# THE EOSINOPHIL AND LYSOPHOSPHOLIPASE RESPONSES IN MICE INFECTED WITH *Trichinella spiralis*: A ROLE FOR THE LYMPHOCYTE AND MACROPHAGE

DISSERTATION

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The relationship among eosinophils, lysophospholipase activity and the immune response in animals infected with Trichinella spiralis was studied using in vivo and in vitro techniques. In an in vivo experiment, anti-thymocyte serum (ATS) was administered to mice infected with T. spiralis and its effects on intestinal lysophospholipase (EC 3.1.1.5.) activity, peripheral blood, bone marrow and intestinal eosinophilia were measured in the same experimental animal. The ATS caused a significant temporally related suppression of both the tissue lysophospholipase response and eosinophilia, in all three compartments. These findings support the hypothesis that parasite-induced eosinophilia is the cause of the increased lysophospholipase activity of parasitized tissue and that the responses are thymus cell-dependent. In vitro experiments demonstrated that the eosinophil was the primary inflammatory cell source of lysophospholipase among eosinophils, neutrophils macrophages and lymphocytes. The role of other cells and antigen in the production of the enzyme by the eosinophil was also

investigated in vitro. Results demonstrated that eosinophils cultured with both T. spiralis antigen and other leukocytes yielded enzyme activities significantly greater than eosinophils cultured alone or with only antigen. More specific experiments showed that T-lymphocytes were the cells responsible for influencing the eosinophils' lysophospholipase activity in the presence of antigen, and that their influence was enhanced by the presence of macrophages. These results suggested that increased lysophospholipase activity present in parasitized tissue was not only due to increased numbers of eosinophils infiltrating parasitized tissue but was also due to each eosinophil synthesizing more of the enzyme. The necessity for antigen and other cells suggests a role for cell cooperation in the production of the enzyme, specifically T-lymphocytes and macrophage interaction with the eosinophil. A lymphocyte soluble factor collected from sensitized lymphocytes stimulated with specific antigen or concanavalin A was found to enhance the eosinophil lysophospholipase activity when added to cultures of eosinophils plus other peritoneal cells. The soluble factor did not stimulate the lysophospholipase activity of pure cultures of eosinophils.

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# CHAPTER I

# INTRODUCTION

## Review of the Literature

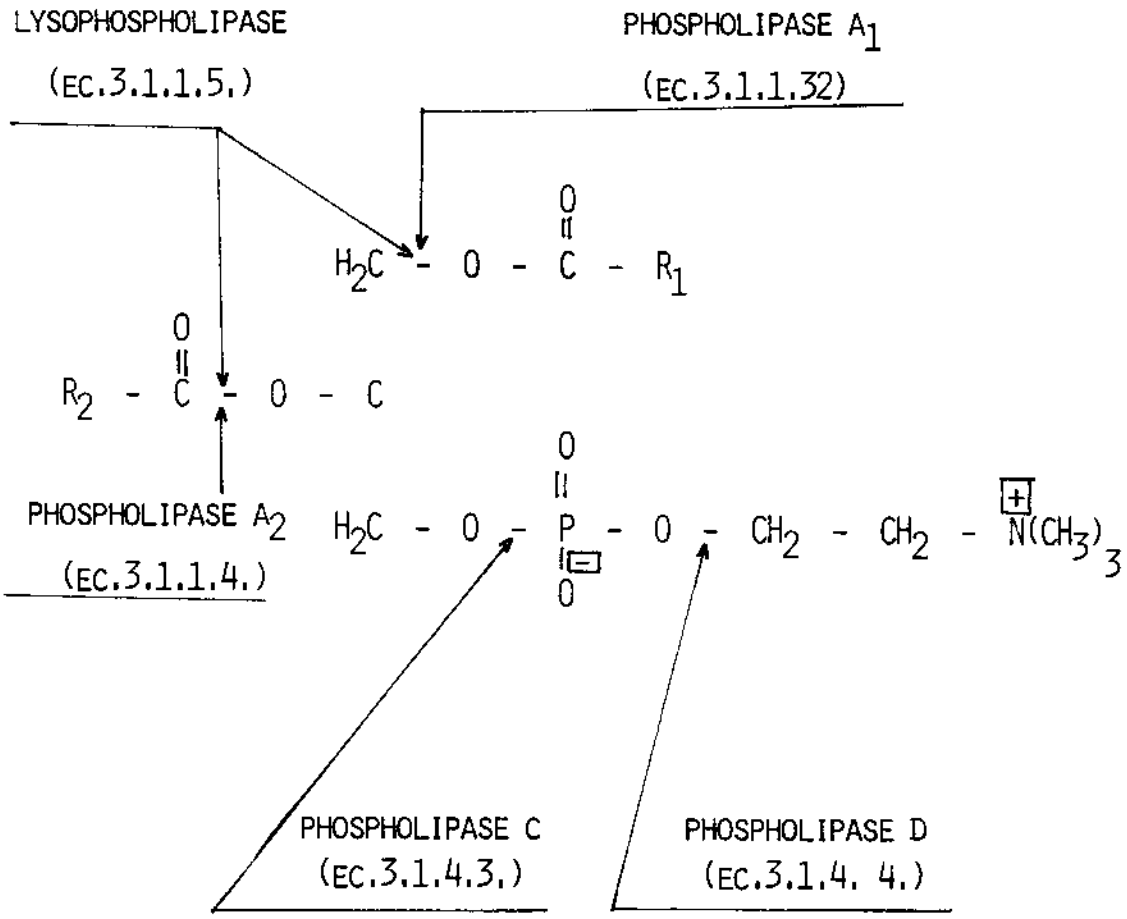
Considerable evidence suggests an association between a variety of parasitic infections, eosinophilia and increased lysophospholipase activity in parasitized tissues. This literature review documents this association.

### Occurrence and Biochemical Properties of Lysophospholipase

Lysophospholipase (EC. 3.1.1.5), also designated lecithinase B, lysolecithinase, lysolecithin acylhydrolase and phospholipase B, is an enzyme that catalyzes the complete hydrolysis of acyl chains from a diacylglycerophospholipid such as phosphatidylcholine. The hydrolysis is carried out in a sequential order, first the 2-acyl group and then the 1-acyl group (Figure 1). Lysophospholipases are also always active towards lysoglycerophosphatides in that they remove the fatty acid esterified in the 1-position from lyso-compounds such as lysolecithin (Robertson and Lands, 1962; Rossiter, 1967; Van den Bosch, 1982).

The presence of lysophospholipase was first described in rice bran (Contardi and Ercoli, 1933), later in molds (Fairbairn, 1948), bacteria (Hayashi and Kornberg, 1954),

Figure 1--The action of lysophospholipase and other phospholipases on phosphatidylcholine.


LYSOPHOSPHOLIPASE
(EC.3.1.1.5.)
PHOSPHOLIPASE A1
(EC.3.1.1.32)
PHOSPHOLIPASE A2
(EC.3.1.1.4.)
PHOSPHOLIPASE C
(EC.3.1.4.3.)
PHOSPHOLIPASE D
(EC.3.1.4. 4.)

and mammalian tissue (Shapiro, 1953; Van den Bosch and Van Deenen, 1965; Elsbach et al., 1965). Recently, lysophospholipase activity has been reported in four species of the protozoan, Trypanosoma, (Hambrey et al., 1981), and the bacterium, Legionella pneumophilia (Thorpe and Miller, 1985).

The presence of lysophospholipase in rat intestinal mucosa was first described by Schmidt et al. (1957), and was later confirmed by Epstein and Shapiro (1959), who showed that lecithin and lysolecithin were hydrolyzed by a component present in rat intestinal mucosa with the liberation of fatty acids and glycerylphosphoryl choline. Marples and Thompson (1960) showed that the enzyme was most active in rodent intestine (ilium), lung, and spleen, and least active in nerve and heart tissues. Recently, lysophospholipase activity has been demonstrated in the liver and kidney of the mouse by use of the electron microscope (Nagata and Iwadare, 1984).

Intestinal lysophospholipase has a higher enzyme activity than that of lysophospholipase present in the spleen, thymus and bone marrow and its activity is unaffected by proteolytic enzymes such as trypsin (Ottolenghi, 1967). Different molecular weights have been found for lysophospholipases purified from various sources using different methods. A lysophospholipase purified from the bacterium Escherichia coli had a molecular weight of

about 39,000 (Doi and Nojima, 1975), while a homogeneous lysophospholipase obtained from baker's yeast, Saccharomyces cerevisiae had a molecular weight of 330,000 (Ichimasa et al., 1984), while a molecular weight of 60,000 was estimated for a lysophospholipase purified from Legionella pneumophilia (Thorpe and Miller, 1985). Intestinal lysophospholipase has a molecular weight of about 17,000 daltons, an optimun pH of 4.5-6.6 and a specific activity in the range of $1x10^{-9}$ mol/min/mg protein (Ottolenghi, 1967; Berezeit et al., 1978).

## Lysophospholipase Activity and Infection

The close association between infection and increased activities of lysophospholipase was first demonstrated in animals infected with helminth parasites. In isolating lysophospholipase for biochemical characterization Ottolenghi (1973a) discovered an unusually high concentration of the enzyme in the small intestines of mice, infected with Hymenolepis nana. Further studies, in which parasite-free mice were infected with H. nana (Ottolenghi 1973 b) showed a relation between increased lysophospholipase activity and the tapeworm infection. It was observed that changes in lysophospholipase concentration were directly related to the migration of adult tapeworms from the most anterior part of the small intestine to the posterior part of the small intestine, where the adult worms eventually reside (Hunninen, 1935). After treatment of mice

with Niclosamide®, an anti-parasitic drug, to remove the tapeworms the enzyme activity returned to normal levels, thus demonstrating that the parasite caused the increase in the enzyme activity.

Subsequently, other host-parasite models were tested which resulted in the documentation of an association between the presence of parasitic worms in tissue and increased concentrations of lysophospholipase in the same sites. Larsh et al. (1974) demonstrated increased enzyme concentrations in the intestines of mice and rats after an initial infection with Trichinella spiralis. Mice infected with 400 larvae developed increased intestinal enzyme activities on the fifth day post-infection and maximum quantities of the enzyme were present on day 14. After a gradual decline, normal activities were reached by day 31. Rats infected with 3,000 larvae showed increased enzyme concentrations at day four that remained high through day 13. After this, the enzyme activity decreased, indicating a return to normal was underway.

In a later paper, Larsh et al. (1975) found that sensitized mice (three stimulations with 200 larvae at three-week intervals) challenged with 400 T. spiralis larvae showed an anamnestic-type response as measured by enzyme concentrations in intestinal tissue. Increased activity was present on day one peaked at day 11 and remained high through 20 days after challenge, returning to the level of

uninfected controls by 25 days post-infection.

Ottolenghi et al. (1975) found elevated lysophospholipase concentrations in the lungs and intestines of nonsensitized and sensitized rats infected or challenged with Nippostrongylus brasiliensis. Rats given an initial infection exhibited increased enzyme activity in the intestinal tissue from day eight through day 22, with peak activity occurring on day 15 after challenge. The intestinal activities of lysophospholipase were greatest in the area with the largest worm population. The proximal half of the small intestine, where most of the worms are found early in the infection, had a greater enzyme level than did the distal half of the intestine. After day 15, at the time of the expulsion of worms from the proximal half of the gut (Brambell, 1965), the enzyme concentration in that half of the intestine declined faster than that in the distal half. An anamnestic-type of response was found in rats sensitized with 1,000 larvae and challenged 40 days later with 1,000 larvae. The lysophospholipase concentrations were elevated on day three and reached a peak by day five (Ottolenghi et al., 1975). Goven (1979a, b) showed that the lysophospholipase activity of the intestines of nonsensitized and sensitized rats was directly related to the number of adult N. brasiliensis worms present in the intestine. Thus, the size of the worm burden influenced the induction and concentration of intestinal lysophospholipase.

Ottolenghi et al. (1977) also found elevated enzyme concentrations in the lungs and brains of rats infected with Angiostrongylus cantonesis during both a primary and secondary infection. Rats with an initial infection had moderately increased levels of lysophospholipase activity in the lungs at 8 and 15 days after infection and greatly increased levels at 35, 43 and 49 days when compared with uninfected controls. The brain tissue contained elevated concentrations at day 15 through 35 days post-infection. These periods of increased activity in the lungs and brain coincided with the migration patterns of the third stage larvae and the adult worms in the host. Sensitized rats demonstrated an anamnestic response in both the lungs and brain after challenge with elevated enzyme concentrations in both organs present on day one through day 35 post challenge.

Goulson et al. (1981) showed that rats given an infection with Strongyloides ratti developed elevated lysophospholipase levels in the lungs and small intestines during the first week of infection which remained elevated through the fourth week. Sensitized rats challenged with S. ratti developed an anamnestic response in both the lungs and intestines. Finally, Laubach (1982) found an increase in intestinal lysophospholipase activity in swine during an infection with Macracanthorhynchus hirudinaceus.

In addition to these helminth models Laubach et al.

(1978) demonstrated elevated levels of lysophospholipase in mice infected with the bacteria, Pasteurella pneumotropia and Mycoplasma pulmonis and Ngwenya and Capaci (1982) demonstrated elevated levels of lysophospholipase in mice infected with the sporozoan, Plasmodium yoelii. Finally, an increased enzyme activity has been demonstrated in swine infected with transmissible gastroenteritis virus (Goven and DeBuysscher, 1985).

These experimental findings demonstrate a relation between parasitic infections and increased lysophospholipase activities of parasitized tissue. It has been suggested that this relation was due to the presence of eosinophils and was part of the inflammatory response of the host found in parasitic infections (Larsh et al., 1975).

## Lysophospholipase and the Eosinophil

Substantial evidence has been found which links the eosinophilic leukocyte with lysophospholipase activity in animal tissues. Elsbach and Rizack (1963) have shown lysophospholipase activity in homogenates of rabbit polymorphonuclear leukocytes from peritoneal exudates. Using histochemical techniques, Ottolenghi et al. (1966) demonstrated the presence of lysophospholipase in various tissues of the rat. In all tissues, the cells associated with lysophospholipase activity possessed a "doughnut" shaped nucleus surrounded by acidophilic cytoplasmic granules and had a strong positive peroxidase reaction, all

characteristic of the eosinophil. Ottolenghi (1970) showed a close correlation between the numbers of eosinophils in histological sections and cell suspensions, and the enzymatic activity of the same preparations. These results demonstrated the existence of a relation between the concentration of lysophospholipase and the number of eosinophilic leukocytes.

Morphological evidence relating eosinophils to lysophospholipase has been supported by results obtained using corticosteroids. Ottolenghi and Barnett (1974a, b) used dexamethasone and cyclophosphamide to vary the number of eosinophils in rat tissues. Dexamethasone produces a rapid decline in the number of peripheral eosinophils while cyclophosphamide produces a decline in the bone marrow eosinophil number. By performing cell counts in tissue sections and cell suspensions, along with determining lysophospholipase activity, it was found that changes in the numbers of eosinophils paralleled changes in the enzyme activity in the intestine and bone marrow. Ottolenghi and Barnett (1974a) also found that eosinophil numbers and elevated enzyme levels could be related in additional organs. They found by injecting rats intavenously with Sephadex beads, which has been shown to induce lung eosinophilia (Walls and Beeson, 1972), that the observed increase in the eosinophil number corresponded to an increase in enzyme activity.

Due to this direct relation between lysophospholipase activity and eosinophils in animal tissues, Larsh et al. (1974; 1975), Ottolenghi et al.(1975; 1977), Goven (1979a,b) and Goulson et al. (1981) found it worthwhile to measure the numbers of eosinophils in the bone marrow while at the same time monitoring enzyme concentrations in animals given parasitic infections.

Larsh et al. (1974) found in mice infected with T. spiralis that an increase in bone marrow eosinophils occurred a few days after the intestinal lysophospholipase had increased. The eosinophil numbers reached a maximum and declined concurrently with the enzyme activity. This same pattern was seen in rats infected with T. spiralis. In addition, Larsh et al. (1975) found that in sensitized rats challenged with T. spiralis, the numbers of eosinophils in the bone marrow increased shortly after the elevation of enzyme concentrations in the intestinal tissue. Both responses were accelerated in comparison with the nonsensitized mice (Larsh et al., 1974). Ottolenghi et al. (1975) and Goven (1979a, b) found a clear relation between eosinophilia and enzyme activity in rats given a primary infection with N. brasiliensis and in sensitized rats given a challenge infection with the same parasite. The increase in intestinal lysophospholipase activity coincided with the increase in intestinal eosinophilia reported by Kelly and Ogilvie (1972). In addition, Goven (1979a, b) demonstrated

that the eosinophil and lysophospholipase responses were both related to the number of N. brasiliensis larvae administered to a host.

Increased numbers of eosinophils in the bone marrow were also found to be related to elevated enzyme levels in the lungs and brains of rats infected with A. cantonensis (Ottolenghi et al., 1977). A biphasic enzyme response was found in the lungs. It was concluded that the first response was due to the larvae migrating through the lungs to the brain, while the second response was due to the reinvasion of the lungs by the worms which become established in the branches of the pulmonary artery. A temporal relation was observed between the biphasic enzyme response and a similar biphasic bone marrow eosinophil response. An anamnestic response for both parameters was observed in sensitized rats after a challenge infection. Bone marrow eosinophils and lysophospholipase activity were also found to be elevated during the same period throughout the course of primary and secondary infections of rats with S. ratti (Goulson et al., 1981).

More direct evidence concerning the relation between eosinophils and lysophospholipase came from experiments which utilized anti-eosinophil serum (AES) and experiments where numbers of eosinophils were enumerated and lysophospholipase activity determined in the same parasitized tissue. When AES was administered to mice

infected with 400 T. spiralis larvae, Goven (1983) observed a significant, temporally related suppression in both peripheral eosinophilia and lysophospholipase activity when compared to responses found in infected mice to which normal rabbit serum was administered. Wilkes and Goven (1984) demonstrated that the accumulation of eosinophils in the small intestine was directly associated with an increased lysophospholipase activity in mice infected with 400 T. spiralis larvae.

## The Eosinophil, Lysophospholipase and the Inflammatory Response

Larsh et al. (1974, 1975) found that the increase in intestinal lysophospholipase concentrations in nonsensitized and sensitized mice challenged with T. spiralis corresponded closely in time and degree with the inflammatory response reported in previous work (Larsh and Race, 1954, 1975).

Larsh and Race (1954) reported that after an initial infection with T. spiralis an acute inflammatory response developed in about four days. By day eight a panmucosal inflammation was present which gradually diminished so that by 14 days after infection a chronic inflammation was present. In sensitized mice challenged with T. spiralis, the response was similar except for the more rapid appearance of the acute inflammation, within seven to 12 hours after infection, which peaked at four days. In nonsensitized mice, Larsh et al. (1974) found the first

increased enzyme concentration on day five, which followed the inflammatory response present on day four. The enzyme levels, which peaked at day 14, were high throughout the known inflammatory time span (14 days) and remained elevated until 29 days after infection. The increased enzyme concentration within 24 hours after T. spiralis challenge in sensitized mice followed the inflammatory response present 12 hours after challenge (Larsh et al., 1975). The enzyme level peaked at day 11 and remained elevated for at least 20 days.

The enzyme concentrations in sensitized mice increased earlier, but did not reach as high an activity or last for as long as those in nonsensitized mice. This was explained from conclusions drawn by Larsh and Race (1975) from studies on the inflammatory pattern found in mice infected with T. spiralis. They found that not only does a direct association exist between acute intestinal inflammation and the expulsion of T. spiralis adults, but that a similar association exists between the degree of sensitivity of the host at challenge and the timing and intensity of inflammation, and the loss of worms. In sensitized animals, the inflammatory response developed much sooner than in nonsensitized animals (Larsh and Race, 1975). Because of the temporal pattern described above, this early inflammatory response was linked to the earlier rise in lysophospholipase activity. Also, since a significant number

of adult worms, which initiate the inflammatory response, was expelled a week earlier in sensitized animals, it was assumed that the inflammation would subside earlier (Larsh et al., 1975). This would account for the lower concentrations and shorter durations of lysophospholipase activity in sensitized animals.

A temporal relation between lysophospholipase concentration and the inflammatory response has also been observed in rats infected with N. brasiliensis (Ottolenghi et al., 1975). Elevated concentrations of lysophospholipase were noted in non-immune rats on day eight post infection, which correlated with the initiation of inflammation. The peak amount was recorded on day 15, which correlated with the period of severe inflammation, after which the enzyme activity declined along with the intensity of inflammation (Ottolenghi et al., 1975). Sensitized rats challenged with N. brasiliensis showed an anamnestic response to a challenge infection (Ottolenghi et al. 1975). A similar temporal association between inflammation and enzyme concentration was reported in the lungs and brains of rats infected with A. cantonesis (Ottolenghi et al., 1977) and the intestines of rats infected with S. ratti (Goulson et al., 1981).

More detailed studies concerning the association between inflammation and enzyme activities have been done by Goven (1979a) working with non-sensitized rats infected with N. brasiliensis. He found that by increasing the size of

worm burden the degree of inflammation went from mild to severe and that this factor influenced the intensity of the bone marrow eosinophilia and lysophospholipase responses. He found an anamnestic response to this pattern in rats sensitized with N. brasiliensis before challenge with various infecting doses of larvae (Goven, 1979b). These results suggest that an increasing inflammatory response was associated with an increased lysophospholipase activity.

In view of the close association between lysophospholipase and the above mentioned inflammation, Larsh et al. (1974, 1975) and Ottolenghi et al. (1975) suggested that increased intestinal enzyme activities found in parasitized tissues were part of the host inflammatory response (delayed hypersensitivity) against a parasite and that the eosinophil was the source of the enzyme. It was further suggested that T-cells, after interaction with parasite antigen, released the stimulus for the increased production of eosinophils by the bone marrow and directed these cells to the site of inflammation where they would undergo morphological changes and release their stores of lysophospholipase, thus increasing the level of the enzyme in parasitized tissue. This would continue until the worms were expelled and the inflammation subsided, thereby lessening the stimulus for bone marrow eosinophil production.

Individual aspects of this hypothesis have been tested

in separate experiments. It has been shown that the suppression of the eosinophil response in T. spiralis infected mice with AES also resulted in a diminished lysophospholipase activity, (Goven, 1983). In addition, elevated intestinal lysophospholipase activity has been directly related to increased numbers of eosinophils in the same tissue (Wilkes and Goven, 1984). Together these experiments indicated the eosinophil as the source of the enzyme. In yet another experiment it has been shown that the lysophospholipase and eosinophil responses are absent in T.spiralis infected athymic mice, thus demonstrating the responses to be apparently dependent on an intact T-lymphocyte population (Goven and Moore, 1980).

## The Eosinophil, Lysophospholipase and the Specific Immune Response against Helminth Parasites

In helminth infections, blood eosinophil counts reach levels 10 to 100 times higher than normal and massive eosinophil accumulation occurs in the tissues around invading parasites. The mechanisms underlying the blood and tissue eosinophil response and the "purpose" of such a reaction have been investigated by several generations of researchers.

The search for factors regulating eosinophilopoiesis led to the discovery of several mediators released by lymphocytes following mitogen or antigen stimulation able to stimulate bone marrow eosinophilopoiesis in vitro and in

vivo. Metcalf et al. (1974) found that pokeweed mitogen stimulated mouse spleen cells release a factor which influenced marrow-eosinophil cell precursors to multiply and differentiate. This factor was named eosinophil colony-stimulating factor (EO-CSF). In later studies, Ruscetti and Chervernick (1975) and Ruscetti et al. (1976) reported the release of EO-CSF-like activity by T. spiralis sensitized T-lymphocytes stimulated by T. spiralis antigens. Similar results have been obtained in other parasite experimental systems such as the Mesocestoides corti-mouse model (Warren and Sanderson, 1985 and Sanderson et al., 1985). The mechanism leading to accelerated eosinophilopoiesis can be summarized as follows. A stimulus results in the antigen-specific priming of T-lymphocytes, after which these cells release factors capable of enhancing, in a nonantigen-specific manner the proliferation and maturation of marrow eosinophil precursors.

Several factors released during parasitic infections have been shown to be chemotactic for eosinophils. Colley (1973, 1976, 1980) and Greene and Colley (1974, 1976) described the production by phytohemagglutinin or antigen stimulated T-cells of a 35,000 to 55,000 molecular weight protein chemotactic for eosinophils. This mediator was referred to as eosinophil stimulation promoter (ESP). Similar additional factors have been shown to exist in other experimental systems. Hirashima et al. (1983) described the

synthesis of a 70,000 dalton factor, called eosinophil chemotactic factor (ECF), by peritoneal exudate cells of guinea pigs that were immunized with dinitrophenyl derivatives of Ascaris extract. An ECF with a similar molecular weight has also been collected from guinea pig mesenteric lymph node (MLN) and spleen cells after an eight hours stimulation with concanavalin A (Hirashima et al., 1984), while in a separate experiment, ESP was shown to be produced by guinea pig T-cells in response to both mitogenic and antigenic stimuli (Lammie et al., 1985). In summary, the existence of lymphokines, which are chemotactic for eosinophils is well documented. Production of these molecules is probably under antigen specific T-cell control. Their possible involvement in the localization of eosinophils in parasitized tissue is supported by the demonstration of their release by lymphocytes obtained from helminth-infected humans and animals (Basten and Beeson, 1970; Colley, 1973, 1976; Greene and Colley, 1974; Butterworth et al., 1976; Mackenzie et al., 1977; Glauert and Butterworth, 1977; and Nawa and Hirashima, 1984).

Several lines of evidence strongly suggest that eosinophils are directly involved in the destruction of helminth parasites. The demonstration that eosinophils can damage helminth larvae was provided by the work of Butterworth and co-workers (Butterworth et al., 1974, 1975, 1976). They demonstrated that eosinophils, in the presence

of heat-inactivated serum from infected individuals attached to the tegument and induced the release of $^{51}Cr$ from isotope labeled schistosomula. Isotope release correlated well with damage to the worm as assessed by phase-contrast microscopy. Using a preparation of 90-98 percent pure human eosinophil, it was confirmed that eosinophils, but not neutrophils could inflict damage to antibody-coated schistosomula in vitro (Vadas et al., 1979). In in vitro experiments, Mahmoud et al. (1973, 1975) were able to show that the abolishment of the eosinophil response by the administration of anti-eosinophil serum resulted in a reduced ability of the mice to destroy a Schistosoma cercarial challenge.

Butterworth's original experiments have been confirmed by others on the ability of eosinophils from humans (Anwar et al., 1979), rats (Mackenzie et al., 1977; McLaren et al., 1977, 1978) and mice (Kassis et al., 1979) to damage schistosomula in vitro. Eosinophils have been shown to adhere to and eventually damage several antibody-coated helminths at one stage or another of their life cycle. These include the newborn larvae of T. spiralis (Mackenzie et al., 1977; Kazura and Grove, 1978; Bass and Szejda, 1979; Kazura and Aikawa, 1980) and the infective larvae of N. brasiliensis (Jarret et al., 1968; McLaren et al, 1977) and Wucheria bancrofti (Higashi and Chowdhury, 1970).

Information strongly suggest that the eosinophil damages parasites through the release of granules or their

contents onto the surface of the parasite (Ackerman et al., 1982). The granular contents are thought to disrupt the parasites external membrane, and in the case of schistosomula of Schistosoma mansoni (Glauert and Butterworth, 1977; McLaren et al, 1978), eosinophils have been shown to actually invade the body of the parasite through a breech in the body wall. The ability of the eosinophil to disrupt the external membrane of the parasite implies the existence of a powerful-active effector system. Eosinophil anti-parasitic effector molecules which may process the ability to disrupt parasite membranes so far include major basic protein, eosinophil peroxidase, eosinophil cationic protein and Charcot-Leydon crystal protein (all reviewed by Ackerman et al., 1982).

The temporal relation between lysophospholipase and the eosinophil raises the question of the role of this enzyme in the host defense against parasites. Two methods by which lysophospholipase could function as part of the immune response of the host for controlling the extent and direction of parasite infection have been suggested. First, it is possible that the enzyme may be indirectly involved in worm expulsion through its role in the synthesis of prostaglandins, which have been shown to cause worm expulsion (Ottolenghi et al., 1975, 1977). Lysophospholipase on interaction with cell membrane phospholipids or membrane phospholipids of damaged cells,

effect the release of free fatty acids, such as arachidonic acid. Arachidonic acid, by the action of cyclooxygenases, can be converted to prostaglandin E and F (PGE and PGF) which have been shown to increase the vascular permeability of tissues and effect smooth muscle contraction (Kelly and Dineen, 1976). It has been shown that an infection of N. brasiliensis in rats could be expelled by the administration of prostaglandin-like factors from ram semen (Dineen et al., 1974). Intraluminal injection of purified PGE has been shown to be highly effective in causing the expulsion of N. brasiliensis from the small intestine of rats (Kelly et al., 1974). Finally it was observed that concentrations of prostaglandins in the small intestine were elevated during a primary infection with N. brasiliensis and showed an anamnestic type response in a secondary infection with the same parasite (Dineen and Kelly, 1976). A comparison of the increase in prostaglandin levels with those for lysophospholipase in animals infected with the same parasite shows that they are temporally related. These findings support the suggestion that lysophospholipase interacts with the phospholipids of cells damaged in the inflammatory response to yield fatty acids, which are synthesized into PGE. Secondly, it is possible that the enzyme acts directly on the worm (Goetzl and Austin, 1977; Kazura and Aikawa, 1980). In vitro studies have demonstrated that following binding via specific antibody, eosinophils flatten and

spread along the parasite tegument, after which the cells release anti-parasitic effector molecules into the space between the cell and the parasite (Ackerman et al.,1982). This has been clearly demonstrated in electronphotomicrographs of eosinophils attached to the tegument of schistosomules (Glauert et al., 1978) and the membrane of T. spiralis larvae (Kazura and Aikawa, 1980). Lysophospholipase could be released and function as an anti-parasitic molecule. Through either direct, indirect or a combination of both actions, lysophospholipase may play an important role in the cascade of events leading to the elimination of parasitic worms from the host.

## Statement of Problem

The overall purpose of this study was to elucidate the mechanisms involved in the production of parasite induced lysophospholipase by the eosinophil using in vivo and in vitro systems. The actions and interactions of parasite antigen and components of the cellular immune response (lymphocytes, macrophages and soluble factors) on the eosinophils production of lysophospholipase were investigated.

The goal of the first phase of the project was to demonstrate the eosinophil as the major inflammatory leukocyte source of lysophospholipase among other inflammatory cells (lymphocytes, macrophages, neutrophils) using an in vitro system.

The specific aim of the second phase of this study was to investigate the eosinophil, lysophospholipase and lymphocyte association in the same animal during a parasitic infection to more precisely define their interrelation and to demonstrate that the eosinophil-lysophospholipase response was thymus cell dependent.

The purpose of the third segment of the problem was to develop an in vitro system to determine if parasite antigen and/or cells or cell-cell interactions influenced the eosinophil lysophospholipase activity.

The fourth phase of this study was designed to demonstrate, in an in vitro system, the dependence of the eosinophil-lysophospholipase response on T-lymphocytes.

The final phase of the study was to attempt to determine if soluble factors influenced the activity of eosinophilic lysophospholipase.

# CHAPTER II

# MATERIALS AND METHODS

## Experimental Animals

Three to four week-old male and female Balb/c mice and three week-old male CD-1 mice were purchased from Harlan Sprague Dawley, Inc., Houston, Texas. Mice were kept in plastic cages (five per cage) at 25°C and fed commercially-prepared rodent chow and water ad libitum.

To maintain a regular supply of Balb/c mice for experimental studies, male and female mice were mated over a period of two weeks and the pregnant females separated into individual cages. Five weeks after birth, the young mice were weaned and separated according to sex. All CD-1 mice were purchased. Experimental mice used were over eight weeks old.

## Source and Maintenance of Trichinella spiralis

The Trichinella spiralis strain (originally obtained from Dr. N. F. Weatherly, Department of Parasitology and Laboratory Practice, University of North Carolina, Chapel Hill) used was maintained in Balb/c mice.

## Isolation and Standardization of Trichinella spiralis Larvae, and Infection of Animals

T. spiralis larvae were isolated according to the

methods of Larsh and Kent (1949) and standardized according to the techniques of Weatherly (1970). Briefly, mice infected with T. spiralis larvae for a minimum of six weeks were killed by cervical dislocation, skinned, evicerated and the carcasses rinsed in cool tap water. Each carcass was minced with scissors and homogenized in a Waring blender with artificial digestive medium, which was composed of one percent hydrochloric acid, 1.5 g pepsin (United States Biochemical Corporation, Cleveland, OH) and one liter tap water. The homogenate was incubated at 37°C for two hours with constant stirring. Subsequent to incubation, the preparation was strained through two layers of cheese cloth into a one liter graduated cylinder and incubated at 37°C for one hour. Approximately 800 ml of supernatant was removed by aspiration and the remainder poured into 50 ml conical centrifuge tubes and further incubated for 15 minutes at 37°C. Using a Pasteur pipette, the freed larvae were removed from the bottom of the centrifuge tubes and were washed in prewarmed (37°C) isotonic saline. Nutrient broth containing 0.8 g of nutrient agar in 100 ml of water and five percent gelatin was prepared by heating the mixture and then cooling it to 37°C. Using a Pasteur pipette, packed larvae were dropped into two ml of nutrient broth. The suspension was mixed thoroughly with a one ml tuberculin syringe fitted with a blunted 18 gauge needle, and a 0.05 ml sample was removed, spread over a glass plate in a straight

line and the larvae present in the sample were counted using a hand tally and dissecting microscope. Larvae or nutrient broth was added using a Pasteur pipette until the desired infecting dose was present in a 0.2 ml volume.

Mice were infected with the desired number of larvae by intubation using a one-ml tuberculin syringe fitted with a blunted 18 gauge needle.

Preparation and Administration of Anti-Thymocyte Serum

Anti-thymocyte serum (ATS) was prepared by immunizing New Zealand White rabbits with thymocytes derived from male CD-1 mice according to the methods of Davis et al. (1969). Mice were bled and thymuses removed into a petri dish containing serum free Roswell Park Memorial Institute (RPMI) 1640 media (Gibco Laboratories,Grand Island, NY). The glands were washed in RPMI 1640 to remove contaminating erythrocytes, minced with scissors and transferred into a Ten Broeck homogenizer (Arthur H. Thomas, Philadelphia, PA) with a Pasteur pipette. The glands were disrupted in the homogenizer and the resultant cell suspension filtered through two layers of gauze and transferred into a siliconized 15 ml conical centrifuge tube. After centrifugation at 250 x g for 30 minutes at 4°C, the cells were washed twice with RPMI 1640, pelleted into a 15 ml centrifuge tube and resuspended in fresh RPMI 1640. Cell counts were made in a Neubauer's improved hemocytometer and the cell suspension adjusted to 10 x $10^8$ cells per ml. The

cells were mixed with complete Freund's adjuvant (Difco Laboratories, Inc., Detroit, MI) to give an equivalent of 5 x $10^8$ thymocytes per ml. One ml of the mixture was injected into each rabbit intramuscularly. Two weeks later, 5 x $10^8$ thymocytes in one ml RPMI 1640 were injected into each rabbit intravenously. One week after the booster injection, blood was collected from each rabbit by cardiac puncture, into 50 ml conical centrifuge tubes and allowed to sit at 4°C for three hours after which the clots were removed. The serum was cleared by centrifugation at 250 x g for 30 minutes at room temperature.

The ATS was considered satisfactory if four hours after intraperitoneal injection of 0.3 ml of the serum into an adult CD-1 mouse, the total lymphocyte count was reduced to 15 percent of the total nucleated blood cell count. The complement mediated cytotoxicity of the serum was determined by the trypan blue dye exclusion technique (Weir, 1973). Thymocytes were collected as described above and the cells adjusted to 1 x $10^7$ cells per ml in Hank's Balanced Salt Solution (HBSS). Doubling dilutions (final volume of 0.1 ml) of ATS were made in HBSS. To each antibody dilution, 0.1 ml of guinea pig complement (Anderson Laboratories Inc, Fort Worth, TX), diluted to 1:4 was added, after which 0.1 ml of cell suspension (1 x $10^6$ cells) was added, the tubes shaken and incubated for 90 minutes at 37°C. Subsequently, 1.6 ml of diluted trypan blue solution (one part of one percent

trypan blue in water, to 14 parts of HBSS) was added and the tubes incubated at room temperature for ten minutes. The cells were subsequently fixed with 0.1 ml of 40 percent formaldehyde (Fisher Scientific, Pittsburgh, PA), and the number of dead cells (those taking up trypan blue dye) were counted in a Neubauer's improved hemocytometer. The titer was determined as the reciprocal of the dilution which killed 50 percent of the lymphocytes The titer was determined as 2560. In addition, the lymphagglutination titer of the serum was tested according to the method of Burrell (1979) and determined to be 1280. The resulting rabbit anti-mouse thymocyte serum was inactivated at 56°C for 30 minutes and stored in three ml aliquots at -70°C.

Normal serum (NRS) was collected from rabbits of the same strain and age as those used to produce ATS, and stored under the same conditions as noted for ATS.

## Enumeration of the Eosinophils

The percentage of bone marrow eosinophils was determined according to the methods of Larsh et al. (1974). A mouse femur was removed, freed of adhering tissue and clipped at the extremities. The femur was flushed with three ml of ice cold isotonic saline into a teflon-glass homogenizer (Arthur H. Thomas, Philadelphia, PA) and dispersed by gentle strokes. The cell suspension was centrifuged at 250 x g for five minutes after which the supernatant was removed and the pellet resuspended in 0.2 ml

of saline. Total white blood cell (WBC) counts were done with WBC diluting fluid using a Neubauer's improved hemocytometer, while eosinophil counts were performed using Discombe's solution (Discombe, 1946). Eosinophils were expressed as percent of the nucleated cells.

The percentage of peripheral eosinophils was determined using standard methodology (Kirk et al., 1975). Mice were bled from the tail while in a restraining cage, and a drop of blood collected on a glass slide. Blood smears were made on slides, air dried and stained with Wright's stain (Fisher Scientifics, Fairlawn, NJ) for three minutes. Wright's buffer was added to the slides for an additional six minutes after which the slides were washed under running water. Slides were air dried and the different leukocyte populations counted under a compound microscope with the eosinophils being expressed as percent of total nucleated cells.

Techniques developed by Ottolenghi et al. (1966) were used for the histological determination of intestinal tissue eosinophils, with slight modification. Tissue samples were collected from the proximal part of both the anterior and posterior part of the small intestine, embedded in frozen tissue embedding media (Fisher Scientific, Fairlawn, NJ) and frozen with Freez-It® (Chemtronics Inc., Hauppauge, NY). Six to ten micron thick sections were cut at -20°C using a Cryo-cut microtome (American Optical Corporation, Buffalo,

NY) and transferred onto microscope slides. The sections were fixed in chilled calcium-formal fixative (1 µg $CaCl_2$ and 1 µg $CaCO_3$ in 100 ml four percent formaldehyde) for ten minutes and then briefly rinsed in two changes of 200 ml of 0.1M Tris buffer at room temperature. Sections were stained in Eosinophil Stain (Blanco Laboratories, Fort Worth, TX) for two minutes, rinsed twice in distilled water, destained for 15 seconds in methanol, dehydrated in ethanol-xylene and mounted using Permount® (Fisher Scientifics, Fairlawn, NJ). Five tissue sections were cut from each of the two segments of the small intestine. The number of eosinophils was determined by averaging the number of eosinophils present in five microscopic fields (X630) from each of the five tissue sections. The numbers of eosinophils from each segment were averaged so that the tissue eosinophil response could be reported for the anterior and posterior region of the small intestine. Fields were chosen at random with one stipulation that the outer muscular wall of the intestine be present in each viewing field.

Preparation of Antigen from Trichinella spiralis Larvae

T. spiralis antigen was prepared according to the methods of Larsh et al. (1969). Freshly digested larvae were collected from infected mice as previously described and washed in isotonic saline. Packed larvae (0.3 ml) were suspended in 5.7 ml of phosphate buffered saline (PBS, Sigma Chemical Company, St. Louis, MO) and transferred into a

motor driven chilled Ten Broeck tissue grinder (Arthur H. Thomas, Philadelphia, PA). The grinder was submerged in an ice bucket and larvae ground at 300 rpm until completely disrupted as verified microscopically. The mixture was transferred into a centrifuge tube. The grinder was washed with approximately four ml of PBS at 300 rpm for five minutes and the resulting solution added to the contents of the centrifuge tube. After an overnight incubation at 4°C, the suspension was centrifuged at 10,000 x g for 20 minutes at 4°C. Using a Pasteur pipette, the supernatant was carefully transferred to a capped 15 ml conical centrifuge tube and held at 4°C.

Protein concentration of the crude antigen was determined by the technique of Waddell (1956). A forty fold dilution of 0.1 ml of the protein containing solution was made with PBS. The diluted antigen was placed in a quartz cuvette (Fisher Scientifics, Fairlawn, NJ) and the protein concentration determined spectophotometrically (Perkin Elmer Analytical Instruments, Norwalk, CT). The absorbance was read at wavelengths of 215nm and 225nm with PBS as the blank. If absorbance at 215nm exceeded 1.5, the solution was further diluted. The absorbance at 225nm was subtracted from that of 215nm and the difference multiplied by 144. This calculation gave the protein concentration in the diluted solution in micrograms per ml. The stock solution was adjusted to a protein concentration of one mg/ml and then

stored in two-ml aliquots at -70°C until needed.

## Collection of Purified and Mixed Populations of Leukocytes

Pure and mixed suspensions of murine eosinophils, lymphocytes, macrophages and neutrophils for use in *in vitro* experiments were obtained as described below. Sensitized neutrophils, lymphocytes and macrophages were collected from mice previously infected with 500 *T. spiralis* larvae, whereas nonsensitized cells were collected from naive animals. All eosinophils were collected from sensitized mice. Inbred Balb/c mice were the source of all cells.

### Eosinophils

Eosinophils were collected using the method outlined by Colley (1973) and Mahmoud et al. (1973), with slight modifications. Briefly, mice infected with 500 *T. spiralis* larvae for two weeks were injected with 1.5 ml of sterile ten percent proteose peptone (Difco Laboratories, Inc., Detroit, MI) intraperitoneally. Peritoneal exudates were collected 48 hours later by peritoneal lavage with five ml of HBSS containing 2.5 I. U. heparin per ml. Exudates were pooled, layered over a solution of Hypaque 50, sodium diatrizoate (Winthrop Laboratories, New York, NY) diluted 1:2 with distilled water and centrifuged at 250 x g for 40 minutes at 4°C. Subsequent to centrifugation the cells were removed and washed twice with HBSS. White blood cell counts were performed with white blood cell diluting fluid (three

percent acetic acid, one drop of Gentian violet) in a Neubaer's hemocytometer and the number of WBC in one ml calculated. Differential counts were done using Wright's stain (Fisher Scientifics, Fairlawn, NJ) to determine the purity of the cell population. This method yielded cells that were 89 percent eosinophil.

## Lymphocytes

Lymphocytes were harvested from mouse spleens using the method of North and Henry (1980). Briefly, mice were killed with carbon dioxide from dry ice and the spleens removed into a petri dish containing ice cold HBSS. A single cell suspension was prepared which was filtered through cheese cloth and layered over Histopaque 1077, (Sigma Chemical, St. Louis, MO) after which the cells were centrifuged at 250 x g for 30 minutes at room temperature to remove erythrocytes and connective tissues. Subsequent to centrifugation, lymphocytes were removed from the Histopaque-HBSS interface into clean 15 ml centrifuge tubes and washed twice with HBSS at 150 x g for 10 minutes. This method gave a 99 percent lymphocyte yield.

## Macrophages

The methods of Unanue (1968) was used to collect macrophages. Briefly, peritoneal exudates were stimulated by intraperitoneal injection of 1.5 ml of sterile ten percent proteose peptone (Difco Laboratories, Detroit, MI). Mice

were killed with carbon-dioxide from dry ice after 72 hours and the peritoneal exudate collected with heparinized HBSS. The exudate was centrifuged at 250 x g for ten minutes at 4°C and the collected cells washed twice with HBSS at 150 x g for ten minutes at 4°C. These exudates contained 94 percent macrophages.

## Neutrophils

Neutrophil rich peritoneal exudates were collected and cells purified using a modification of the technique described by Simpson and Ross (1971).The peritoneal cavity of mice was stimulated with an intraperitoneal injection of three ml of sterile three percent proteose peptone followed 15 hours later by a second intraperitoneal injection of 1.5 ml. Three hours later, mice were killed with carbon-dioxide from dry ice, skinned and three ml of heparinized HBSS was injected into the peritoneal cavity. Peritoneal exudates were transferred to 15 ml centrifuge tubes and centrifuged at 250 x g for ten minutes at 4°C and the collected cells washed twice with HBSS at 150 x g for ten minutes each at 4°C. The suspension contained 94 percent neutrophils.

## Mixed Population of Leukocytes

Mixed populations of peritoneal cells were obtained from mice in the following manner. Mice were infected with 500 *T. spiralis* larvae, 12 days after which 1.5 ml of ten percent proteose peptone was injected intraperitoneally.

Peritoneal exudates were collected 48 hours later, with three ml of heparinized HBSS. Collected cells were washed three times in HBSS and resuspended in RPMI 1640 so that a one ml cell suspension contained 2 x $10^6$ cells of which approximately 50 percent were eosinophils, 20 percent were lymphocytes, 15 percent neutrophils and 15 percent macrophages.

## Preparation of Lysolecithin

L-α-lysophosphatidylcholine (Sigma Chemical Company, St. Louis, MO) from egg yolk was dissolved in double distilled water to give a concentration of 20 µmole per ml and stored in aliquots of five ml at -10°C. Prior to use, a frozen sample was thawed in water bath and brought to a temperature of 37°C.

## Determination of Lysophospholipase Activity in Individual Leukocyte Populations

Lymphocytes,neutrophils,macrophages and eosinophils were all assayed in the same manner, thus only the method for the eosinophils is given. Eosinophils were washed three times in HBSS and adjusted to the desired cell concentration. The methods of Dole (1956) and Larsh et al. (1974) with slight modification were employed for determining the enzyme concentration of various numbers of cells. Samples of packed cells were brought to a volume of 0.6 ml with reacting medium (12.5 percent glycerol, 0.1M potassium phosphate, 5 X $10^{-3}$M MgCl , 2 X $10^{-3}$M

ethylenediaminetetracetic acid, pH 6.6). To all the tubes, 0.1 ml of 0.1 percent trypsin (Sigma Chemical Company, St. Louis, MO) was added to ensure linear reaction rates. After equilibration to 37°C for four minutes, 0.3 ml of $2 \times 10^{-2}$M lysolecithin was added and incubation continued for one hour or until a precipitate was formed. The reaction was stopped by addition with mixing of 0.1 ml of 2M $H_2SO_4$, 1.0 ml isopropanol and 0.4 ml distilled water. Free fatty acids, a reaction product, were extracted by mixing two ml heptane in the reaction tube. One ml of the heptane layer containing fatty acids was removed and titrated with 0.01N sodium hydroxide by using a syringe microburet (Micrometric Instrument Company, Cleveland, OH) to which a volume displacement syringe capable of delivering one microliter per division was fitted. Thymol blue (Sigma Chemical Company, St. Louis, MO) was used as an indicator. One ml of palmitic acid (Eastman Kodak Company, Rochester, NY) in heptane (two micromole per ml) was used as a reference standard for sodium hydroxide. Nitrogen gas was bubbled through the solution during titration. The nitrogen gas served to expel carbon dioxide and to ensure proper mixing of the two phases during titration. The end point of titration was reached when the color changed from pink to blue. The lysophospholipase activity was expressed as micromoles of lysolecithin hydrolysed per number of cells per hour.

## Determination of Eosinophils' Lysophospholipase Activity in Mixed Cell Culture

Suspensions of eosinophils only or eosinophil cultured with neutrophils, macrophages, lymphocytes or a mixture of lymphocytes and macrophages; or eosinophil rich peritoneal exudates ($2 \times 10^6$ cells consisting of approximately 50 percent eosinophils, 20 percent lymphocytes, 15 percent macrophages and 15 percent neutrophils) were assayed for lysophospholipase activity by a modification of the method of Laubach (1984). Cells were cultured in 0.5 ml RPMI 1640 (containing five percent fetal calf serum (FCS), one percent non-essential amino acids, one percent penicillin streptomycin) with or without an equal volume of various concentrations of T. spiralis antigen in serum free RPMI 1640 at 37°C, five percent $CO_2$ for 12, 24, 36 or 48 hours. At the end of the incubation period, 0.1 ml of each cell culture ($2 \times 10^5$ cells, $1 \times 10^5$ eosinophils) was brought to a total volume of 0.6 ml with reaction medium, and lysophospholipase activity assayed as previously described. Lysophospholipase was expressed as micromoles of lysolecithin hydrolysed per $10^5$ eosinophils per hour.

## Determination of Lysophospholipase Activity in Intestinal Tissue

Lysophospholipase activity was assayed for in intestinal tissue according to the methods of Larsh et al. (1974). Mice were killed by cervical dislocation and the small intestine removed and placed on ice. The small

intestine was freed of adhering tissue, the contents extruded and the intestine cut into two equal segments (anterior and posterior ). The first centimeter of tissue from each segment was removed and embedded in Tissue-Tek II® (Miles Laboratories, Naperville, IL) for tissue eosinophil enumeration as described above. The remaining tissue was minced with scissors and throughly mixed. A one g portion was weighed out and homogenized in a teflon-glass homogenizer with 19 volumes of ice cold reaction medium . Fifty to 100 microliter aliquots of intestinal tissue were placed into 15 ml centrifuge tubes and brought to a final volume of 0.6 ml with reaction medium. To all the tubes, 0.1 ml of 0.1 percent trypsin was added and the tubes equilibrated at 37°C for four minutes. Subsequently, 0.3 ml of $2x10^{-2}M$ of lysolecithin was added and the tubes incubated for one hour or until a precipitate formed. The reaction was terminated by addition of 0.1 ml of 2M $H_2SO_4$ , 1.0 ml isopropanol and 0.4 ml distilled water with constant mixing. Free fatty acids were extracted with two ml heptane. One ml of the heptane layer was removed and titrated against 0.01N sodium hydroxide using thymol blue as the indicator. Lysophospholipase activity was expressed as micromoles lysolecithin hydrolyzed per g of tissue per hour.

### Preparation and Enumeration of Pure T- and B-lymphocyte Populations

Spleens were removed from donor Balb/c mice and single

cell suspensions made by teasing and aspirating in HBSS. The cell suspensions were filtered through sterile gauze, to remove debris, and then layered over Histopaque 1077 (Sigma Chemical Co., St. Louis, MO) and centrifuged at 250 x g for 30 minutes at 25°C. Using a Pasteur pipette, viable lymphocytes were removed from the Histopaque-HBSS interphase into a sterile centrifuge tube and the cells washed twice in HBSS at 4°C. Cells were resuspended in HBSS-fetal calf serum (Gibco Laboratories, Grand Island, NY) and stored at 4°C until they were ready to be fractionated on nylon wool columns as described by Julius et al. (1973).

Scrubbed nylon fiber (Fenwal Laboratories, Deerfield, IL) was washed by boiling for ten minutes in a beaker of double deionized (DDI) water. After cooling to room temperature, the wool was drained and washed six times with DDI water. Excess water was squeezed out with cheese cloth and the wool placed in a drying oven for four days. Approximately 0.6 g and 1.8 g of teased dry washed nylon wool was placed into ten ml and 35 ml syringes (Monoject, Sherwood Medical Industry Inc., St. Louis, MO) respectively for preparation of a syringe column. All syringe columns were autoclaved.

The syringe column, to which a compression screw and a capped 22-gauge needle (Monoject, Sherwood Medical Industries, Inc., St. Louis, MO) were attached, was mounted on a ring stand, and washed with 20 ml of prewarmed HBSS

containing five percent heat inactivated FCS. Excess medium was allowed to drain off and the syringe covers replaced and sealed with parafilm to prevent evaporation. The column was placed in an incubator at 37°C for one hour before loading cells, which was done under a tissue culture hood (The Baker Company, Sanford, ME). In a total volume of two ml, 1 x $10^8$ lymphocytes were loaded onto the column and one ml of warm medium was added. The column was resealed and placed back in the incubator for 45 minutes.

The column was washed slowly with warm medium (20 ml for the ten ml and 60 ml for the 35 ml column) so as not to generate a fluid head, the effluent containing the non adherent cells (T-cells) was collected into 15 ml conical centrifuge tubes. Cells were pelleted at 200 x g for ten minutes at 4°C. Further depletion of Ig bearing cells was accomplished by incubating the non-adherent cells (1 x $10^7$ cells in 0.5 ml serum free RPMI 1640) with 0.1 ml anti-mouse polyvalent immunoglobulin, 200 µg protein (Sigma Chemical Company, St. Louis, MO) at 37°C for 30 minutes and 0.1 ml of diluted (1:4) guinea pig complement (Anderson Laboratories, Inc., Fort Worth, TX) as described by North and Henry (1980). The resultant lymphocyte population was shown by indirect immunofluorescence to be 97 percent Thy 1.2 positive.

B-lymphocyte enrichment was accomplished by recovering the adherent lymphocytes from the nylon wool column by

adding cold saline (0.85 percent NaCl, w/v) after which the cells and fluid were forcibly pushed out with the plunger. The cells were washed twice at 200 x g for ten minutes at 4°C in serum free RPMI 1640 and then resuspended in the appropriate medium. The contaminating T-cells were removed by treating the cell suspension (1 x $10^7$ cells in 0.5 ml serum free RPMI 1640) with 0.25 ml monoclonal anti-mouse Thy 1.2 serum (Miles Scientific, Napperville, IL) and guinea pig complement (0.1 ml) diluted to 1:4 with distilled water using the method of Harte et al. (1985). The resultant lymphocyte population was shown to be 94 percent surface Ig-positive by indirect immunofluorescence.

## Indirect Immunofluorescent Assay

The purity of T- and B-lymphocyte suspensions was examined by a modified version of the indirect immunofluorescence technique of Julius et al. (1973), and Harte et al. (1985). T and B lymphocyte suspensions, (one ml) containing 1 X $10^7$ cells were placed in a centrifuge tube and spun at 200 x g for ten minutes at 4°C. The appropriate cells were resuspended in 0.25 ml of either monoclonal anti-mouse thy 1.2, 0.5 mg/ml (Miles Scientific, Napperville, IL) (T-lymphocytes) or goat anti-mouse polyvalent immunoglobulins, 1.5 mg/ml (Sigma Chemical Co., St. Louis, MO) (B-lymphocytes) and incubated at 4°C for 20 minutes. The mixtures were subsequently layered over four ml of neat FCS and centrifuged at 200 x g for 20 minutes at

room temperature. The pelleted cells were washed in two ml of medium, after which the T-lymphocytes were resuspended in 0.25 ml of fluorescein-isothiocyanate (FITC) conjugated goat anti-mouse thy 1.2, 0.2 mg/ml, (Miles Scientific, Napperville, IL) and the B-lymphocytes resuspended in 0.25 ml of FITC conjugated goat anti-mouse immunoglobulins, 0.2 mg/ml, (Sigma Chemical Co., St. Louis, MO) at 4°C for 20 minutes. The cell suspensions were again pelleted through four ml of neat FCS and washed in two ml of medium. The pelleted cells were subsequently resuspended in 0.05 ml of neat FCS. Smears of the cells were made on microscope slide, air dried for 30 minutes at room temperature and fixed for 20 seconds in 95 percent ethanol at room temperature. Following air drying, slides were mounted by addition of a small drop of glycerol-phosphate buffer (9:1 glycerol-phosphate buffered saline) covered and sealed. Cells were examined with dark field illumination using a Zeiss fluorescent microscope (Carl Zeiss, Inc., Thonwood, NY).

Preparation of Eosinophil Stimulatory Factor

Spleens were removed aseptically from mice that were previously infected with 500 T. spiralis larvae according to the methods of Colley (1973) and Hirashima et al. (1984). Spleens were minced in RPMI 1640 and distrupted using a teflon homogenizer. The resulting single cell suspension was filtered through two layers of sterile cheese cloth to

remove cell debris. The cells were washed three times with HBSS at 200 x g for ten minutes at 4°C and resuspended in serum free RPMI 1640 supplemented with one percent streptomycin and one percent penicillin. Cell viability was estimated by the trypan blue exclusion method, and was found to be 85-90 percent. Lymphoid cells (5 X $10^6$ per ml) were cultured with T. spiralis antigen (one µg per ml) or with concanavalin A (5 µg/ml, Miles Scientific, Naperville, IL) at 37°C for either four or eight hours in a humidified atmosphere with five percent $CO_2$ and 95 percent air. At the end of incubation, cells were removed by centrifugation (200 x g for ten minutes at 4°C), resuspended in serum free culture medium and incubated for 24 hours under the same condition. For control cultures, lymphoid cells (5 X $10^6$ per ml) were incubated without Con A or T. spiralis antigen under the same condition for 24 hours. Thirty minutes before the end of the incubation period, either 5 µg/ml Con A or 1 µg/ml T. spiralis antigen was added to the cultures. At the end of incubation, cells were removed by centrifugation (250 x g for ten minutes at 4°C) and the supernatant collected. Con A and T. spiralis antigen were removed from control culture fluid by dialysis against HBSS before assays. The culture fluid was concentrated by centrifugation at 12,000 x g for 30 minutes at 4°C, in an Amicon 10 microconcentrator (Amicon Corporation Scientific Systems Division, Danvers, MA) and stored at -70°C until needed.

Stimulatory effects of the culture fluid on the eosinophils lysophospholipase activity were examined by a modification of the technique of Larsh et al. (1974) as previously described. Eosinophil rich cell suspensions (2 x $10^6$ cells/ml) containing approximately 50 percent eosinophil, 20 percent lymphocyte, 15 percent macrophages and 15 percent neutrophil in a 0.5 ml of RPMI 1640 containing five percent FCS were placed in petri dishes. To each dish, 0.5 ml of eosinophil stimulatory factor (ESF) from either antigen or mitogen stimulated cells was added and the plates incubated for 48 hours at 37°C, 95 percent humidity and five percent $CO_2$. At the end of the incubation period, lysophospholipase was assayed for as previously described. Control culture supernatant was prepared from sensitized lymphocytes not exposed to antigen or mitogen. Two groups of experimental control cultures were prepared. One group was prepared with cultures of pure eosinophils to which ESF was added, while another group was prepared with eosinophil peritoneal exudate cells cultured with T. spiralis antigen.

Protein concentration of the cell free lymphocyte culture fluid was done according to the Bradford method (Bradford, 1976) and as modified by Pierce Chemical Company, Rockford, IL). Culture fluid (0.1 ml) was pipetted into a test tube, protein assay reagent (5.0 ml) (Pierce Company) was added and the contents mixed throughly. Absorbance was

read at 595nm with DDI water as blank. All assays were read in duplicate. The protein concentration was determined from a standard curve.

## Statistical Analysis

The Student t-test was used to determine the statistical significance of the observed differences in the experimental and control specimen. A probability greater than 0.05 was considered not significant.

# CHAPTER III

## RESULTS

### Determination of the Inflammatory Cell Source of Lysophospholipase

To determine the major inflammatory cell source of lysophospholipase for use in later *in vitro* experiments, populations of eosinophils, neutrophils, macrophages and lymphocytes were collected from Balb/c mice and their enzyme activity determined. Cells sensitized to *T. spiralis* antigen were collected from mice previously infected with 500 *T. spiralis* larvae, while nonsensitized cells were collected from naive animals.

Table I illustrating the lysophospholipase activity of $10^5$ cells shows that eosinophils contain the majority of lysophospholipase activity among the cell types tested. Table II which gives the gross enzyme activity among the various populations of each cell type, shows that the eosinophil is the primary source of lysophospholipase among the cells cultured, and that enzyme concentration increases with increasing numbers of eosinophils.

TABLE I

LYSOPHOSPHOLIPASE ACTIVITY* PRESENT IN DIFFERENT POPULATIONS OF MOUSE LEUKOCYTES COLLECTED FROM NONSENSITIZED MICE AND MICE SENSITIZED BY INFECTION WITH 500 Trichinella spiralis LARVAE

| Cell type | Source of cells | | Purity of cell population (%) | Lysophospholipase activity*** |
|---|---|---|---|---|
| | Uninfected | Infected | | |
| Neutrophils | + | | 99 | 0.04 ±0.02 |
| | | + | 99 | 0.03 ±0.01 |
| Macrophages | + | | 97 | 0.15 ±0.02 |
| | | + | 94 | 0.23 ±0.02 |
| Lymphocytes | + | | 99 | 0.00 ±0.00 |
| | | + | 99 | 0.14 ±0.01 |
| Eosinophils | | + | 89 | 3.05 ±0.01 |

*Measured as micromoles of lysolecithin hydrolyzed per $10^5$ leukocytes per hour.

**Each value represents the average of three ±SD determinations for each cell type.

TABLE II

LYSOPHOSPHOLIPASE ACTIVITY OF MURINE LEUKOCYTES COLLECTED FROM MICE SENSITIZED TO *Trichinella spiralis*

| Cell Number | Lysophospholipase activity* | | | |
|---|---|---|---|---|
| | Neutrophils | Macrophages | Lymphocytes | Eosinophils |
| $1x10^5$ | 0 | 0.93±0.04 | 0.01±0.01 | 3.52±0.34 |
| $5x10^5$ | 0.58±0.02 | 0.13±0.03 | 0.01±0.01 | 11.50±1.49 |
| $1x10^6$ | 1.04±0.04 | 0.12±0.06 | 0.02±0.01 | 23.15±4.81 |
| $5x10^6$ | 2.49±0.12 | 0.18±0.01 | 0.01±0.01 | --- |

*Measured as micromoles of lysolecithin hydrolysed per number of cells per hour. Each value represent the average ±SD of 3 determinations.

## The Relation Between Thymus Dependent Cells, Eosinophils and Lysophospholipase as Compared in the Same Animal

This study was designed to demonstrate that the eosinophil lysophospholipase response was thymus cell dependent. To do this, it was decided to investigate the effects of the depletion of lymphocytes, using anti-thymocyte serum (ATS), on bone marrow, peripheral blood and intestinal eosinophils and intestinal lysophospholipase activity of animals during a parasitic infection. In this experiment, 70 male CD-1 mice were divided into four groups: 21 experimental mice injected intraperitoneally with 0.3 ml of ATS four days before and every day after infection with 100 T. spiralis larvae (Group 1); 21 mice treated with the same volume and dose of NRS and infected with 100 T. spiralis larvae (Group 2); 21 untreated mice infected with the same number of larvae (Group 3); and seven untreated, uninfected mice (Group 4). After infection three mice were killed, from each of Groups 1, 2, and 3, on the days indicated in Figure 2 and the bone marrow, peripheral blood and intestinal tissue eosinophilia determined along with the lysophospholipase activity of the anterior and posterior segments of small intestine. The results were expressed as the mean ±SD of three animals. A single control mouse was killed on each day of the experiment and the results expressed as the mean ±SD of all seven animals.

Figure 2--Design of experiment to determine the relation between bone marrow, blood and tissue eosinophilia, and lysophospholipase activity in the same mouse treated with anti-thymocyte serum or normal rabbit serum and infected with 100 Trichinella spiralis larvae.

| DAYS POST INFECTION | GROUP 1 INFECTED, ATS TREATED* | GROUP 2 INFECTED, NRS TREATED** | GROUP 3 INFECTED UNTREATED | GROUP 4 UNINFECTED UNTREATED |
|---|---|---|---|---|
| 0 | 100 larvae | 100 larvae | 100 larvae | 0 larvae |
| 2 | B,T,P,L,LY*** | B,T,P,L,LY | B,T,P,L,LY | B,T,P,L,LY |
| 5 | B,T,P,L,LY | B,T,P,L,LY | B,T,P,L,LY | B,T,P,L,LY |
| 8 | B,T,P,L,LY | B,T,P,L,LY | B,T,P,L,LY | B,T,P,L,LY |
| 12 | B,T,P,L,LY | B,T,P,L,LY | B,T,P,L,LY | B,T,P,L,LY |
| 15 | B,T,P,L,LY | B,T,P,L,LY | B,T,P,L,LY | B,T,P,L,LY |
| 21 | B,T,P,L,LY | B,T,P,L,LY | B,T,P,L,LY | B,T,P,L,LY |
| 28 | B,T,P,L,LY | B,T,P,L,LY | B,T,P,L,LY | B,T,P,L,LY |

*Mice injected with 0.3 ml ATS from -4 through 28 day post infection.

**Mice injected with 0.3 ml NRS from -4 through 28 day post infection.

***"B"--bone marrow eosinophil count, "L"--peripheral lymphocyte count, "LY"--lysophospholipase count, "P"--peripheral blood eosinophil count, "T"--intestinal tissue eosinophil count

The titer of the ATS was determined to be 2560 by complement mediated lysis and 1280 by lymphagglutination. The data presented in Table III show the peripheral lymphocyte pool in infected animals treated with ATS (Group 1) to be significantly lower than that found in infected NRS treated (Group 2) or infected untreated (Group 3) mice on all days of the experiment, thus providing evidence that the ATS was an effective in vivo lymphocyte suppressant.

The data reported in Tables IV, V, VI, and VII illustrate the eosinophils' response in infected mice treated with ATS. The values demonstrate that animals infected with 100 T. spiralis larvae and treated with ATS (Group 1) developed a significantly lower bone marrow (Table IV), peripheral blood (Table V) and tissue eosinophil response (Tables VI and VII) when compared with either the infected, NRS treated (Group 2) or the infected, untreated (Group 3) mice on the days indicated in the appropriate tables.

The data presented in Tables VIII and IX show the lysophospholipase activity in infected animals treated with ATS (Group 1) to be significantly lower than that found in infected NRS treated (Group 2) or infected untreated (Group 3) mice on all days of the experiment.

Tables VI through IX show a relation exist between the lysophospholipase activity and numbers of eosinophils in the anterior and posterior segments of the small intestine. The

TABLE III

PERCENTAGE OF PERIPHERAL LYMPHOCYTES IN UNTREATED MICE AND MICE TREATED WITH ANTI-THYMOCYTE SERUM OR NORMAL RABBIT SERUM AND INFECTED WITH 100 *Trichinella spiralis* LARVAE

| | Percentage of peripheral lymphocytes | | |
|---|---|---|---|
| | Group 1* | Group 2* | Group 3* |
| Days post infection | Infected, ATS treated | Infected, NRS treated | Infected, untreated |
| 2 | 13.3±0.5*** | 43.0±18.0 | 57.0±2.0 |
| 5 | 13.0±4.1*** | 34.5±2.5 | 58.5±7.5 |
| 8 | 9.6±4.2*** | 41.0±9.0 | 60.0±7.4 |
| 12 | 14.7±4.2*** | 52.6±5.5 | 51.0±4.0 |
| 15 | 15.7±4.6*** | 63.0±7.0 | 53.0±1.6 |
| 21 | 15.6±5.4*** | 75.0±0.0 | 64.5±5.5 |
| 28 | 36.3±2.5*** | 69.5±1.5 | 52.5±1.5 |

Group 4** : Uninfected, untreated 72.3±7.8

*Numbers represent an average of three mice ±SD.

**Numbers represent an average of seven uninfected mice ±SD, one killed on each day of the experiment.

***Statistically significant from corresponding values in Groups 2 and 3, Student t-test, $P<0.05$.

TABLE IV

PERCENTAGE* OF BONE MARROW EOSINOPHILS IN UNTREATED MICE AND MICE TREATED WITH ANTI-THYMOCYTE SERUM OR NORMAL RABBIT SERUM AND INFECTED WITH 100 Trichinella spiralis LARVAE

| | Percentage of bone marrow eosinophils | | |
|---|---|---|---|
| | Group 1 | Group 2 | Group 3 |
| Days post infection | Infected, ATS treated | Infected, NRS treated | Infected, untreated |
| 2 | 2.9±0.6*** | 4.5±0.6 | 4.4±0.8 |
| 5 | 5.4±0.1*** | 8.1±0.6 | 8.1±0.6 |
| 8 | 8.2±0.6*** | 10.5±1.7 | 11.5±1.4 |
| 12 | 9.6±0.7*** | 14.7±2.7 | 14.4±3.0 |
| 15 | 4.6±0.7*** | 8.5±0.2 | 9.2±1.4 |
| 21 | 2.6±0.8*** | 5.0±0.3 | 5.8±1.1 |
| 28 | 2.5±0.2 | 3.2±1.1 | 2.7±0.9 |

Group 4:** Uninfected, untreated 3.0±0.7

*Numbers represent an average of three mice ±SD.

**Numbers represent an average of seven uninfected mice ±SD, one killed on each day of the experiment.

***Statistically significant from corresponding values in Groups 2 and 3, Students t-test, $P<0.05$.

TABLE V

PERCENTAGE* OF PERIPHERAL BLOOD EOSINOPHILS IN UNTREATED MICE AND MICE TREATED WITH ANTI-THYMOCYTE SERUM OR NORMAL RABBIT SERUM AND INFECTED WITH 100 *Trichinella spiralis* LARVAE

| | Percentage of peripheral blood eosinophils | | |
|---|---|---|---|
| | Group 1 | Group 2 | Group 3 |
| Days post infection | Infected, ATS treated | Infected, NRS treated | Infected, untreated |
| 2 | 2.8±1.5 | 2.7±1.2 | 3.3±1.2 |
| 5 | 3.0±2.0*** | 7.0±1.0 | 6.0±2.0 |
| 8 | 7.7±0.6***[a] | 11.0±2.0 | 12.0±1.0 |
| 12 | 8.7±3.2***[a] | 16.0±2.0 | 15.3±3.1 |
| 15 | 3.0±1.0***[a] | 7.0±1.7 | 7.3±1.2 |
| 21 | 1.3±0.6***[a] | 6.0±1.7 | 6.0±1.7 |
| 28 | 1.7±0.6***[a] | 3.3±1.1 | 3.3±1.2 |

Group 4:** Uninfected, untreated 1.7±1.0

*Numbers represent an average of three mice ±SD.

**Numbers represent an average of seven uninfected mice ±SD, one killed on each day of the experiment.

***Statistically significant from corresponding values in Group 2, Student t-test, $P<0.05$.

[a]Statistically significant from corresponding values in Group 3, Student t-test, $P<0.05$.

TABLE VI

NUMBERS* OF TISSUE EOSINOPHILS IN THE ANTERIOR SEGMENT OF THE SMALL INTESTINE OF MICE TREATED WITH ANTI-THYMOCYTE SERUM OR NORMAL RABBIT SERUM AND UNTREATED MICE AFTER INFECTION WITH 100 Trichinella spiralis LARVAE

| | Numbers of intestinal tissue eosinophils | | |
|---|---|---|---|
| | Group 1 | Group 2 | Group 3 |
| Days post infection | Infected, ATS treated | Infected, NRS treated | Infected untreated |
| 2 | 4.1±1.8 | 6.0±2.2 | 6.9±2.0 |
| 5 | 5.3±2.6*** | 10.5±4.3 | 11.4±2.9 |
| 8 | 12.3±3.2*** | 25.3±1.4 | 29.7±9.6 |
| 12 | 7.3±3.1*** | 16.3±1.1 | 16.6±3.3 |
| 15 | 6.7±4.4*** | 15.7±2.7 | 16.6±2.2 |
| 21 | 5.1±2.6*** | 9.2±1.5 | 9.1±1.8 |
| 28 | 4.6±2.0 | 7.3±2.6 | 5.7±3.5 |

Group 4:** Uninfected, untreated 5.0±3.6

*Numbers represent an average of three mice ±SD.

**Numbers represent an average of seven uninfected mice ±SD one killed on each day of the experiment.

***Statistically significant from corresponding values in Groups 2 and 3, Students t-test, $P<0.05$.

TABLE VII

NUMBERS* OF TISSUE EOSINOPHILS IN THE POSTERIOR SEGMENT OF THE SMALL INTESTINE OF MICE TREATED WITH ANTI-THYMOCYTE SERUM OR NORMAL RABBIT SERUM AND UNTREATED MICE AFTER INFECTION WITH 100 *Trichinella spiralis* LARVAE

| | Numbers of intestinal tissue eosinophils | | |
|---|---|---|---|
| | Group 1 | Group 2 | Group 3 |
| Days post infection | Infected, ATS treated | Infected, NRS treated | Infected, untreated |
| 2 | 5.5±3.1 | 7.1±3.6 | 9.3±2.4 |
| 5 | 6.9±2.5[a] | 7.9±2.5 | 10.2±4.0 |
| 8 | 8.2±2.6***[a] | 20.2±8.6 | 25.6±4.5 |
| 12 | 9.1±4.1***[a] | 18.4±4.5 | 16.6±3.3 |
| 15 | 7.4±3.4***[a] | 18.2±5.8 | 19.0±4.7 |
| 21 | 6.6±2.7***[a] | 14.7±2.7 | 13.0±3.5 |
| 28 | 5.5±2.0 | 9.5±2.1 | 7.5±3.9 |

Group 4:** Uninfected, untreated 7.5±3.9

*Numbers represent an average of three mice ±SD.

**Numbers represent an average of seven uninfected mice ±SD, one killed on each day of the experiment.

***Statistically significant from corresponding values in Group 2, Students t-test, $P<0.05$.

[a]Statistically significant from corresponding values in Group 3, Students t-test, $P<0.05$.

TABLE VIII

LYSOPHOSPHOLIPASE ACTIVITY* IN THE ANTERIOR SEGMENT OF THE SMALL INTESTINE OF MICE TREATED WITH ANTI-THYMOCYTE SERUM OR NORMAL RABBIT SERUM AND UNTREATED MICE AFTER INFECTION WITH 100 Trichinella spiralis LARVAE

| | Lysophospholipase activity** | | |
|---|---|---|---|
| | Group 1 | Group 2 | Group 3 |
| Days post infection | Infected, ATS treated | Infected, NRS treated | Infected, untreated |
| 2 | 126±10 | 430±96 | 610±324 |
| 5 | 1,544±658 | 20,476±8,585 | 12,681±5,356 |
| 8 | 41,954±15,54[a] | 89,684±12,155 | 89,195±11,376 |
| 12 | 27,232±1,401[a] | 48,074±4,897 | 50,217±8,273 |
| 15 | 18,052±3,969[a] | 47,251±8,462 | 52,194±14,111 |
| 21 | 948±118 | 1,978±1,077 | 2,059±1,026 |
| 28 | 307±49 | 261±145 | 185±140 |

GROUP 4:*** Uninfected, untreated 257±223

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

**Numbers represent an average of three mice ±SD.

***Numbers represent the average of seven uninfected mice ±SD, one killed on each day of the experiment.

[a]Statistically significant from corresponding values in Groups 2 and 3 Student t-test, $P<0.05$.

TABLE IX

LYSOPHOSPHOLIPASE ACTIVITY* IN THE POSTERIOR SEGMENT OF THE SMALL INTESTINE OF MICE TREATED WITH ANTI-THYMOCYTE SERUM OR NORMAL RABBIT SERUM AND UNTREATED MICE INFECTED WITH 100 Trichinella spiralis LARVAE.

| | Lysophospholipase activity** | | |
|---|---|---|---|
| | Group 1 | Group 2 | Group 3 |
| Days post infection | Infected, ATS treated | Infected, NRS treated | Infected, untreated |
| 2 | 1,774±1,622 | 566±47 | 864±560 |
| 5 | 731±175[a] | 5,302±3,328 | 15,369±9,910 |
| 8 | 26,643±13,098[a] | 71,888±26,682 | 63,670±16,548 |
| 12 | 38,786±9,676[a] | 58,173±11,321 | 63,271±8,240 |
| 15 | 29,378±6,904[a] | 58,116±15,807 | 62,471±18,420 |
| 21 | 3,134±1,032 | 11,015±7,935 | 10,642±7,553 |
| 28 | 510±98 | 726±164 | 635±129 |

Group 4:*** Uninfected, untreated 54±173

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

**Numbers represent an average of three mice ±SD.

***Numbers represent the average of seven uninfected mice ±SD, one killed on each day of the experiment.

[a]Statistically significant from corresponding values in Groups 2 and 3, Student t-test, $P<0.05$.

results illustrate that both the numbers of tissue eosinophils (Tables VI and VII) and enzyme activity (Tables VIII and IX) in the ATS treated animals (Group 1) were significantly lower than that found for the infected control groups (Groups 2 and 3) over the same time period in both the anterior and posterior segments of small intestine. Uninfected control animals averaged 5.0±3.6 (anterior segment) and 7.5±3.9 (posterior segment) for tissue eosinophil number and 257±223 (anterior segment) and 548±173 (posterior segment) micromoles lysolecithin hydrolysed/g wet tissue/hour for lysophospholipase activity.

The results demonstrate that the treatment of mice, infected with 100 T. spiralis larvae, with ATS (Group 1) caused a significant reduction in eosinophilia which was temporally related to a decrease in the lysophospholipase response. The results indicate that the eosinophil and lysophospholipase responses, as measured in the same animal, in the small intestine were thymus dependent.

## Development of an in vitro System to Investigate Influences on the Eosinophil-Lysophospholipase Response

An in vitro system was developed to study the effects that parasite antigen and/or other cells might have on the production of lysophospholipase by the eosinophil. In the first experiment, eosinophil rich peritoneal exudates consisting of approximately 50 percent eosinophils, 20 percent lymphocytes, 15 percent macrophages and 15 percent

neutrophils were obtained from Balb/c mice infected with 500 T. spiralis larvae. The cells were cultured in 0.5 ml RPMI 1640 supplemented with five percent FCS, one percent non-essential amino acids and one percent penicillin streptomycin, with or without an equal volume of various concentrations of T. spiralis antigen for 48 hours. After incubation, the enzyme activity of the eosinophils in the culture was determined.

The results presented in Table X demonstrate that the eosinophil can vary in its lysophospholipase activity. The level of enzyme activity associated with the eosinophil appears to be influenced by the presence of parasite antigen and other cells. The data show that the culture of T. spiralis sensitized peritoneal exudate cells with T. spiralis antigen resulted in increased enzyme activity of the eosinophils. Stimulation of lysophospholipase activity was greatest when peritoneal cell mixtures were incubated with concentrations of T. spiralis antigen between 0.01 and 10.0 µg/ml with 1.0 µg/ml providing the greatest stimulus ($34.79 \pm 1.80$ per $1 \times 10^5$ eosinophils). Incubation of only eosinophils with concentrations of antigen, or peritoneal cells containing eosinophils without antigen, resulted in no significant increase in lysophospholipase activity over that found for eosinophils alone reported in Table I ($3.05 \pm 0.01$).

A second experiment was carried out to determine the effect of incubation time on the enhancement of

TABLE X

LYSOPHOSPHOLIPASE* ACTIVITY OF MURINE PERITONEAL EOSINOPHILS AFTER 48 HOUR INCUBATION WITH OR WITHOUT Trichinella spiralis ANTIGEN AND POPULATIONS OF LEUKOCYTES

| Trichinella spiralis antigen (µg protein/ml) | Lysophospholipase activity** | |
|---|---|---|
| | Eosinophils only*** | Eosinophils and other cells[a] |
| 100 | 7.64±0.32 | 16.96±2.97 |
| 10 | 6.95±0.80 | 26.53±5.04 |
| 1 | 6.10±0.63 | 34.79±1.80 |
| 0.1 | 6.76±0.83 | 28.72±3.58 |
| 0.01 | --- | 25.00±0.74 |
| 0.001 | --- | 6.61±1.53 |
| 0 | 5.73±0.91 | 4.28±0.79 |

*Measured as micromoles of lysolecithin hydrolyzed per 1 x $10^5$ eosinophils per hour.

**Each value represents the average of four ±SD determinations from cells exposed to each concentration of T. spiralis antigen.

***Cultures contained 1 x $10^6$ eosinophils.

[a]2 x $10^6$ leukocytes: 50 percent eosinophils, 20 percent lymphocytes, 15 percent neutrophils, 15 percent macrophages obtained from Trichinella spiralis infected mice.

the eosinophils' lysophospholipase activity after antigen stimulation. As shown in Table XI, lysophospholipase activity was obtained after various periods of incubation (12, 24, 36 and 48 hours). Generally, the results demonstrate that as the period of incubation was lengthened, enzyme activity increased. At 12 hours after incubation, a substantial increase in enzyme activity (15.07±0.38) was present. This increase continued through 24 (31.06±0.52) and 36 hours (39.34±1.27) after incubation resulting in the formation of remarkably high levels of lysophospholipase. A slight decrease in enzyme activity was observed at 48 hours after incubation (33.83±0.28).

### Determination of Cells that Influence the Eosinophils' Lysophospholipase Activity

This series of experiments was done to find out which peritoneal cells influenced the eosinophils' lysophospholipase activity and to determine the importance of antigen priming. To do this, individual populations of neutrophils, macrophages, lymphocytes and a mixture of lymphocytes and macrophages harvested from uninfected or T. spiralis infected Balb/c mice were cultured with eosinophils with or without T. spiralis antigen (Figure 3). The data in Table XII show that only those eosinophils cultured with sensitized lymphocytes or a mixture of sensitized lymphocytes and macrophages harvested from T. spiralis infected mice cultured in the presence of 1.0 µg T. spiralis

TABLE XI

LYSOPHOSPHOLIPASE ACTIVITY OF MURINE PERITONEAL EOSINOPHILS CULTURED WITH OR WITHOUT *Trichinella spiralis* ANTIGEN AND POPULATIONS OF LEUKOCYTES

| Incubation period (hr) | Lysophospholipase activity* | | | |
|---|---|---|---|---|
| | Cell**+Ag[a] | Cells** | Eosinophils[b] | Eosinophils+Ag[ab] |
| 12 | 15.07±0.38 | 4.31±0.08 | --- | --- |
| 24 | 31.06±0.52 | 5.16±0.48 | --- | --- |
| 36 | 39.34±1.27 | 3.53±0.49 | --- | --- |
| 48 | 33.83±0.28 | 4.10±0.11 | 5.73±0.81 | 6.10±0.63 |

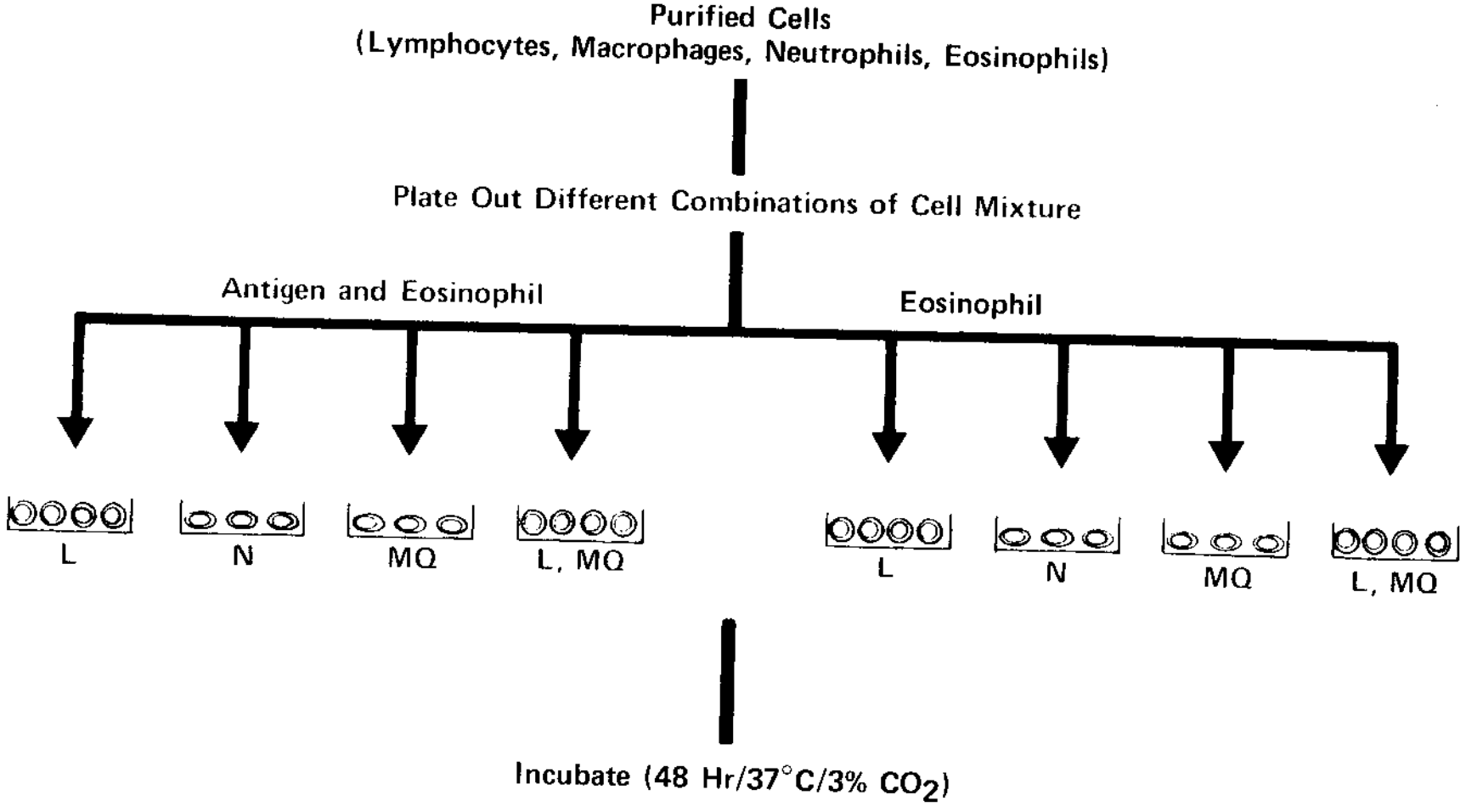
*Measured as micromoles of lysolecithin hydrolysed per $10^5$ eosinophils per hour. Each value represents the average ±SD of 4 determinations.

**Cultures contained 2 x $10^6$ cells: 50 percent eosinophils, 20 percent lymphocytes, 15 percent macrophages, 15 percent neutrophils.

[a] Antigen concentration equaled 1.0 µg *T. spiralis* protein per ml.

[b] Cultures contained 1 x $10^6$ eosinophils.

Figure 3--Design of experiment to determine the cells that influence the eosinophils lysophospholipase activity.




L = Lymphocyte N = Neutrophil MQ = Macrophage

TABLE XII

LYSOPHOSPHOLIPASE ACTIVITY OF MURINE EOSINOPHILS AFTER 48 HOUR INCUBATION WITH OR WITHOUT *Trichinella spiralis* ANTIGEN AND POPULATIONS OF LEUKOCYTES FROM NONSENSITIZED MICE OR MICE SENSITIZED BY INFECTION WITH 500 *T. spiralis* LARVAE

| Cell type* incubated with eosinophil | Source of cells | | Antigen** | Lysophospholipase activity*** |
|---|---|---|---|---|
| | Uninfected | Infected | | |
| Neutrophils | + | | - | 2.43 ± 0.23 |
| | | + | - | 4.86 ± 0.56 |
| | + | | + | 7.46 ± 1.68 |
| | | + | + | 5.18 ± 0.35 |
| Macrophages | + | | - | 3.56 ± 1.00 |
| | | + | - | 2.59 ± 0.92 |
| | + | | + | 4.19 ± 0.77 |
| | | + | + | 5.87 ± 1.36 |
| Lymphocytes | + | | - | 3.24 ± 0.46 |
| | | + | - | 4.32 ± 0.19 |
| | + | | + | 5.18 ± 1.62 |
| | | + | + | 11.87 ± 0.81[a] |
| Macrophages, lymphocytes | + | | - | 5.83 ± 0.65 |
| | | + | - | 6.04 ± 1.78 |
| | + | | + | 10.47 ± 3.90 |
| | | + | + | 26.23 ± 2.88[a,b] |

TABLE XI--Continued

*Cultures contained 5 x $10^5$ of each cell type indicated incubated with 1 x $10^6$ eosinophils.

**1 µg protein of T. spiralis antigen per ml of culture fluid.

***Measured as micromoles of lysolecithin hydrolyzed per 1 x $10^5$ eosinophils per hour. Each value represents the average three ±SD determinations.

[a]Statistically significant from lysophospholipase values for eosinophils cultured alone or with antigen reported in Tables I, X and XI, Student's t-test, $P<0.05$.

[b]Statistically significant from lysophospholipase values for eosinophils incubated with sensitized lymphocytes and T. spiralis antigen, (Table XII), Student's t-test, $P<0.05$.

antigen developed lysophospholipase activity significantly greater than those found for eosinophils cultured alone (Table XI, 5.73±0.91) or eosinophils cultured with only T. spiralis antigen (Table XI, 6.1±0.63). Although the culture of eosinophils in the presence of antigen with only sensitized lymphocytes resulted in a significant increase in lysophospholipase activity (11.87±0.81) the addition of macrophages from sensitized mice resulted in the eosinophil developing over twice this enzyme activity (26.23±2.88). The presence of either neutrophils or macrophages alone with eosinophils had no influence on the eosinophils lysophospholipase activity whether or not antigen was present.

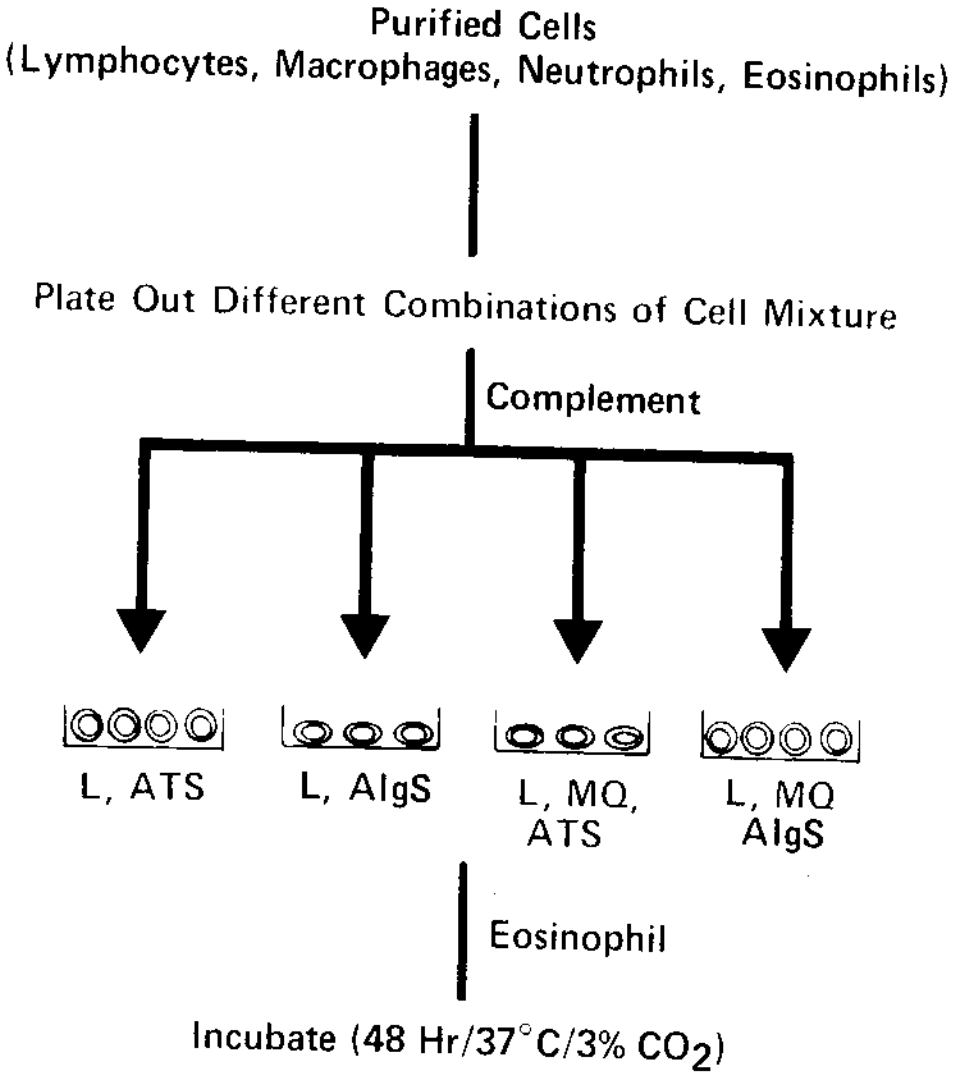
Overall, the results from Table XII illustrate that sensitized lymphocytes influence the eosinophil in its production of lysophospholipase, in addition, the data indicate that some form of synergism or cell cooperation between macrophages and lymphocytes takes place to further enhance the activity of the enzyme.

Having established that the enhanced activity of lysophospholipase by the eosinophil was lymphocyte dependent, an experiment was performed to determine which sub-population of lymphocytes interacted with macrophages and influenced the eosinophils' production of lysophospholipase. In doing this, T. spiralis sensitized splenic lymphocytes were depleted of B-lymphocytes by

passage through a nylon wool column and by treatment with anti-IgG plus guinea pig complement. This yielded a population of 97 percent Thy 1.2 positive cells as shown by indirect immunofluorescence. B-lymphocytes were prepared by removing the adherent lymphocytes from a nylon wool column and treating them with monoclonal anti-mouse Thy 1.2 serum plus guinea pig complement. This resulted in a yield of 94 percent Ig-bearing cells as demonstrated by indirect immunofluorescence. The viability of the column effluent cells as assessed by trypan blue exclusion test was greater than 95 percent. The resulting sub-populations of lymphocytes were added to cultures containing eosinophils or eosinophils and macrophages plus T. spiralis antigen and the lysophospholipase activity assayed (Figure 4).

The results (Table XIII) indicate that the lysophospholipase activity of eosinophils cultured with T. spiralis antigen and sensitized B-lymphocytes (5.59±1.13) was only equal to values found in cultures containing only eosinophils (5.73±0.91, Table XI). Eosinophils cultured with T. spiralis antigen and sensitized T-lymphocytes, however developed enhanced lysophospholipase activities (12.67±0.37) that were equal to those containing a complete population of sensitized lymphocytes (11.93±2.18). The data in Table XIII also demonstrate that the incubation of sensitized B-lymphocytes and macrophages with T. spiralis antigen and eosinophils does not cause an enhanced production of

Figure 4--Design of experiment to determine the sub-population of lymphocytes involved in cooperation with the eosinophil to synthesize increased levels of lysophospholipase.




L = Lymphocyte N = Neutrophil MQ = Macrophage

ATS = Monoclonal anti-mouse Thy 1.2 antibody
AIgS = Anti-mouse polyvalent immunoglobulin

TABLE XIII

LYSOPHOSPHOLIPASE ACTIVITY OF MURINE EOSINOPHILS AFTER 48 HOUR INCUBATION WITH *Trichinella spiralis* ANTIGEN AND SUBPOPULATIONS OF LYMPHOCYTES AND MACROPHAGES FROM *T. spiralis* SENSITIZED MICE

| Cell type* incubated with eosinophils and antigen (1 μg protein/ml) | Lysophospholipase activity** |
|---|---|
| B lymphocytes | 5.59±1.13 |
| T lymphocytes | 12.67±0.37*** |
| Lymphocytes | 11.93±2.19*** |
| B lymphocytes and macrophages | 7.80±2.42 |
| T lymphocytes and macrophages | 29.96±0.06***[a] |
| Lymphocytes and macrophages | 30.20±1.33***[a] |

*Cultures contained 5 x $10^5$ of each cell indicated with $10^6$ eosinophils.

**Measured as micromoles of lysolecithin hydrolysed per $10^5$ eosinophils per hour. Each value represents an average three ±SD determinations.

***Statistically significant from lysophospholipase values for $10^5$ eosinophils reported in Tables I and IX, Student's t-test, $P<0.05$.

[a]Statistically significant from lysophospholipase values for $10^5$ eosinophils incubated with sensitized lymphocytes and *T. spiralis* antigen, Student's t-test, $P<0.05$

lysophospholipase by the eosinophil (7.84±2.42). The results do, however, show that the culture of sensitized T-lymphocytes and macrophages with T. spiralis antigen and eosinophils results in a significantly enhanced lysophospholipase activity (29.96±0.06) equal to that reported for cultures containing sensitized macrophages and a complete population of sensitized lymphocytes (Table XII, 26.23±2.88; Table XII, 30.20±1.33).

### Effect of an Eosinophil Stimulatory Factor on the Eosinophil Lysophospholipase Response

This phase of the study attempted to demonstrate the influence of a lymphocyte soluble factor on eosinophil lysophospholipase activity. To do this, antigen and other cells, specifically T-lymphocytes and macrophages were replaced by an eosinophil stimulatory factor (ESF) in culture and the effect on lysophospholipase activity determined. ESF was produced from Balb/c splenic cells exposed to either T. spiralis antigen (ESF-Ag) or concanavalin A (ESF-ConA) for four or eight hours followed by resuspension in a serum free culture medium and incubation for 24 hours at 37°C. A 0.5 ml aliquot of ESF was added to an equal volume of RPMI 1640 containing either eosinophils only or eosinophils and other leukocytes (50 percent eosinophils, 20 percent lymphocytes, 15 percent neutrophils, 15 percent macrophages) and the cultures incubated for 48 hours at 37°C. The results of this

experiment (Table XIV) show that the activity of lysophospholipase from eosinophils incubated with either ESF-Ag (20.67±1.40) or ESF-ConA (27.34±0.20) and other peritoneal leukocytes appeared to be enhanced equally with those previously observed when cultured with T. spiralis antigen (Table XIV, 22.79±2.2). The results showed that eight hours stimulation with either antigen or Con A yielded an ESF that was functionally better than that produced after four hours of stimulation. Incubation of eosinophils only with ESF did not result in any increase in enzyme activity. ESF activity was not found in control culture fluids (ESF-control) obtained from T. spiralis infected splenic cells that were exposed to Con A or T. spiralis antigen for the last 30 minutes of incubation and followed by dialysis against HBSS (8.2±0.2). Protein concentration by Bradford assay was found to be 0.78 µg/ml for ESF-Ag, 1.5 µg/ml for ESF-con A and 0.38 µg/ml for ESF-control after four hours of stimulation, while eight hours of stimulation gave protein concentrations of 1.3 µg/ml for ESF-Ag, 1.7 µg/ml for ESF-con A, and 0.59 µg/ml for ESF-control.

TABLE XIV

LYSOPHOSPHOLIPASE ACTIVITY OF MURINE EOSINOPHILS AFTER 48 HOUR INCUBATION WITH OR WITHOUT *Trichinella spiralis* ANTIGEN OR EOSINOPHIL STIMULATORY FACTOR AND POPULATIONS OF LEUKOCYTES FROM MICE SENSITIZED TO *T. spiralis*

| Method of lymphocyte stimulation | | | | Lysophospholipase activity** | |
|---|---|---|---|---|---|
| Agent | Stimulation period(hr) | ESF added | Ag* added | Eosinophils only*** | Eosinophils and other cells[a] |
| Con A[b] | 4 | + | - | 4.73±0.9 | 16.32±2.6 |
| Con A[b] | 8 | + | - | --- | 27.32±0.2 |
| Ag* | 4 | + | - | 4.99±1.8 | 9.62±0.1 |
| Ag* | 8 | + | - | --- | 20.67±1.4 |
| none | 0 | + | - | --- | 8.20±0.2 |
| - | - | - | + | 6.10±0.6 | 22.79±2.2 |
| - | - | - | - | 5.73±0.9 | 4.70±0.65 |

*Antigen concentration equaled 1.0 microgram *T. spiralis* protein per ml culture fluid.

**Measured as micromoles of lysolecithin hydrolysed per $10^5$ eosinophils per hour. Each value represents an average of 3 ±SD determinations.

***Cultures contained 1 x $10^6$ eosinophils.

[a]Cultures contained 2 x $10^6$ cells: 50 percent eosinophils, 20 percent lymphocytes, 15 percent macrophages, 15 percent neutrophils.

[b]Concentration equaled 5 microgram per ml concanavalin A per ml culture fluid.

# CHAPTER IV

## DISCUSSION

When inflammatory cells other than the eosinophils were assayed as possible sources of lysophospholipase, the eosinophil was found to be the primary cell among those assayed (neutrophils, lymphocytes, macrophages and eosinophils) that could be present in a parasite induced inflammation to possess lysophospholipase activity. Eosinophils could only be collected from mice given a parasitic infection which was needed to stimulate their production. Because of this the enzyme activity from non-stimulated eosinophils collected from uninfected animals could not be determined. A direct comparison of enzyme activity from eosinophils, however was made with neutrophils, macrophages and lymphocytes harvested from T. spiralis infected mice. Minor enzyme activity present in cells other than the eosinophil might be explained by the presence of a small number of contaminating eosinophils or the activity may represent the cells' native lysophospholipase activity. In any event, it appears that the eosinophil is the major inflammatory cell source of lysophospholipase in both in vivo and in vitro experimental systems.

The existence of a relation between T-lymphocytes,

eosinophils and lysophospholipase has been shown by interpreting the results from several studies. One goal of this investigation was to measure all of the parameters in the same animal. To do this, the effect of treatment with ATS on the bone marrow, peripheral blood, intestinal tissue eosinophilia and intestinal lysophospholipase activity was studied in mice infected with 100 T. spiralis larvae. The results demonstrated that the specific immunologic reduction of a host animals' lymphocyte responses with ATS during a T. spiralis infection caused a temporally related suppression of both the eosinophil and intestinal lysophospholipase activity responses. The data reported in Table IV illustrated that the bone marrow developed a significantly lower level of eosinophilopoiesis, the peripheral blood (Table V) showed a significantly reduced eosinophilia and the numbers of tissue eosinophils (Tables VI and VII) were significantly lower in ATS treated mice (Group 1) compared to control animals (Groups 2 and 3). The reduction in the numbers of tissue eosinophils through the duration of the infection correlated directly with the reduced intestinal lysophospholipase activity reported in Tables VIII and IX. Together, these data provide support for the hypothesis that thymus derived lymphocytes influence the tissue concentration of lysophospholipase via their action on the eosinophil and that an increase in the number of tissue eosinophils was necessary for an increase in enzyme

activity.

In addition, a comparison of the data for numbers of tissue eosinophils found in infected animals treated with either NRS (Group 2) or untreated (Group 3), (Tables VI and VII) with their corresponding tissue lysophospholipase activity (Tables VIII and IX) demonstrate a direct relation between increasing tissue eosinophil numbers and rising enzyme activities, thus providing additional evidence for the eosinophil being the source of the elevated enzyme response in parasitized tissues.

The results of this experiment suggest that one component necessary for the development of an increase in parasitized tissue lysophospholipase, thymus dependent lymphocytes, was hampered by the administration of ATS. I propose that the ATS treated mice lacked an intact or normal thymus dependent lymphocyte population to interact with T. spiralis antigens. This resulted in a lower than normal eosinophilopoiesis in the bone marrow, resulting in a lower peripheral blood eosinophilia. The reduced eosinophilopoiesis and the probable reduction in lymphocyte generated chemotactic factors are the probable reasons for the diminished tissue eosinophil numbers. Consequently, tissue lysophospholipase activity, which has been related to numbers of tissue eosinophils, did not increase in mice treated with ATS during infection with T. spiralis. In essence, this experiment showed that T-lymphocytes are

necessary for the development of an elevated lysophospholipase activity in parasitized tissue. The experiment did not, however rule out or define the involvement of other cell types.

The above mentioned hypothesis detailing the relation between eosinophils, lysophospholipase and thymus dependent cells has been totally supported by most studies (Larsh et al., 1974, 1975; Ottolenghi et al., 1975; Goven and Moore, 1980; Goulson et al., 1981 and Goven, 1983). Some experimental results, however have only partially supported the hypothesis. Wilkes and Goven (1984) compared numbers of intestinal eosinophils and lysophospholipase activity of the same tissue over the course of a primary infection of mice with T. spiralis. It was hypothesized that the tissue eosinophils number and lysophospholipase activity would increase in a parallel manner. They found instead, that the enzyme activity increased at a far faster rate than did the number of tissue eosinophils so that between days ten and 14 post-infection, a reversal of the expected eosinophil number/lysophospholipase activity took place. In experiments where sensitized mice were challenged with T. spiralis an anamnestic response for both numbers of tissue eosinophils and enzyme activity developed when compared to results of a primary infection. Both responses demonstrated an increase by day three post infection, however the peak enzyme response (day four) occurred well before the peak tissue

eosinophil response (day seven) (Wilkes, 1984). Together these results suggested that increased tissue lysophospholipase activity was due to more than just an increase in the number of infiltrating eosinophils releasing their stores of lysophospholipase as proposed by Larsh et al. (1974, 1975) and Ottolenghi et al. (1975).

An explanation for these in vivo findings can be seen in the results of three in vitro experiments presented in this report. These experiments were performed to determine the influence of parasite antigen, lymphocytes, macrophages and neutrophils on the eosinophils' lysophospholipase activity using an in vitro system. The results of the first experiment (Tables I and II) describe the lysophospholipase activity of populations of leukocytes that characterize the cells infiltrating the intestine during the host inflammatory reaction to T. spiralis. The data indicate that some intestinal enzyme activity is probably due to infiltrating eosinophils, while neutrophils, macrophages and lymphocytes would contribute at most, minor amounts of the enzyme. These results do not, however, explain the rapid increase in enzyme activity compared to the slower increase in eosinophil numbers found in the above mentioned in vivo experiments. The second experiment describes the roles that peritoneal cells and antigen play in the eosinophils production of lysophospholipase. The results (Tables X and XI) demonstrate that the same number of eosinophils can vary

in their lysophospholipase activity. This capacity appears to be dependent upon the presence of antigen and seems to be regulated by other cells. The data suggest that part of the lysophospholipase activity assayed in the in vivo experiments of Wilkes and Goven (1984) and Wilkes (1984) was due to eosinophils actively synthesizing the enzyme over time in response to influences from other cells and antigen. These results suggest a role for cell-cooperation in the eosinophi-lysophospholipase response. The third experiment was done to determine which cells influence the eosinophils lysophospholipase production. The results (Table XII) clearly indicate that lymphocytes, in the presence of antigen, cooperate with or influence the eosinophils' production of lysophospholipase. Furthermore, it can be seen that lymphocytes and macrophages together with antigen have a synergistic effect on the eosinophils' enzyme production.

These in vitro experiments clarify the above in vivo experiments and partially explain the mechanism of the eosinophil-lysophospholipase response in tissue. I propose that the post-infection reversal of the eosinophil number/lysophospholipase activity during a primary infection, found by Wilkes and Goven (1984), was partly due to each eosinophil increasing its enzyme activity in response to influences from other cells (lymphocytes and macrophages) after their interaction with parasite antigens. This would explain the increase in enzyme activity, above

what would be expected if the response was entirely due to increasing numbers of eosinophils infiltrating parasitized tissue. The expulsion of the adult worms, and thus the removal of the stimulating antigen would result in the eosinophil number/lysophospholipase activity relation returning to normal. In sensitized animals challenged with T. spiralis (Wilkes, 1984), I propose that the interaction of worm antigens with memory or sensitized lymphocytes resulted in the rapid release of eosinophil mediators, which caused an early release of stored eosinophils from the bone marrow and their early migration to parasitized tissue resulting in an almost immediate increased lysophospholipase activity, again, the increased tissue enzyme activity would partly be due to each eosinophil becoming enzymatically more active. This is supported by the data in Table XII which illustrate that eosinophils when stimulated by sensitized lymphocytes or a mixture of lymphocytes and macrophages in the presence of antigen increase their lysophospholipase activity, while the result presented in Table XI demonstrates that this increase in enzyme activity can be found as early as 12 hours after the start of incubation and high levels could be reached within 36 hours. An explanation for the peak tissue eosinophil response (day seven) occurring after the peak enzyme response (day four) may be that the tissue eosinophilia represents an accumulation of eosinophils from the bone marrow compartment into the

parasitized tissues after the worm expulsion had taken place. I propose that the early expulsion of worms from immune animals (Larsh and Race, 1954), and thus the removal of the antigen stimulus for lymphocytes, results in the tissue eosinophils not being influenced to synthesize or possibly release lysophospholipase.

In summary these results support a role for cell cooperation in the eosinophils production of lysophospholipase during a parasitic infection. The data suggest that the increased lysophospholipase activity in parasitized tissues is not only due to an increase in the numbers of tissue eosinophils attracted to such tissues as originally proposed, but is also due to the lysophospholipase activity of each eosinophil increasing after cooperation with lymphocytes, macrophages and parasite antigen.

The eosinophil-lysophospholipase response has been indirectly shown to be dependent on an intact thymus dependent population of lymphocytes (Goven and Moore, 1980). I have demonstrated that the eosinophil lysophospholipase response was directly dependent on the presence of lymphocytes. In order to more clearly define the role of the lymphocyte, an experiment was done to determine which sub-population was involved in the eosinophil-lysophospholipase response. The results (Table XIII) clearly indicate that T-lymphocytes, in the presence of

antigen, cooperate with or influence the eosinophils' production of lysophospholipase. Furthermore, the data indicate that T-lymphocytes and macrophages together with antigen have a synergistic effect on the eosinophils' enzyme production equal to that found for a complete lymphocyte population. It appears that B-lymphocytes have no effect on the eosinophils' lysophospholipase response. In light of these results, I speculate that the T-lymphocyte after interaction with the macrophage and parasite antigen releases a soluble product, which causes a selective change in the biochemical effector function of murine eosinophils, which in this case is an increase in lysophospholipase activity.

To determine if a soluble factor was produced by lymphocytes, a final experiment was run in which sensitized lymphocytes were stimulated with specific antigen or concanavalin A (Con A) and the effect of factors collected from the culture medium determined. An analysis of the results (Table XIV) shows that sensitized lymphocytes when stimulated with specific antigen or Con A released a factor into the culture fluid that stimulated the eosinophils' lysophospholipase activity only when eosinophils were in the presence of other leukocytes, probably macrophages and/or lymphocytes. No significant stimulatory activity was detected when the soluble factor was added to suspensions of purified eosinophil.

Based on the data reported in Table XIV, it is not possible to ascertain whether the function described for this lymphocyte derived soluble factor is newly observed or has been previously described. Since the soluble factor failed to stimulate eosinophil-lysophospholipase activity in the absence of other leukocytes one must speculate that the soluble factor acted on the lymphocytes and/or macrophages present in the culture. After such activation, they in turn may release a product(s) which triggered an increase in the eosinophils' lysophospholipase activity. This is supported by the fact that the soluble factor can replace specific antigen, which was shown in this report (Tables X, XI, XII) to be necessary for the stimulation of the lysophospholipase activity of eosinophils cultured with peritoneal cells, lymphocytes or a mixture of lymphocytes and macrophages.

# CHAPTER V

## SUMMARY

The results obtained from this investigation led to the following conclusions:

1. The eosinophil is the primary inflammatory cell source of lysophospholipase;

2. The eosinophil develops an enhanced lysophospholipase activity upon incubation with antigen and specifically sensitized peritoneal cells;

3. The development of an eosinophil-lysophospholipase response is dependent on an intact thymus cell compartment;

4. Sensitized T-lymphocytes, but not B-lymphocytes, in the presence of specific antigen stimulate the eosinophils' lysophospholipase activity;

5. A cooperation between sensitized lymphocytes and macrophages in the presence of specific antigen results in a synergistic effect as measured by the enhancement of eosinophilic lysophospholipase activity; and

6. A lymphocyte soluble factor produced in response to a specific antigen or mitogen stimulation can replace specific antigen for the enhancement of eosinophilic

lysophospholipase activity in the presence of other leukocytes, probably macrophages and lymphocytes.

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