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Effects of TRYPANOSOMA MUSCULI Kendall, 1906 Immune Serum on the Reproduction of TRICHINELLA SPIRALIS (Owen, 1835) Railliet, 1896 in Mice

Samuel L. Chafin

ABSTRACT

EFFECTS OF TRYPANOSOMA MUSCULI KENDALL, 1906
IMMUNE SERUM ON THE REPRODUCTION OF TRICHINELLA SPIRALIS
(OWEN, 1835) RAILLIET, 1896 IN MICE

by Samuel L. Chafin

The possible effects of ablastin, a reproduction-inhibiting antibody which is produced during the infection of the blood parasite, Trypanosoma musculi in the mouse, were investigated by fractionating the parasite into a supernatant fraction (designated as Group A test mice) and a pellet fraction (Group B test mice). These groups A and B mice were injected with the respective fractions, plus Freund's Complete Adjuvant (FCA). Control mice were injected with FCA only. Sera from these three groups of mice were removed after the Group A and Group B mice were shown to be negative to T. musculi challenge, and Group A and Group B mice were given their passive immune serum injections. The FCA control mice were similiarily given the FCA serum injections. These FCA control mice were given the same dose inoculum of Trichinella spiralis larvae by gastric gavage. On day 26 post T. spiralis gavaging, all of the mice were sacrificed, and the nematode larvae digested from the muscles of each mouse. The mean number of larvae recovered from the FCA control mice was significantly higher

(46,050) than the means of both Group A (12,427) and Group B (11,144), thus suggesting that the immune serum containing ablastin transferred to the test mice, suppressed the development of larvae as compared to the FCA control mice. A group of control mice which received normal mouse serum only, and given larvae by gavage at the same dosage level and at the same time, showed a markedly lower mean number of larvae (5,669) as compared to the FCA control mice, and about half as many larvae as the test mice, Groups A and B. The mechanism in operation which permits the FCA to greatly enhance the larval development in the host, possibly by suppressing the expulsion of the adult worms from the host's intestine, is not known. However, it must be considered as playing a major role, and suggests an area of future study. When the mean larval count of the control mice, which received only normal mouse serum, is compared with the mean larval counts of Groups A and B, it is noted that there was a 2-fold increase in the test mice. This agrees with findings of a pilot study as part of this investigation of a concurrent infection of mice with T. musculi and T. spiralis. In addition another worker observed a significant increase in Schistosoma mansoni adult worms recovered in mice concurrently infected with T. musculi and the blood fluke, as compared with the control mice. This suggests a change in the host's intestinal environment which tends to suppress adult worm expulsion.

LOMA LINDA UNIVERSITY

Graduate School

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Each person whose signature appears below certifies that he has read this thesis and that in his opinion it is adequate in scope and quality as a thesis for the degree Master of Science.

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INTRODUCTION

Two species of mammalian blood parasites, Trypanosoma lewisi found in rats, and Trypanosoma musculi found in mice, are known to cause a special type of acquired immunity in these rodent hosts, as described below. Taliaferro (1924) first described the appearance in the blood serum of rats, infected with T. lewisi, a substance which inhibits the reproduction of the parasite. Approximately 10 days after an established infection, parasite reproduction ceases.

Taliaferro (1924,1932) and Coventry (1929) described two types of antibodies present in the serum which have different functional characteristics. One type, called ablastin, inhibits reproduction at an early stage of infection, occuring at about day 10 post-infection, referred to as a crisis. At this time no reproduction occurs, but the parasites continue to persist in the blood. Furthermore, these parasites retain their infectivity when transferred to another rat. The adult forms persist for several weeks during which time their number gradually decreases. This is followed by a sudden second crisis, at which time all of the adult forms are destroyed, and the rat is immune to future infections with T. lewisi. This second substance is referred to as a trypanocidal antibody. Ablastin differs in one way from the trypanocidal antibody in that it can not be adsorbed from serum. Both are specific humoral antibodies (Taliaferro, 1941) and are probably produced by the lymphoid-macrophage or reticuloendothelial systems.

Thillet and Chandler (1957) showed that the immunity to infection with T. lewisi was due to the metabolic products of the trypanosomes. It was possible to actively immunize rats with these metabolic products, because when the parasite-free antiserum was injected into uninfected rats, passive immunity developed.

Taliaferro, et al.(1958) found that ablastin acts by inhibiting the synthesis of protein and nucleic acid. Chandler and Read (1961) stated that this could be either a cause, or more likely, a result of inhibition of reproduction which is attributable to interference with the process of nutrition and secondarily with respiration.

Pizzi and Taliaferro (1960) reported that the inhibition of division and growth by ablastin in T. lewisi was accompanied by an essentially complete inhibition of nucleic acid synthesis and a marked inhibition of protein synthesis.

In ablastin-inhibited trypanosomes the level of enzymes outside the Krebs cycle (D'Alesandro,1966) and glusose utilization (Patton,1975) are reduced. Patton (1975) provides evidence that ablastin arrests cell division as well as new evidence that the primary effect of ablastin is on membrane function. He also states that, except for an exceptionally low avidity, ablastin has the usual properties of antibodies as previously reported by D'Alesandro (1970).

D'Alesandro (1962) demonstrated ablastic activity in vitro and clearly showed that the anti-reproducing activity in immune serum is titratable.

The antigen inducing ablastin is called ablastinogen by Chandler (1958) and is found in the triturated bodies of trypanosomes. Even though ablastin (antibody) and ablastinogen (antigen) are demonstratable (ablastinogen is released by T. lewisi as a metabolic product) the location of ablastinogen, and thus the site of ablastin's action, are still unknown. Bawden (1975) states that certain properties of ablastinogen suggest that it has protein components: The antigen is inactivated by low and high pH treatment (pH 2.5 and pH 11.5); the antigen is destroyed by heating to 100 ^{o} C; the antigen is not detectably altered by ether treatment. Bawden (1975) suggests that ablastinogen is part of the plasma membrane, perhaps a part of the transport system, and that it is not entirely exposed to the outside surface of the membrane. Thus, ablastic antibody does not bind avidly to ablastinogen. He also suggests that ablastinogen is present in triturated trypanosomes because the procedure yields pieces of plasma membrane which contain ablastinogen. Patton (1975) reports that one of the most important features of trypanosome infection is that both reproducing and adult forms are susceptible to ablastin, which suggests that ablastinogen is a common antigen.

Taliaferro and Paulinova (1936) found that the general course of infections involving T. musculi (=duttoni) in mice were similar to T. lewisi in the rat. The passive transfer of immune serum to mice before infection with T. musculi extended the prepatent period and reduced the parasitemia (Wilson et al. 1973). In contrast to T. lewisi ,

irradiation of mice lowered the innate resistance to infections by T. musculi (Jaroslow, 1955). Dusanic (1975) reports that splenectomy before infection delays the synthesis of ablastin.

Patton (1972) showed that Ouabain, a cardioglycoside whose physiological action is the inhibition of the sodium pump, inhibits the reproduction of T. lewisi in vitro and that the effect is indistinguishable from reproduction inhibition produced by ablastin in vitro. He concluded that inhibition of the membrane ATPase by Ouabain is the primary mechanism by which the drug inhibits reproduction in the parasite, and that this (Na^++K^+) activated ATPase was sensitive to Ouabain and to the IgG rich fraction of immune rat serum. Thus it is likely, as D'Alesandro (1966, 1970) suggested, that ablastin acts by interferring with the transport of essential substances across the plasma membrane.

Targett and Viens (1975) reported the following experimental results of T. musculi infections. The parasite closely resembles T. lewisi both morphologically and in its reproductive characteristics. After a latent period which is determined by the size of the parasite inoculum, there is a phase of rapidly increasing parasitemia with dividing forms (mostly epimastigotes with a few trypomastigotes) present in the blood, although the most active reproduction occurs in the kidney capillaries (Wilson, 1971). The level of parasitemia becomes stabilized after 6-7 days and gives rise to a plateau phase lasting about 10 days. During this period the blood forms are

monomorphic, consisting entirely of long, slender adult trypomastigotes. The onset of the plateau phase with the elimination from the blood of all reproductive forms of the parasite is called the first crisis by analogy with a similar response in T. lewisi-infected rats. A second crisis at the end of the plateau phase produces an abrupt fall in parasitemia and the parasites disappear from the blood within a few days. Viens, et al. (1972) have shown, however, that multiplicative infective parasites are still present in the vasa recta of the kidneys of mice which had recovered from T. musculi infection about one year previously.

Targett and Viens (1975) using thymectomized mice, reported that the parasite T. musculi, continues to show multiplication throughout the infection, and this implies that T- cell deprivation has removed the factor (ablastin) responsible for inhibition of reproduction of the parasite, and that ablastin production is thus thymus dependent. They stated further that the anti-parasitic antibody (trypanocidal) which was unaffected by T-cell deprivation is therefore thymus independent, and was found by ultracentrifugation to be an IgM antibody (17s protein).

In their summary, Targett and Viens (1975) suggest that dividing parasites stimulate two systems. 1) B-lymphocytes synthesize antibody (IgM?) which has either a direct trypanocidal effect or an indirect action involving macrophages. 2) The second system is thymus dependent, sensitized T-cells activating B-cells to produce a

reproduction-inhibiting ablastin (IgG1?).

The immunity to the nematode Trichinella spiralis, as reported by Gonzalez (1940), is directed towards preventing the establishment of new adults in the intestine, but how this is effected is not known. In addition to that defensive mechanism of the intestine, there is also an immunity of the musculature which does not allow the few larvae that may reach it, to live and develop. This suggests a humoral mechanism for the immunity in both of these sites, as the serum from these immune animals has been proven protective. Furthermore, the immune serum derived from infected animals seems to have a stronger action on the larvae than on the adults.

According to Despommier, et al. (1977), the precise ways in which the host interrupts the biology of T. spiralis, thereby causing the reduction of muscle larvae, have not yet been fully elucidated.

Chandler (1935) reported the results of his experiments on the rat hookworm, Nippostrongylus muris. White rats infected with N. muris were injected with the immune serum of T. lewisi. No passive immunity to the nematode was demonstrated. Also he noted that the course of infection in the rat is not influenced by the immune serum.

Larsh (1967) reports that humoral antibodies at best play only a minor role in the elimination of trichina worms. Nevertheless, they are important in the total immunity, in that they exert deleterous effects on the worms, such as interference with growth and reduction of the reproductive potential.

Culbertson and Kaplan (1938) pointed out that the action of the antibody of trichinella immune serum appears to be directed specifically against the ingested larvae which are maturing to adult worms in the intestine of the infected animal.

Rappaport (1943) reported that the lethal T. spiralis dose was quite variable. He noted that in a few cases a dose as low as 30 larvae per g of body weight was lethal to mice.

T. spiralis larvae encysted in host musculature excyst in the new host's stomach 3-4 hr after ingestion. They then migrate to the small intestine of the host. Within the small intestine the adults are enbedded in the mucosa and crypts. Soon after copulation the males perish or are passed out of the bowel, and the females actively penetrate the crypts of Lieberkuhn. Here they deposit larvae and may persist for 4 to 16 weeks before they die. Approximately 2 days after birth the larvae begin to pass into the lymphatics and bloodstream, enroute to skeletal muscles. There is maximum invasion of muscle fibers by day 10. Day 14 shows a decrease in larvipositing. By day 17, larvae in the muscles are mature but not encapsulated. However, encapsulation is under way by day 21 and the bloodstream is practically free of larvae by the 25th day. Encapsulation is essentially complete at the end of one month.

Perrudet-Baboux and Binaghi (1978) state that following an oral infection with living muscle larvae of T. spiralis, animals develop an active immunity against subsequent infections. The animals become

resistant to new infections, but the larvae encysted in the muscle after the initial infection are not affected. The reasons for this paradoxical situation are not known, and may be related to the stage specificity of the immune response to T. spiralis.

Based on the literature cited above ablastin appears to be the substance that causes the inhibition of reproduction of trypanosomes in rats and mice. The direct mode of action of ablastin has not beeen formulated, partially because of its non-adsorbability. However, ablastin has caused essentially complete inhibition of nucleic acid synthesis and a marked reduction of protein synthesis. Ablastin has also reduced enzyme activity and glucose utilization. Evidence has been presented that ablastin arrests cell division, and that the primary effect is on membrane function. It has been reported that ablastin interferes with the transport of nutrients and other essential substances across the plasma membrane. The exact origin of ablastinogen is unknown, although it is presumed by at least one author to be in the plasma membrane.

Despite the few criticisms of ablastic immunity it is generally accepted that a true inhibition of reproduction occurs and that ablastin is responsible, but the nature of the antigen and the site of antibody activity have yet to be determined.

A pilot study was done by this author in which an attempt was made to determine the effects of ablastin on T. spiralis in mice. Mice were immunized with live T. musculi at various time intervals, then infected

concurrently with T. spiralis. The results (Table I) showed a significant increase in the number of larvae recovered from the test mice when compared to the controls. Also, those mice which received a killed antigen of T. musculi showed a much higher larval recovery as compared to the controls. A drawback of concurrent infections is that separation of ablastin from trypanocidal antibodies is impossible.

Few attempts have been made to determine the effects of ablastin on other species of parasites (Cestodes and Trematodes). Rigby and Chobotar (1966) studied the effects of ablastin on the cestode parasite, Hymenolepis diminuta, in a concurrent infection of the tapeworm and T. lewisi in the rat. When the test rats were very heavily inoculated with the trypanosome, there was a reduction in the mean net weight of the tapeworm as compared to the rats receiving a light inoculum of the blood parasite. Andeberhan (1976) found that in a concurrent infection with T. musculi and Schistosoma mansoni, the number of adult schistosomes recovered from the test mice was as high as or higher than the controls, and that therefore, ablastin as produced in the mouse in a natural infection, has no effect upon this blood fluke. Furthermore, when immune serum containing ablastin (obtained from a rat source infected with T. lewisi) was used in the mice to produce a passive immunity, the number of adult schistosome worms recovered from these mice was higher than for the controls.

Therefore, a research plan was designed in an attempt to determine the effects of passive immune serum (Ablastin) on the

nematode parasite, Trichinella spiralis, in mice and at the same time, attempt to determine the fraction in which the antigen (ablastinogen) is located.

MATERIALS AND METHODS

Fifty female albino swiss webster mice, weighing 19-21 g were obtained from Simonsen laboratories, Incorporated, Gilroy, California. They were housed on pine shavings in metal cages and fed with Purina laboratory chow meal. The feed was dispensed from stainless steel feeders suspended from the cage lids. These mice were used for the final phase of the study, involving the Trichinella spiralis test mice and the control mice. In addition, the same strain and sex of mice which were bred in this laboratory were used for the procurement of parasites and immune sera.

The Trichinella spiralis strain was obtained from Dr. Lawrence Ash of the University of California, Los Angeles, from a rat infected with 500 larvae. This rat was sacrificed, the muscles digested with artificial gastric juice and the larvae collected and used to infect the test mice.

Preparation of Trypanosomes

The Lincicome (L) strain of Trypanosoma musculi was maintained in a freezing solution consisting of 70 parts of 0.85% saline and 30 parts glycerine. The parasites were frozen and stored at -60°C to -70°C in a 1:1 mixture of freezing solution and blood, taken from heavily infected mice. For use, the frozen blood was thawed at 37°C. For the source of trypanosomes for the initial phase of the test, 0.1 ml of blood was injected intraperitoneally into each of 15 mice (Fig. 1). After 4 days the mice were bled by cardiac puncture with a heparinized needle and syringe. The blood was pooled and centrifuged to obtain the buffy coat

which contained the parasites. A Pasteur pipette was used to aspirate the live trypanosomes. After washing in normal saline, the trypanosomes were resuspended in normal saline and disrupted by sonication. The resulting solution was centrifuged at 18000 rpm for 30 min. The supernatant was mixed with an equal volume of Freund's complete adjuvant (FCA) (solution A). The remaining pellet was resuspended in normal saline and mixed with an equal volume of Freund's complete adjuvant (solution B).

Group A (13 mice), group B (13 mice) and the Freund's complete adjuvant control (2 mice) were given 0.2 ml of their respective solutions by intraperitoneal injections. Two boosters of the same volume at 3-day intervals were given to all groups. After 12 days representative mice from groups A and B were challenged with 0.1 ml of live trypanosomes, and the blood checked daily for parasites by the tail snip method to determine immunity. All the immune mice were bled in groups by cardiac puncture. The blood was allowed to clot, then stored at $4^{o}C$ overnight before centrifugation.

Fifty mice were divided into 4 groups: Group A (16 mice), group B (16 mice) group C, (controls 16 mice) and Freund's complete adjuvant controls (2 mice).

Group A, group B and the Freund's complete adjuvant controls were given 0.1 ml intraperitoneal injections of their respective sera. The group C (controls) were given 0.1 ml normal mouse serum, intraperitoneally, which was obtained by cardiac puncture of 6

uninfected mice.

Preparation of Trichinella

A rat infected with Trichinella spiralis was sacrificed with ether, skinned and eviscerated. The muscle tissue was cut into small pieces and put into an aqueous artificial digestion (gastric) solution containing 0.6% pepsin and 0.8% HCL. This muscle tissue and some gastric juice were added to a Waring blender for maceration. The resulting suspension of digestive juice and macerated rat muscle were incubated for 8 hrs. at 35°C. The mixture was then filtered through 3 layers of moist coarsely-meshed cheesecloth suspended over a funnel, and the larvae collected by sedimentation at the bottom of a centrifuge tube. From the centrifuge tube, the larvae were transferred to a nutrient broth solution consisting of 10-20% gelatin and 2% tryptose in water, maintained as a liquid suspension at 40°C.

The larvae were counted so that the gastric gavage dose could be established. Each mouse received 14.4 larvae/g body weight. The mean weight of the mice was 20g, so that each mouse received approximately 288 larvae.

All mice receiving Trichinella spiralis larvae were sacrificed 26 days post infection. They were skinned, eviscerated and the muscles of each mouse digested individually by groups in artificial digestion solution. After filtering with coarsely-meshed cheesecloth, the larvae were suspended in nutrient broth and counted in order to determine the total number of larvae digested from the tissues of each mouse.

The total larval counts were recorded for each mouse in groups A and B, and the FCA controls and statistically treated by the randomization test.

The Ouchterlony double diffusion test (Ouchterlony, 1949.) was utilized to determine if the immune serum was precipitatable by the sonicated fractions of groups A and B. The lymphocyte stimulation test (Pellegrino, et al. 1973), which can determine ablastic activity, was done in vitro using phytohemagglutin, concanavalin A and pokeweed mitogen. Tritiated thymidine was used as the tracer.

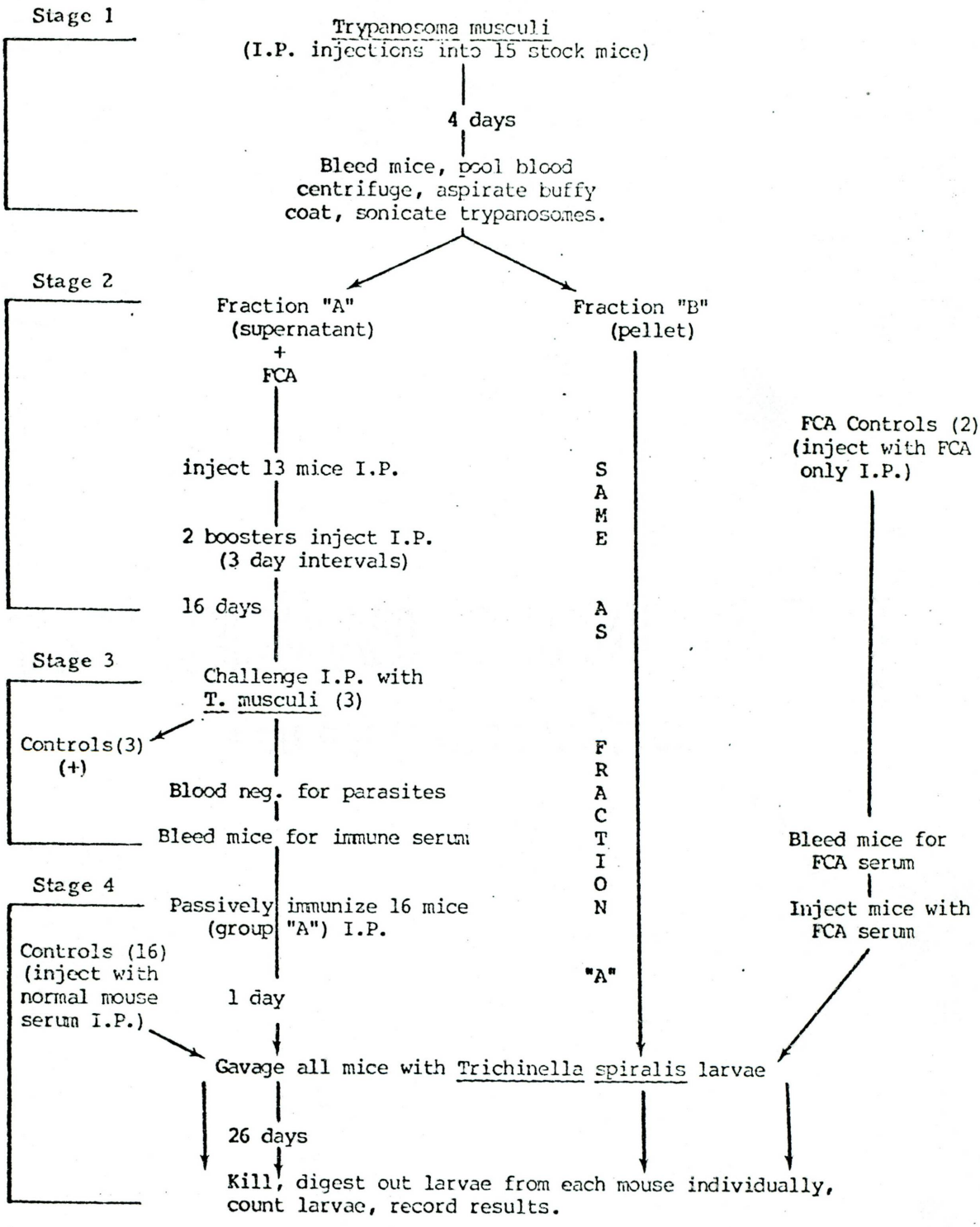

Figure 1. Flowsheet detailing the experimental design by stages 1 through 4.

RESULTS AND DISCUSSION

The results of this study are shown in tables II and III. The mean larval counts recovered from group A injected with immune serum produced using Freund's complete adjuvant + the supernatant fraction, and group B injected with immune serum produced using Freund's complete adjuvant + pellet fraction, were found to be much higher than the normal serum controls injected with normal mouse serum only. However, the counts from groups A and B were found to be significantly lower than the counts found in animals injected with serum produced by injection of FCA only.

The variance in larval counts in this study has been encountered in previous studies. Rappaport (1943) concluded that there were considerable variations in the proportions of larvae recovered to larvae fed to the test animals.

The mean count of the FCA control mice was 46,050 compared with 12,427 for group A, and 11,144 for group B, the normal mouse control count was 5,065. Unfortunately, there were only 2 FCA controls used. Both of these FCA control counts were higher than counts in either group A or B. The probability of this occuring purely by chance is less than 1/1100.

The mean larval count for the FCA controls is approximately 8 times that of the mean normal mouse control.

This indicates that there is a marked difference between the normal mouse serum controls and the controls containing FCA only. All

mice injected with serum derived from FCA-treated animals (FCA controls, group A, group B) have higher larval counts than the mean normal mouse control. Among the mice injected with FCA serum (3 groups), the control means are approximately 4 times the means of groups A and B, which were injected with fractions of the Trypanosoma musculi ablastin serum.

Parashar et al. (1977), working with Plasmodium berghei in mice, observed that immune serum in high doses (0.5 ml/mouse) enhanced parasitaemia, and when used in smaller doses (0.1 ml/mouse) afforded a greater degree of protection. He concludes that the production of specfic antibody is dose-dependent. He further states that the explanation for the dose-dependent variation in levels of parasitaemia will have to be determined by estimating the actual level of protective antibody in the serum transferred and the presence of the antigen-antibody complex.

In order to determine if this ablastin serum truly inhibits cell blastogenesis, the lymphocyte stimulation test in vitro was performed using tritiated thymidine as a tracer. The results (Table IV) show that the number of radioactive counts of the immune serum was substantially lower than the number of control radioactive counts, which did not receive the ablastin serum. This indicates that cell blastogenesis is inhibited by ablastin. Cell division is arrested and therefore, the tritiated thymidine is not being incorporated into the cells. The Ouchterlony test did not show any precipitation using the

immune serum and the antigenic fraction from groups A and B. This is consistent with the finding that ablastin is nonadsorbable.

The data show that the mean number of larvae recovered from groups A and B is significantly lower than the mean number of larvae recovered from the FCA controls. This reduction in the number of larvae recovered is assumed to be directly due to the effect of ablastin, inhibiting the reproduction of the T. spiralis parasite in the mouse.

Groups A and B and the FCA control mice have much higher larval counts than the control using normal mouse serum only. This indicates that Freund's complete adjuvant plays an important role in enhancing the number of larvae produced in the mouse, and/or interferes with the host's natural immunity.

As compared to the FCA controls, the reduction in number of larvae recovered in group A was 73% and for group B 76%. Although the reduction percentages between the two groups are not significantly different, the membrane fraction did appear to reduce the mean larval count slightly more than did the supernatant fraction.

The results of this study suggest that additional follow-up work be initiated to clarify the role of Freund's complete adjuvant in passive immunity. In such a test, control mice would be used with and without Freund's complete adjuvant, and these would be compared to T. musculi injected without Freund's complete adjuvant.

Table I. Larval counts of mice concurrently infected with Trypanosoma musculi and Trichinella spiralis at various time intervals before and after the mice were gavaged with the nematode parasite larvae. All mice were gavaged at the same time, designated as day zero.

Live T. musculi I.P. injections given days before (+) or days after (-)T. spiralis gavaging.	No. Mice	Mean No. Larvae	Range
Day + 8	3	9,050	1,750 - 13,400
Day 0	3	37,317	25,100 - 56,350
Day - 4	4	15,550	7,150 - 24,550
Formalin-killed T. musculi Antigen			
Day + 10 (3 boosters)	3	14,400	4,250 - 18,500
Day 0 (3 boosters)	3	31,600	20,550 - 42,950
Control mice (T. spiralis only)	4	4,575	3,400 - 5,600

TABLE II. Larval counts showing the effects of *Trypanosome musculi* fractions on *Trichinella spiralis* in mice

Mouse no.	FCA Controls	Group A	Group B
1	47,833	12,000	1,900
2	44,267	24,616	16,850
3		5,717	14,767
4		4,167	14,050
5		23,333	6,000
6		2,617	37,200
7		1,183	1,917
8		1,000	517
9		9,282	1,733
10		5,250	34,133
11		18,050	2,683
12		2,350	4,267
13		39,500	12,383
14		5,567	7,483
15		31,770	19,417
16			3,000
Mean	46,050	12,427	11,144
% reduction relative to FCA controls		73	76

TABLE III. Means ±(SE) of Square root of larval counts

	Means	±(SE)*	p value^
FCA Controls	46,050	36.7	
Group A	12,427	13.4	0.0147
Group B	11,144	13.0	0.0131

* Based on the pooled estimate of the variance of these 3 groups

^ p values were obtained from a Randomization test comparing groups A and B with the FCA Controls

Table IV. Results of Human lymphocyte mitogen stimulation test using phytohemagglutin (PHA), concanavlin A (CON A) and pokeweed (PW). Tritiated thymidine is used as a tracer. Percent reduction calculated from group/control index.

		COUNTS	MEAN COUNT	% REDUCTION VS. CONTROL
CONTROL	PHA:	198570		
		165448	191248	
		209725		
	CON A:	144766		
		147224	146507	
		147531		
	PW:	31760		
		28848	30398	
		30585		
GROUP A	PHA:	46491		
		36365	46867	84
		57744		
	CON A:	43605		
		44849	46211	80
		50180		
	PW:	30812		
		10684	20261	62
		19286		
GROUP B	PHA:	51869		
		44081	51944	94
		59883		
	CON A:	56025		
		42490	41746	90
		26723		
	PW:	17711		
		15151	14571	10
		10850		

SUMMARY

The results of this study demonstrate an effect of Trypanosoma musculi immune sera fractions containing ablastin on the production of T. spiralis in mice. The mice receiving immune serum produced using Freund's complete adjuvant (FCA) + the supernant fraction and those receiving the immune serum produced using Freund's complete adjuvant + the pellet fraction of T. musculi, had significantly fewer muscle larvae recovered than the FCA control mice. The percent reduction for groups A and B are 73 and 76 respectively. However, these two groups and their controls, all of which received Freund's complete adjuvant, had significantly greater numbers of larvae recovered than the mice receiving normal mouse serum only.

A major finding is that serum from mice treated with Freund's complete adjuvant appears to suppress the host's immune system and/or greatly enhances reproduction of the nematode parasite in mice.

The mechanism may be related to the changed condition of the host intestine and consequently the greater suppression of adult worm expulsion. An alternative view is that seen in the results in the concurrent infection (pilot study) test using T. musculi and T. spiralis, as well as that of the work by another investigator, (Andeberhan 1976), in which T. musculi and Schistosoma mansoni infections occured simultaneously in mice. In both instances, the number of larvae or adult worms recovered , respectively was greater than in the control mice. This is supported in this study by comparing

the number of larvae recovered from the control mice receiving only mormal mouse serum, and test mouse groups A and B.

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