The immunology of spontaneous cure in the Nippostrongylus brasiliensis-mouse system

by William Hiram Benjamin

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Microbiology
Montana State University
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Abstract:
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The serum factor was found to precipitate in 50% saturated NH4(SO4)2, was resistant to 56 C for 30 minutes, was dialyzable, was freeze-thaw resistant and apparently didn't bind to larvae in a way that adaptively transferred immune cells could act on the larvae. Protective serum was more effective when given on day 3 of a larval infection than when given on day 0 only. Protective serum injected intraduodenally on day 4 of an infection had no effect on the patency of the worm infection.

Expulsion of a N. brasiliensis larval infection by mice is very radio-resistant as shown by normal expulsion after 350 rads on day -1 only slightly delayed expulsion after 450. rads, The lesion created by 450-550 rads given before a larval infection can be repaired by 1 x 10^8 immune mesenteric lymph node cells and worms are expelled nearly at the normal time in mice given 550 rads and normal thymocytes on day -1.

Attempts were made to transfer immunity to nude mice with protective immune serum or immune mesenteric lymph node cells or a combination of the two, none of which transferred any apparent protection.

Evidence is presented that suggests that the expulsion of N. brasiliensis from mice isn't as prostaglandin dependent as the N. brasiliensis rat system. Neither aspirin nor in-domethacin delayed expulsion times. Experiments in which prostaglandins were injected intraduodenally also had little effect on an ongoing infection which is different than had been shown in the rat system.

The role of macrophages in the expulsion of a N. brasiliensis infection in mice was approached by using a macrophage lysozyme inhibitor, trypan blue. Larval brasiliensis infections in mice pretreated with trypan blue, then maintained on a chronic treatment schedule maintained their worms longer than controls. Mice given normal adult worms intra-duodenally after pretreatment and continued chronic treatment with trypan blue expelled their worm burden about the same time as non-trypan blue treated mice. These results suggest that trypan blue interfered in an afferent vs. effector immune function.

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Date 5 Oct 1979

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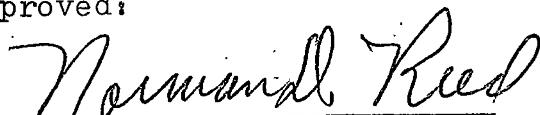
Approved:



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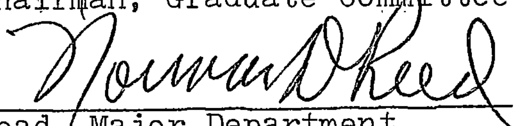

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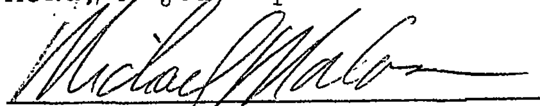

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ABSTRACT

Either cells or serum from immune animals has been found to be able to abrogate the patency of a larval N. brasiliensis infection when given to naive mice. All pools of cells that could cause abrogation of a larval patency were mesenteric lymph node cells collected from mice on days 9-12 after a primary N. brasiliensis infection. This correlated with a lymphadenopathy that was shown to occur at about days 6-12 after larval infection. No cell pools collected from secondary infections demonstrated the ability to protect a naive mouse to this extent nor did cells collected before or after this period. Pools of serum from mice 13 days after a second infection were commonly found to be able to transfer as high a level of protection as protective cells. A lot of variability in the degree of protectivity was found in both the serum and cell pools, even in pools that were apparently prepared in the same manner.

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INTRODUCTION

There are several reasons to study the immune response of mammalian hosts to intestinal nematodes. The most obvious is the large number of humans and their economically important domestic animals that are infected with nematodes (18,32). Some of the more common human intestinal nematodes are: *Ascaris*, which infects approximately one in every four people in the world; hookworms, which infect approximately 400 million people; and *Trichuris*, which infects approximately 350 million people worldwide (53). Intestinal nematodes aren't just a third world problem as shown by Warren's estimates of human infection in the United States (64). Other studies show half or more of children in some low-income rural communities in the Southeast U.S. are infected with *Ascaris* or *Trichuris* (1). A surprising amount of disease--both chronic disease, such as anemia and malnutrition, and the more acute medical emergencies, which are usually caused by *Ascaris*--occurs in the U.S. as a result of intestinal nematode infection (1).

The immunological response of humans to these nematodes isn't well studied. The apparent lack of expulsion of the adult worms and even the apparent lack of developed resistance to subsequent larval infection is well known (47) (although normal adults in *Ascaris* endemic areas seem to be

more resistant to challenge than their counterparts from nonendemic areas (37)). The lack of protection is evident even in apparently normal adult individuals, even though the individuals have antibodies of several classes to several antigens released by or present on the parasites (37,38). Some researchers have demonstrated cell-mediated immunity to be active in intestinal nematode infections in humans without evidence of protective immunity (59).

Currently, the best way to control most of these intestinal infections, at least in humans, is education and higher living standards. However, considering the poor response to this approach, vaccines seem to be a useful, possible adjunct. Because of the parasite-related problems incurred in livestock management, nematode vaccines will always be in demand (47). Much effort has been made in trying to prepare vaccines in nematode-host systems with very few usable results (5).

The first vaccines tested for nematode diseases were patterned after the bacterial vaccines. Attenuated (irradiated) worms and various preparations of killed worms have been tried. These have multiple deficiencies such as: lack of effective protection, being hard to prepare and store, and requiring adjuvants and large volumes.

The next step in preparing vaccines against intestinal nematodes seems to be finding out how these parasites can be acted upon by the host, the host response which is responsible for protection, and the antigen that stimulates this protective response (5). The best system in which to study this problem would be a parasite that stimulates long-lasting and complete immunity in its host. Several such systems with laboratory animals as the host are known (62), but even in the best characterized of these systems, neither the mechanism of expulsion nor the protective antigen(s) is known.

The Nippostrongylus brasiliensis rat system is probably the most completely studied of all of the intestinal nematode-host systems. I will now confine most of my remarks to this system because the system I am using is the analogous system of N. brasiliensis in mice. The rat system has been extensively reviewed (10,11,18,27,42,43,44) in the fairly recent literature and I won't attempt to duplicate this. The biology, host, geographical distribution, systematics and life cycle have been adequately reviewed by Haley (10,11). I will briefly give the life cycle here.

N. brasiliensis was first described in 1914 by Travassos and has been recovered in the wild from Rattus norvegi-

cus, Rattus rattus and Mus muscularis. Typical of all nematodes, there are four moults during the life cycle of N. brasiliensis. The immature eggs are passed in the feces of the host and, in proper conditions, embryonate and hatch in about 1 day. The first moult occurs on about day 1, with the second shortly thereafter. These first two larval stages, called rhabditoid larvae, feed on live bacteria in soil-feces or feces-charcoal mixtures. The third larval stage, which is the filariform or infective stage, crawl up to a high point where they wait until a prospective host passes by. The larvae, which are actively thermotactic, penetrate normal rat skin and feed their way to the lungs via the bloodstream and/or lymphatic channels in 11-15 hours. The larvae feed on blood in the lungs and moult at about 36 hours after infection. They are then coughed up and swallowed and the fourth stage larvae reach the small intestine where they take up residence and begin the fourth moult in about 48 hours (42). Fertile eggs are found in the feces of the host at 6 days post infection. The adult worms live in the duodenum and jejunum where they feed on host tissue but cause little in the way of specific pathologic lesions in light infections.

The infection in rats is essentially terminated after

12 days (42). The worms are eliminated by spontaneous cure which may leave a small residual worm burden but which confers complete immunity to subsequent infections. This phenomenon is probably the best studied expression of host immunity to a gut nematode and has been reviewed extensively (18,42,43,44).

Most of the work conducted to determine the active components and mechanisms of action in the expulsion of the worms during spontaneous cure has been done in rats. Studies have shown the immune expulsion of larval infection in mice closely parallels or is identical to that seen in rats, except it occurs a little earlier in mice (day 9 or 10) than in rats (day 12)(28,29). This work was done with a rat strain of N. brasiliensis. It was found that mice reject both the rat strain and the mouse adapted strain of N. brasiliensis on the same day but a greater percentage of the mouse adapted strain reach the adult stage (66).

One approach to understanding the mechanisms of expulsion and to affirm the immunological nature of spontaneous cure is to try to inhibit expulsion of worm burdens by immunosuppression. Some tools that delay expulsion are neonatal thymectomy, cortisone derivative treatment, lethal x-irradiation, thoracic duct drainage, and antithymocyte

serum (27). Recently, it has been shown that athymic mice (nude) don't expel a worm burden unless they are thymus grafted or injected with thymocytes (15,16,34). Taken together, these results suggest a thymus dependent step is necessary in the development of immune expulsion of N. brasiliensis.

Rats were observed to retain their worm burden longer than normal when infected during two special periods of the normal rat life cycle. One was during the neonatal period before 7-9 weeks of age, depending on the strain (40), and the other was during lactation (4). When the worms remaining after day 14 from either of these cases are examined carefully, it is evident that they are abnormal; these worms are given the label "damaged". Damaged worms are rapidly eliminated from normal rats if transferred directly into the duodenum. Normal worms, when transferred, are eliminated in 8-10 days, but damaged worms are eliminated in 5-6 days. The damaged worms have altered gut structure, the cells are vacuolated and the gut is distended. There are changes in isoenzyme patterns, reduced feeding on host tissue as measured by P^{32} uptake from the host, and the female worms have a reduced number of eggs in their uteri. During an infection in a normal rat, the worms are found to

be damaged by day 11 also, but are then rapidly eliminated from the host (44). In mice, the damage occurs earlier and is evident by day 6 (28).

A second method used to determine the mechanism of self-cure is to attempt to passively immunize naive animals with components from immune animals. Several different investigators have found that some degree of protection can be achieved with certain serum pools (41,52). Large amounts of serum are required and the passive protection is generally not nearly as effective as active immunity in eliminating worms. In a large group (48 pools) of sera tested, 1 out of 3 pools was somewhat protective (41). Hyperimmunization of donors with repeated larval infections didn't seem to increase the probability of a pool being protective or increase the titer of protection (41). A few cell preparations from immune rats and mice have been found which will protect nonimmune animals when transferred alone into them. However, protection approaching that seen in immune animals challenged with a larval infection has been achieved reproducably only by giving a rat or mouse both immune cells and immune serum before challenge (28,29).

Combining the two tools of passive and adoptive immunization and the neonatal and lactating rat models which are

defective in worm expulsion, a hypothesis of two step requirement for expulsion has been put forth (42). Both neonatal rats and lactating rats can cause the phenotypic changes in worms which is characteristic of damage, but in both of these systems the adult worms are expelled very slowly. Immune cells transferred into either of these systems from adult mice will cause expulsion in normal animals. Serum transferred from neonatal and lactating rats has been shown to cause increased protection of recipients against larval challenge. From this work it is felt that there is a humoral factor (antibody) that damages the worms which must precede the step requiring lymphocytes which causes the rapid expulsion (42,44).

Several groups of researchers then attempted to define the damaging factor(s) and the expulsive factor(s). To study the expulsive factor, a truncated infection was used. Worms were left in one rat until damaged, then transferred to the experimental host to determine what cells were required for the second rat to eliminate the worms (42,44).

Using this approach, damaged worms were transferred into rats irradiated at sublethal (440 rads) or lethal (750 rads) levels. These rats were then reconstituted to find out the cellular requirment for expulsion. It was found

that the lethally-irradiated animals required both an immune lymph node cell, presumably lymphocytes, and a bone marrow component to effect expulsion within 4 days. The sublethally irradiated animal needed only the immune lymph node cell (6,25). Several cell types were postulated as being the one supplied by the bone marrow. One of the more favored possibilities was the monocyte or macrophage (44).

It recently has been shown that rats lethally irradiated with 650-750 rads, then given damaged worms intraduodenally, can be reconstituted to expel the worms in 3 days if given Ig^- thoracic duct lymphocytes collected from rats infected 7-8 days or 13 days before collection. One of two pools of MLNC also caused expulsion from lethally irradiated animals (45).

Another interesting thing shown by this work is that even though there are in thoracic duct lymph on days 7 and 8 cells capable of causing expulsion, the worms normally wouldn't even be damaged until day 10 or 11. These results suggest that in a primary infection with <u>N. brasiliensis</u> the damaging step actually occurs after there are cells capable of effecting the expulsion in the thoracic duct lymph, making the damaging step the rate limiting step. It is well documented that pools of cells from like infections

have different activity when transferred (23,41).

The mechanism of action of the cell causing the expulsion is unknown. It is felt that the worms aren't killed in the small intestine but die in the adverse environment of the colon (42). Therefore, the cell that causes expulsion would only have to cause the worm to lose its hold for awhile. Because no cell has been seen to attach to the worm in the lumen of the intestine, it is thought to be a substance released by cells or an environmental change in the gut that causes expulsion (46).

Workers in this system have assumed that the humoral damaging factor is antibody and have tried to find the class that was produced at the right time in an infection to cause damage. One interesting thing about parasitic infections has been the high IgE levels, both specific for worm antigen and specific for unrelated antigens the animal comes in contact with (43). The role of IgE in passive protection and its active amine-releasing role in active infections has largely been discounted in damage or expulsion of N. brasiliensis (43,44). Recently, worm-specific IgA has been found in the gut of infected rats at the time that damage is occuring. The importance of this class in the host-parasite relationship hasn't been defined (49,54).

One study (20) demonstrated that the *in vivo* protective fraction of protective sera fractionated on one type of an ion exchange column contained mostly antibody of the IgG_1 subclass and protective fractions were found that contained little IgE, IgM or IgA.

One real problem with studying the damaging antibody is that it can only be demonstrated to damage worms *in vivo*. Worms live just as long in immune protective serum as they do in normal rat serum *in vitro* (21,31). Another reason that the effect of antibody can't be monitored *in vitro* is because, even in the optimum cultural conditions, after the worm is kept *in vitro* for 3 days, it looks exactly like *in vivo* damaged worms in all respects (31).

Recently, non-steroid anti-inflamatory agents such as aspirin and indomethacin have been demonstrated to delay expulsion of worms in a rat larval infection. Because a known activity of these agents is to inhibit prostaglandin synthesis, workers in Australia (9) injected purified prostaglandins directly into the gut on day 6 of an infection and found that this increased expulsion. This work interpreted with some even more recent work suggests that prostaglandins by themselves cause damage to the worm (50).

This inconclusive evidence, coupled with the fact that

a serum protective capacity doesn't correlate with any known antibody activity and the fact that sera from multiply infected rats don't protect more often nor at higher dilutions than sera from animals infected only once, suggests a factor other than antibody being the "damaging antibody". This is also suggested by the inconsistency of analogous pools of antiserum to transfer passive protection (41). Some work done in the mouse model also suggests that the damaging factor may be other than immunoglobulin. Mice panspecifically immunosuppressed with anti-μ expelled their worms at the same time as the normal control suggesting that specific antibody isn't necessary for expulsion (17).

This thesis will address the following areas of the <u>N. brasiliensis</u> system:

1) The macrophage has been suggested as a possible active participant in the expulsion of <u>N. brasiliensis</u> from rats. I will investigate the role of the macrophage in the self-cure phenomenon in mice by using the macrophage lysosome inhibitor, trypan blue.

2) The role of prostaglandins in the expulsion of <u>N. brasiliensis</u> will be investigated using prostaglandin inhibitors and synthetic prostaglandins.

3) I believe that the humoral damaging factor has been

incompletely studied. Other groups have just assumed that it is antibody and tried to find out what class of immunoglobulin is active or the antigenic specificity of the protective antibody. I propose that the humoral protective factor may not be immunoglobulin and will attempt to find protective sera pools from immune mice and characterize the factor.

MATERIALS AND METHODS

Parasites

A mouse-adapted strain of _N. brasiliensis_ was used in all experiments. This strain was derived from a rat strain by Dr. R. B. Wescott, Washington State University, Pullman, WA. (56,66) and has been passaged serially through at least 400 generations in mice.

N. brasiliensis was maintained in our laboratory by injecting 900 IL_3 in 0.1 ml saline subcutaneously on the dorsal aspect of the mouse. Feces were then collected on days 6, 7 and 8 post-infection by keeping mice in cages with 0.8 cm mesh wire bottoms over moist paper towels. The feces were then comminuted with water and mixed with moist granular animal bone charcoal (VWR Scientific, San Francisco) in a feces to charcoal ratio of 1:4 V:V. The mixture was divided between petri dishes and incubated at room temperature. Moisture was maintained throughout the incubation period. Larvae were used between days 5 and 20 after the culture was initiated.

The larvae were harvested from charcoal cultures by placing 40 C 0.85% NaCl in a small funnel with a screen (30 mesh/cm) which was covered by 2 layers of Kimwipes (Kimberly Clark Co., Neenan, Wisconsin). The larvae

migrated through the Kimwipes and were recovered after 1 hour by opening a clamp on the end of the funnel. The larvae were then washed twice in 0.85% NaCl by centrifuging at 200 g for 1.5 minutes. A suspension of 3,000 or 9,000 IL_3/ml was prepared by counting worms in 0.1 ml of suspension under a stereoscope, then resuspended in the correct volume of saline. Mice were then infected with 300 or 900 IL_3 as above.

Fecal Examinations

The modified McMaster technique of Whitlock (67) was further altered for use in estimating the number of worm eggs per gram of feces (EPG). Fecal material was comminuted with saturated NaCl (sp. gr. 1.20) in a ratio of 1 gm feces to 30 ml saline. Commonly, 0.25 gm feces was used with 7.5 ml saline. A portion of this suspension was rapidly transferred to a fecal egg counting chamber (Cutler-Haver-Lockhart Laboratories, Shawnee Mission, KS.). Counts of eggs from both grids of the chamber were averaged and corrected by a dilution factor of 200 to arrive at the EPG count.

Animals

All mice used in this work are either BALB/c raised in

this institution or a BALB/c Bom. line carrying the nude (nu) gene. The latter line was bought from Bomholtgoard, Ltd., Ry, Denmark and maintained in this laboratory by mating heterozygotes (+/nu) to obtain nude (nu/nu) congenitally thumus-deficient mice and their littermates (hereafter designated LM) which are one-third wild type (+/+) and two-thirds heterozygotes (+/nu). All mice were maintained on Wayne Lab blox and acidified water *ad libitum*. All animals were between 8- and 12-weeks old at the beginning of an experiment unless otherwise noted. The rats used to donate worms were Lewis strain females.

Necropsy and Worm Recovery

Mice were killed by cervical dislocation and the entire small intestine was quickly removed. The entire length of the small intestine was opened with scissors and placed in 37 C 0.8% NaCl on 1 thickness of gauze suspended in a plastic 250 ml specimen cup. The temperature was maintained in a water bath for 1-2 hours. Intermittent mixing was used to allow the adult worms to migrate to the bottom of the cup. The gut was then examined for residual worms. The worms that had migrated to the bottom of the cup were then counted under a stereoscope at about 10X as they were

picked up with a Pasteur pipette on a 10 ml syringe.

EP♀

The number of eggs in individual female worms (EP♀) was counted under a microscope at 63X on lined microscope slides. EP♀ from 20 to 50 female worms were counted from each mouse and the mean was calculated. EP♀ from mice which had less than 20 female worms weren't recorded.

Cell Transfer

Cells for transfer were prepared by dissecting out the organ of interest--either the mesenteric lymph node or thymus--and gently screening through a 100 mesh stainless steel screen to prepare single cell suspensions. The cells were then counted in a hemocytometer, washed once in balanced salt solution and resuspended to the correct concentration and, unless otherwise indicated, 1×10^8 cells were injected IV in a 0.5 ml volume.

Trypan Blue

Trypan blue was purchased from Kallestad Labs, Inc., Minneapolis, Minnesota and prepared according to Hibbs (13). The trypan blue was dissolved in double-distilled water and dialized against water for 72 hours at 4 C.

The dialyzed material was then lyophilized and stored at room temperature until used. Trypan blue was resuspended in 0.85% NaCl to a concentration of 10 mg/ml. Mice in the trypan blue treated groups received 3 mg IP 24 hours or 48 hours before the beginning of the experiment and 0.5 mg or 1 mg IP 3 hours or 1 day before the experiment. Mice in all treated groups were then given 0.5, 1 or 2 mg every third day subcutaneously until the experiment was terminated. Dosage protocols are indicated in footnotes to tables and figure legends.

Aspirin

Aspirin was acquired in tablet form from Bayer Company, New York, N.Y. The percentage active ingredient (acetylsalicilic acid) was determined. Two hundred mg of the active ingredient were dissolved in 2 ml of 95% ethyl alcohol then diluted to 50 ml with a 0.5% Tween 80 in water solution. Thus, the final concentration of acetylsalicilic acid was 4 mg/ml and 20 gm mice were given 0.25 ml, which is approximately 50 mg/kg, twice daily. Control groups were given the diluent without aspirin (3,24).

Indomethacin

Capsules of Indocin were obtained from Merck, Sharp &

Dohme. It was found that the active ingredient (indomethacin) was 10% of the capsule content's weight. The drug was dissolved in 0.125% Tween 80 to an active ingredient concentration of 0.04 mg/0.2 ml which was given by stomach tube twice daily beginning on day 0 (2).

Per Os Treatment Procedure

Mice were given drugs *per os* using intramedic polyethylene tubing (Clay Adams, Parsippany, N.J.) with outside diameter of 0.048 inches. A 1 ml tuberculin syringe with a 21 G needle inserted into the tubing was used to inject the drug. A tongue depressor was cut to 7 mm in width on one end which would fit crosswise in the mouse's mouth. A hole was drilled in the tongue depressor just large enough to allow the tube to pass through. It was positioned so the tube was guided down the esophagus when the awake mouse was held to maintain a straight neck. The tubing was inserted with a gentle twisting motion until it reached the stomach and the contents of the syringe were then emptied into the mouse's stomach.

Ammonium Sulfate Precipitation of Serum

Saturated ammonium sulfate was added dropwise to serum that had been diluted 1:1 with 0.85% NaCl until a volume

equal to the diluted serum had been added. Precipitation was then allowed to proceed at 4 C for 30 minutes. Then, the precipitate was separated by centrifuging 800 g for 30 minutes at 4 C. The precipitate was resuspended in phosphate buffered saline pH 7.2 (PBS) and both the precipitate (globulin-rich) and the supernatent (albumin-rich) fractions were dialized against 200 volumes PBS for 12 hours. Each fraction was reconstituted to the original serum volume and given to test mice, 0.5 ml IV and 0.5 ml IP (65).

Irradiation of Mice

Mice were irradiated using a ^{60}CO source while individually immobilized in a slowly-revolving plastic container. Source to skin distance was always 70 cm; the final dose received is listed in the appropriate places in the text.

SRBC Plaque Assay

The antibody response of mice was quantitated by a slide modification (33) of Jerne's (19) localized hemolysis-in-gel technique. Mice were plaqued on day 4 after being immunized IV with 0.25 ml of a 10% SRBC suspension.

Laporotomy for Inserting Adult Worms Intraduodenally

Normal rat worms (day 7 post-infection) were transferred to the duodenum of mice using the method of Jacobson & Reed (16). Briefly, the mouse's stomach and proximal duodenum were exteriorized through a 3-5 mm ventral incision. A purse string suture was placed in the greater curvature of the stomach and a Pasteur pipette containing 150 worms was inserted into a puncture wound in the area circumscribed by the purse string. The 150 worms were deposited in the duodenum approximately 1-2 cm from the pyloric sphincter. The pipette was removed and the purse string suture was tightened.

Intraduodenal Injections

The duodenum was exteriorized as in worm transfer and the drug or serum was then injected intralumenally into the duodenum within 1 cm of the pyloric sphincter with a 30 G needle.

Serum

Mice were bled retroorbitally while under ether anesthesia. The blood was then allowed to clot at room temperature for 2 hours, then it was refrigerated for 24 hours.

The serum was then separated from the clot and centrifuged to separate cells from the serum. The serum was then passed through a 0.45 μm millipore filter and stored at -20 C until it was used, when it was thawed at 37 C.

Freezing, Thawing and Heating Serum

Serum was frozen in a methanol bath at -50 C and thawed on ice. This was repeated 5 times. Serum was then used in the appropriate experiments. A second sample of serum was frozen, thawed and then maintained in a 56 C water bath for 60 minutes, then used in the appropriate experiments.

Cyclophosphamide

Cyclophosphamide (Mead, Johnson & Co., Evansville, Indiana) was dissolved in PBS to a concentration of 4 mg/ml. One-half ml was given to mice on day 2 of a larval infection (34).

PCA

IgE responses were measured in Lewis female rats using heterologus passive cutaneous anaphylaxis (PCA) assay (48). Mouse serum specimens were diluted serially 2-fold and 0.1 ml of these dilutions were injected intradermally into the shaved back of a rat. Four serum specimens could be titrat-

ed to 2^8 or 2^{10} in one rat. Forty-eight hours later the rats were given 1 ml IV of a solution containing 2,000 worm equivalents of N. brasiliensis antigen and 5 mg of Evans blue. The rats were killed after 30 minutes and the results were read as positive where a blue circle could be seen on the underside of the skin. The antigen used in this test was prepared by collecting worms from rats infected with 4,500 IL_3 8 days before collection. These worms were counted and macerated thoroughly in a glass tissue homoginizer. The preparation was then centrifuged at about 700 g for 10 minutes and the supernatent was frozen and used as antigen in the PCA test.

Prostaglandins

Synthetic prostaglandins used in this study were kindly donated by the UpJohn Company, Kalamazoo, Michigan. The prostaglandins PGE_1, PGE_2 and $PGF_{2\alpha}$ were received as crystalline solids and were dissolved in 95% ethyl alcohol, 10 mg in 1 ml. This was further diluted 1:10 with 0.2 M sodium phosphate buffer, pH 7.4. One-tenth ml of this was injected intraduodenally for a dosage of 100 µg/mouse (24).

Statistical Treatment

Worm recovery data are usually given as arithmatic mean

± standard deviation (SD). Because of the variance seen in this system, the number of worms recovered from individual mice is also listed most of the time. The method used to get EPG data precluded any statistical treatment as feces from all mice in a group were collected on the same tray. The Student T test was used to assess the significance of differences noted between means and a probability (p) value of less than 0.05 indicated significance.

RESULTS

Chronic Trypan Blue Treatment of Mice and the Elimination of *N. brasiliensis*

The role of macrophages in elimination of an infection of *N. brasiliensis* was approached by the method of inhibiting macrophage lysosomal enzymes with trypan blue (TB). This chemical inhibition was chosen because of its apparent lack of marked effect on the afferent and efferent limbs of the acquired immune response, except for the killing by macrophages of tumor cells (13,26).

The experimental design of the first TB experiment is shown below:

	Day -3	Day -1	Day 0	Day 3 and Every 3rd Day Thereafter
Group 1	-	-	300IL_3	-
Group 2	3mg TB IP	0.5mg TB IP	300IL_3	0.5mg TB SQ

Four of the mice in the experimental group died before monitoring of egg count began. Egg counts per gram (EPG) feces were monitored daily starting on day 6 (Figure 1).

The egg counts in the TB group were sustained at a high level for about 6 days longer than those in the control group. There was a definite high plateau in the EPG counts which suggests that the worm damage phase was delayed.