THE EOSINOPHIL RESPONSE IN MICE INFECTED WITH *Trichinella spiralis* OR *Trichinella pseudospiralis* AS INDICATED BY PHOSPHOLIPASE B ACTIVITY

DISSERTATION

Presented to the Graduate Council of the University of North Texas in Partial Fulfillment of the Requirements For the Degree of

DOCTOR OF PHILOSOPHY

By

Shing-Chien (Jane) Hsu, B.S., M.S.
Denton, Texas
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The host eosinophil response was compared in mice infected with either *T. spiralis* or *T. pseudospiralis* by determination of levels of splenic and intestinal phospholipase B, a marker enzyme for eosinophils. Primary infection of naive mice and challenge infection of homologously sensitized mice with *T. pseudospiralis* resulted in significantly lower tissue phospholipase B activities than infection with *T. spiralis*. Mice homologously challenged with *T. pseudospiralis* did exhibit an anamnestic eosinophil response compared to mice given a primary *T. pseudospiralis* infection. This anamnestic response, however, was significantly lower than the eosinophil response seen in sensitized mice given a homologous *T. spiralis* challenge. Mice sensitized to *T. spiralis* or *T. pseudospiralis* and heterologous challenge demonstrated an elevated eosinophil response compared to mice given a primary infection with either parasite. The heterologous challenge response, however, was not as intense as found for sensitized mice given a homologous challenge. These results
indicate that *T. spiralis* and *T. pseudospiralis* share antigens necessary for eosinophil stimulation, but quantitative and/or qualitative antigenic differences probably exist. Biochemical experiments indicated that eosinophils collected from *T. pseudospiralis* or *T. spiralis* infected mice possess equivalent cellular enzyme activity. Histological experiments demonstrated that infection with *T. pseudospiralis* induced fewer eosinophils to migrate into parasitized tissue than found for *T. spiralis*. Overall the suppression of the eosinophil response in host infected with *T. pseudospiralis* compared to *T. spiralis* indicates that the immunoregulatory effects described for *T. pseudospiralis* extend to the eosinophil. Results suggest that suppression of the eosinophil response is due to an inhibition of the migration of this cell into parasitized tissues.
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CHAPTER I

INTRODUCTION

*Trichinella spiralis* was discovered and described by Paget and Owen in 1835. It has been recognized as an important human pathogen worldwide, with its primary focus being in the United States, Canada, and eastern Europe (Gould, 1970). *Trichinella* is a highly successful parasite in that it can establish, develop, and reproduce in a wide range of vertebrate hosts.

The genus *Trichinella* is somewhat unique among parasitic nematodes in that all stages of the life cycle occur within a single host. Infection is initiated by the consumption of raw or insufficiently cooked meat containing the encysted larvae. After ingestion, larvae are liberated by the action of digestive enzymes and are transported by peristalsis to the upper small intestine, where they penetrate the epithelium and undergo four molts during a 30-hour period at the end of which they are adult worms. Five days after copulation females begin to deposit newborn larvae (NBL) that migrate through the lamina propria into either the mesenteric lymphatics and then the circulation, or directly into the bloodstream. Larvae eventually become distributed throughout the body. Larvae emerge from
capillaries and penetrate skeletal muscle cells where further growth and development takes place along with host cell biochemical and ultrastructural alteration. Twenty days after penetration, the larvae become enclosed within a collagenous capsule. Larvae that have penetrated tissue other than striated muscle fail to induce capsule formation either re-enter capillaries or become surrounded by inflammatory cells and eventually die. The encapsulated larvae which are infective for a new host, survive for years before eventually becoming calcified. The life cycle of the parasite is completed in approximately 30 days (Katz et al., 1982).

Taxonomically, *T. spiralis* has long been regarded as the only member of the family Trichinellidae, however over the last 20 years studies dealing with host spectrum, parasite morphology, cross-immunity and geographic distribution, among other factors, have led some authors (Nelson et al., 1961; Britov, 1969; Read and Schiller, 1969; Garkavi, 1972; Komandarev et al., 1975; Dick and Belosevic, 1978; Sukhdeo and Meerovitch, 1978; Belosevic and Dick, 1979) to propose species status for several organisms, especially *T. pseudospiralis*, (e.g., *T. nativa* Britov, 1971a, b; *T. nelsoni* Britov and Boev, 1972; *T. pseudospiralis* Garkavi, 1972).

*Trichinella pseudospiralis*, first reported by Garkavi in 1972, was isolated from the muscles of a raccoon (*Procyon*
lotor) in the northern Caucasus. Since then numerous studies have focused on its biological characteristics and the immunological response of infected host. Much of this work has been conducted in the murine host and has involved comparative studies of *T. pseudospiralis* with *T. spiralis* isolates. *T. pseudospiralis* is considered by some to be a distinct species because of differences in adult and larval morphology, host spectrum, the absence of a capsule surrounding the infective muscle larvae (Garkavi, 1972; Geller et al., 1977), a dramatically less intense inflammation accompanying invasion of host muscle (Bessonov et al., 1975), and the fact that this species has been reproductively isolated from *T. spiralis* (Bessonov et al., 1975). Madsen (1976), however, has maintained that there is not sufficient evidence to designate *T. pseudospiralis* as a species. He has suggested that further morphological, physiological, biochemical and genetic analysis must be conducted before assignment of species status to any strain or variety in the Trichinellidae is justified. However, in 1988 the international commission on *Trichinella* declared *T. pseudospiralis* is a new species (Stewart, 1989).

This research focuses on the comparison of the eosinophil response of the murine host during infection with *T. spiralis* and/or *T. pseudospiralis* by assaying tissue phospholipase B activity. Phospholipase B (E.C.3.1.1.5) has been biochemically characterized and has been associated
with eosinophils present in a number of animal tissues. A close association between the presence of parasitic worms and increased phospholipase B activity in parasitized host tissues has been demonstrated in mice infected with *Hymenolepis nana* and *T. spiralis*, and in rats infected with *T. spiralis*, *Nippostrongylus brasiliensis* and *Angiostrongylus cantonensis*. Although this enzyme has been extensively studied, its activity in tissues parasitized by *T. pseudospiralis* is unknown. Dramatic differences exist in terms of host immune response to *T. pseudospiralis* and *T. spiralis* (Stewart, 1989). The purpose of the present study was to focus specifically on the eosinophil response mounted against these two parasites in an effort to more clearly define the role played by their key granulocytes in host immune reaction to these two helminths.

Biology of *T. spiralis* and *T. pseudospiralis*

Biologically, *T. pseudospiralis* can be differentiated from *T. spiralis* by its smaller size in both the larval (Boev, Britov and Orlov, 1979) and adult (Dick, 1983) stages, its lack of a capsule surrounding the muscle phase, lower fecundity (Stewart, 1989), consistent establishment of larvae invading host muscle over time, and dose during primary infections (Kramar, 1981; Despommier, 1977), and the lower infectivity of infective first stage (L1) larvae isolated by pepsin-HCl digestion (Stewart and Deford, 1989).
Experimental infection of *T. pseudospiralis* in a variety of avian species demonstrates that, unlike *T. spiralis*, they can complete their life cycle in birds (Bober and Dick, 1983; Stewart, 1989). The fact that a greater fecundity results when *T. pseudospiralis* is incubated in vitro at 42°C rather than at 37°C reflects its adaption to parasitism of birds (Stewart, Kramar and Charniga, 1982). Additionally, the isolation of *T. pseudospiralis* from wild birds and mammals in the USSR (Dick, 1983) and in North America (Wheeldon, Dick and Schulz, 1983) suggest that they may have a broader geographic distribution.

Evidence suggests that *T. pseudospiralis* is less virulent than *T. spiralis* with the minimum lethal dose of *T. pseudospiralis* infective L1 larvae in outbred mice being over 20,000, while that for *T. spiralis* is approximately 4,500 (Flokhart, 1986; Dick, Currans and Klassen, 1985; Przyjalkowski, 1983 and 1985; Stewart, 1985, 1988 and 1989). Pig and human isolates of *T. spiralis* show similar levels of virulence in mice, while isolates from wild animals vary widely in virulence, and thus the use of different host strains and different parasite isolates makes comparisons difficult (Dick, 1983; Stewart, 1989).

During the adult phase of infection, with either *T. spiralis* or *T. pseudospiralis*, an inflammation of the small intestine develops characterized by a decrease in the villus/crypt ratio, an increase in the epithelial miotic
index and an infiltration of neutrophils, eosinophils, lymphocytes, plasmocytes, mast cells and macrophages. Although both parasites induce an inflammation, *T. pseudospiralis* has been shown to produce significantly less intestinal pathology, with infection resulting in a less severe enteritis compared to that for *T. spiralis* (Stewart, 1985 and 1988).

Stpiczynska (1986) demonstrated that the severe intestinal malabsorption of D-xylose evident in *T. spiralis* infection was not seen in rats infected with *T. pseudospiralis* (Stpiczynska and Starzenska, 1986; Stewart, 1989), which, surprisingly, have higher levels of D-xylose absorption than uninfected animals. This effect may be related to elevations in host-plasma corticosterone (Stewart, 1988, 1989), which may also underlie the weight gains seen in mice during the chronic phase of infection with *T. pseudospiralis*. Ten months following infection with *T. pseudospiralis* mice weigh more than uninfected controls, while mice infected with *T. spiralis* weigh less (Al Karmi and Faubert, 1980; Stewart 1989).

Approximately five days after copulation female worms begin to deposit live larvae which gain access to host lymphatic and circulatory systems. Larvae eventually enter host striated muscle cells and mature into infective larvae. *T. spiralis* larvae preferentially invade slow-twitch striated fibers, whereas those of *T. pseudospiralis* invade
both fast and slow-twitch fibers (Bagheri, 1986). During this period of infection, dramatic changes occur in the microarchitecture of the myofibers, which in *T. spiralis* infections are localized to the sarcoplasmic zones surrounding the larva. Both infected and neighboring cells become basophilic (Stewart, 1983), a feature associated with the disappearance of muscle fibrils and development of an extensive network of membrane organelles (Despommier, 1975). An altered cell enclosing the larvae which has been called a "nurse cell" develops (Purkerson and Despommier, 1975). Approximately 14 days after the invasion of muscle cells by *T. spiralis* larvae, a capsule of host origin (Stewart, 1983) begins to surround the nurse cells. There is no exchange of metabolites between the host and the encysted larvae.

Although the development of larvae of *T. pseudospiralis* is accompanied by similar basophilic transformation of infected muscle, a capsule never develops around the larvae, even during chronic stages of infection. This lack of a capsule surrounding *T. pseudospiralis* larvae may allow for the diffusion of parasite molecules involved in the modulation of host muscle and immunologic cells, since an inhibited cellular immune response has been observed around the larvae. Lack of a capsule has also raised several questions concerning the ability of the larvae to survive the putrefactive processes in carrion and survive the
inflammation that accompanies invasion of host muscle (Stewart, Wood and Boley, 1985).

Crude muscle larvae antigen from infective larvae and surface antigens from adult T. spiralis and T. pseudospiralis have been shown to be different as determined by biochemical analysis (Almond et al., 1986). Gamble and Murrell (1980), however, demonstrated that surface proteins of neonatal larvae were indistinguishable and excretory-secretory products from T. pseudospiralis bound antibodies isolated from T. spiralis-infected pig serum. Antigenic differences of infective larvae and adult worms may affect the biology of the two worms by influencing host immune responses against these parasitic stages.

Host Immunological Response to T. spiralis and T. pseudospiralis Infection

The most striking feature of the host response to infection with either T. pseudospiralis or T. spiralis is the resulting intestinal inflammation. Smith and Castro (1978) demonstrated that enteritis accompanying infection with T. spiralis in the rat could be quantified by determining peroxidase activity in the mucosa of the small intestine. Kramar et al. (1981) demonstrated murine intestinal myeloperoxidase activity during infection with T. spiralis to be greater than that found in mice infected with T. pseudospiralis. Stewart, Charniga and Boley (1982)
demonstrated the direct linkage of the inflammatory response with increased enzyme activity by showing a positive correlation between the presence of granulocytes infiltrating infected muscle and peroxidase activity.

The cellular responses of a host to infection with *T. pseudospiralis* are markedly less than those in *T. spiralis* infections. Stewart et al. (1985, 1988) showed a *T. pseudospiralis* dose-dependent suppression of inflammation in diaphragm and heart muscle, the small intestine, as well as a non-specific inflammation response to subcutaneous implanted cotton string. They demonstrated that an increase in numbers of *T. spiralis* larvae in host muscle was accompanied by a rise in myositis, but an increase in numbers of *T. pseudospiralis* larvae in host muscle was accompanied by a dramatic decline in myositis. Additionally, as infective doses of *T. pseudospiralis* were increased (500, 1000, 2000) in mice infected concurrently with 500 *T. spiralis*, myositis declined dramatically. These decreases in myositis during concurrent infections were not due to a decline in the overall number of larvae present in muscles or to a decline in the presence of *T. spiralis* larvae recoverable from host muscle.

Stewart et al. (1988) demonstrated that the suppression of the host inflammatory response in mice infected with *T. pseudospiralis* was associated with host plasma corticosterone levels that were significantly higher than
those seen in uninfected mice or in mice infected with T. *spiralis*. They found a significant elevation in enteritis in adrenalectomized T. *pseudospiralis* infected mice, but no influence of adrenalectomy on enteritis in mice infected with T. *spiralis*. Moreover, the severe myositis normally accompanying infection with T. *spiralis* was suppressed by concurrent infection with 1000 or 2000 T. *pseudospiralis* larvae to levels equivalent to those seen in animals receiving 0.15 and 0.41 mg cortisone acetate/25 g mouse/day, respectively. In addition, Stewart et al. (1982a) and Bell et al. (1983) have shown that a decreased intestinal response during trichinosis in cortisone-treated animals is accompanied by higher fecundity in adult T. *spiralis*. Therefore, it has been suggested that T. *pseudospiralis* modulates enteritis via induction of corticosterone production during concurrent infection with T. *spiralis* leading which accounts for an observed increase in the number of T. *spiralis* larvae becoming established in host muscle over that seen in infection with T. *spiralis* alone (Stewart et al., 1985, 1988).

Ritterson (1959) suggested that cortisone was responsible for prolonging the structural integrity of the injured muscle fibers infected by T. *spiralis*. Stewart et al. (1988) suggested that T. *pseudospiralis* may elaborate an endocrine modulatory substance that has a direct action at some point on the host neuroendocrine system, which may lead
to enhanced adrenal activity. On the other hand, it is possible that *T. pseudospiralis* induces the synthesis/release of a monokine or lymphokine having ACTH-like activity or pituitary-stimulating activity. Thus, the parasite may manipulate host neuroendocrine function by influencing molecular communication between the immune and neuroendocrine systems of the host.

The importance to muscle stage larvae of this anti-inflammatory effect was demonstrated in mixed infections with *T. pseudospiralis* and *T. spiralis* where recruitment of inflammatory cells around *T. spiralis* muscle-stage larvae was markedly diminished (Stewart et al., 1985, 1988) and significantly greater number of larvae established in host muscle (Stewart et al., 1985; Przyjalkowski et al., 1983).

In a comparative study of macrophages collected from *T. spiralis* and *T. pseudospiralis* peritoneal compartments, Dikon (unpublished or personal communication) found greater numbers of macrophages during infection with *T. pseudospiralis*, however cells collected from *T. spiralis* infected animals possessed greater phagocytic activity. Production of interleukin-1 (IL-1) by macrophages isolated from *T. pseudospiralis* infected mice was delayed although the production of prostaglandin E2, which is known to play a negative regulatory role in immune/inflammatory responses (Oppenheim et al., 1986), occurred earlier and at higher levels than in mice infected with *T. spiralis*. Thus, during
early stages of infection with *T. pseudospiralis*, alterations occur in macrophage function that may play a role in modulation of the host's responses to this parasite (Stewart, 1989).

A dramatic potentiation of natural killer (NK) cell activity is evident in the lungs of mice infected with *T. pseudospiralis* (Niederkon, 1988). Although infection with *T. spiralis* stimulates pulmonary NK activity, this effect is not consistently observed. NK cells do not appear to be protective in infections with *Trichinella*, but as the down-regulation of other components of the host cellular response occur in infections with *T. pseudospiralis*, NK cell activity may allow the host to deal with other pathogens or malignant neoplasms.

The reactivity of splenic lymphocytes collected from *T. pseudospiralis* infected mice to T- and B- cell mitogens is depressed (Tanner and Faubert, 1978). The cytolytic response of immune T-cells against allogenic antigens in *T. pseudospiralis* infected mice is similar to that of non-immune control mice and lower than that of immunized, uninfected or immunized, *T. spiralis* infected mice. The cytolytic T-cell response to tumor cells is not impaired in mice infected with *T. pseudospiralis* (Stewart, 1989).

There is disagreement among workers with regard to the levels of parasite-specific antibodies in mice infected with *T. pseudospiralis*. Some reports describe lower antibody
responses to *T. pseudospiralis* than to *T. spiralis* (Przyjalkowski et al., 1983; Van Knappen et al., 1981; Golinska et al., 1986), while others show the opposite (Al Karmi and Fabuert, 1985). Stewart et al., (1989) has shown that the level of total IgE in sera from mice infected with *T. pseudospiralis* is more than twice that of mice infected with *T. spiralis*. Additionally, Stankiewicz and Teska (1981) indicated that the L1 larvae of both species fix the C3 component of complement on their cuticle surface following exposure to normal rabbit serum. This event appears to opsonize larvae and help mediate the adherence of peritoneal phagocytes to the worm.

Several investigators have reported cross-resistance in mice to *T. spiralis* and *T. pseudospiralis* (Palmas et al., 1985), although the worm rejection in heterologous challenge of immune animals is much slower than that occurring during homologous challenge (Wassom et al., 1988). Furthermore, the proliferation of mesenteric lymph node cells from infected mice is significantly stronger in response to homologous antigen than heterologous antigens.

*T. pseudospiralis* adults are rejected more slowly during primary infection in a slow-responder mouse strain (B10G) than are *T. spiralis* adults, but both parasites are expelled at similar rates in a rapid-responder strain (NIH) of inbred mice (Palmas et al., 1985). In contrast, a more recent study using the same strain of rapid-responder mice
showed a longer survival and reduced fecundity in adult worms and a weaker and later stimulation of mesenteric lymph nodes of the host during infection with *T. pseudospiralis* (Stewart, 1989).

**Chemical Properties and Sources of Phospholipase B**

Phospholipase B (E.C.3.1.1.5.), also designated lecithinase B, lysolecithinase and lysolecithin acylhydrolase, is an enzyme which hydrolyzes phospholipids at α and β positions of the fatty acid ester bond (Lehninger, 1970). The action of phospholipase B and similar phospholipases is displayed below utilizing phosphatidylcholine as a substrate.

![Diagram of phospholipase action](image)

*Van den Bosch, 1982*
Phospholipase B can remove both acyl chains from a diacylglycerophospholipid such as phosphatidylcholine. Phospholipase B is always most active towards lysoglycerophosphatides, thus it possesses lysophospholipase activity in that it can remove the fatty acid esterified in the α position from lyso-compounds such as lysolecithin (Robertson and Lands, 1962; Rossiter, 1967). An example of the reaction catalyzed is as follows:

\[
\text{lysolecithin} + \text{H}_2\text{O} \rightarrow \text{Glycerol Phosphorylcholine} \\
\quad + \text{A Fatty Acid Anion}
\]

(Lehninger, 1970; Florkin and Stotz, 1973)

Phospholipase B was first recognized in rice bran (Contardi and Ercoli, 1933) and was subsequently demonstrated in certain molds (Faribairn, 1948) and bacteria (Hayaishi and Kornberg, 1954). Shapiro (1953) obtained active enzyme preparations from glycerol extracts of pancreas, and Dawson (1956) found liberated glycerol phosphorylcholine when extracts of rat kidney, spleen, brain and blood were incubated with lysolecithin.

The presence of phospholipase B in rodent intestine was first reported by Epstein and Shapiro (1959) and confirmed by Marples and Thompson (1960), Robertson and Lands (1962), and Ottolenghi (1964). In a quantitative assessment of enzymatic activity in rodent tissue, Marples and Thompson
(1960) found that intestinal, lung and splenic tissues were the most active, whereas nervous tissue and heart muscle were the least active. Comparing these findings on the whole ileum (i.e., mucosa and muscle) with those of Epstein and Shapiro (1959), who studied only the mucosa, it appears that the greater part of intestinal enzyme activity is associated with the mucosa.

**Effects of Parasitic Infections on Host Phospholipase B Levels**

The association between parasitic infections of the small intestine and increased phospholipase B activity was first demonstrated by Ottolenghi (1973a,b). In isolating phospholipase B for biochemical characterization, unusually high concentrations of the enzyme were found in the small intestines of mice which subsequently were found to be infected with *Hymenolepis nana*. Other host-parasite models have confirmed the association between the presence of parasitic worms in tissue and increased phospholipase B activity in the same sites. Larsh et al. (1974) demonstrated increased enzyme activities in the intestines of mice and rats after an initial infection with *T. spiralis*. In a subsequent paper, Larsh et al. (1975) found that sensitized mice challenged with *T. spiralis* showed an anamnestic-type response as measured by enzyme activity in intestinal tissue.
Ottolenghi et al. (1975) found elevated phospholipase B concentrations in the lungs and intestines of nonsensitized and sensitized rats after challenge with *Nippostrongylus brasiliensis*. Ottolenghi et al. (1977) also found elevated enzyme concentrations in the lungs and brains of rats infected with *Angiostrongylus cantonensis* during both a primary and secondary infection. Goulson et al. (1981) showed that rats given an infection with *Strongyloides ratti* developed elevated phospholipase B levels in the lungs and small intestines in both primary and secondary infections.

In addition to these helminth models Ngwenya and Capaci (1982) demonstrated elevated levels of phospholipase B in mice infected with the sporozoan *Plasmodium yoelii* and Laubach et al. (1982) demonstrated increased enzyme activity in the lungs of rats infected with the bacteria *Pasteurella pneumotropia* and *Mycoplasma pulmonis*.

The experimental findings described above demonstrate a relation between parasitic infections and increased phospholipase B activity and led to the suggestion that this relation was due to the presence of eosinophils infiltrating parasitized tissues.

**Association of Phospholipase B Activity and Eosinophilia**

It has been shown that increases in phospholipase B in tissues is associated with eosinophils. As examples,
Elsbach and Rizack (1963) have shown phospholipase B activity in homogenates of rabbit polymorphonuclear leukocytes from peritoneal exudates. Using histochemical techniques, Ottolenghi et al. (1966) demonstrated the presence of phospholipase B in various tissues of the rat. Cells exhibiting a positive stain were found in the lamina propria of the villi and crypts of the intestines, in the spleen, in the interstitial tissues of the lungs, liver, thymus, and in smears of bone marrow and peritoneal exudates. These tissues all contained the same type of positive cells that possessed acidophilic cytoplasmic granules surrounding a "doughnut" shaped nucleus and a strongly positive peroxidase reaction. This cellular staining and morphology corresponded to eosinophilic leukocytes. These findings demonstrated a close correlation between numbers of eosinophils in histological sections and cell suspensions with the enzymatic activity of the same preparations (Ottolenghi, 1970). Bone marrow preparations demonstrated a lower ratio of enzyme activity to the number of eosinophils. This may have been due to the fact that the bone marrow contained many immature eosinophils that lacked the enzyme. The morphological evidence that related eosinophils and the enzyme has also been demonstrated by Ottolenghi and Barnett (1974a, b).

A temporal parallelism has been shown to exist between elevated enzyme activity, parasite induced inflammation and
increased production of eosinophils. Based on these findings, and the reported close association between phospholipase B and eosinophils (Ottolenghi, 1970), Larsh et al. (1974) and Ottolenghi et al. (1975) developed a working hypothesis which suggested that elevated enzyme activities were due to parasite induced inflammation and that eosinophils were the source of the enzyme.

In testing this hypothesis Goven (1983) found that mice infected with T. spiralis and administered anti-eosinophil serum developed a significantly lower percentage of peripheral blood eosinophils and a corresponding lower intestinal enzyme activity as compared to infected mice not administered anti-eosinophil serum. The results of this study demonstrated that specific immunologic reduction of the host eosinophil response in a T. spiralis infection resulted in a diminished phospholipase B response, thereby providing support for the hypothesis that the eosinophil was the source of the enzyme.

Adewusi and Goven (1987) provided biochemical evidence concerning the leukocyte source of the enzyme by measuring the phospholipase B activity of pure populations of murine leukocytes. In their study, lymphocytes, macrophages, neutrophils and eosinophils collected from mice sensitized with T. spiralis antigen, and from nonsensitized mice, were assayed for enzyme activity. Results indicated that the only murine inflammatory cell type to test positive for
phospholipase B activity among individual cell populations was the eosinophilic leukocyte.

**Increased Phospholipase B Activity As a T Cell Dependent Function**

The proposed hypothesis of Larsh et al. (1974) and Ottolenghi et al. (1975) further suggested that T cells, after interaction with worm antigens, provided the stimulus for increased production and chemotaxis (possibly through the production of lymphokines) of eosinophils, which as terminal cells, underwent morphologic alterations at the site of inflammation and released their stores of phospholipase B. This suggested that the phospholipase B response was a T-cell dependent function, which as part of the complex immune response of the host, helped to limit the extent and the degree of infection by parasites.

It has been shown that congenitally athymic (nude) mice, which lack a T-lymphocyte population, infected with *T. spiralis* do not expel the parasite, thus demonstrating that immune expulsion is a thymus dependent phenomenon (Ruitenberh and Steerehberg, 1974). In light of these findings, Goven and Moore (1980) used *T. spiralis* infected athymic (nude) mice to test the hypothesis that the eosinophil and phospholipase B responses were dependent on thymus cells. In this study athymic (nude) mice, as well as, heterozygous mice were infected with 200 *T. spiralis*
larvae. Bone marrow eosinophils and intestinal phospholipase B levels were quantitated and compared to uninfected nude and heterozygous mice. The results showed that infected congenitally athymic mice did not develop an eosinophilia or an increased intestinal phospholipase B activity at any time during the experiment, while heterozygous mice infected with 200 larvae developed a marked eosinophilia and increased phospholipase B activity.

These data demonstrated that thymus-derived cells are necessary for the development of both bone marrow eosinophilia and for increased enzyme activity in an animal infected with *T. spiralis*. The rationale behind the lack of either response is the absence of a T-lymphocyte population in these animals. These results supported the hypothesis which suggested that a T-cell population is necessary to interact with worm antigens to provide a stimulus for increased eosinophil production and a chemotactic substance that directs these cells into areas of inflammation, where they release their stores of phospholipase B (Goven and Moore, 1980).

The dependency of eosinophil production and phospholipase B activity upon a T-lymphocyte population was further supported by Adewusi and Goven (1987) in a study which involved administration of anti-thymocyte serum to mice infected with 100 *T. spiralis* larvae. Bone marrow eosinophils, peripheral blood eosinophils and intestinal
phospholipase B activities were quantitated to determine the effect of anti-serum on these parameters as compared to those in animals infected but not given anti-serum, and to uninfected animals both with and without administration of anti-serum. The results demonstrated significantly lower levels of peripheral eosinophils, bone marrow eosinophils, and intestinal phospholipase B activity in infected mice treated with anti-thymocyte serum compared to untreated infected mice. Therefore, giving further evidence to support the hypothesis that thymus derived lymphocytes influenced the tissue concentration of phospholipase B via their action on eosinophils.

Adewusi and Goven (1987) also provided information on the mechanism of T-lymphocyte involvement in the eosinophil-phospholipase B - parasite association. They demonstrated that the eosinophil can vary in its phospholipase B activity and that the level of enzyme activity per cell was influenced by parasite antigen and other cells. Specifically, when cultured in vitro with T-lymphocyte and macrophages, eosinophilic phospholipase B activity increased fivefold over that of cells cultured alone or with other combinations of cells. This suggested that a lymphokine (possibly eosinophil stimulation promoter) could stimulate eosinophils to synthesize phospholipase B. Numerous investigations have demonstrated that factors released from T-lymphocytes regulate the production, migration and
antiparasitic function of eosinophils. These factors, characterized as lymphokines, including eosinophil colony-stimulating factor (EO-CSF), eosinophil stimulating promotor (ESP), and eosinophil chemotactic factor (ECF).

Role of the Eosinophil in Host Defense against *Trichinella*

Eosinophilia is characteristic of a mammalian host response to tissue invasion by helminths. Grove et al. (1977) found that eosinophil depletion in *T. spiralis* infected mice did not affect the rate of expulsion of adult worms but did result in increases in numbers of muscle stage larvae. He suggested that eosinophils may play a crucial role in resistance against larval but not adult stages of the parasite. Kazura et al. (1980, 1981) examined the association of granulocytes to *Trichinella spiralis* NBL. Eosinophils and neutrophils were found to attach to NBL and exert a cytotoxic effect manifested as complete morphologic destruction of the organisms. Assessment of the time course of neutrophil and eosinophil attachment and cytotoxicity indicated that fewer neutrophils than eosinophils maintained stable physical contact with NBL. They also indicated that the role of eosinophils in parasite destruction was dependent on stage-specific IgG antibodies which developed at high titer within the first three weeks of infection, and only reacted with the NBL.
Eosinophil-mediated damage of *T. spiralis* initially involves intimate contact of the cell surface with the larval cuticle followed by the development of long cytoplasmic projections that spread over the surface of the parasite. The major components of the cuticle of NBL are collagen and phospholipids such as phosphatidylcholine and phosphatidylethanolamine. Quantitative differences of these constituents in the cuticles of NBL and other stages of *T. spiralis* may explain the differential susceptibility of various stages of the parasite life cycle to eosinophil-mediated damage (Castro and Fairbairn, 1969).

Several characteristics of the eosinophil may relate to its particular capacity to induce larval damage. As eosinophils contain large amounts of phospholipase B (Kater, Goetzl and Austen, 1976; Ottolenghi, 1970), they may be capable of damaging a cuticle rich in phospholipids. Additionally, Ottolenghi et al. (1975, 1977) speculated that increased amounts of phospholipase B resulting from parasitic infections were probably indirectly involved in worm expulsion. It is possible that the enzyme is involved in the synthesis of prostaglandins, which have been shown to have a direct role in parasite expulsion (Kelly and Dineen, 1972). The reaction of the enzyme with cell membrane phospholipids results in the production of free fatty acids. These fatty acids, such as arachidonic acid, could be
converted to primary prostaglandins of the PGE, and PGF series by prostaglandin synthesis (Kelly and Dineen, 1972).

Major basic protein, a constituent of the crystalloidal granules of eosinophils, may be toxic to multicellular organisms, although other basic proteins possess similar cytotoxic properties (Butterworth et al., 1979). Eosinophils also contain multiple lysosomal enzymes, peroxidase being among the most abundant (Cohn and Hirsch, 1960). This enzyme, in the presence of H$_2$O$_2$ and a source of halide iron, has been shown to lead to the death of *Schistosoma mansoni* schistosomula. In addition, eosinophils incubated with antibody-treated schistosomula release H$_2$O$_2$ (Kazura, Blumer and Mahmoud, 1979).

Since the eosinophil has been strongly linked to the host immune response against parasitic infections, it would be of interest to compare the host eosinophil response during infection with *T. pseudospiralis* with that of *T. spiralis*. Phospholipase B, an enzyme uniquely associated with the eosinophil, can be used to assay for the eosinophil response.

The following questions were addressed in this research.

I. How does the host eosinophil response, as measured by tissue phospholipase B activity, compare in mice given a primary infection with either *T. spiralis* or *T. pseudospiralis*?
II. How does the host eosinophil response, as measured by tissue phospholipase B activity, compare in immune mice given a challenge infection with either *T. spiralis* or *T. pseudospiralis*?

III. How does immunization with a primary *T. spiralis* or *T. pseudospiralis* infection effect the host eosinophil response on cross challenge with either *T. spiralis* or *T. pseudospiralis*, as measured by tissue phospholipase B activity?

IV. How does infection with *T. spiralis* or *T. pseudospiralis* effect synthesis of phospholipase B by eosinophils?

V. How does the infiltration of eosinophils into tissue compare with the sensitized tissue phospholipase B activity mice given either a *T. spiralis* or *T. pseudospiralis* infection?
CHAPTER II

MATERIALS AND METHODS

Mice

Eight to 10 wk old male ICR (Southwest Breeding Laboratory, Inc., Midlothian, Texas) or BALB/c x ICR mice (bred at UNT) were used in all experiments. Mice were housed in a temperature controlled animal room in plastic cages with five mice in each cage. Purina Laboratory Rodent Chow and water were provided ad libitum.

Parasites

The strains of *T. spiralis* and *T. pseudospiralis* were maintained in adult ICR mice and were obtained from the University of Texas at Arlington. The techniques of Larsh and Kent (1949), Weatherly (1970) and Stewart (1989) were used for isolation, collection, standardization of the inocula, and infection of experimental animals.

*T. spiralis* larvae were isolated from the muscles of mice infected at least 45 days previously with 500 *T. spiralis* larvae. Mice infected with *T. spiralis* were killed via cervical dislocation, after which the carcass was skinned, eviscerated and rinsed in cool tap water.
carcass was coarsely minced with scissors and homogenized in digestion medium (10 g Merck granular pepsin, 1% HCl, 1000 ml H₂O) using a blender. Following digestion at 37°C for one hr with constant agitation, the mixture was strained through two layers of cheese cloth into a 1 l graduated cone and incubated for an additional 20 min to allow settling of the larvae. About 800 ml of supernatant was carefully aspirated off and saline was added to the remaining 200 ml containing the larvae to wash and to further facilitate settling of the larvae. After a 20 min sedimentation at room temperature, the supernatant was aspirated down to 10 ml, being careful not to disturb the larvae pellet at the bottom of the tube. The whole suspension was transferred to a small beaker containing a stirring bar. A 100 µl aliquot containing larvae was transferred from the gently stirring mixture to a counting plate and counted using a hand tally and stereoscopic microscope. Larvae were added or removed from the mixture until 10% of the desired number were present in a 0.01 ml sample; 0.1 ml represented the standard inoculum.

Instead of pepsin HCl digestion medium, PBS (pH 7.0) was used for the isolation of *T. pseudospiralis* (Stewart et al., 1989). Infective larvae were obtained from skinned, eviscerated mouse carcasses, which had been rinsed in PBS and diced into 0.5 to 1 cm³ pieces. These preparations were
placed on plastic screens (7 openings/cm) mounted on plastic platforms supported approximately 5 cm above the bottom of a large glass bowl. The platforms containing the tissue was placed in the bowl with sufficient PBS (pH 7.0) to cover the tissue on the screen. This apparatus was placed in a water bath with water approximately 2 cm deep and incubated at 37°C for one hr without shaking. The mixture was strained through two layers of cheese cloth and the larvae concentrated by gravity sedimentation for 20 min. The total number of infective larvae was determined as above for T. spiralis. Larvae that were tightly coiled and moving were considered viable.

Source and Preparation of Lysolecithin

Lysolecithin (Sigma Chemical Company, St. Louis, Missouri) was dissolved in distilled water to give a concentration of 2 x 10^{-2} M and stored in aliquots of 4 ml at -10°C. The frozen lysolecithin was thawed in a water bath at 37°C immediately before used.

Determination of Phospholipase B Activity

The method of Larsh et al. (1974) was used to prepare and assay intestinal and splenic tissue samples for phospholipase B activity. Briefly, mice were killed by cervical dislocation on the days indicated in the tables and
figures. Spleens and intestines were removed, freed from adhering tissues, and in the case of the intestine, the contents extruded. Tissues removed were weighed and placed on ice to conserve enzyme activity. After weighing, each tissue sample was minced with scissors and mixed thoroughly. One g of each sample was then homogenized in 9 ml (1:10 w/v dilution) of ice-cold 12.5% glycerol buffer medium (12.5 % glycerol, 0.1 M K₂HPO₄, 5 x 10⁻¹ M MgCl₂, 2 x 10⁻³ M ethylenediaminetetraacetic acid (EDTA), pH 6.6) in a motor driven, chilled Teflon glass homogenizer. After homogenization the intestinal mixture was poured into separate 50 ml centrifuge tubes and placed on ice. A 50 μl aliquot of the homogenated intestinal mixture was transferred to a test tube containing 4.95 ml of glycerol buffer and placed on ice. This represented a 1:100 diluted of the homogenate which was originally diluted 1:10 for homogenation. Thus, the final dilution of the intestinal homogenate was 1:1000. The entire spleen of each mouse was weighed and homogenized in the same dilution medium as for the intestine. Splenic tissue samples were diluted at a 1:20 (w/v) ratio. After homogenization of both spleen and intestine, 40 μl of the homogenate was transferred with a micropipette to 15 ml glass centrifuge tubes, and brought to a final volume of 0.6 ml with glycerol buffer (pH 6.6). Two test tubes were prepared for each sample. Duplicate tubes
of each sample were incubated at 37°C in a water bath for 4 min to allow for temperature equilibration. The reaction in one tube was started by adding 0.3 ml of lysolecithin solution (20 µ mol/ml) prewarmed at 37°C with gentle mixing. The other tube served as a reaction blank. Incubation was continued after mixing until the reaction was stopped after 30 or 60 min, or at the first signs of formation of a cloudy white precipitate. The reaction was terminated by the addition with mixing of 0.2 ml of 2 N H2SO4, followed by 1 ml of isopropyl alcohol and 0.4 ml of deionized, distilled water. After the reaction was stopped, fatty acids were extracted by addition of 2 ml of heptane. To enhance the extraction of fatty acids, tubes were vortexed using a Vortex-Genie (Scientific Industries Incorporated, Bohemia, New York) for 1 min. Tubes were then centrifuged at 500 x g for 10 min to enhance the separation of the heptane layer containing the fatty acids. After centrifugation, exactly 1 ml of the heptane layer containing the fatty acid solution was carefully transferred by micropipette to a clean 10 ml glass test tube for titration. The reaction blank tubes were treated in the same manner.

The technique described by Dole (1956) was used for titration of the extracted fatty acids. Thymol blue (Difco Laboratories, Detroit, Michigan), used as indicator, was made fresh each day by mixing 1 ml of stock solution (500 mg
thymol blue, 500 ml absolute ethyl alcohol) with 40 ml of isopropyl alcohol. One ml of thymol blue was added to each experimental and control tube for titration. The extracted fatty acids were titrated with 0.01 N NaOH using a syringe microburet (Micro Metric Instrument Company, Cleveland, Ohio). The 0.01N NaOH was prepared fresh each day from a stock solution and calibrated by titration against 1.0 ml of palmitic acid reference standard in heptane (2 μmol/ml) (Eastman Kodak Company, Rochester, New York).

Nitrogen gas was bubbled into the test tubes throughout the titration procedure to enhance the mixing of the solution and to expel any carbon dioxide. The end point of the titration was reached when the color changed from pink to blue. The phospholipase B activity was expressed as μmol of lysolecithin hydrolyzed per gram of wet tissue per hr.

Eosinophil Collection and Phospholipase B Activity

Eosinophils were collected as described by Mahmoud (1973), Colley (1974) and Kazura (1978) from ICR mice infected with either 500 T. spiralis or 500 T. pseudospiralis larvae on day 12 post-infection.

On day 12 post-infection, 10 T. spiralis-infected mice and 10 T. pseudospiralis infected mice were each given an intraperitoneal injection (i.p.) of 1.5 ml sterile 10 %
protease peptone to stimulate production of peritoneal exudate cells. After 48 hr mice were killed using carbon dioxide gas, after which, 3.0 ml of heparinized Hank’s Balanced Salt Solution (HBSS) was injected intraperitoneally. The abdomen was gently massaged to free peritoneal cells, after which the skin was excised and the exudates collected. The intestine and spleen of each mouse were kept for determination of phospholipase B activity. Cells from each animal were washed three times with HBSS by centrifugation at 100 x g for 10 min at 4°C. After the final wash, cells were counted and adjusted to a concentration of 1 x 10^6 eosinophils per ml. Approximately 30 % of the cells were eosinophils for both _T. spiralis_ and _T. pseudospiralis_ infected animals as determined by differential counts with Discombe’s dilution fluid (0.1 g aqueous eosin, 10.0 ml acetone, 90.0 ml water) and Wright’s stains. Cells were centrifuged and resuspended in saline such that each tube contained 1 x 10^6 eosinophils suspended in 1.0 ml saline. Tubes were centrifuged, the saline removed and the cell button resuspended in 0.6 ml glycerol buffer containing _T. spiralis_ or _T. pseudospiralis_ antigen (1 µg/ml) and 0.3 ml lysolecithin. Control tubes were centrifuged and resuspended in 0.6 ml glycerol buffer containing _T. spiralis_ or _T. pseudospiralis_ antigen (1 µg/ml) but not lysolecithin. All tubes were incubated in a
37°C water bath for phospholipase B assay as previously described.

Intestinal Eosinophil Enumeration

BALB/c x ICR mice were randomly divided into two groups with one group being infected with 500 T. spiralis and the other with 500 T. pseudospiralis. Mice were killed by cervical dislocation on day 12 post-infection. The entire small intestine was removed, freed of adhering tissues, the contents extruded and placed on ice. The first several centimeters of the anterior segment of each intestine were removed and embedded in Tissue-Tek II (Miles Laboratories, Napeville, Illinois) for tissue-eosinophil enumeration. Tissue samples were frozen using Cryo-Quick (International Equipment Company, Nedham, Massachusetts) and maintained frozen (-20°C) until sectioning. The remaining small intestine was used for phospholipase B determination. This technique allowed the numbers of tissue eosinophils and phospholipase B to be compared in the same animal. The method used to prepare and assay intestinal tissue samples for enzyme activity were as described above.

For tissue eosinophil enumeration, 6 to 10 μm thick sections were cut at -20°C using a Cryo-cut microtome (American Optical Corporation, Buffalo, New York). The sections were transferred to microscope slides and
maintained frozen until fixed in chilled calcium-formal fixative (1 g CaCl₂, 1 g CaCO₃, 100 ml 4% formaldehyde) for 10 min and rinsed briefly in two changes of 200 ml of 0.1 M Tris-acetate buffer at room temperature (Ottolenghi et al., 1966). Sections were stained using Eosinophil Stain (Banco Laboratories, Fort Worth, Texas) for 5 min, rinsed twice in distilled water and destained for 15 sec in methanol. After destaining, sections were dipped directly into a dehydration series consisting of: Absolute alcohol (1 min); a mixture of equal parts of absolute alcohol and xylene (1 min); and two rinses in pure xylene (1 min). Excess xylene was removed and a drop of mounting medium was placed on the slide and a cover slip added. The mean number of eosinophils was calculated by averaging the number of eosinophils present in five microscopic fields (x630) from five tissue sections per animal.

**Statistical Analysis**

Data were presented as the mean (X) ± standard error (SE) and were analyzed with appropriate statistical tests. When two groups were compared either parametric independent t or nonparametric Mann-Whitney U test was applied depending on the distribution of the data. The level of significance
(α) was selected as 0.05. Statistics with an α lower than 0.05 indicated significant differences between or among data tested.
CHAPTER III

RESULTS

Tissue Phospholipase B Activity in Mice
Given a Primary Trichinella Infection

This experiment determined the host eosinophil response
to a primary infection with T. spiralis or T. pseudospiralis
by measuring the phospholipase B activity of intestinal and
splenic tissues. The data presented in Figures 1, 2 and 3
illustrate that mice given a primary infection with 250 and
500 T. spiralis larvae developed significantly higher
intestinal phospholipase B activity than mice infected with
the same numbers of T. pseudospiralis larvae on days 6, 9,
and 12 post-infection. Infection with 1000 T. spiralis
larvae resulted in significantly greater intestinal
phospholipase B activity over T. pseudospiralis infected
mice on days 3, 6, 9 and 12 post-infection. Infection with
all doses of larvae for both parasites, except that for 1000
T. spiralis, resulted in intestinal enzyme activity in the
range of control animals on days 1 and 3 post-infection. A
dose response was demonstrated for T. spiralis infection
with increasing larvae exposure resulting in increasing
enzyme activity. Infection with 500 or 1000 T.
pseudospiralis larvae resulted in approximately the same
FIGURE 1. Phospholipase B activity in the small intestines of mice infected with 250 T. spiralis larvae (Ts) or T. pseudospiralis larvae (Tp). Each point represents the mean of three animals ± SE. An asterisk indicates a significant difference between the two groups. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
INTESTINAL PHOSPHOLIPASE B ACTIVITY (X 1000)

μMole Lyssolecithin Hydrolyzed/Gm Tissue/Hr

Nonsensitized 250 Ts
Nonsensitized 250Tp
uninfected

DAYS POST INFECTION

0 1 3 6 9 12
FIGURE 2. Phospholipase B activity in the small intestines of mice infected with 500 *T. spiralis* larvae (Ts) or *T. pseudospiralis* larvae (Tp). Each point represents the mean of three animals ± SE. An asterisk indicates a significant difference between the two groups. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
INTESTINAL PHOSPHOLIPASE B ACTIVITY (X 1000)

- ■ Nonsensitized 500 Ts
- □ - □ Nonsensitized 500 Tp
- ● ● ● uninfected

µmol/g/hr

0 1 3 6 9 12
DAYS POST INFECTION
FIGURE 3. Phospholipase B activity in the small intestines of mice infected with 1000 *T. spiralis* larvae (Ts) or *T. pseudospiralis* larvae (Tp). Each point represents the mean of three animals ± SE. An asterisk indicates significant difference between the two groups. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
INTESTINAL PHOSPHOLIPASE B ACTIVITY (X 1000)

μmol/g/hr

DAYS POST INFECTION

- Nonsensitized 1000 Ts
- Nonsensitized 1000 Tp
- uninfected

* denotes statistical significance.
intestinal enzyme activity, however, this activity was higher than found for infection with 250 larvae.

Infection with *T. spiralis* resulted in higher splenic phospholipase B activity than found for animals infected *T. pseudospiralis* on days 9 and 12 post-infection in animals given 250 larvae (Figure 4), on all days post-infection in animals given 500 larvae (Figure 5), and on days 1, 3 and 6 in animals given 1000 larvae (Figure 6).

**Tissue Phospholipase B Activity in Sensitized Mice Given a Challenge *Trichinella* Infection**

This experiment compared the effect of immunizing infections with *T. spiralis* and *T. pseudospiralis* on the eosinophil response after homologous challenge as determined by intestinal and splenic phospholipase B activity. Phospholipase B activity in the intestines and spleens of mice sensitized with 500 *T. spiralis* larvae and challenged 45 days later with *T. spiralis* (250, 500 or 1000 larvae) (Table I) or sensitized with 500 *T. pseudospiralis* and challenged 45 days later with *T. pseudospiralis* (250, 500 or 1000) (Table II) demonstrated a secondary tissue enzyme response when compared to results of mice receiving a primary infection with the same number and species of parasite. This secondary response was characterized by the presence of a significant increase in phospholipase B activity early in the challenge infections, and an earlier peak and decline in enzyme activity compared to values
FIGURE 4. Phospholipase B activity in the spleens of mice infected with 250 *T. spiralis* larvae (Ts) or *T. pseudospiralis* larvae (Tp). Each point represents the mean of three animals ± SE. An asterisk indicates significant difference between the two groups. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
Nonsensitized 250 Ts
Nonsensitized 250 Tp
uninfected

SPLENIC PHOSPHOLIPASE B ACTIVITY
μmol/g/hr

DAYS POST INFECTION

0 1 3 6 9 12

*
FIGURE 5. Phospholipase B activity in the spleens of mice infected with 500 T. spiralis larvae (Ts) or T. pseudospiralis larvae (Tp). Each point represents the mean of three animals ± SE. An asterisk indicates significant difference between the two groups. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
FIGURE 6. Phospholipase B activity in the spleens of mice infected with 1000 *T. spiralis* larvae (Ts) or *T. pseudospiralis* larvae (Tp). Each point represents the mean of three animals ± SE. An asterisk indicates significant difference between the two groups. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
SPLenic PHOSPHOLIPASE B Activity

\[ \mu \text{mol/g/hr} \]

- ▲ ▲ Nonsensitized 1000 Ts
- △ △ Nonsensitized 1000 Tp
- ■ ■ uninfected

DAYS POST INFECTION

0 1 3 6 9 12
TABLE I. Phospholipase B activity in the intestines and spleens of mice given a primary or challenge infection with 250, 500 or 1,000 *Trichinella spiralis* larvae.

<table>
<thead>
<tr>
<th>Larval Days</th>
<th>Intestine$^b$</th>
<th>Spleen$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>PI</td>
<td>Primary</td>
</tr>
<tr>
<td>250</td>
<td>1</td>
<td>926 ± 132</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1,666 ± 685</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>55,478 ± 1,520</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>78,527 ± 14,666</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>87,365 ± 1,777</td>
</tr>
<tr>
<td>500</td>
<td>1</td>
<td>985 ± 64</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1,310 ± 708</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>63,287 ± 4,218</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>95,727 ± 5,304</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>129,639 ± 15,222</td>
</tr>
<tr>
<td>1000</td>
<td>1</td>
<td>4,217 ± 1,597</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>16,731 ± 616</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>84,408 ± 10,813</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>121,000 ± 7,211</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>172,500 ± 7,500</td>
</tr>
</tbody>
</table>

Uninfected controls

| 235 ± 23$^d$ | 38 ± 13$^d$ |

---

*a* Measured as micromoles of lysolecithin hydrolyzed per gram wet tissue per hour.

*b* Each value represents an average from three mice ± SE.

*c* Significantly different from values for primary infections.

*d* Mean ± SE of 15 uninfected mice (three assayed per experimental day).
**TABLE II.** Phospholipase B activity in the intestines and spleens of mice given a primary or challenge infection with 250, 500 or 1,000 *Trichinella pseudospiralis* larvae.

<table>
<thead>
<tr>
<th>Larval Days</th>
<th>Intestine&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Spleen&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary</td>
<td>Challenge</td>
</tr>
<tr>
<td>Dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>1</td>
<td>178 ± 91</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>685 ± 685</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2,362 ± 693</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>21,627 ± 188</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>50,767 ± 884</td>
</tr>
<tr>
<td>500</td>
<td>1</td>
<td>126 ± 29</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>949 ± 259</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>35,811 ± 4,060</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>71,970 ± 6,829</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>87,578 ± 12,649</td>
</tr>
<tr>
<td>1000</td>
<td>1</td>
<td>316 ± 188</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1,934 ± 1,716</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>57,352 ± 3,169</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>72,619 ± 4,811</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>94,151 ± 13,834</td>
</tr>
</tbody>
</table>

Uninfected controls

235 ± 23°  38 ± 13°

---

<sup>a</sup> Measured as micromoles of lysolecithin hydrolyzed per gram wet tissue per hour.

<sup>b</sup> Each value represents an average from three mice ± SE.

<sup>c</sup> Significantly different from values for primary infections.

<sup>d</sup> Mean ± SE of 15 uninfected mice (three assayed per experimental day).
obtained for the primary infections.

Although challenge infections resulted in anamnestic enzyme responses at all infection levels for both parasites, challenge with *T. pseudospiralis* resulted in significantly lower intestinal enzyme activity when compared to values found for *T. spiralis* (Figures 7-9). Splenic enzyme activities in *T. pseudospiralis* infections began lower than those for *T. spiralis* at all infection levels before becoming greater on days 9 and 12 post-infection during challenge with 250 larvae, and equivalent on day 12 post-infection during challenge with 500 and 1000 larvae (Figures 10-12).

**Tissue Phospholipase B Activity in Mice Sensitized with a Primary *Trichinella spiralis* or *Trichinella pseudospiralis* Infections and Given a Cross (Heterologous) Challenge *Trichinella* Infection**

This experiment determined how immunization with a primary *T. spiralis* (500 larvae) or *T. pseudospiralis* (500 larvae) infection influenced the host eosinophil response, as measured by intestinal and splenic phospholipase B activity, on cross or heterologous challenge (45 days following primary infection) with either *T. spiralis* (250, 500, 1000) or *T. pseudospiralis* (250, 500, 1000) larvae. Results were compared to those obtained from mice given primary infections with *T. spiralis* or *T. pseudospiralis* larvae (250, 500, 1000) and to mice immunized by a primary infections with *T. spiralis* or *T. pseudospiralis* (500...
FIGURE 7. Phospholipase B activity in the small intestines of mice sensitized with an infection of 500 *Trichinella spiralis* larvae and challenged 45 days later with 250 *T. spiralis* larvae, and mice sensitized with an infection of 500 *Trichinella pseudospiralis* larvae and challenged 45 days later with 250 *T. pseudospiralis* larvae. Each point represents the mean of three animals ± SE. An asterisk indicates a significant difference between the two groups. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
INTESTINAL PHOSPHOLIPASE B ACTIVITY (X 1000)

µmol/g/hr

- - - - - 500 Ts Sensitized, 250 Ts Challenged

O - - - - 500 Tp Sensitized, 250 Tp Challenged

uninfected

DAYS POST INFECTION
FIGURE 8. Phospholipase B activity in the small intestines of mice sensitized with an infection of 500 *Trichinella spiralis* larvae and challenged 45 days later with 500 *T. spiralis* larvae, and mice sensitized with an infection of 500 *Trichinella pseudospiralis* larvae and challenged 45 days later with 500 *T. pseudospiralis* larvae. Each point represents the mean of three animals ± SE. An asterisk indicates a significant difference between the two groups. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
INTESTINAL PHOSPHOLIPASE A2 ACTIVITY (X 1000)

- 500 Ts Sensitized, 500 Ts Challenged
- 500 Tp Sensitized, 500 Tp Challenged
- uninfected

µmol/g/hr

DAYS POST INFECTION

0 1 3 6 9 12
FIGURE 9. Phospholipase B activity in the small intestines of mice sensitized with an infection of 500 Trichinella spiralis larvae and challenged 45 days later with 1000 T. spiralis larvae, and mice sensitized with an infection of 500 Trichinella pseudospiralis larvae and challenged 45 days later with 1000 T. pseudospiralis larvae. Each point represents the mean of three animals ± SE. An asterisk indicates a significant difference between the two groups. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
INTESTINAL PHOSPHOLIPASE B ACTIVITY (X 1000)

Δ — △ 500 Ts Sensitized, 1000 Ts Challenged
Δ—Δ 500 Tp Sensitized, 1000 Tp Challenged

uninfected

μmol/g/hr

DAYS POST INFECTION

0 1 3 6 9 12

0 20 40 60 80 100 120 140 160 180 200
FIGURE 10. Phospholipase B activity in the spleens of mice sensitized with an infection of 500 *Trichinella spiralis* larvae and challenged 45 days later with 250 *T. spiralis* larvae, and mice sensitized with an infection of 500 *Trichinella pseudospiralis* larvae and challenged 45 days later with 250 *T. pseudospiralis* larvae. Each point represents the mean of three animals ± SE. An asterisk indicates a significant difference between the two groups. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
FIGURE 11. Phospholipase B activity in the spleens of mice sensitized with an infection of 500 *Trichinella spiralis* larvae and challenged 45 days later with 500 *T. spiralis* larvae, and mice sensitized with an infection of 500 *Trichinella pseudospiralis* larvae and challenged 45 days later with 500 *T. pseudospiralis* larvae. Each point represents the mean of three animals ± SE. An asterisk indicates a significant difference between the two groups. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
SPLENIC PHOSPHOLIPASE B ACTIVITY

μmol/g/hr

DAYS POST INFECTION

0  1  3  6  9  12

uninfected

500 Ts Sensitized, 500 Ts Challenged

500 Tp Sensitized, 500 Tp Challenged

63
FIGURE 12. Phospholipase B activity in the spleens of mice sensitized with an infection of 500 *Trichinella spiralis* larvae and challenged 45 days later with 1000 *T. spiralis* larvae, and mice sensitized with an infection of 500 *Trichinella pseudospiralis* larvae and challenged 45 days later with 1000 *T. pseudospiralis* larvae. Each point represents the mean of three animals ± SE. An asterisk indicates a significant difference between the two groups. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
SPLENIC PHOSPHOLIPASE A2 ACTIVITY

µmol/g/hr

500 Ts Sensitized, 1000 Ts Challenged

500 Tp Sensitized, 1000 Tp Challenged

uninfected

DAYS POST INFECTION

0 1 3 6 9 12

*
larvae) and challenged 45 days later with the same parasites.

Tissue phospholipase B activity for *T. pseudospiralis* sensitized mice challenged with 250, 500 or 1000 *T. spiralis* larvae are illustrated in Figures 13, 14 and 15 for intestinal and Figures 16, 17 and 18 for the splenic tissues. Also illustrated are tissue enzyme activities for mice given a primary infection with *T. spiralis* and mice sensitized and challenged with *T. spiralis*. Phospholipase B activities present in mice given a primary and sensitized mice given a homologous *T. spiralis* infection were as previously described and reported in Tables I and II.

There was little direct association between the intestinal phospholipase B activity of mice sensitized with *T. pseudospiralis* and challenged with *T. spiralis*, and mice given a primary infection with *T. spiralis* or mice primed and challenged with *T. spiralis*. This is especially evident early (days 1 and 3 post-infection) and later (days 9 and 12 post-infection) in the infection. *T. pseudospiralis* primed, *T. spiralis* challenged mice developed an earlier intestinal phospholipase B activity than found for a primary *T. spiralis* infection, a characteristic of an anamnestic response, however, the enzyme activity did not reach the levels found for the *T. spiralis* primed, *T. spiralis* challenged mice. On day 12 post-infection, the final day assayed, a similar association was found. Intestinal
FIGURE 13. Phospholipase B activity in the small intestines of mice: 1) Given a primary infection with 250 T. spiralis larvae; 2) sensitized with 500 and challenged with 250 T. spiralis larvae; or 3) sensitized with 500 T. pseudospiralis larvae and challenged with 250 T. spiralis larvae. Each point represents the mean of three animals ± SE. Labels "A, B and C" indicate a "significant difference" between groups: 1 and 2; 1 and 3; or 2 and 3, respectively. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
INTESTINAL PHOSPHOLIPASE B ACTIVITY (X 1000)

μmol/g/hr

- - - Nonsensitized 250 Ts (1)
- - - 500 Ts Sensitized, 250 Ts Challenged (2)
- - - 500 Tp Sensitized, 250 Ts Challenged (3)

Uninfected

DAYS POST INFECTION

0 1 3 6 9 12

A B C A B C
FIGURE 14. Phospholipase B activity in the small intestines of mice: 1) Given a primary infection with 500 *T. spiralis* larvae; 2) sensitized with 500 and challenged with 500 *T. spiralis* larvae; or 3) sensitized with 500 *T. pseudospiralis* larvae and challenged with 500 *T. spiralis* larvae. Each point represents the mean of three animals ± SE. Labels "A, B and C" indicate a "significant difference" between groups: 1 and 2; 1 and 3; or 2 and 3, respectively. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
FIGURE 15. Phospholipase B activity in the small intestines of mice: 1) Given a primary infection with 1000 *T. spiralis* larvae; 2) sensitized with 500 and challenged with 1000 *T. spiralis* larvae; or 3) sensitized with 500 *T. pseudospiralis* larvae and challenged with 1000 *T. spiralis* larvae. Each point represents the mean of three animals ± SE. Labels "A, B and C" indicate a "significant difference" between groups: 1 and 2; 1 and 3; or 2 and 3, respectively. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
INTESTINAL PHOSPHOLIPASE B ACTIVITY (X 1000)

- Nonsensitized 1000 Ts
- 500 Ts Sensitized, 1000 Ts Challenged
- 500 Tp Sensitized, 1000 Ts Challenged
- Uninfected

µmol/g/hr

DAYS POST INFECTION
FIGURE 16. Phospholipase B activity in the spleens of mice: 1) Given a primary infection with 250 T. spiralis larvae; 2) sensitized with 500 and challenged with 250 T. spiralis larvae; or 3) sensitized with 500 T. pseudospiralis larvae and challenged with 250 T. spiralis larvae. Each point represents the mean of three animals ± SE. Labels "A, B and C" indicate a "significant difference" between groups: 1 and 2; 1 and 3; or 2 and 3, respectively. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
SPLENIC PHOSPHOLIPASE B ACTIVITY

µmol/g/hr

DAYS POST INFECTION

- Nonsensitized 250 Ts (1)
- 500 Ts Sensitized, 250 Ts Challenged (2)
- 500 Tp Sensitized, 250 Ts Challenged (3)
- Uninfected
FIGURE 17. Phospholipase B activity in the spleens of mice: 1) Given a primary infection with 500 T. spiralis larvae; 2) sensitized with 500 and challenged with 500 T. spiralis larvae; or 3) sensitized with 500 T. pseudospiralis larvae and challenged with 500 T. spiralis larvae. Each point represents the mean of three animals ± SE. Labels "A, B and C" indicate a "significant difference" between groups: 1 and 2; 1 and 3; or 2 and 3, respectively. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
SPLenic PHOSPHOLIPASE B ACTIVITY

μmol/g/hr

- Nonsensitized 500 Ts (1)
- 500 Ts Sensitized, 500 Ts Challenged (2)
- 500 Tp Sensitized, 500 Ts Challenged (3)

Uninfected

DAYS POST INFECTION

0 1 3 6 9 12
FIGURE 18. Phospholipase B activity in the spleens of mice: 1) Given a primary infection with 1000 T. spiralis larvae; 2) sensitized with 500 and challenged with 1000 T. spiralis larvae; or 3) sensitized with 500 T. pseudospiralis larvae and challenged with 1000 T. spiralis larvae. Each point represents the mean of three animals ± SE. Labels "A, B and C" indicate a "significant difference" between groups: 1 and 2; 1 and 3; or 2 and 3, respectively. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
phospholipase B activities of T. pseudospiralis primed, T. spiralis challenged animals were less than found for a primary T. spiralis infection, again a characteristic of an anamnestic response, however the enzyme activities were greater than found for the T. spiralis primed, T. spiralis challenged mice.

Mice primed with a T. pseudospiralis infection and challenged with 250 T. spiralis larvae (Figure 16) developed splenic phospholipase B activities that were greater than those found for a primary infection with the same number of T. spiralis larvae on days 1, 3, 6 and 9 post-infection. A reversal occurred on day 12 where the enzyme activity of mice given a primary infection was greater. These results are characteristic of an anamnestic response. However, their enzyme activities were less than those found for T. spiralis primed and challenged (250 larvae) mice on days 1, 3 and 6 post-infection and greater than those found on days 9 and 12, indicating that the splenic enzyme response was somewhat between the normal primary and challenge infections with T. spiralis. Mice primed with a T. pseudospiralis infection and challenged with 500 (Figure 17) or 1000 (Figure 18) T. spiralis larvae developed a complex splenic phospholipase B activity pattern characterized by lower enzyme activities early in the infection (day 1 and 3 post-infection) compared to those for mice given a primary or
challenge infection with the same number of *T. spiralis* larvae.

Tissue phospholipase B activity for *T. spiralis* sensitized mice challenged with 250, 500 or 1000 *T. pseudospiralis* larvae are illustrated in Figures 19, 20 and 21 for intestinal and Figures 22, 23 and 24 for splenic tissues. Also illustrated are tissue enzyme activities for mice given a primary infection with *T. pseudospiralis* and mice sensitized and challenged with *T. pseudospiralis*. Phospholipase B activities in both of these infections were as previously described and reported in Tables I and II.

*T. spiralis* primed, *T. pseudospiralis* challenged mice demonstrated an anamnestic intestinal phospholipase B response when compared to the enzyme activities of mice given a primary infection with *T. pseudospiralis*. This response was characterized by enzyme actives greater on days 3, 6 and 9 post-infection for animals challenged with 250 larval and on days 6 and 9 post-infection for animals challenged with 500 or 1000 larvae. All challenged animals processed lower intestinal enzyme activities on day 12 post-infection when compared to animals given a primary *T. pseudospiralis* infection.

The anamnestic phospholipase B response in animals sensitized with *T. spiralis* and challenged with *T. pseudospiralis* followed a pattern strikingly similar to that for animals primed and challenged with *T. pseudospiralis*
even though some differences in enzyme activities are noted in the figures.

Mice primed with *T. spiralis* and challenged with *T. pseudospiralis* larvae developed splenic phospholipase activities that were greater than those found for a primary infection with the same number of larvae on days 3 and 6 post-infection for 250 larvae (Figure 22), day 3, 6 and 9 post-infection for 500 larvae (Figure 23), and day 3 for 1000 larvae (Figure 24).

A reversal in this pattern occurred later in the infection with *T. spiralis* primed *T. pseudospiralis* challenged animals developing lower enzyme activities (days 9 and 12 for 250 and 1000 larvae, and day 12 for 500 larvae) than mice given a primary *T. spiralis* infection. These results are characteristic of an anamnestic response in that enzyme activities in the challenged animals both increased and declined earlier than enzyme activities present in animals given a primary infection.

Splenic phospholipase B activities in animals sensitized by a primary infection with *T. spiralis* and challenged with *T. pseudospiralis* were lower than animals sensitized and challenged with *T. pseudospiralis* on all days except day 6 in animals challenged with 250 larvae and day 9 in animals challenged with 500 larvae.
FIGURE 19. Phospholipase B activity in the small intestines of mice: 1) Given a primary infection with 250 *T. pseudospiralis* larvae; 2) sensitized with 500 and challenged with 250 *T. pseudospiralis* larvae; or 3) sensitized with 500 *T. spiralis* larvae and challenged with 250 *T. pseudospiralis* larvae. Each point represents the mean of three animals ± SE. Labels "A, B and C" indicate a "significant difference" between groups: 1 and 2; 1 and 3; or 2 and 3, respectively. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
INTESTINAL PHOSPHOLIPASE B ACTIVITY (X 1000)

μmol/g/hr

- Nonsensitized 250 Tp
- 500 Tp Sensitized, 250 Tp Challenged
- 500 Ts Sensitized, 250 Tp Challenged

Uninfected

DAYS POST INFECTION

0 1 3 6 9 12
FIGURE 20. Phospholipase B activity in the small intestines of mice: 1) Given a primary infection with 500 *T. pseudospiralis* larvae; 2) sensitized with 500 and challenged with 500 *T. pseudospiralis* larvae; or 3) sensitized with 500 *T. spiralis* larvae and challenged with 500 *T. pseudospiralis* larvae.

Each point represents the mean of three animals ± SE. Labels "A, B and C" indicate a "significant difference" between groups: 1 and 2; 1 and 3; or 2 and 3, respectively. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
INTESTINAL PHOSPHOLIPASE B ACTIVITY (X 1000)

μmol/g/hr

- - - Nonsensitized 500 Tp (1)
- - - 500 Tp Sensitized, 500 Tp Challenged (2)
- - - 500 Ts Sensitized, 500 Tp Challenged (3)
- Uninfected

DAYS  POST  INFECTION
0  1  3  6  9  12

A B C
FIGURE 21. Phospholipase B activity in the small intestines of mice: 1) Given a primary infection with 1000 *T. pseudospiralis* larvae; 2) sensitized with 500 and challenged with 1000 *T. pseudospiralis* larvae; or 3) sensitized with 500 *T. spiralis* larvae and challenged with 1000 *T. pseudospiralis* larvae. Each point represents the mean of three animals ± SE. Labels "A, B and C" indicate a "significant difference" between groups: 1 and 2; 1 and 3; or 2 and 3, respectively. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
INTESTINAL PHOSPHOLIPASE B ACTIVITY (X 1000)

- Nonsensitized 1000 Tp (1)
- 500 Tp Sensitized, 1000 Tp Challenged (2)
- 500 Ts Sensitized, 1000 Tp Challenged (3)
- Uninfected

μmol/g/hr

DAYS POST INFECTION

0 1 3 6 9 12

A B A B A B
FIGURE 22. Phospholipase B activity in the spleens of mice: 1) Given a primary infection with 250 T. pseudospiralis larvae; 2) sensitized with 500 and challenged with 250 T. pseudospiralis larvae; or 3) sensitized with 250 T. spiralis larvae and challenged with 250 T. pseudospiralis larvae.

Each point represents the mean of three animals ± SE. Labels "A, B and C" indicate a "significant difference" between groups: 1 and 2; 1 and 3; or 2 and 3, respectively. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
SPLENIC PHOSPHOLIPASE B ACTIVITY

μmol/g/hr

- - - Nonsensitized 250 Tp (1)
- - - 500 Tp Sensitized, 250 Tp Challenged (2)
- - - 500 Ts Sensitized, 250 Tp Challenged (3)

Uninfected

DAYS POST INFECTION

0 1 3 6 9 12
FIGURE 23. Phospholipase B activity in the spleens of mice: 1) Given a primary infection with 500 *T. pseudospiralis* larvae; 2) sensitized with 500 and challenged with 500 *T. pseudospiralis* larvae; or 3) sensitized with 250 *T. spiralis* larvae and challenged with 500 *T. pseudospiralis* larvae.

Each point represents the mean of three animals ± SE. Labels "A, B and C" indicate a "significant difference" between groups: 1 and 2; 1 and 3; or 2 and 3, respectively. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
DAYS POST INFECTION

SPLENIC PHOSPHOLIPASE B ACTIVITY

- Nonsensitized 500 Tp (1)
- 500 Tp Sensitized, 500 Tp Challenged (2)
- 500 Ts Sensitized, 500 Tp Challenged (3)

Uninfected

μmol/g/hr
FIGURE 24. Phospholipase B activity in the spleens of mice: 1) Given a primary infection with 1000 *T. pseudospiralis* larvae; 2) sensitized with 500 and challenged with 1000 *T. pseudospiralis* larvae; or 3) sensitized with 250 *T. spiralis* larvae and challenged with 1000 *T. pseudospiralis* larvae. Each point represents the mean of three animals ± SE. Labels "A, B and C" indicate a "significant difference" between groups: 1 and 2; 1 and 3; or 2 and 3, respectively. Hatch-marks represent the mean ± SE from 5 uninfected mice (one assayed per experimental day). SE bars that fell within the symbols were omitted for clarity.
Phospholipase B Activity of Eosinophils Collected from Mice Infected with *Trichinella spiralis* or *Trichinella pseudospiralis* larvae

This experiment compared eosinophil cellular phospholipase B activity and tissue (intestinal and splenic) enzyme activity in animals infected with *T. spiralis* or *T. pseudospiralis*. Eosinophils collected from mice infected with either 500 *T. spiralis* or 500 *T. pseudospiralis* were found to possess equivalent phospholipase B activities on day 12 (44.2 vs 35.7 μ moles/10^6 eosinophils/hr, p=0.3849, Mann-Whitney U test).

Although both species of *Trichinella* influenced eosinophil cellular phospholipase B activity in a similar manner, infection with *T. spiralis* resulted in significantly greater enzyme activity in both intestines and spleens on day 12 post-infection compared to values found in animals infected with *T. pseudospiralis* (intestine: p=0.001, spleen: p=0.040, Independent t test) (Figure 25).

Relation Between Intestinal Phospholipase B Activity and Numbers of Intestinal Eosinophils in Mice Infected with *Trichinella spiralis* or *Trichinella pseudospiralis* Larvae

Figure 26 illustrates the association between tissue phospholipase B activity and numbers of eosinophils in the small intestines of uninfected mice, and mice infected with either 500 *T. spiralis* or 500 *T. pseudospiralis*. Both intestinal tissue enzyme activity and numbers of eosinophils
FIGURE 25. Intestinal, splenic and eosinophilic phospholipase B activities of mice infected with 500 T. spiralis (filled boxes) or 500 T. pseudospiralis (open boxes) larvae on day 12 post-infection.
FIGURE 26. Relation between intestinal phospholipase B activity and numbers of intestinal eosinophils in uninfected mice (open boxes) and mice infected for 12 days with 500 T. spiralis (filled boxes) or 500 T. pseudospiralis larvae (slanted lines).
in animals infected with either parasite were significantly elevated over values found for uninfected controls on day 12 post-infection (p < 0.01 for all four groups, Independent t test). Although infection with T. pseudospiralis resulted in both an elevation of tissue phospholipase B activity and eosinophilia over uninfected control animals these increase were significantly lower than the corresponding values found for T. spiralis infected mice (p = 0.0140, Independent t test).
CHAPTER IV

DISCUSSION

Biochemical and histochemical evidence has shown the eosinophil to be the major inflammatory cell source of phospholipase B (Adewusi and Goven, 1986; Pirkle and Goven 1988). An association has been shown to exist between nematode infections and an increased phospholipase B activity of parasitized tissues (Larsh et al., 1974, 1975; Ottolenghi et al., 1975; Goven 1979a, b; Goulson et al., 1981). Parasite-induced tissue inflammation, specifically the infiltration of eosinophils into parasitized host tissues, has been shown to be responsible for this increased enzyme activity (Larsh et al., 1974; Ottolenghi et al., 1975; Goven 1983; Wilkes and Goven, 1984). Further, the eosinophil-enzyme response has been shown to be a T-lymphocyte dependent function (Goven and Moore, 1980; Adewusi and Goven, 1986, 1987). With respect to these findings, it was hypothesized that after interaction with worm antigens, T-lymphocytes provided the stimulus for increased production and chemotaxis of eosinophils, and enhanced eosinophil production of phospholipase B for release at sites of parasite induced inflammation.

The purpose of the present study was to evaluate the host eosinophil response, as measured by tissue
phospholipase B activity, in mice infected with *T. pseudospiralis* or *T. spiralis* and to compare this aspect of the host immune response against these parasites. Initial studies in this investigation examined the splenic and intestinal phospholipase B activities, and thus the eosinophil response, of nonsensitized mice given a primary infection and sensitized mice given a homologous challenge infection with *T. spiralis* or *T. pseudospiralis* larvae. The tissues assayed were selected because of their involvement in the infection scheme, with the spleen indirectly indicating the eosinophil response in the circulatory system and the intestine indicating eosinophil infiltration into the site occupied by adult worms.

The phospholipase B activity reported for primary (Figures 1-6) and homologous challenge (Figures 7-12) infections demonstrated that animals infected with *T. spiralis* developed greater splenic and intestinal enzyme activities than animals infected with *T. pseudospiralis*. Comparison of tissue enzyme activity during either primary or challenge infection with *T. pseudospiralis* to values from uninfected control animals does, however, indicate that *T. pseudospiralis* infection stimulated phospholipase B activity, and thus eosinophils. Additionally, homologous challenge of *T. pseudospiralis* sensitized mice induced an anamnestic enzyme response characterized by an earlier increase, peak and decline of phospholipase B activity.
This anamnestic response, although less pronounced than that found during a homologous *T. spiralis* challenge, infection paralleled the pattern seen in this study for *T. spiralis* and that reported by Larsh et al. (1975). These results suggest that a similar host response occurred on homologous challenge of sensitized mice with either *T. spiralis* or *T. pseudospiralis*, although the host response to *T. pseudospiralis* was somewhat diminished.

In this regard, challenge infection with *T. pseudospiralis* may initiate immune mechanisms responsible for increased phospholipase B activity similar to those hypothesized by Larsh et al., (1975), Ottolenghi et al., (1975) and Goven (1979a, b) for *T. spiralis* and other nematode parasites. They suggested that upon challenge there was an interaction of worm antigens with memory or sensitized T-lymphocytes which resulted in the release of lymphokines, and that parasite mediated tissue injury triggered the inflammatory response. Together, these acted as stimuli for the immediate production and migration of eosinophils to sites where they released their stores of phospholipase B. Although the same mechanism as hypothesized for *T. spiralis* may be responsible for the increased phospholipase B activity (or eosinophil response) during primary and challenge infection with *T. pseudospiralis*, results of this study demonstrated that the enzyme response was significantly lower. These results
suggest that infection with *T. pseudospiralis* may stimulate fewer eosinophils to infiltrate tissues compared to *T. spiralis* infection, or that the eosinophils possess lower enzyme activity, or a combination of both these factors.

The second series of experiments were performed to determine if a primary infection with *T. spiralis* or *T. pseudospiralis* sensitized the host to common *Trichinella* antigens resulting in the ability of the immune system to mediate a secondary or anamnestic eosinophil response upon heterologous or cross challenge.

The eosinophil response (as determined by tissue phospholipase B activity) in *T. pseudospiralis* sensitized-*T. spiralis* challenged animals (Figures 13-18) demonstrated that an immunizing infection with *T. pseudospiralis* provided partial immunity to challenge with *T. spiralis* larvae. This partial protective response was characterized by *T. pseudospiralis* sensitized-*T. spiralis* challenged animals displaying an earlier increase and decline in phospholipase B activity than that found for animals given a primary *T. spiralis* infection. This pattern of enzyme activity indicates that *T. pseudospiralis* sensitization induced the proliferation of T-lymphocytes recognizing *T. pseudospiralis* antigens and that upon challenge some *T. spiralis* antigens were able to activate the *T. pseudospiralis* sensitive memory cells. Memory cell activation mediated an earlier and more intense immune response resulting in the earlier expulsion
of the \textit{T. spiralis} challenge infection. Aspects of this immune response were the rapid activation of eosinophils and their early decline as the worms (antigen) were eliminated from the host.

Results suggest that sensitization with \textit{T. pseudospiralis} only provided partial immunity to challenge with \textit{T. spiralis} since the early increases in enzyme activities of this heterologous challenge were significantly lower than that those in homologous \textit{T. spiralis} challenged animals. Additionally, the enzyme response did not decline as rapidly as seen in the homologous challenged animals indicating that the elimination of antigen (worm expulsion) was not as rapid. This indicates that although \textit{Trichinella} share antigens there may be qualitative or quantitative difference in the repertoire of immunogens possessed by the two species of worms.

Eosinophils in the tissue of \textit{T. spiralis} sensitized, \textit{T. pseudospiralis} challenged animals (Figures 19-24) demonstrated a response similar to that described above for the \textit{T. pseudospiralis} sensitized-\textit{T. spiralis} challenged mice (Figures 13-18). The data, however, indicate that on day 12 post-challenge the intestinal enzyme activity of the \textit{T. spiralis} sensitized-\textit{T. pseudospiralis} challenged animals was similar to that of the homologous challenged \textit{T. pseudospiralis} mice. This is different than that found for the \textit{T. pseudospiralis} sensitized-\textit{T. spiralis} challenged
animals (Figures 13-14), which had a greater enzyme activity on day 12 post-challenge than the homologous challenged *T. spiralis* animals. Indeed, the intestinal enzyme activity of the *T. spiralis* sensitized-*T. pseudospiralis* challenged animals was similar to values found for the homologous challenged *T. spiralis* animals. Since a low enzyme activity on day 12 in immunized animals is indicative of the early expulsion of parasites, it appears that immunization with a *T. spiralis* infection provided greater protection than did *T. pseudospiralis* as demonstrated on heterologous challenge. This is probably due to the immunizing infection with *T. spiralis* resulting in an activation of a far greater number of immune cells when compared to a primary infection with *T. pseudospiralis*, which has been shown to suppress T- and B-lymphocytes, and macrophages (Tanner et al., 1978; Oppenheim et al., 1986; Stewart, 1989).

A third series of investigations was aimed at determining if the tissue eosinophil response present in *T. pseudospiralis* infections was due to a lower eosinophil cellular enzyme activity by comparing the eosinophil and tissue (intestinal and splenic) enzyme activities in ICR mice infected with either 500 *T. spiralis* or 500 *T. pseudospiralis* larvae. The data presented in Figure 25 illustrates that infections with either parasite resulted in eosinophils possessing equivalent enzyme activities. Tissue enzyme activities, however, were significantly different on
day 12 post-infection, with the *T. spiralis* infection resulting in both intestinal and splenic phospholipase B activities being significantly greater than those found for mice infected with *T. pseudospiralis*.

In this study eosinophils were collected from the peritoneal cavity of infected mice in a manner similar to that used by Adewusi and Goven (1986, 1987). While in the peritoneal cavity eosinophils were influenced by a complete population of leukocytes including T-lymphocytes, B-lymphocytes, macrophages and neutrophils. In addition, eosinophils and other peritoneal cells were exposed to parasite antigens released during the natural infection process. Although cell populations were enriched for eosinophils on collection, all cells shown to be necessary for enhanced eosinophil phospholipase B production by Adewusi and Goven (1987) were present in culture before enzyme assay. Additionally, *T. spiralis* or *T. pseudospiralis* muscle larvae antigens were added to the appropriate cultures after cell collection. Enzyme activities of eosinophils obtained from *T. spiralis* and *T. pseudospiralis* infected mice in this experiment were equivalent to values reported by Adewusi and Goven (1987). Eosinophils could only be collected from mice given a parasitic infection which was needed to stimulate their production. Because of this enzyme activity from non-stimulated eosinophils collected from uninfected animals
could not be determined.

The eosinophil-phospholipase B response has been indirectly shown in in vivo experiments to be dependent on an intact thymus derived population of lymphocytes (Goven and Moore, 1980). More recent experiments by Adewusi and Goven (1986, 1987), using an in vitro system, demonstrated that culturing eosinophils with T-lymphocytes in the presence of T. spiralis antigen enhanced the phospholipase B activity of eosinophils 5-fold over eosinophils cultured alone. Furthermore, it was demonstrated that culturing eosinophils with T-lymphocytes and macrophages, together with T. spiralis antigen, had a synergistic effect on eosinophil enzyme activity resulting in a 10-fold increase over eosinophils cultured alone and equal to that found for a complete population of peritoneal cells. Adewusi and Goven (1986, 1987) speculated that T-lymphocytes after interaction with macrophages and parasite antigen released a soluble product, that caused a selective change in the biochemical effector function of murine eosinophils, which in this case resulted in an increased phospholipase B activity.

Results herein, indicate that eosinophils collected from mice infected with either T. spiralis or T. pseudospiralis possess equivalent enzyme activities. This suggests that eosinophil enzyme activity was influenced in a similar manner during both infections and that T. spiralis
and T. pseudospiralis may contain or elicit similar antigens that stimulate T-lymphocytes and/or macrophages, as discussed above, to release soluble factors which influence the phospholipase B activity of the eosinophil. Support for these results is found in the heterologous challenge experiments in the present study which indicate that immunization with a primary T. spiralis or T. pseudospiralis infection results in an anamnestic eosinophil response on heterologous challenge. This suggests that Trichinella possess a common antigen capable of stimulating eosinophils.

These results, however, are in contrast to those of Rickert et al. (1992) who have demonstrated a reduction in the oxidative burst, an enzyme mediated response, in neutrophils and eosinophils, and reduced peroxidase activity in eosinophils collected from T. pseudospiralis-infected mice compared to cells from uninfected animals. Rickert et al. found that the lower enzyme activities were accompanied by lower concentrations of serum gamma interferon (IFN-γ), interleukin-4 (IL-4) and interleukin-5 (IL-5). IFN-γ has been shown to activate neutrophils and IL-5 is known to stimulate eosinophil growth and differentiation and to activate eosinophils in such a way that they can kill helminths (Abbas et al., 1991). IL-4 has been shown to be a growth factor for mast cells, which produce eosinophil chemotactic factor (ECF), and thus could indirectly influence eosinophils. The results of the present study,
however, indicate that these negative effects of *T. pseudospiralis* infection on cytokines do not influence eosinophil cellular phospholipase B activity, however, they could effect infiltration of eosinophils into parasitized tissue.

The final study was performed to determine if differences in the eosinophil response in mice infected with *Trichinella* were due to dissimilarities in the migration of eosinophils into parasitized tissue. The relation between intestinal phospholipase B activity and numbers of intestinal tissue eosinophils was studied in BALB/c x ICR mice infected with either 500 *T. spiralis* or 500 *T. pseudospiralis* larvae. It is apparent from the data (Figure 26) that infection with *T. spiralis* resulted in both a greater number of eosinophils infiltrating intestinal tissue and an associate higher tissue phospholipase B activity compared to infection with *T. pseudospiralis*.

Results obtained for intestinal eosinophil numbers and enzyme activity for *T. spiralis* in this experiment were similar to those found by Wilkes and Goven (1984). Different infective doses (200 vs 500) used in the studies probably account for any minor discrepancies. Agreement of the present *T. spiralis* data with that of Wilkes and Goven (1984) confirms that the experimental techniques used in this study were valid.

Kramar et al. (1981) demonstrated that intestines of
mice infected with *T. pseudospiralis* possessed significantly lower myeloperoxidase activities than those of mice infected with *T. spiralis*. Myeloperoxidase activity indicates the level of enteric inflammation and is a marker enzyme for granulocytes and mononuclear phagocytes. This enzyme is thus reflective of levels of leukocyte infiltration into tissue. Additionally, Stewart et al. (1991) demonstrated that infection with *T. pseudospiralis* inhibited the inflammatory response as measured by the myeloperoxidase activity of granuloma tissue found forming around cotton strings subcutaneously implanted in mice. Together these studies support the present findings that demonstrate a reduced eosinophil infiltration into *T. pseudospiralis* parasitized tissues and a reduced phospholipase B activity compared to the values found for mice infected with *T. spiralis*.

Overall, the results of the present study support evidence that *T. pseudospiralis* has a broad range of immunoregulatory effects on its host. Demonstrated immunoevasive strategies employed by *T. pseudospiralis* that could effect the intestinal eosinophil response against the host include: Induction of host plasma corticosterone synthesis (Stewart et al., 1988); suppression of the host T-lymphocyte mediated delay-type hypersensitivity response (Stewart et al., 1991); and inhibition of the ability of T-lymphocytes to synthesize and/or release cytokines (IL-4, IL-5) known to
influence eosinophil activity (Rickert et al., 1992).

A specific role has been assigned to the eosinophil in the killing of and resistance to *T. spiralis* (Grove et al., 1977; Kazura and Grove 1978, Wakelin, 1984; Gansmuller et al., 1985; Abbas et al., 1991). The data herein, demonstrated that the first 12 days of the intestinal tissue phase of infection with *T. pseudospiralis* induces a significantly lower intestinal phospholipase B activity than found during *T. spiralis* infection. Phospholipase B, an enzyme marker for eosinophils and tissue enzyme activity, indicates the level of eosinophil infiltration. Thus, tissue enzyme activity demonstrated that *T. pseudospiralis* infection induced fewer eosinophils to infiltrate parasitized tissue than *T. spiralis* infection. Biochemical and histological experiments support these findings. Enzymatic assays indicate that eosinophils collected from *T. pseudospiralis* and *T. spiralis* infected mice possessed equivalent phospholipase B activities. Histological experiments, in which intestinal eosinophils were enumerated, indicated that *T. pseudospiralis* infection induced fewer eosinophils to migrate into parasitized tissue than *T. spiralis*. These findings provide evidence that the host response to *T. pseudospiralis* differs from *T. spiralis*.


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