the pathogenesis of hepatosplenic schistosomiasis in the experimental animal.[1-3] With the intent of providing a means of preventing the development of overt disease in animals with schistosomiasis, the effect of immunosuppressive drugs on the granulomatous reaction to *Schistosoma mansoni* eggs was studied.[4] The demonstration that these drugs significantly suppressed granuloma formation led to a later series of experiments to determine whether the granuloma formation was an immunologic response. The observation that a specific sensitization phenomenon could be induced, which could be transferred by cells and not by serum, suggested that the schistosome egg granuloma was a form of delayed hypersensitivity.[5] Neonatal thymectomy was then found to result in markedly diminished granuloma formation around schistosome eggs.[6] In the present study heterologous antilymphocyte serum caused virtual abolition of the granulomatous reaction to *S. mansoni* eggs in the unsensitized animal. Neither neonatal thymectomy[6] nor antilymphocyte serum had any effect on the smaller, more rapidly forming granuloma around plastic beads.

Materials and Methods

Preparation of Serums

Rabbit anti-mouse lymphocyte serum (RAMLS) was prepared in White New Zealand rabbits (2–3 kg.) according to the method of Gray *et al.*[7] using lymph nodes from female C3H/HEJ mice as a source of lymphocytes. Normal rabbit serum (NRS) was obtained from blood drawn from nonimmunized rabbits. Prior to use, both the

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Table 1. Protocol for Study of Effect of NRS and RAMLS on Granuloma Formation Around Schistosoma mansoni Eggs or Divinyl-benzene-copolymer Beads Injected via Tail Vein into Lungs of Mice

Experimental conditions	NRS or RAMLS * (0.25 ml. I.P.)	Experimental animals (No.)		Days after egg injection on which lungs were removed for study *
		NRS	RAMLS	
S. mansoni eggs (1000) injected into lungs				
Unsensitized mice				
During course of granuloma formation †	Start Day −1, 3×/wk. until Day 32	21	24	4, 8, 16, 32
Onset at 9 days	Start Day 9, 3×/wk. until Day 32	15	15	8, 16, 32
Withdrawal at 8 days	Start Day −1, 3×/wk. until Day 8	18	18	8, 16, 32
Single dose of serum	Day −17	12	12	8, 16
Sensitized mice ‡				
During course of granuloma formation	Days −7, 6, 5, 4, 3, 2, 1, 0, & 3×/wk. until Day 8	10	10	1, 8
During induction of sensitization	Start Day −1 of I.P. sensitizing injection, 3×/wk. for 3 wk.	12	12	1, 8
Divinyl-benzene-copolymer beads (6000) injected into lungs	Start Day −1, 3×/wk. until Day 8	12	12	2, 4, 8

* Day 0 was the time at which eggs or beads were injected into the lungs.
† C3H/HEJ female mice used for this experiment; Swiss albino females used in all other experiments.
‡ S. mansoni eggs (6000) in 0.5 ml. saline injected I.P. 4 or 5 weeks prior to injection of eggs into lungs.

antilymphocyte and normal serums were treated at 56° C. for 30 min. to inactivate complement.

Technic for Studying Granuloma Formation

In the studies outlined in Table 1, the schistosome eggs used to elicit granuloma formation were isolated by the method of Coker and von Lichtenberg[8] from the livers of Swiss albino female mice infected 8 weeks previously with a Puerto Rican strain of *S. mansoni*. The reaction to a foreign body of a size similar to the schistosome egg was studied with divinyl-benzene-copolymer beads (Bio-Rad Labs) which were screened through a Size 70 steel mesh seive and triple-washed with normal saline. A total of 1000 schistosome eggs or 6000 plastic beads suspended in 0.5 ml. of normal saline were injected into the lungs of mice via a tail vein. At various time intervals after injection (Table 1), groups of mice were anesthetized, 1 ml. of 10% buffered formalin was injected intratracheally into each animal, and the lungs were removed and placed in a container of the same solution. Three sections from each lung, 5 μ in thickness and at least 250μ apart, were stained with hematoxylin and eosin and examined for the presence of schistosome eggs or plastic beads. The size of each egg or bead, including the reaction around it, was determined by measuring 2 diameters at right angles to each other with a Vickers-A.E.I. image-splitting eyepiece. The mean diameter of approximately 50–100 such lesions for each time period was then calculated. After the mean granuloma diameter for each experimental group was determined, the sections were searched for a granuloma representative of the mean diameter for the group. This granuloma was marked and then photographed (Fig. 1–3).

Hepatosplenic Disease Study

A total of 30 young Swiss albino female mice were each exposed, by the method of Olivier and Stirewalt,[9] to 40 cercariae of a Puerto Rican strain of *S. mansoni* obtained from a pool of infected *Australorbis glabratus* snails. These infected animals and 30 similar but uninfected mice were divided into 6 equal groups consisting of uninfected and infected mice which were either (1) untreated, or (2) treated with NRS or RAMLS (0.25 ml. intraperitoneally, 3 times a week from the fourth until the eighth week of infection). At that time the mice were anesthetized, and the body, liver, and spleen weights, portal pressure, presence of esophageal varices, and hematocrit were determined as previously described.[1] Measurements of schistosome granulomas were performed as described above for the lungs. The mean worm load harbored by the infected mice was determined, by the method of Duvall and DeWitt,[10] in 10 additional mice infected at the same time as those above.

Results

Rabbit Anti-mouse Lymphocyte Serum

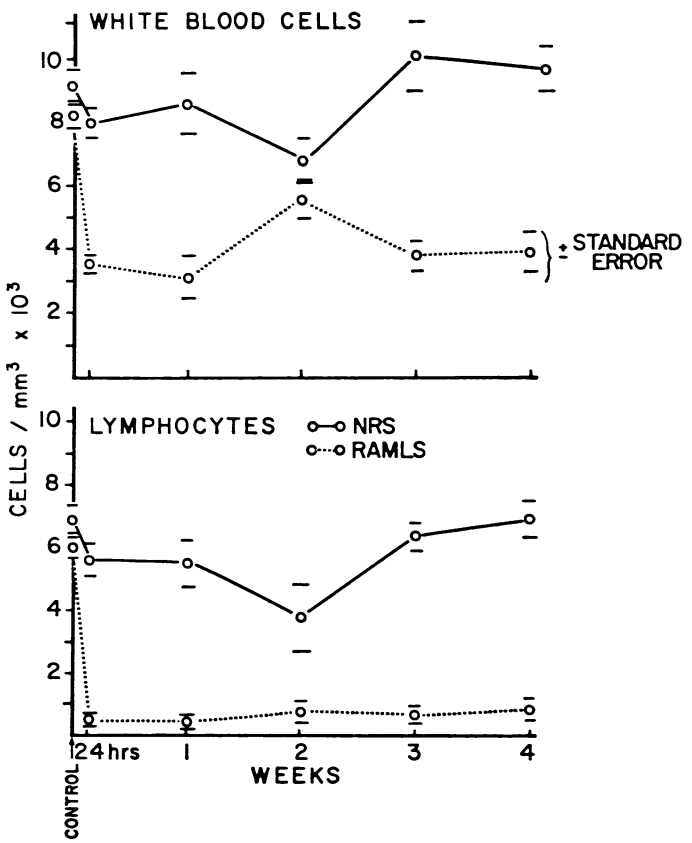
Using a system consisting of equal volumes of phosphate-buffered saline containing 5×10^6 lymphocytes from C3H/HEJ or Swiss albino mouse lymph nodes and serial dilutions of serums,[7] leukoagglutination was observed up to a dilution of 1:1024 with RAMLS. NRS and saline controls did not show lymphocyte agglutination at any dilution (Table 2). RAMLS, but not NRS, given intraperitoneally at a dose of 0.25 ml. per mouse 3 times a week, produced a 90% drop in the lymphocyte count in

groups of 5–29 animals at time periods from 24 hr. to 28 days after the onset of treatment (Text-fig. 1). Neither the hematocrit nor the polymorphonuclear cell count (Text-fig. 1) was affected. After a single in-

Table 2. Leukoagglutination Titration of RAMLS (Prepared with C3H/HEJ Lymph Node Cells) with Lymph Node Cells from C3H/HEJ and Swiss Albino Mice

	Titer *									
Suspension	4	8	16	32	64	128	216	512	1024	2048
Saline control										
C3H/HEJ cells	—	—	—	—	—	—	—	—	—	—
Swiss albino cells	—	—	—	—	—	—	—	—	—	—
NRS										
C3H/HEJ cells	—	—	—	—	—	—	—	—	—	—
Swiss albino cells	—	—	—	—	—	—	—	—	—	—
RAMLS										
C3H/HEJ cells	+	+	+	+	+	+	+	+	±	—
Swiss albino cells	+	+	+	+	+	+	+	+	±	—

* Expressed as the reciprocal of the dilution.



TEXT-FIG. 1. Total white blood cell and lymphocyte counts in mice given intraperitoneal injections of 0.25 ml. of NRS or RAMLS 3 times a week for 4 weeks.

jection of RAMLS, the lymphocyte count returned to about 50% of its preinjection level after 16 days, and to the preinjection level by 30 days. Enlargement of the spleen and lymph nodes was noted 2 weeks after RAMLS administration, with microscopic changes characterized by destruction and depletion of germinal centers and lymphoid cells and, in many animals, increase of reticular cells.

Effect on Granuloma Formation in Unsensitized Mice

A reproducible pattern of granulomatous response follows the injection of schistosome eggs into the lungs of unsensitized mice,[5,6] and a similar series of reactions was noted when such mice were treated with NRS. One day after their injection, schistosome eggs in the pulmonary arterioles were free of any reaction. Four days later a reaction consisting primarily of macrophages accompanied by some round cells and eosinophils surrounded approximately 70% or more of the eggs (Fig. 1). By 8 days the reaction was much larger; the eggs were surrounded with a halo of inflammatory cells consisting of epithelioid cells, macrophages, large numbers of eosinophils, occasional multinucleated giant cells, and lymphocytes. The reaction reached its peak size at 16 days, being almost 3–3.6 times the diameter of the egg alone, so that in terms of volume this presumably spherical lesion was 12 times larger than the egg itself. By 32 days the granuloma had decreased in size so that it was slightly smaller than the lesions seen at 8 days. In contradistinction to the NRS-treated animals, the mice injected with RAMLS had almost complete suppression of granuloma formation, with the exception of the 4-day time interval, at which time the granuloma was comparable to that in the NRS-treated animals (Fig. 1). For example, at 16 days there was a 217% increase in the average granuloma diameter relative to egg diameter (mean granuloma diameter — mean egg diameter/mean egg diameter $\times$ 100) in the mice treated with NRS as compared to a 33% increase in the animals injected with RAMLS. Furthermore, while 99% of the eggs in the NRS-treated mice were surrounded by inflammatory cells, only 27% of the eggs in the RAMLS-treated animals had any reaction whatsoever around them (Table 3, Fig. 1).

In the above experiment, the administration of control serum and RAMLS was carried on from the day prior to egg injection into the lungs until 32 days thereafter. Two further experiments were performed, one in which the serum treatment was terminated early in the course of granuloma formation (at 8 days), and the other in which the first administration of serum was delayed until 9 days after the eggs were injected into the lungs. In the former study RAMLS treatment resulted in marked suppression of the host reaction until it was withdrawn at 8 days, whereupon

Table 3. Effect of NRS and RAMLS on Granulomatous Reaction around Schistosoma mansoni Eggs Injected into Lungs of Mice

Parameters	Serum	Days after egg injection			
		4	8	16	32
Mice studied (No.)	NRS	6	5	5	5
	RAMLS	6	6	6	6
Lesions measured—eggs with or without granulomatous reactions (total No.)	NRS	81	130	121	125
	RAMLS	81	130	130	114
Diameter (μ) of lesions (mean ± S.E.)*	NRS	78 ± 2.1	162 ± 4.8	190 ± 6.3	160 ± 4.0
	RAMLS	79 ± 2.4	75 ± 2.1	80 ± 2.5	67 ± 1.0
Eggs with granulomatous reactions (%)	NRS	81	95	99	100
	RAMLS	68	25	27	0

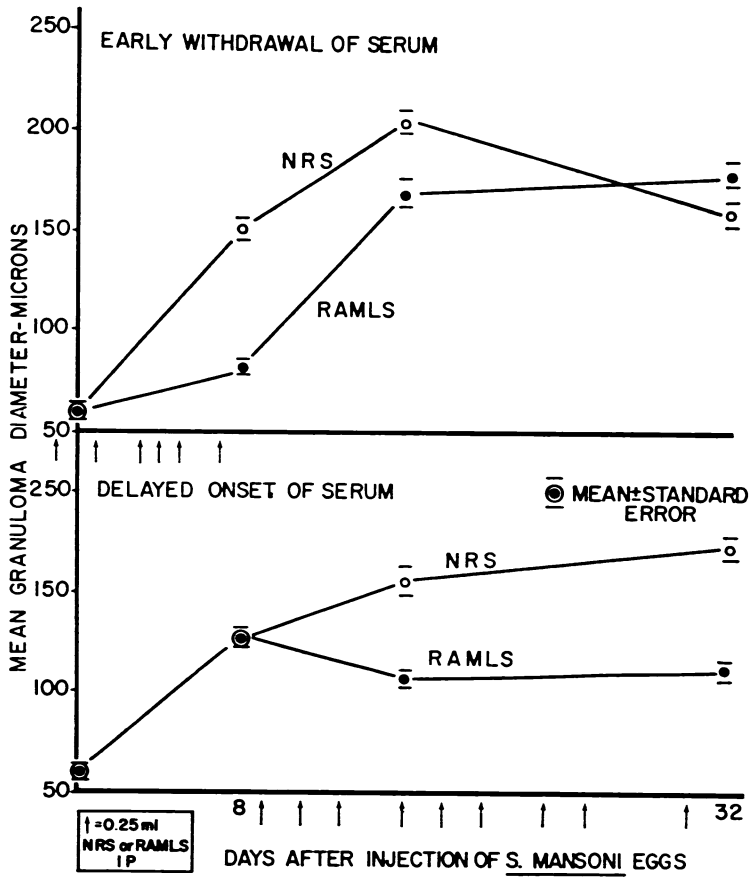
* Mean diameter of eggs alone = 60 μ.

granuloma size more than doubled by 16 days (Text-fig. 2). In the study in which serum treatment was delayed until after the granuloma had partially formed (9 days), NRS had essentially no effect on further granuloma development, but RAMLS resulted in a significant decrease in the size of the lesions by 16 days ($p = 0.0025$); the suppression was maintained through 32 days, the granuloma remaining the same size as at the previous measurement period (Text-fig. 2).

Another group of mice was given a single injection of RAMLS. Serial white cell counts were performed until the lymphocytes returned to 50% of the pretreatment levels (16 days), and then the mice received schistosome eggs intravenously. Eight days later the mean granuloma diameter of 119 ± 6 μ in these animals was significantly smaller than that of 169 ± 8 μ in NRS controls ($p = < 0.0005$). The absolute lymphocyte counts reached normal levels 16 days after egg injection in the mice treated with RAMLS 32 days previously; nevertheless, the mean granuloma diameter of 151 ± 6 μ in these animals still was smaller than that of 176 ± 7 μ in the NRS controls ($p = < 0.005$).

Effect on Granuloma Formation in Sensitized Mice

Intraperitoneal injection of schistosome eggs has been demonstrated to induce sensitization to eggs subsequently injected intravenously as evidenced by (1) a significant reaction occurring around approximately 70% of the eggs 24 hr. after their injection, a time at which no reactions are seen in unsensitized mice; and (2) a more rapidly developing granuloma reaching a much greater peak size than that seen in unsensitized mice.[5] Although this response was not abolished by RAMLS, it was greatly at-



TEXT-FIG. 2. Granuloma formation around *S. mansoni* eggs injected into lungs of mice treated with NRS and RAMLS: effects of early withdrawal and delayed onset of treatment.

tenuated as compared to that observed in the sensitized animals treated with NRS (Table 4, Fig. 2). In addition, 63% of the eggs in the sensitized NRS-treated animals showed reactions 24 hr. after receiving the injection, as compared with only 22% of the eggs in the sensitized RAMLS-treated animals.

Effect on Induction of Sensitization

When RAMLS was administered during the initial intraperitoneal exposure to schistosome eggs, it did not prevent the induction of sensitization. Thus, mice which had been treated with RAMLS immediately prior to and for 3 weeks after the primary intraperitoneal injections of schistosome eggs had, on subsequent intravenous egg injection, reactions with a mean diameter of 90 $\pm$ 3 μ around 79% of the eggs at 1 day as com-

Table 4. Effect of NRS and RAMLS on Granuloma Formation Around Schistosoma mansoni Eggs Injected into Lungs of Mice Previously Sensitized by I.P. Injection of Homologous Eggs

Parameter	Serum	Days after egg injection 1	8
Mice studied (No.)	NRS	5	5
	RAMLS	5	5
Lesions measured—eggs with or without granulomatous reactions (total No.)	NRS	90	100
	RAMLS	65	72
Diameter (μ) of lesions * (mean $\pm$ S.E.)	NRS	81 ± 3	226 ± 6
	RAMLS	63 ± 2	130 ± 8
Eggs with granulomatous reactions (%)	NRS	63	100
	RAMLS	22	74

* Mean diameter of eggs alone = 60 μ.

pared to 86% of the eggs and a reaction of 95 ± 3 μ mean diameter in the mice which had been treated with NRS at the period of the primary injection. Eight days after the secondary egg injection in these animals, the RAMLS-treated mice had a mean granuloma diameter of 214 ± 6 μ as compared to 205 ± 7 μ in the NRS-treated mice.

Effect on Granuloma Formation Around Plastic Beads

Since the schistosome egg is a living, antigen-secreting agent eliciting an immune type of granulomatous response,[5] the effect of RAMLS on a foreign body granuloma induced by plastic beads is characterized by rapid evolution with peak reactions at 2–4 days and a decline by 8 days. The mean diameter of the cellular response in relation to the size of the inciting agent is much less with plastic beads than with schistosome eggs (a 128% vs. a 217% peak increase in mean granuloma diameter relative to mean bead or egg diameter). RAMLS had only a slight suppressant effect on the granulomatous response to plastic beads; this suppression was significant as compared to NRS-treated controls at 2 and 8 days, but not at 4 days following bead injection (Table 5, Fig. 3).

Effect on Development of Hepatosplenic Disease

On perfusion following exposure to 40 *Schistosoma mansoni* cercariae each, the mice were heavily infected, with a mean total worm burden of 22 ± 3 and mean number of worm pairs of 9 ± 1. Although RAMLS appeared to diminish granuloma formation significantly ($p = < 0.0005$) as compared to untreated and NRS-treated controls, the effect was relatively small. In comparison with their respective controls, the

Table 5. Effect of NRS and RAMLS on Granuloma Formation Around Divinyl-benzene-copolymer Beads Injected into Lungs of Mice

Parameter	Serum	Days after bead injection 2	4	8
Mice studied (No.)	NRS	4	4	4
	RAMLS	4	4	4
Lesions measured—beads with or without granulomatous reaction (total No.)	NRS	100	100	100
	RAMLS	100	100	100
Diameter (μ) of lesions * (mean ± S.E.)	NRS	121 ± 4	101 ± 3	90 ± 2
	RAMLS	103 ± 3	94 ± 3	79 ± 3

* Mean diameter of beads alone = 53 μ.

RAMLS-treated animals developed only slightly less pronounced changes in each of the parameters of hepatosplenic disease studied than did the untreated and NRS-treated mice (Table 6).

Discussion

Recent studies on the pathogenesis of schistosomiasis mansoni in experimental animals indicate that the clinical manifestations of this para-

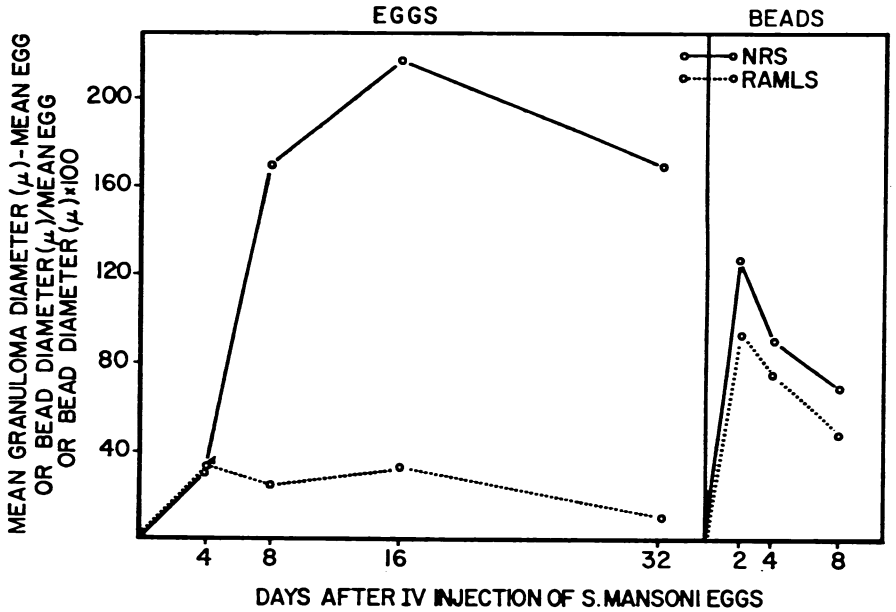
Table 6. Effect of NRS and RAMLS on Development of Hepatosplenic Disease in Mice Infected with Schistosoma mansoni

Parameter	Uninfected Untreated	NRS	RAMLS	Infected Untreated	NRS	RAMLS
Body wt. ± S.E. (gm.)	27.4 ± 0.6	26.2 ± 0.2	27.2 ± 0.5	28.2 ± 0.6	25.4 ± 0.7	29.7 ± 0.8
Liver wt. as % of body wt. ± S.E.	4.4 ± 0.1	4.9 ± 1.6	5.5 ± 0.1	7.8 ± 0.2	7.8 ± 0.4	8.2 ± 0.4
Spleen wt. as % of body wt. ± S.E.	0.5 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	1.3 ± 0.1	1.8 ± 0.1	1.6 ± 0.1
Portal pressure (cm. saline) ± S.E.	4.8 ± 0.4	4.6 ± 0.1	5.9 ± 0.4	10.0 ± 0.4	7.8 ± 0.6	8.5 ± 0.7
Hematocrit (%) ± S.E.	48 ± 0.5	45 ± 0.8	43 ± 0.8	38 ± 0.8	35 ± 1.9	38 ± 1.1
Mice with esophageal varices (%)	0	0	0	50	30	20
Diam. (μ) of liver granulomas (mean ± S.E.)	—	—	—	295 ± 4.9	323 ± 6.1	258 ± 6.3

sitic disease may have an immunologic etiology. Not only has the parasite egg been shown to be necessary for the development of overt murine hepatosplenic schistosomiasis,[11,12] but the host granulomatous response to the egg appears to be an essential pathogenic factor.[1-3] This host reaction has recently been demonstrated to be an immunologic response of the delayed hypersensitivity type in that a specific sensitization reaction occurs which can be transferred by cells and not by serum.[5] Desensitization, characterized by accelerated but markedly diminished granuloma formation, has also been demonstrated in animals with natural schistosome infections and those given repeated intraperitoneal injections of schistosome eggs.[13] In addition, the partial suppression of granuloma formation by immunosuppressive drugs[4] and neonatal thymectomy[6] has provided support for the concept of the granuloma as an immunologic entity. In the present study the effect on the granulomatous reaction of heterologous antilymphocyte serum, which has been claimed to be the most powerful immunosuppressive agent known,[14] was investigated for two reasons: (1) to confirm the immunologic status of the schistosome granuloma, and (2) to provide definitive evidence for the role of the granuloma in the pathogenesis of hepatosplenic schistosomiasis.

Heterologous antilymphocyte serum has been shown to prolong allografts and xerografts,[15] to depress delayed hypersensitivity to tuberculin, purified diphtheria toxoid, and contact allergens, and to protect against experimental allergic encephalomyelitis[16] and lymphocytic choriomeningitis,[17] diseases whose fatal effects are believed to be due to delayed hypersensitivity. Virtually complete suppression of the murine granulomatous response to schistosome eggs by treatment with RAMLS may now be added to the above effects. In contrast, antilymphocyte serum had only a negligible effect on the so-called foreign body granulomas induced by plastic beads. The lack of effect on the beads suggests that the slight reaction seen around *S. mansoni* eggs at 4, 8, and 16 days in antilymphocyte serum-treated animals may have been due to a foreign body type of reaction. Waksman, Arbouys, and Arnason[16] noted a similar dissociation in the effect of antilymphocyte serum on an immunologic and presumably nonimmunologic tissue reaction: In guinea pigs treated with heterologous (rabbit) antilymphocyte serum there was a pronounced suppression of the delayed skin reaction to tuberculin, diphtheria toxoid, and contact allergens, but only moderate inhibition of the nonspecific skin reaction following the intradermal injection of turpentine. Thus, in view of the markedly suppressive effect of antilymphocyte serum on the schistosome egg granulomas and its lack of effect on the plastic bead granulomas, it appears that the first goal—confirming the immunologic status of the former lesion—was achieved (Text-fig. 3).

Further aspects of the effect of antilymphocyte serum on the schistosome granuloma are worthy of discussion. Withdrawal of this serum early in the course of granuloma formation resulted in the rapid development of the granuloma to a size equaling those in animals treated with NRS. On the other hand, one dose of antilymphocyte serum had an inhibitory



TEXT-FIG. 3. Effect of NRS and RAMLS on granuloma formation around *S. mansoni* eggs as compared to divinyl-benzene-copolymer beads.

effect on granuloma formation around eggs injected at a later time, when the lymphocyte count had returned to 50% of its pretreatment level. This difference in effect might be explained by the occurrence, in the former case, of either a rebound phenomenon following the sudden release of immunosuppression, or antigenic stimulation of some cells which may still have been unaffected by antilymphocyte serum administered only 1 day prior to schistosome egg injection.

When antilymphocyte serum was administered after the onset of granuloma formation, it not only prevented the further development of the lesion, but also resulted in a marked diminution in granuloma size. This observation is in apparent contrast with the allograft system in which the onset of rejection is not prolonged if antilymphocyte serum is given 7 days after grafting,[15] but the gross observation of skin grafts may have little in common with granulomatous events which transpire at a microscopic level.

The anamnestic response to schistosome eggs was attenuated by antilymphocyte serum to a degree somewhat greater than that previously observed in neonatally thymectomized animals.[6] In both cases, however, the lesser effectiveness in suppressing the secondary rather than the primary reaction is obvious. This situation is similar to that occurring in graft rejections in which secondary allografts slough more rapidly in spite of immunosuppressive measures.[15] In addition, antilymphocyte serum did not prevent the induction of sensitization when administered for a 3-week period at the time of the primary intraperitoneal injection of schistosome eggs. This may be explained by the persistence of antigen secretion by the eggs which were protected by their shells. Furthermore, in the antilymphocyte serum-treated animals, the eggs were relatively unexposed to attack by the host cells.

The decreased ability of antilymphocyte serum to suppress the secondary as compared to the primary reaction, and its inability to prevent the induction of sensitization may explain the failure to fulfill the second purpose of these studies: to prevent granuloma formation in mice infected with *S. mansoni*. There was a slight reduction in granuloma size in the antilymphocyte serum-treated animals concomitant with slight decreases in severity (with respect to the relevant controls) in all of the disease parameters studied. The animals in this study were inordinately heavily infected, but even in mildly infected animals, neonatal thymectomy did not reduce granuloma formation to a level at which its effect on overt disease development could be studied.[6] The continuous output of eggs in the infected animals probably serves a role similar to repeated booster antigen administration, thus overcoming the effect of immunosuppressive measures.

The attempts to inhibit granuloma formation around schistosome eggs, which have thus far been unsuccessful, are continuing because the granulomas have not only been shown to be responsible for the anatomic changes,[1] but more indirect evidence suggests that they are also responsible for the pathophysiologic changes observed in hepatosplenic schistosomiasis.[2,3] Definite proof of this hypothesis would be provided by complete suppression of the granulomatous response. The prevention by potent immunosuppressive agents (including heterologous antilymphocyte serum) of disease in which the morbidity and lethal effects are the results of immune phenomena has already been demonstrated in the case of lymphocytic choriomeningitis[17] and allergic encephalomyelitis.[16] Perhaps the parasitic agent responsible for the disease syndromes of schistosomiasis may be bypassed in a similar manner, simply by preventing the host reaction to its presence.

Summary

The effect of RAMLS on granuloma formation around *Schistosoma mansoni* eggs injected intravenously into the lungs of mice was investigated. As compared to mice treated with NRS in which granulomas developed that reached a peak diameter 16 days after egg injection (217% greater than that of the egg alone), RAMLS-treated animals had virtually no granulomatous reactions. When RAMLS was withdrawn early in the course of granuloma formation, the lesions rapidly reached the size of those seen in NRS-treated animals. Onset of RAMLS treatment after granuloma formation began caused a rapid recession in their size. RAMLS did not completely prevent granuloma formation around eggs injected into the lungs of mice previously sensitized by an intraperitoneal injection of eggs, but the lesions were much smaller than in NRS-treated controls. The antilymphocyte serum had a negligible effect on the "foreign body" granulomas which formed around divinyl-benzene-copolymer plastic beads and caused only a slight diminution in granuloma size around eggs in the livers of mice infected with *Schistosoma mansoni* and in the various parameters of hepatosplenic disease studied in these animals.

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[*Illustrations follow*]

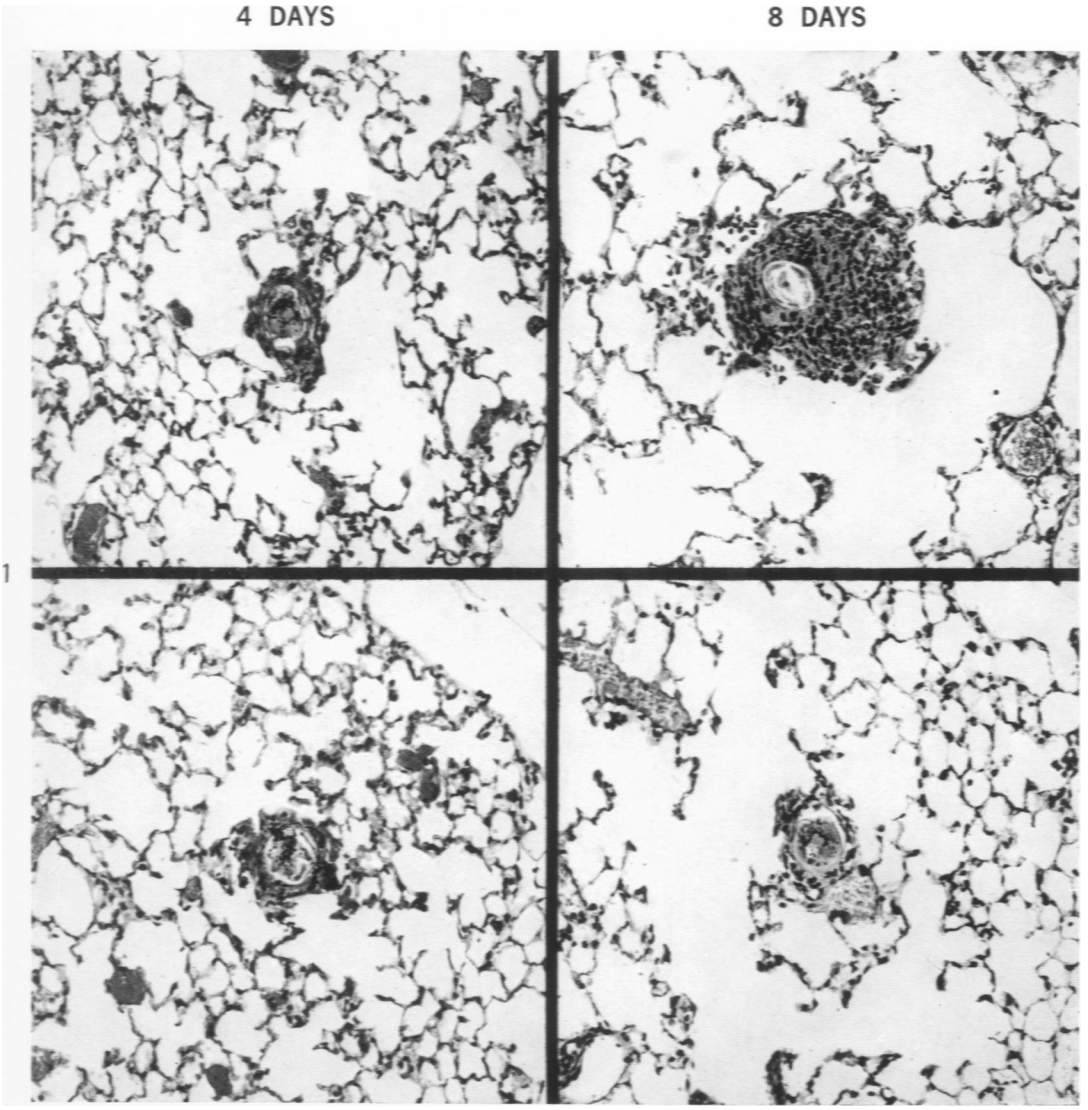
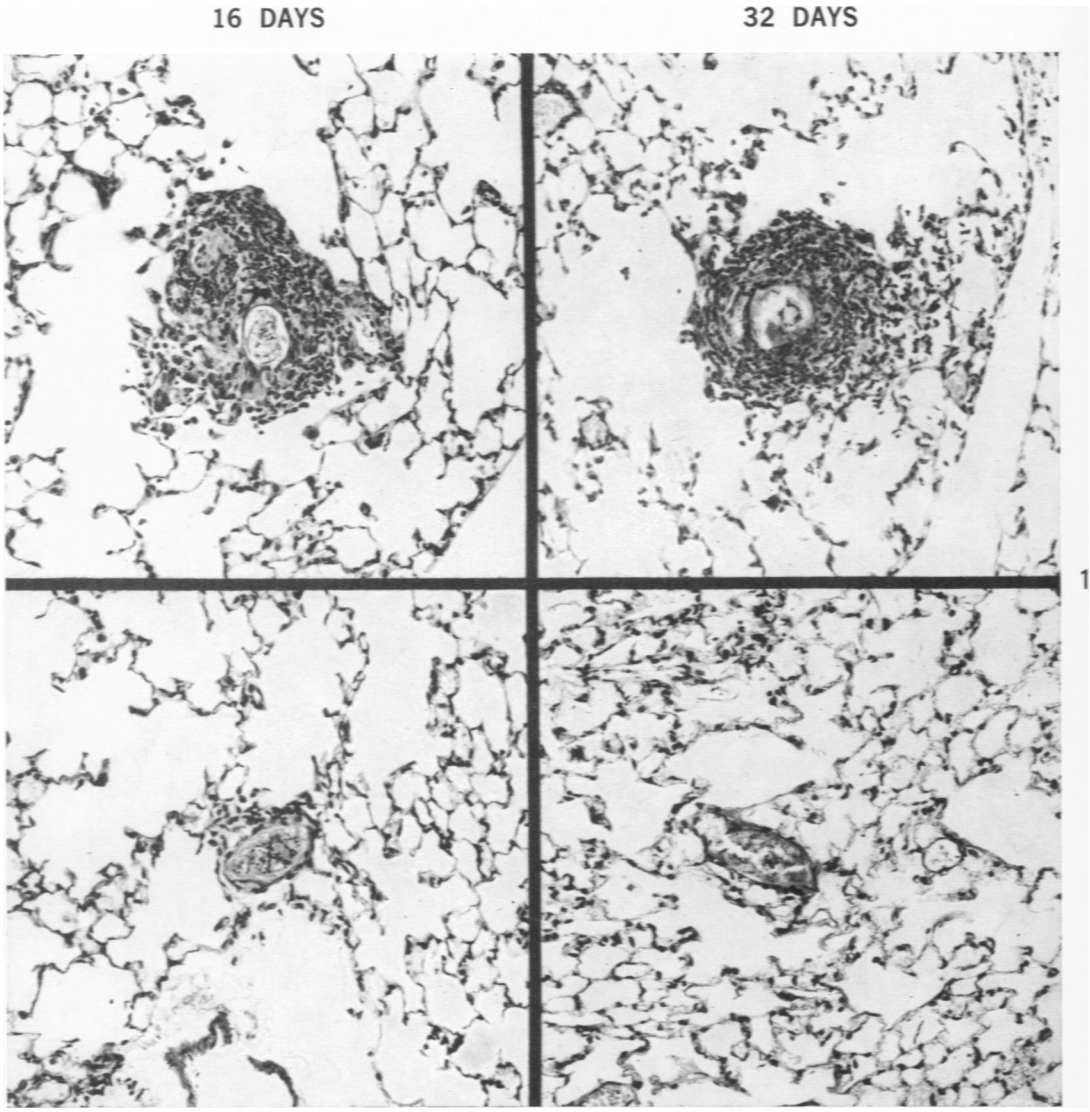


Fig. 1. Representative granulomas after I.V. injection of S. mansoni eggs into lungs of mice treated with NRS (top) or RAMLS (bottom). Hematoxylin and eosin. × 180.


16 DAYS
32 DAYS

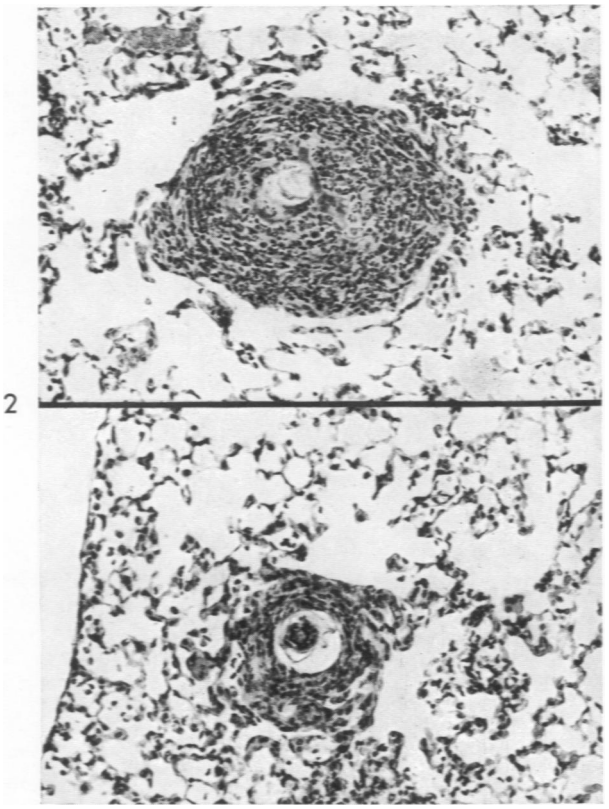


Fig. 2. Representative granulomas 8 days after I.V. injection of S. mansoni eggs into lungs of mice previously sensitized by I.P. injection of S. mansoni eggs and treated with NRS (top) or RAMLS (bottom). Hematoxylin and eosin. × 180.

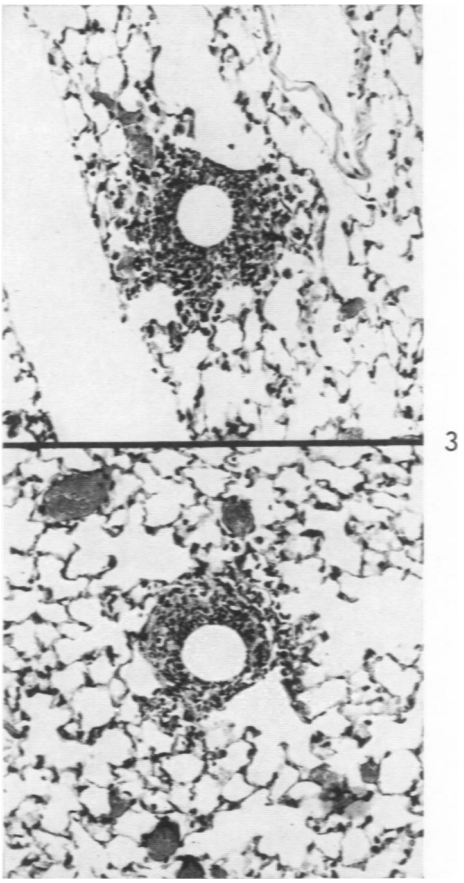


Fig. 3. Representative granulomas 2 days after I.V. injection of divinyl-benzene-copolymer beads into lungs of mice treated with NRS (top) or RAMLS (bottom). Hematoxylin and eosin. × 180.