An investigation of factors involved in the development of eosinophilic responses in rodents

by

Gary Entrican

THESIS
for the
Degree of Doctor of Philosophy

Department of Immunology, University of Glasgow

September, 1985.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

ACKNOWLEDGEMENTS

I would like to express my gratitude to everyone who has helped me during the course of this project, particularly my supervisors: Professor D.M.V. Parrott, Dr. R.G. Bruce and Dr. R.P. Eady for their help and guidance. I also thank Professor K. Vickerman for allowing me to have laboratory space in the Department of Zoology.

I am indebted to my various laboratory colleagues for their support and companionship, especially the staff in the Department of Life Sciences at Fisons, Loughborough. I thank Miss Pauline Hannan and Mr Hector Cairns for helping me with my histology, and Mrs Elizabeth Gault for typing this thesis.

Finally, I would like to thank my parents for all the sacrifices they have made on my behalf during my studies.

CONTENTS Page

CONTENTS Page

CONTENTS Page

CONTENTS Page

ABBREVIATIONS

ACTH	adrenocorticotropic hormone
AES	anti-eosinophil serum
ALS	anti-lymphocyte serum
AM	arithmetic mean
ANS	anti-neutrophil serum
anti-TSWI	rabbit serum directed against a whole *T. spiralis* infection
anti-TSAG	rabbit serum directed against TSAG
ATS	anti-thymocyte serum
BGG	bovine gamma globulin
BSA	bovine serum albumin
CPM	counts per minute
CRH	corticotropic releasing hormone
ECF	eosinophil chemotactic factor
ECF-A	eosinophil chemotactic factor of anaphylaxis
ECP	eosinophil cationic protein
EDTA	ethylene diamine tetraacetic acid
EPP	eosinophilopoietin
ERF	eosinophil releasing factor
ES	excretory/secretory products
ESP	eosinophil stimulation promoter
FCA	Freund's complete adjuvant
GAR/Ig/FITC	fluoroscein conjugated goat anti-rabbit immunoglobulin
GM	geometric mean
HETE's	monohydroxyeicosatetraenoic acid
HGG	human gamma globulin
HPF	high power field
HI	heat inactivated
IEP	immunoelectrophoresis
IFAT	indirect fluorescent antibody technique

IL-1	interleukin-1
IL-2	interleukin-2
IU	international units
kD	kilo daltons
MBP	major basic protein
MW	molecular weight
NAG	N-acetyl-D-glucosamine
NRS	normal rabbit serum
OID	Ouchterlony immunodiffusion
PAF	platelet activating factor
PBS	phosphate buffered saline
PGD_2	prostaglandin D2
PHA	phytohaemagglutinin
RT	room temperature
SD	standard deviation
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SISS-T	soluble immune suppressor of T cell proliferation
SRBC	sheep red blood cells
SRS-A	slow reacting substance of anaphylaxis
TSAG	T. spiralis antigen

LIST OF TABLES Page

LIST OF FIGURES

LIST OF FIGURES

LIST OF PLATES

SUMMARY

Elevated numbers of blood and tissue eosinophils were induced in mice and rats by parasitic and non-parasitic means. Mice infected with the nematode Trichinella spiralis developed a peripheral blood eosinophilia which peaked approximately 21 days post infection. Histological examination of various tissues showed that the blood does not always reflect total body changes in eosinophil distribution. The numbers of eosinophils in skeletal muscle continued to increase at a time when the numbers of eosinophils in the blood were decreasing.

The involvement of T. spiralis antigens in eliciting this eosinophilia was investigated using an artificial method to localize parasitic material in tissue. A homogenate of T. spiralis larvae was shown to be antigenic by precipitation with serum from a T. spiralis-infected rabbit. This antigenic preparation, designated TSAG, was also capable of eliciting a humoral response when injected into a rabbit. Although not a pure antigenic preparation, the TSAG did contain antigenic components which could bind to inert latex particles. This was demonstrated in vitro by agglutination and fluorescent techniques.

When these TSAG-coated particles were injected into mice in such a manner as to result in their embolization in the pulmonary vasculature, they elicited a greater peripheral blood eosinophilic response than uncoated particles. However, similar studies on mice injected with latex particles coated with a non-parasite antigen (BGG) induced a comparable eosinophilia to that induced by parasite antigen-coated particles. The eosinophilia could not, therefore, be directly related to TSAG. Histological measurements of pulmonary cellular

accumulations around the embolized particles also showed that TSAG was not more effective than BGG at inducing pulmonary cell accumulations, and furthermore, that uncoated particles also induced cell accumulations.

The response to uncoated particles suggested a non-specific mechanism for the induction of eosinophilic responses. This latex model was used to investigate the ability of athymic (Rnu/Rnu) nude rats to develop an eosinophilia following pulmonary embolization of BGG-coated or uncoated latex particles. The number of circulating eosinophils increased in both cases, with the greater response occurring in rats injected with latex-BGG. A similar response was observed in euthymic (Rnu/+) littermates. Pretreatment of both Rnu/Rnu and Rnu/+ rats with antithymocyte serum altered the magnitude of the response but did not totally abrogate it.

Studies on interleukin-1 activity in lung lavages recovered from these rats showed that injection of latex particles did not enhance alveolar macrophage activity 48 hours after their pulmonary embolization. The level of activity was similar in both Rnu/Rnu and Rnu/+ rats suggesting that macrophages were not involved in the response in Rnu/Rnu rats which are T cell deficient. The results show that the possibility of a residual T cell population in these rats cannot be excluded.

In addition to these responses to physical manipulations, evidence is provided for inherent fluctuations in the number of circulating eosinophils, emphasizing the importance of careful experimental design when investigating haematological phenomena.

GENERAL INTRODUCTION

Discovery of the eosinophil

The discovery of the eosinophil is credited to Paul Ehrlich in 1879. Up until this time the fixation processes used on blood smears destroyed the integrity of the granules of eosinophils, but Ehrlich found that the best results were obtained when the blood was spread as thinly as possible, dried at room temperature and then stained. He commented on the presence of granulated cells and described eosinophils which were characterized by their affinity for a range of acid coal tar dyes but not for basic aniline dyes. In 1900, he postulated that the granulations were secretory products which the cell releases to the environment as needed (Hirsch and Hirsch, 1980).

Distribution of eosinophils

It has been recognised since the beginning of this century that eosinophils are abundant in tissues which have epithelial surfaces exposed to the environment, for example the gastro-intestinal tract and lungs, thereby having the possibility of being subjected to antigenic challenge, but are less abundant in internal organs, such as the heart, kidneys and liver (Opie, 1904a; Rytomaa, 1960). The number in the bone marrow however, equals or exceeds the combined number in all other tissues and estimates put the ratio of bone marrow: blood: tissue eosinophils at 300-400:1:200-300 (Weller and Goetzl, 1979).

Cellular properties of eosinophils

Eosinophils show certain characteristics in different species. Immediately noticable is nuclear morphology. Human eosinophils have a bilobed nucleus whereas those of mouse and rats have a ring-shaped nucleus. Eosinophils may be secretory, releasing their granule contents when appropriately stimulated (Spitznagel, Dalldorf, Leffell, Folds, Welsh, Cooney and Martin, 1974) or they may act as phagocytes, ingesting extracellular material (Baehner and Johnston, 1971; De Chatelet, Migler, Shirley, Muss, Szejda and Bass, 1978).

As noted by Ehrlich, the most characteristic feature of eosinophils is their acidophilic granules. In mature circulating eosinophils they are ellipsoidal membrane-bound structures which measure 0.5-1.5 μm in their longer diameter and 0.3-1.0 μm in their shorter diameter (Zucker-Franklin, 1980). When viewed under the electron microscope, eosinophil granules consist of an electron-dense core and an electron-radiolucent matrix (Miller, de Harven and Palade, 1966). In addition to the crystalloid-containing granules, eosinophils also contain smaller membrane-bound granules (Archer and Hirsch, 1963).

Eosinophil granules contain a number of substances, including major basic protein (Gleich, Loegering and Maldonado, 1973), peroxidase (Klebanoff, Jong and Henderson, 1980), phospholipase D (Weller, Wasserman and Austen, 1980), histaminase (Zeiger, Yurdin and Colten, 1976), arylsulphatase B (Archer and Hirsch, 1963) and eosinophil cationic protein (Venge, Dahl, Hallgren and Olsson, 1980). These substances are involved in eosinophil function, in both regulatory and effector roles.

The IgE/mast cell/eosinophil response

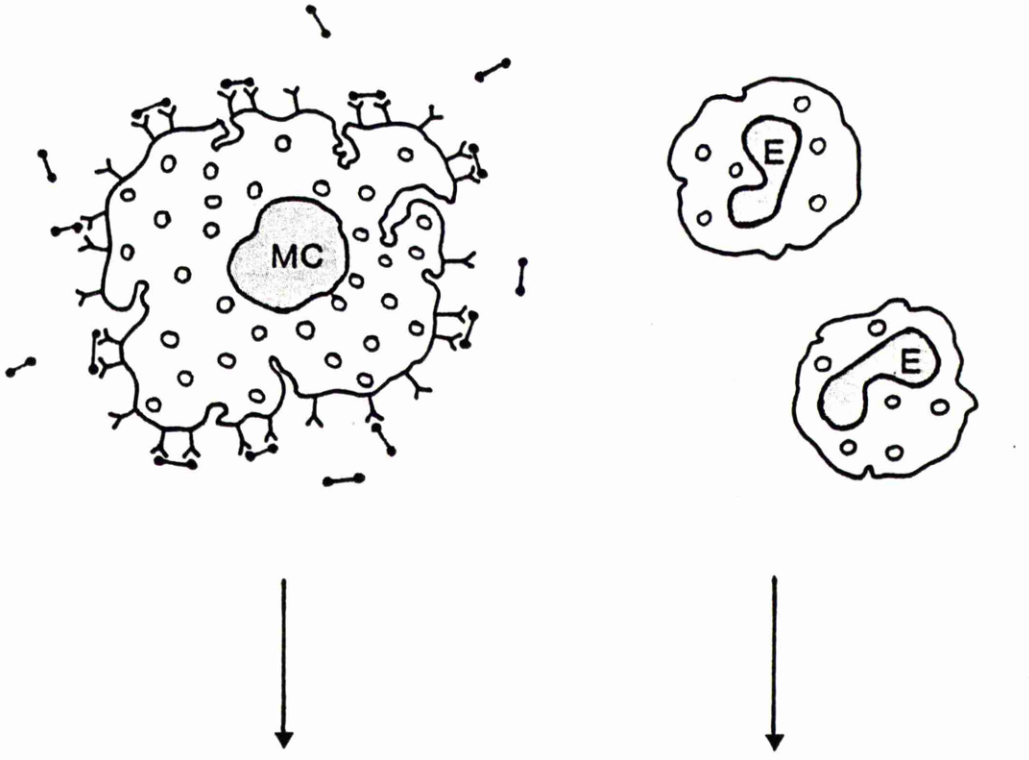
This system plays an important role in the defence against certain antigens, particularly those which gain access through epithelia, for example the respiratory and gastrointestinal tracts (Katz, 1978). The lungs are also the site of the manifestation of many allergic responses, such as hay fever and asthma. Allergic asthma occurs as a result of altered sensitivity in the lung to foreign allergic material. Initial exposure to the sensitizing protein results in IgE production which will bind to, and thereby sensitize, mast cells. Subsequent exposure to the same allergen results in crosslinking of the IgE molecules bound to the mast cell surface. This results in mast cell degranulation and the release of the mediators of immediate hypersensitivity which induce bronchospasm in the lung (Orr, 1973; Crofton and Douglas, 1981). These mediators fall into two groups. Primary mediators exist in a preformed state, and include histamine, platelet activating factor (PAF), heparin and eosinophil chemotactic factor of anaphylaxis (ECF-A). Secondary mediators are produced as a result of the action of substances released from sensitized mast cells following exposure to antigen and include leukotrienes, also known as slow reacting substance of anaphylaxis (SRS-A), prostaglandin D_2 (PGD_2) and monohydroxyeicosatetraenoic acids (HETE's) (Crofton and Douglas, 1981).

Eosinophils are attracted to the site of the allergen-IgE-mast cell reaction by the chemotactic activity of histamine, ECF-A, PGD_2 and HETE's (Gallin, Weinstein, Cramer and Kaplan, 1980; Goetzl and Austen, 1980; Goetzl, 1980). There, they are stimulated to release

Figure 1.

Eosinophil regulation of immediate hypersensitivity reactions.

Adapted from Roitt (1977).



MC : Mast cell

E : Eosinophil

: Allergen

: IgE

their granular contents, some of which are capable of neutralizing mast cell products (Weller and Goetzl, 1979). This is shown in Fig. 1 (adapted from Roitt, 1977).

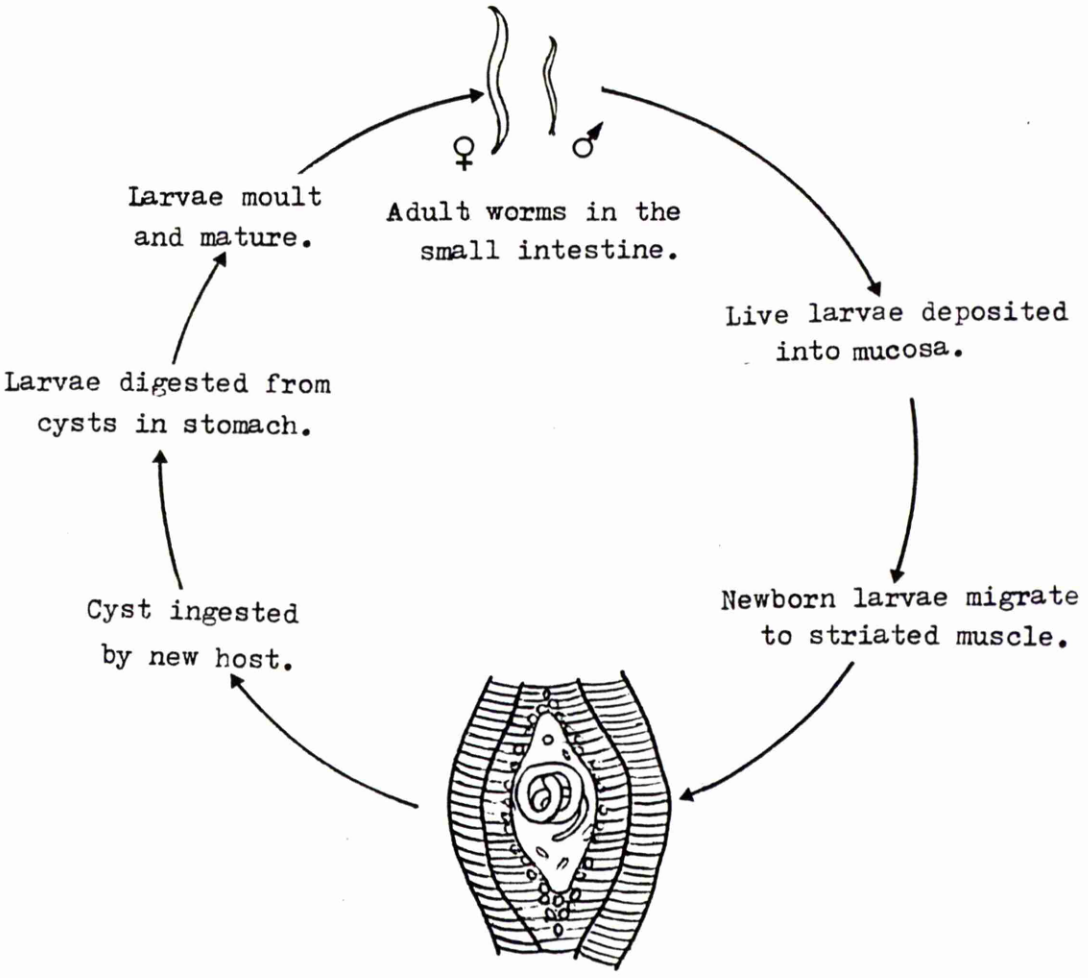
This system is not exclusively associated with conditions such as asthma. High levels of serum IgE are often found in the hosts of parasitic helminths and this, plus an infiltrate of mast cells, basophils and eosinophils, is frequently a hallmark of parasitic infestations (Ogilvie, 1967). There is a relative abundance of IgE-producing plasma cells in the intestine and respiratory tract and since the respiratory tract is derived embryologically from the foetal gut, this may account in part for these organs having similar immunological defence mechanisms (Kay, 1979). However, contrary to their regulatory role in the control of immediate hypersensitivity reactions, eosinophils may be regarded as effector cells in the host response to parasites (Weller and Goetzl, 1979). The parasite used for the work presented in this thesis was the nematode Trichinella spiralis.

The life cyle of T. spiralis

The nematode T. spiralis was first described in 1835, and according to the classification proposed by Soulsby (1968), Trichinella spiralis belongs to the phylum Nematoda, subclass Aphasmidia, suborder Trichurata, family Trichinellidae. The life cycle is detailed in Fig. 2. Following ingestion of muscle containing T. spiralis cysts, the larvae excyst in the gut of the new host and burrow under the columnar epithelium of the anterior half of the small intestine. There, they undergo four moults within 36 hours prior to

Figure 2. The life cycle of Trichinella spiralis.

Adapted from Manson Bahr (1966) and Wakelin (1984).



becoming adults (Ali Khan, 1966). The adult worms are sexually distinct and can be identified by morphological differences. Females are 3-4 mm long and 60 /um in diameter on average, while males are smaller, averaging 1.6 mm in length and 40 /um in diameter. The males can also be identified by the presence of posterior papillae (Manson Bahr, 1966). The adults mate just below the columnar epithelium and the female worms will begin to produce live larvae by days 5-6 post infection, which then progress to the lamina propria (Gardiner, 1976). The newborn larvae then enter a draining lymphatic or blood vessel in the lamina propria and are carried to the arterial circulation from which they migrate to penetrate skeletal muscle cells (Harley and Gallichio, 1971). After entering the muscle fibres the worms undergo a ten-fold increase in size, growing to approximately 1 mm in length. They then roll into a spiral and become infective after 17-18 days. The larvae remain in the cysts until ingested by a new host (Chandler and Read, 1961).

Immunity to T. spiralis

Immunity to T. spiralis appears to involve a complex series of components. Following infection, pronounced inflammatory changes in the gut serve to make it an inhospitable environment for the worms. These changes include increased peristalsis, alterations in brush border enzymes and a net fluid excretion into the gut as opposed to net absorption (Wakelin, 1984). Intestinal mast cells increase on days 10-13 post-infection, coinciding with worm expulsion from the gut. Eosinophils accumulate in the intestinal mucosa on days 5-7, during migration of newborn larvae through the intestine (Tronchin, Dutoit, Vernes and Biguet, 1979). In 1931, McCoy found that a

T. spiralis infection in rats produced a strong protection against reinfection. He later proposed that the accelerated worm expulsion from immune animals is one of the main mechanisms of defence against reinfection. This was based on his observations that the majority of challenge larvae are expelled from immune rats within 12 hours of infection (McCoy, 1940). This may be an immediate hypersensitivity reaction, but recent attempts at inhibiting rapid expulsion by administration of inhibitors of immediate hypersensitivity have been unsuccessful (Bell, McGregor and Adams, 1982).

Specific immunity to T. spiralis can be transferred to recipients with either antiserum or mesenteric lymph node cells from immune animals, and a combination of both will have an enhanced effect (Love, Ogilvie and McLaren, 1976).

The excretory/secretory antigens of T. spiralis

In 1938 Sarles was one of the first workers to show that animals mounted a specific humoral response to substances excreted or secreted (ES) by parasites. He demonstrated precipitates around the oral, anal and excretory pore openings of larval developmental forms of Nippostrongylus muris when the helminths were incubated in the serum of previously infected animals. Similar observations were noted for T. spiralis two years later by Oliver-Gonzales (1940), who found oral precipitates around T. spiralis larvae incubated in immune serum. In addition to an oral precipitate, he found that anal and vulval precipitates developed around adult worms. The crucial demonstration of a role for metabolic products eliciting a protective immune response came from Campbell (1955). He studied the course of

infection in mice following injection of ES products of T. spiralis larvae cultured in vitro, and found that the injected mice exhibited a more rapid loss of adult worms and harboured fewer larvae in their muscles after infection compared to controls.

Since the response appeared to be directed against material orally extruded by infective larvae, studies began to focus on the stichosome as the source of the antigens. The stichosome of mature muscle larvae consists of a single row of 45 to 55 stichocytes, each approximately 25 µm in diameter and possessing a single nucleus, mitochondria, rough endoplasmic reticulum, structures resembling Golgi-complexes, and one of two types of membrane-bound secretory granules, designated α and β (Despommier and Miller, 1976). The α granules are 800 nm in diameter, have a granular appearance, and are confined to the final 10 to 13 stichocytes at the posterior end of the stichosome. The β granules are smaller, being 600 nm in diameter and are homgeneous in appearance. A duct leads from each of the stichocytes to the lumen of the oesophagus, and both types of stichocyte secrete their granule products during the enteral phase of infection (Despommier, 1974). Using hyperimmune rabbit serum, antigens from both types of granule have been found to cross-react with those present in the excretory/secretory products of living muscle larvae (Despommier and Miller, 1978). Fluorescent studies using serum from rats immunized with excretory/secretory products have shown the presence of these antigens in the stichosome in situ (personal unpublished observations). However, the antigenic repetoire of T. spiralis is not restricted to stichosome products.

Stage-specific antigens of T. spiralis

In 1941, Oliver-Gonzales reported that two types of antibody appear in the serum of rabbits infected with T. spiralis, one directed against the adults and one directed against the larvae. These findings were disputed by Jackson (1959) who reported that he could not find distinct anti-larval and anti-adult activity in the serum of immune rabbits. The stage-specificity of T. spiralis was confirmed by Mackenzie, Preston and Ogilvie (1978). They found that cell populations consisting of 60-80% eosinophils from normal rats adhered to the surfaces of infective larvae, adult worms and newborn larvae via heat-stable factors present in immune serum, i.e. antibodies. Using this cell adherence technique, antibodies to infective larvae were found to appear in the serum 6-7 days post infection, rise to a peak around day 10 and to be present at a high titre for many weeks thereafter. Antibodies to adult worms appeared around the same time and also peaked on day 10, but diminished in titre more rapidly than antibodies to infective larvae. Antibodies to newborn larvae did not appear until day 13, peaked around day 20 and were almost undetectable by day 40.

A more detailed study of the surface components was performed by Philipp, Parkhouse and Ogilvie (1980) who surface-labelled infective larvae, intestinal worms and newborn larvae with ^{125}Iodine and analysed the reduced components by SDS-PAGE. They found that infective larvae expressed four main surface proteins with MW of 105 kilo Daltons (kD), 90 kD, 55 kD and 47 kD. Adult worms expressed four different surface proteins of MW's 56 kD, 40 kD, 33 kD and 20 kD, which changed in relative abundance as the worms developed. The 56 kD

band decreased in intensity while the 40 kD and 20 kD bands became more intense. Newborn larvae showed two major bands of 64 kD and 58 kD and two faint bands at 30 kD and 28 kD. These proteins also vary in expression during development initially only the 64 kD protein is expressed, the other appearing as the larvae mature (Jungery, Clark and Parkhouse, 1983). These reports demonstrate that whereas qualitative changes in antigen expression occur between stages, quantitative changes occurs within a stage.

These proteins also constitute part of the material described as metabolic antigens. Radiolabelled surface proteins from intestinal stage worms and infective larvae were found to be released from the worms *in vitro* (Philipp *et al*, 1980). Further studies revealed these proteins to be antigenic and to be expressed during the course of an infection, with antibodies precipitating the surface proteins of adult worms and infective larvae appearing approximately five days before antibodies to newborn larvae (Philipp, Taylor, Parkhouse and Ogilvie, 1981). The somatic antigens of *T. spiralis* have also been shown to be stage-specific, with infective larvae having the greatest number of distinct components (Parkhouse and Clark, 1983).

Different stages of *T. spiralis* also differ in their ability to activate complement. Mackenzie *et al* (1978) found that cells will adhere to the surfaces of infective larvae and adult worms in the presence of normal serum. The same was not found to be true for newborn larvae. This adherence could be markedly reduced by pre-heating the serum at 56^{o}C for one hour, indicating that the surfaces of infective larvae and adult worms activate complement, but that of newborn larvae does not. Jungery *et al* (1983) showed that the cuticle begins to acquire the ability to activate complement as the newborn

larvae mature and develop.

Eosinophils in T. spiralis infections

An eosinophilia associated with trichinosis has been documented since the end of the last century when Brown (1898) proposed that eosinophilia could be used as a diagnostic indicator of parasitic infestations. At the beginning of this century Opie (1904b) noticed that administration of T. spiralis larvae to guinea pigs induced an increase in the number of blood eosinophils which was accompanied by certain characteristic changes in the bone marrow. These changes included an increase in the number of eosinophil myelocytes undergoing mitotic division, from which he postulated that the bone marrow was the seat of eosinophil multiplication. This early theory has been confirmed, although eosinophil production may occur in the liver, spleen, lymph nodes and thymus during neonatal development (Foot, 1963; Yoffey and Burghan, 1964; Sin and Sainte-Marie, 1965).

Eosinophils have been shown to have a role in the host defence against T. spiralis infection, both in vivo and in vitro. Using anti-eosinophil serum (AES), Grove, Mahmoud and Warren (1977) found that the depletion of eosinophils in mice infected with T. spiralis did not affect the expulsion of adult worms from the small intestine, but did result in almost twice the number of larvae being deposited in the muscles compared to controls. These observations implied that eosinophils act against the migratory phase of infection, and this has been substantiated by the observations of Kazura and Grove (1978), Bass and Szajda (1979), and Kazura and Aikawa (1980), who found that eosinophils can kill newborn T. spiralis larvae in vitro. Kazura and

Grove (1978) were the first to attribute this killing to eosinophils. They found a correlation between the proportion of eosinophils in the effector cell population and the degree of killing. Preincubation of eosinophil-rich peritoneal exudates with AES and complement abrogated the killing, while an eosinophil-enriched population recovered from a density gradient resulted in enhanced killing compared to the eosinophil depleted fraction. Bass and Szedja (1979) noted that the killing was reduced when cells from individuals with chronic granulomatous disease were used; a disease characterized by a failure to mount a normal oxidative response, implying a role for hydrogen peroxide in the killing. Eosinophil peroxidase has subsequently been shown to have larvicidal activity in combination with hydrogen peroxide and chloride ion (Buys, Wever, van Stigt and Ruitenberg, 1981). In addition to this killing mechanism, eosinophil MBP has also been shown to damage newborn larvae in vitro (Wassom and Gleich, 1979). Because of this strong association with eosinophilia, T. spiralis has been widely used to investigate the mechanisms controlling eosinophil production and function.

The thymus dependency of eosinophilia

In 1970 Basten and Beeson discovered that neonatal thymectomy of rats significantly reduced the peripheral blood eosinophilia normally observed in thymus intact rats following T. spiralis infection. They also found that pre-treatment with antilymphocyte serum (ALS) could reduce the eosinophil response to a subsequent intravenous injection of T. spiralis larvae, all of which implied a role for lymphocytes, and in particular T cells, in the induction of eosinophilic responses.

They confirmed this by showing that thoracic duct lymphocytes taken from rats 3-5 days after infection with T. spiralis were capable of eliciting a blood eosinophilia in recipients. A similar thymus dependency of the eosinophil response to T. spiralis larvae has been shown in mice. Walls, Basten, Leuchars and Davies (1971) injected normal, thymectomized and thymus-reconstituted mice with larvae and found that a rise in the number of circulating eosinophils occurred four to six days later in the normal and thymus-reconstituted mice, but not in thymectomized mice. They also showed that eosinophilic and neutrophilic responses have separate control mechanisms, since the thymus-deprived mice were found to be capable of mounting a neutrophilic response to pyrogenic infection.

The response to T. spiralis has also been studied in congenitally athymic (nu/nu) mice and their euthymic (nu/+) littermates (Ruitenberg, Elgersma, Kruizinga and Leenstra, 1977). The nu/+ mice showed a considerable increase in the number of circulating eosinophils which peaked 21 days post infection. No such increase was observed in nu/nu mice.

This phenomenon is not exclusive to T. spiralis infections. The typical blood eosinophilia normally observed 8-10 weeks after infection of mice with Schistosoma mansoni was found to be much reduced in thymectomized and congenitally athymic mice (Fine, Buchanan and Colley, 1973; Phillips, DiConza, Gold and Reid, 1977). The granulomas around S. mansoni eggs are smaller in nu/nu mice than in nu/+ or nu/nu thymus-reconsituted mice and histological examination has shown that the major cause for this decrease was a reduction in the number of eosinophils (Phillips et al, 1977). Antithymocyte serum

has been found to result in not only a diminished number of lymphocytes in the granulomas which form around S. mansoni eggs injected intravenously into previously infected mice, but also to cause a concomitant 75% reduction in the number of eosinophils (Olds and Mahmoud, 1980).

These experiments demonstrated that lymphocytes were involved in eosinophilic responses, but not how they exerted their effect. In their work with the transfer of cells from T. spiralis infected rats, Basten and Beeson (1970) showed that thoracic duct lymphocytes from infected rats could induce an eosinophilia in normal recipients even when implanted in cell impermeable chambers. This implied that the effect was being mediated by a diffusable substance.

Eosinophil chemotactic factor

In 1971, Cohen and Ward showed that when lymphocytes from sensitized animals were stimulated in vitro with homologous antigen in the presence of specific antibody, an eosinophil chemotactic activity was produced. This was termed eosinophil chemotactic factor (ECF) and was shown to be immune complex-dependent, since stimulation of the cells with antigen alone did not result in chemotactic activity. This factor was also shown to be active in vivo, by its ability to induce an eosinophilic cellular infiltration when injected intradermally into guinea pigs.

Eosinophil stimulation promoter

A lymphokine activity which does not require the presence of a specific antibody for its action has also been reported. Colley

(1973) described a factor which was released from sensitized lymphocytes following challenge with specific antigen or mitogen stimulation. This activity was designated the term eosinophil stimulation promoter (ESP). In vitro experiments showed that this factor is capable of enhancing eosinophil migration out of agarose droplets. The production of this factor is thymus dependent. It was found that spleen cells from nude mice do not produce ESP, and spleen cells from mice treated with ATS show diminished ESP production (Greene and Colley, 1978).

ESP may play a role in the host eosinophil response to parasite infestations, since it has been shown to be released by cells from individuals infected with T. spiralis (Warren, Karp, Pelley and Mahmoud, 1976) and S. mansoni (Kazura, Mahmoud, Karb and Warren, 1975) when challenged in vitro with T. spiralis larval antigen or S. mansoni soluble egg antigen respectively. ESP may be responsible for the recruitment of eosinophils in the host granulomatous response to certain parasitic life-stages. James and Colley (1978) isolated S. mansoni egg granulomas from the livers of infected mice, cultured them in vitro, and found that the culture supernatants contained ESP activity without the addition of exogenous antigen. These culture fluids were also found to stimulate eosinophils to destroy S. mansoni eggs in vitro. Further work has shown that ESP can increase the oxidative activity in eosinophils and also stimulate them to release increased amounts of arylsulphatase B (Rand and Colley, 1982). Supernatants produced by stimulation of lymphocytes with specific antigen or mitogen can enhance a number of eosinophil functions, such as chemotaxis, phagocytosis, microbicidal activity and glycolysis

(Sher, Wadee and Joffe, 1983). Recently, mitogenic stimulation of lymphocytes has been shown to result in the production of a factor with eosinophil chemotactic activity *in vivo* (Hirashima, Tashiro, Sakata and Hirashima, 1984). These reports present a role for lymphocyte products in the recruitment of pre-existing eosinophils, but give no indication of any possible role for lymphocytes in the control of eosinophilopoiesis.

Eosinophilopoietin

By definition, an eosinophilopoietic substance is one which will stimulate *de novo* eosinophil production. Using AES to deplete eosinophils in mice, Mahmoud, Stone and Kellermeyer (1977) found that the eosinopoenia was followed by a dramatic increase in the number of circulating eosinophils 3 days after the final injection of AES. This was accompanied by a steady increase in the number of eosinophils in the bone marrow. Serum recovered from mice injected with AES was found to increase the number of circulating and bone marrow eosinophils when injected into recipients. This activity was termed eosinophilopoietin (EPP). To investigate any possible influence of T lymphocytes on eosinophilopoiesis, EPP production was studied in nude mice and their heterozygous littermates. Serum EPP levels were found to increase in nu/+ mice following infection with *T. spiralis* or eosinophil depletion by AES. However, nu/nu mice did not produce EPP following either of these manipulations, and subsequently did not develop the characteristic peripheral blood eosinophilia which normally follows *T. spiralis* infection. This implied that T cells are either the source of EPP or are at least necessary for its production,

its action on bone marrow eosinophils, however, does not require the presence of T cells, since serum EPP activity will increase the number of bone marrow eosinophils in nude mice (Mahmoud, 1980).

Spleen cells from parasite-infested animals will release eosinophilopoietic substances when challenged with homologous antigen. Using cell chambers, Miller, Colley and McGarry (1976) showed that spleen cells from S. mansoni infected mice produce a diffusible factor capable of stimulating eosinophilopoiesis following challenge with soluble egg antigen, and that spleen cells from uninfected mice do not stimulate eosinophilopoiesis to the same degree when similarly treated. Bartlemetz, Dodge and Bass (1980) showed that media conditioned by the challenge of spleen cells from T. spiralis mice with excretory/secretory products of the larvae could stimulate eosinophil production in syngeneic bone marrow cells in culture. In addition to stimulating increased eosinophil production, a T. spiralis model has been used to demonstrate a mechanism for increased release of these cells from the bone marrow.

Eosinophil releasing factor

Basten, Boyer and Beeson (1970) found that intravenous injection of T. spiralis larvae produced a peripheral blood eosinophilia. This response differed to that following infection by peaking earlier and being much shorter lived, with eosinophils returning to normal levels two weeks after injection. Using this model, Spry (1971a;b) found that the injection of larvae increased the number of bone marrow eosinophils, reduced their marrow transit time, and accelerated their emergence into the circulation, culminating in a peripheral blood

eosinophilia. He also found that serum recovered from rats 3 and 6 hours after injection of larvae was capable of increasing the number of circulating eosinophils in recipients. He termed this activity eosinophil releasing factor (ERF). This activity was not found in the serum 24 hours post injection of larvae, so the factor exerts its effect much more rapidly than EPP. The kinetics of this response suggests that it involves the mobilization of existing eosinophils. Although these factors provide mechanisms for eosinophil mobilization and recruitment they do not explain what qualities a stimulus possesses which determine that an eosinophilic response is elicited.

The tissue localisation of antigen

Basten et al (1970) observed that a peripheral blood eosinophilia followed intravenous injection of rats with T. spiralis larvae. They noted that rats injected with larvae on two occasions 21 days apart developed an enhanced eosinophilic response following the second injection of larvae, but animals injected with homogenized larvae which were ground up into fragments small enough to pass through the pulmonary capillary bed failed to develop a peripheral blood eosinophilia. These experiments indicated that the tissue sequestration of antigens seems to be an important prerequisite for the elicitation of an eosinophilic response. Such localisation can result in granuloma formation around the particulate material, and parasite-induced granulomatous responses have been studied extensively, most notably in laboratory models of S. mansoni infections.

Parasite-induced granulomas

Mammals harbouring S. mansoni are characterized (among other things) by hepatosplenic portal hypertension which is related to the host immune response to the parasite eggs which lodge in the capillaries of the liver (Warren, 1972). Granulomas may also form around eggs in other organs, including Peyers patches and the intestine (Weinstock and Boros, 1981).

Studies on the life cycle of S. mansoni have revealed that the worms reach maturity 4 to 6 weeks after cercarial penetration of the mammalian host, and each female worm will subsequently produce, on average, 300 eggs a day (Moore and Sandground, 1956). Approximately half of these eggs are excreted in the faeces, but the remainder are retained in the host tissues (Cheever, 1968). Those eggs which are retained will elicit a cell mediated delayed hypersensitivity response induced by antigenic materials secreted by the eggs (Warren, Domingo and Cowan, 1967).

Artificial introduction of S. mansoni eggs into the pulmonary vasculature of mice also results in the development of granulomas around the eggs (von Lichtenberg, 1962). In 1967, Warren et al reported that granuloma formation was accelerated around eggs injected into previously infected animals compared to uninfected controls and that this property could be adoptively transferred by cells, but not by serum, into syngeneic recipients. It was later shown that mice with chronic S. mansoni infections show a spontaneous decrease in the size of the granulomatous response to newly generated schistosome eggs, a phenomenon termed modulation (Colley, 1975).

The induction of modulation is dependent on the length of

infection. Domingo and Warren (1968) injected eggs into the pulmonary vasculature of mice infected for periods ranging from 2 to 32 weeks. Animals infected for 2 weeks (which is prior to egg production) developed granulomas similar to uninfected mice, but mice infected for 8 weeks (after egg production has begun) showed an accelerated, enhanced granulomatous response following egg injection. Mice infected for 16, 24 and 32 weeks still developed granulomas more rapidly than uninfected animals, but the size of the granulomas was reduced, so much so that the lesions in mice infected for 24 and 32 weeks were smaller than those in uninfected animals.

Organ-dependent granuloma characteristics

The magnitude and kinetics of the granulomatous responses to S. mansoni eggs seems to be site-specific, since modulation occurs earlier during the course of an infection in the colon and Peyer's patches compared to the liver, while granulomas in the ileum are smaller than those in other organs and do not show modulation during chronic infection. Different lymphocyte-dependent control mechanisms have been proposed to explain these differences (Weinstock and Boros, 1981).

There are organ-dependent differences in the cellular composition of granulomas in S. mansoni infected mice. Nine weeks after infection, liver granulomas are composed of 54% eosinophils, 29% macrophages and 14% lymphocytes. Granulomas in the colon are composed of 44% eosinophls, 48% macrophages and 8% lymphocytes. Macrophages account for 76% of the cells in ileal granulomas, the remainder of the cells being eosinophils (21%) and lymphocytes (3%). Neutrophils and

mast cells account for a very small proportion of the cells in each case (Weinstock and Boros, 1983).

Pulmonary granulomas

Pulmonary granulomas generally only occur naturally in advanced stages of S. mansoni infection (Warren, 1964), but a lung granuloma model has been developed (von Lichtenberg, 1962). Using this model, Olds and Mahmoud (1980) have evaluated the cell populations important in granuloma development. Mice were sensitized by subcutaneous injection of S. mansoni eggs then treated with normal rabbit serum (NRS), anti-neutrophil serum (ANS), AES or ATS, before being reinjected with eggs intravenously. The relative cell proportions in the granulomas of mice injected with NRS were: 58% eosinophils, 13% macrophages, 24% lymphocytes and 5% neutrophils. ANS and NRS treatment did not affect granuloma size, but AES- and ATS-treated mice had significantly reduced granulomas, indicating the importance of eosinophils and lymphocytes in this response.

The experiments presented in this thesis were designed to investigate the induction of eosinophilia by parasitic and non-parasitic techniques and to determine some of the factors which may regulate an eosinophilic response.

Monitoring changes in the numbers of circulating eosinophils involves taking blood samples on a regular basis, which in itself could affect the eosinophil population. The initial experiments were therefore conducted in order to record any changes in the numbers of circulating eosinophils due to regular bleeding or environmental influences prior to any attempt to specifically alter those numbers.

In addition, before studying the effects of parasite-derived antigenic material in mice, the tissue and blood eosinophilia following infection with T. spiralis had to be recorded.

The next step involved the development of an antigenic preparation derived from T. spiralis which could be used to coat inert latex particles for tissue localization in mice. It was not essential that this material was pure, so long as it was antigenic and could be reproduced in sufficient quantities to make subsequent experimental procedures feasible. When a preparation which fulfilled these requirements was obtained, it was localized in the pulmonary capillary bed of mice using latex particles and the number of circulating eosinophils recorded for 3-4 weeks following antigenic exposure. To investigate the possibility that the parasite-derived antigen was a more potent stimulus of eosinophilia than non-parasite-derived antigenic material, mice were similarly injected with BGG-coated latex particles and the resulting eosinophilia monitored.

The final group of experiments set out to investigate a possible thymus-independent eosinophilic response in nude rats. The object was to determine whether or not a residual population was responsible for the eosinophilic response observed in these animals. To do this, rats were injected with a dose of ATS which could be shown to reduce a thymus-dependent response in euthymic rats, namely the contact sensitivity response to oxazolone. Once found, this dose was used to pretreat rats prior to injection with latex-BGG particles and the resulting change in the number of circulating eosinophils recorded. As an additional monitor of local cellular activity, lung lavages recovered from these animals were assayed for the presence of interleukin-1.

MATERIALS AND METHODS

Animals

NIH mice were obtained from Hacking and Churchill Ltd, England. Only male mice were used, and were 10-12 weeks old at the time of purchase. NZW/ LOP 2.5-3.0 kg female rabbits were used for raising antisera and as a source of normal rabbit serum (Ranch Rabbits, England). Rowett nude rats (Rnu/Rnu) and their heterogygote (Rnu/+) littermates were bred in the licenced laboratories of Fisons plc. Only male rats 10-16 weeks old were used. CBA mice were also obtained from Fisons plc. All animals were maintained in a daily cycle of 12 hours artificial light and 12 hours darkness.

Chemicals

Unless stated otherwise, standard chemicals were obtained from BDH Chemicals Ltd, Poole, and were of analytical grade.

Preparation of vials for collecting mouse blood

The dipotassium salt of ethylenediame tetraacetic acid (EDTA) was dissolved in distilled water to a concentration of 1 mg/ml. 200 µl of this solution was dispensed into 1 ml reaction vials (Scotlab Instruments, Scotland) and allowed to evaporate in an oven leaving a coating of EDTA at the bottom of each tube. This is sufficient to prevent coagulation of approximately 100 µl of mouse blood.

Collection of blood samples

Mice were warmed with the heat from a lamp for 5-10 minutes to induce vasodilation prior to being bled. Blood samples were obtained

by piercing the lateral tail vein with a sterile 26G needle and collecting a few drops of blood in the prepared tubes which were gently shaken to dissolve the EDTA. Rats were similarly bled into 0.5 ml collection vials (Teklab, England).

Counting blood eosinophils

Eosinophils were visualized by diluting 20 µl of blood in 180 µl of Discombes fluid (Discombe, 1946), consisting of:

1% aqueous eosin	1 ml
acetone	1 ml
distilled water	18 ml

This stain was prepared fresh each week. The diluted blood was allowed to stain for 5 minutes then counted in a Modified Fuchs Rosenthal chamber, 1/400 mm^2 x 0.1 mm (Weber, England). All cell counts are expressed as $\pm$ one standard deviation (SD) of the geometric mean (GM).

Counting total white blood cells

20 µl of blood was diluted in 180 µl of Kimura's stain (Kimura, Moritani and Tanizaki, 1973). This consists of:

1.	0.05% toluidine blue solution	11.0 ml
2.	0.05% light green solution	0.8 ml
3.	Saturated saponin (Sigma, Poole) in 50% ethanol	0.5 ml
4.	0.1 M PBS, pH 6.4	5.0 ml

The toluidine blue solution was composed of:

toluidine blue (Michrome, High Wycombe)	0.05 g
1.8% sodium chloride	50.0 ml
95% ethanol	22.0 ml

which were mixed and made to 100 ml with distilled water. The stain was made fresh each week from the four stock components (1-4). Diluted cells were counted and expressed as described for eosinophils.

Recovery of T. spiralis larvae

Infected mice were skinned and eviscerated. Excess fat was removed from the carcasses to prevent clogging of the sieves later in the procedure. A digestive solution consisting of 10 Anson units of pepsin A powder (1% w/v) and 10 ml of concentrated hydrochloric acid (1% w/v) dissolved in 1 litre of tap water was prepared for every two carcasses and warmed to 37°C. The carcasses were processed in an MSE Atomix blender in the digestive solution for approximately 1 minute. The resulting mixture was incubated at 37°C in a water bath for 1-1.5 hours with constant stirring to release the larvae from skeletal muscle.

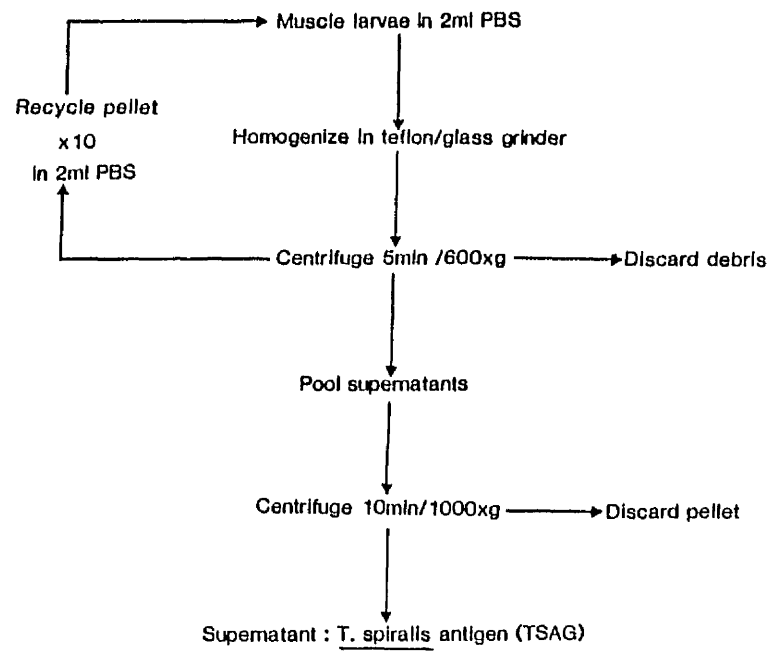
At the end of the incubation period the mixture was passed through a coarse sieve, 250 /um mesh (Endecotts Ltd, London) to remove lumps of bone or gristle, then through a finer sieve, 38 /um mesh (Endecotts Ltd) which retains the larvae. After backwashing with tap water the larvae were collected from the sieve and washed by repeated sedimentation in a 100 ml measuring cylinder.

Infecting mice with T. spiralis

The washed larvae were resuspended in a solution of 0.1% agar in distilled water to a concentration of approximately 1400/ml. The agar prevents the larvae sedimenting, thereby ensuring an even distribution of larvae in each inoculum. Mice were infected by oral intubation with 0.3 ml of the larval suspension, a dose of approximately 400 viable larvae per mouse.

Preparation of T. spiralis larval antigen

Larvae were recovered from T. spiralis infected mice as described, however, following collecting the larvae were subjected to a secondary digestion stage of 45 minutes to minimise contamination with mouse material. The recovered larvae were washed x4 by sedimentation in tap water, followed by 2 washes in PBS. The antigen was prepared using a slight modification of the method described by Despommier and Laccetti (1980):



Motor driven homogenization was performed over crushed ice. The final preparation was dispensed in 200 µl aliquots into 0.5 ml glass ampoules (Jencons, England) and freeze-dried in an EF03 Edwards freeze dryer. Following secondary constriction the ampoules were tested with an Edwards STM4 spark tester to ensure that a vacuum had been maintained. Upon reconstitution with distilled water, insoluble material was removed by centrifugation at 1000 xg. An estimation of total protein per ampoule was performed using the Bio-Rad assay (Bio-Rad Labs, California).

Analysis of TSAG by SDS-PAGE

This technique was performed on 10% slab gels using the buffers, reagents and equipment recommended by Pharmacia (Sweden). TSAG, reduced with 2-mercaptoethanol, was applied to the gel and run against standards of BSA (66 kD), egg albumin (45 kD), glyceraldehyde-3(P) dehydrogenase (36 kD), and carbonic anhydrase (29 kD) (all obtained from Sigma), for 1.5 hours at 12 mAmps, 180 volts. The gel was stained with a mixture of

methanol	225 ml
glacial acetic acid	50 ml
distilled water	500 ml
Coomassie brilliant blue (Raymond Lamb, England)	1.25 g

which were stirred for 30 minutes then filtered through Whatman's No.1 filter paper. The gel was destained with a mixture of

methanol	250 ml
glacial acetic acid	75 ml
distilled water	675 ml

until the background became clear.

Raising antiserum to TSAG

Immunization was based on the method described by Vaitukaitis (1981). 200 µg of TSAG was solubilized in 2 ml PBS and emulsified with 2 ml of Freund's complete adjuvant (FCA) (Difco, U.S.A.) containing 5 mg/ml Mycobacterium butyricum (Difco) by repeated passage through a double-hubbed 19G needle. Emulsion was considered complete when the mixture formed discrete droplets in water. The rabbit received 40 intradermal injections each of 50 µl of emulsion into its shaven back. This contained a total of 100 µg TSAG. The rabbit was exsanguinated after 7.5 weeks and the blood allowed to coagulate at room temperature (RT) for 2 hours. The serum was collected, aliquoted and stored at -20°C. This serum was designated anti-TSAG.

Production of hyperimmune serum

A rabbit was infected by oral intubation with 4000 viable T. spiralis larvae on 3 occasions 10 weeks apart, then challenged with 13,500 viable larvae 4 weeks after the third infection. Blood was collected at various intervals by puncturing the ear vein, and serum recovered as described above. This serum was designated anti-TS WI (whole infection).

Immunoprecipitation by OID

A 1% gel was prepared by heating 0.2 g agarose and 2 mg sodium azide in 20 ml PBS. This was poured into 9 cm plastic petri dishes (Sterilin, England) to a depth of 2-3 mm. The gel was allowed to set and chilled to 4°C before the pattern was cut. The wells were filled with antigen or antiserum then left at RT for 48 hours to allow precipitation. The gel was washed with several changes of 0.9% sodium chloride solution to remove unprecipitated protein prior to staining. The staining and destaining procedure used was that described for SDS-PAGE.

Formalinization of SRBC

90 ml of sheep blood was collected into heparinized tubes (Becton Dickenson and Co., U.S.A.) and washed x5 with PBS. The buffy coat was removed during the washes and the cells packed after the final wash. A length of 5-25/32" dialysis tubing (Medicell International, London) was filled to two-thirds its volume with 50 ml formalin adjusted to pH 5.5-6.0 with 1N sodium hydroxide. After excluding excess air the tubing was tied and immersed in 25 ml of the packed cells resuspended to 200 ml with PBS. This was gently agitated at RT for 3 hours by a magnetic stirrer then the dialysis tubing was punctured and stirring continued overnight. The resulting suspension was filtered through 3 layers of muslin to remove serum and debris then washed x5 with PBS to remove the formalin. The cells were finally made up as a 50% suspension in PBS with 1% formalin as preservative and stored at 4°C.

The tanned cell technique

1 ml packed SRBC were washed x3 with PBS then resuspended to 2 ml with PBS. 0.2 ml of this suspension was pelletted into each of 2 tubes and resuspended to 2 ml with tannic acid (0.5 mg/ml of PBS). The cells were incubated in a water bath for 15 minutes at 37°C then washed with PBS and packed. Before coating the tanned cells with antigen, potential agglutinins in the antigen solution were removed. This was done by packing 0.5 ml of the cell suspension in a tube then resuspending the packed SRBCs in 16 ml of a 100 µg/ml TSAG solution. This was left for 45 min at RT then the SRBC spun down and discarded. NRS, which acts as a stabilizer for the cells in the final washes to balance out the agglutinating effect of the tannic acid, was HI at 56°C for 30 min then twice absorbed with a quarter of its volume of packed SRBC at RT for 45 min to remove any agglutinins. One tube of the tanned SRBC containing 0.1 ml packed cells was resuspended in the absorbed TSAG solution. The other was resuspended in PBS to provide tanned, uncoated cells for controls. The tubes were incubated in a 37°C water bath for 30 min, washed x3 in PBS/1% NRS then finally resuspended in 10 ml PBS/1% NRS to give a 1% cell suspension for use in the assay.

An initial 1:10 dilution of the sera to be examined was made by adding 450 µl of the 1% suspension of tanned, uncoated SRBC to 50 µl of serum. This was left at RT for 30 min to remove potential agglutinins, then the cells spun down and discarded. Serial doubling dilutions of 50 µl of sera were performed in 50 µl PBS in the wells of a 'U'-bottom microtitre plate (Flow Labs, Irvine). 50 µl of tanned, coated SRBC were added to each dilution. To confirm the removal of

heterophile agglutinins from the sera a control consisting of serum at the initial dilution and tanned, uncoated SRBC was performed for each titration. To check for autoagglutination, controls of coated and uncoated cells in PBS alone were also set up. The plate was left at RT overnight before being read.

Agglutination of latex particles

1×10^5 SD-26 latex particles, 27.5 µm in diameter (Sigma) were incubated in 1 ml TSAG solution or PBS alone for 1 hour at RT then washed x3 with PBS/1% BSA and the concentration adjusted to 2×10^4 particles/ml. Serial doubling dilutions of anti-TSAG serum or NRS were performed in 50 µl volumes in the wells of a 'U'-bottom microtitre plate using PBS/1% BSA as diluent. 50 µl of latex suspension containing 1×10^3 particles was added to each well and left at RT overnight.

Identification of antigens by fluorescence

All serum and conjugate dilutions, worked out by titration, were performed in PBS. Lyophylized reagents were reconstituted with distilled water, aliquotted, and stored at -20°C. Latex particles were incubated in BGG (Sigma), TSAG or PBS as previously described. Approximately 1×10^3 particles were pelletted in a reaction vial for every titration performed. The particles were then incubated in 100 µl of a 1:10 dilution of anti-BGG serum (Sigma), anti-TSAG serum or NRS for 30 min at RT before being washed x3 with PBS/1% BSA and pelletted. They were resuspended in 100 µl of a 1:40 dilution of fluorescein-conjugated goat anti-rabbit immunoglobulin (GAR/Ig/FITC)

(Nordic Labs, Netherlands) for 30 min at RT, washed x3 with PBS and pelletted. The particles were finally taken up in 20 µl of a glycerol (Merck, Darmstadt):PBS (50:50) mixture and applied to an area of an alcohol-cleaned glass slide marked out with 'Texpen' and mounted. The particles were examined with a Leitz Ortholux II microscope with incident light fluorescence, an HBO 50 high-pressure mercury vapour lamp, a 2xKP 490 (exciting) and a K515 (suppressing) filter.

Injection of animals with latex particles

Mice were injected with particles 27.5 µm in diameter. Rats were injected with particles 45 µm in diameter (Dow Chemicals, Illinois). Latex particles were incubated in 3 mg/ml BGG or 1 mg/ml TSAG solution for 1 hour at RT then thoroughly washed x3 with PBS to eliminate free antigen. The concentration was adjusted to 3.5×10^5 particles/ml in PBS. Animals were warmed under a lamp for 5-10 min to induce vasodilation prior to injection of the particle suspension via the lateral tail vein. Mice were injected with 0.1 ml of suspension (3.5×10^4 particles) on two occasions thirteen days apart. Rats were similarly injected with 1.0 ml of suspension (3.5×10^5 particles). To avoid blockage of the needle or unequal dosage of the animals the syringe was freshly filled and a new needle was used for each injection.

Investigation of antigen on embolized particles in situ

Fresh lung samples were placed in 'Cryomould' vinyl moulds (Lab-Tek, Illinois), covered with OCT compound (Lab-Tek) and frozen to -

20°C. Tissue sections of 8 µm thickness were cut in a Slee cryostat chamber (model X4031/2) maintained at a constant temperature of -18°C. The sections were transferred to clean glass slides and stored in sealed plastic bags at -20°C until used. The sections were analysed by an indirect fluorescence antibody technique (IFAT). The reaction area was marked out with 'Texpen' then the sections were rehydrated in PBS/1% BSA for 15 min. The sections were drained, then 25 µl of anti-TSAG serum, anti-BGG serum or NRS all diluted 1:10 with PBS was added to the reaction area and the slides incubated for 30 min in a humid chamber. The slides were then rinsed and washed in PBS for 30 min. The slides were drained, and 25 µl of various dilutions of GAR/Ig/FITC added to the reaction areas and incubated in a humid chamber for 30 min. The slides were then rinsed and finally washed for 1.5-2 hours in several changes of PBS to remove free conjugate. The slides were drained, mounted in glycerol:PBS (50:50) and examined as previously described.

Testing ATS activity in vitro

The ATS was a gift from Dr. R.P. Eady. It was prepared by immunization of a rabbit with rat thymus cells. After collection it was absorbed x3 with rat RBCs, aliquotted into 1 ml volumes and stored at -20°C.

The thymus and mesenteric lymph nodes were removed from an Rnu/+ rat, disrupted on metal grids with the plunger of a 1 ml syringe, and washed with RPMI 1640/HEPES (Flow Labs) by centrifugation at 400 xg for 10 min. The cells were resuspended in medium and the concentration adjusted to 1.0×10^8 cells/ml. 100 µl of cell

suspension was added to each well of a flat-bottomed microtitre plate (Flow Labs). 25 µl ATS and 75 µl NRS (as a source of complement) were added to experimental wells. 100 µl NRS was added to control wells. Cell viability was estimated at various times by trypan blue exclusion (Flow Labs). 50 µl of cell suspension was aspirated from each well and added to 50 µl of a fresh 1:2 dilution of trypan blue in PBS, and counted in a 1/400 mm^2 x 0.1 mm chamber.

Testing ATS activity in vivo

ATS was administered in 0.2 ml doses to Rnu/+ rats intramuscularly on four occasions in alternate left and right flanks. Injections were given on days -7, -5, -3 and 0 prior to sensitization with oxazolone. The animals were sensitized by applying 200 µl of 10% 4-ethoxymethylene-2 phenyl oxazolone in acetone onto their shaven abdomens. Primary challenge took place 7 days after sensitization by applying 25 ul of 5% oxazolone to the dorsal surface of the right ear. Secondary challenge, performed as described for primary challenge, took place a further 10 days later. The thickness of both ears was measured using a Mitutoyo Dial Gauge graduated in 0.01 mm divisions immediately prior to primary challenge and at regular intervals after both primary and secondary challenges. The differences in thickness due to challenge was calculated on the following basis:

Ear thickness prior to challenge		Ear thickness post challenge	
Left	Right	Left	Right
+	-	-	+
1	2	3	4

The difference in thickness corresponded to the sum of 1 + 4 minus the sum of 2 + 3. Results are expressed in units of 10^{-2} mm, $\pm$ one SD of the arithmetic mean (AM).

Lung lavage of rats

Animals were killed by intravenous injection of 30 mg Sagatal (May and Baker, Dagenham). The trachea was exposed by dissection, and a small transverse nick made between the cartilage. A length of PE 260 polyethylene tubing (Becton, Dickenson and Co.) was inserted into the trachea and secured with thread. 7.5 ml RPMI 1640 (Flow Labs) was introduced into the lungs via the tubing and withdrawn again after 1 min. The volume recovered was measured, then passed through a coarse metal grid which retained lumps of mucus. The lavages were spun at 400 xg for 10 min to bring down the cells. The supernatants were decanted, filtered through a 0.45 µm membrane (Flow Labs), shell-frozen in 500 ml round bottomed flasks (Gallenkamp) in liquid nitrogen then freeze-dried. The pelleted cells were resuspended in 0.5 ml of fresh medium and counted with Discombes fluid and Kimura's stain as described for blood cells. The number of cells per ul lavage was calculated as follows:

$$\frac{\text{Count per µl} \times 500 \times \frac{\text{volume injected}}{\text{volume recovered}}}{7500}$$

Assaying lavage supernatants for IL-1 activity

A modification of the technique described by Lachman, Hacker and Handschumacher (1977) was used to assay for IL-1 activity in the lung lavages. The freeze-dried lung lavage supernatants were recovered from the flasks by reconstitution with 300 µl distilled water. Prior to use in the assay, the reconstituted supernatants were made isotonic with the culture medium by passage down a 9 cm Sephadex G-25 column (Pharmacia). The elution volumes of supernatant protein and concentrated salts were estimated by applying 300 µl of a mixture of Blue Dextran 2000 (Pharmacia) and phenol red (which mimicked the behaviour of the supernatant proteins [high MW] and salts [low MW], on the column respectively) to a sample column and eluting with PBS. The columns were washed free of merthiolate with 50 RPMI 1640 before the concentrated lavage supernatants were applied. The columns were run under sterile conditions and the eluted products were passed through disposable 0.45 µm sterile filters before being stored at -20°C in 0.5 ml aliquots. Dilutions of the supernatants were made in flat-well microtitre plates in 50 µl volumes using RPMI 1640 as diluent. Each dilution was performed in triplicate.

Thymuses from 3-5 week old CBA mice were mashed in a teflon-glass homogenizer, sieved and washed in sterile RPMI 1640 with sodium bicarbonate and supplemented with 100 IU penicillin (Flow Labs), 100 µg streptomycin (Flow Labs) and 2 mM L-glutamine (Flow Labs). The cells were resuspended in this medium to a concentration of 1.5×10^7 cells/ml. 100 µl of this suspension was added to each well in the assay to give 1.5×10^6 cells/well. Phytohaemagglutinin (PHA; Difco) was added in 40 µl volumes to each well to give a total of 0.01 µg

PHA/well. 50 mM N-acetyl-D-glucosamine (NAG; Sigma) was added to wells in 10 µl volumes were appropriate. The plates were incubated in conditions of 37°C/5% CO_2/100% humidity for 48 hours. After this time, 0.5 µCi of ^3H thymidine (Amersham, England) was added to each well in 10 µl volumes and the cells incubated for a further 24 hours. The cells were harvested onto glass fibre filters (Dynatech, England) by a Dynatech Automash harvester. The filters were dried then placed in 4 ml Fisofluor liquid scintillation (Fisons). The amount of ^3H was measured in a Packard 460 scintillation counter. Results were calculated as the GM of the counts per minute (CPM) $\pm$ 1 SD for each set of triplicates.

Histological Analysis of Tissues

Tissues were fixed in 10% formalin, wax embedded and cut into sections 6 µm thick. The sections were stained by a variety of techniques (see Appendix). Eosinophils were counted by random selection of ten high power fields (HPF) under oil immersion per section using a Leitz SM Lux microscope. Results are expressed as $\pm$ 1 SD of the GM. Pulmonary granulomas were measured by calibration of the microscope at a magnification of x400. Results are expressed as $\pm$ 1 SD of the AM.

Statistical Analysis

Results were analysed using the Student's t-test. Values with a probability of <0.05 are considered to be significantly different, and those <0.001 are considered to be highly significantly different.

CHAPTER 1

THE NUMBERS OF CIRCULATING EOSINOPHILS IN UNSTIMULATED MICE

Introduction

The number of circulating eosinophils in normal animals undergoes a cyclic variation. Colley (1976) has examined this diurnal fluctuation in mice maintained under standard conditions of 9.5 hours light, from 0700 hours to 1630 hours, each day. He found that the lowest number of circulating eosinophils occurred around midnight. The greatest number of eosinophils, which could be as much as six times the midnight value, occurred at noon each day.

Eosinophils are not the only biological system to undergo diurnal fluctuation. The level of circulating corticosteroids also varies in a 24 hour cycle due to a diurnal rhythm in corticotropic releasing hormone (CRH) secretion. This appears to be influenced by the rest-activity cycle of the animal and the adaptive significance of this becomes clear when the energy-mobilizing consequences of these hormones are considered. Glucocorticoids promote glucose synthesis in the liver, leading to hyperglycaemia and thereby increasing the availability of quick energy to muscle. In humans, glucocorticoids are maximal in the early hours of the morning prior to wakening, thus ensuring an energy source at the onset of activity (Eckert, 1978). The diurnal fluctuations of serum corticosteroids have been studied in mice maintained in a daily photocyle of 12 hours light (0600 to 1800 hours) and 12 hours darkness (1800 to 0600 hours) (Ungar, 1964). Minimum steroid levels were recorded at 0400 hours, increasing by 200% to a peak at 1600 hours, approximately 2 hours prior to the onset of darkness. This can be accounted for by the fact that mice are nocturnal and will be entering their period of activity at this time.

A direct link between adrenal stimulation and haematological

effects was demonstrated by Hills, Forsham and Finch (1948) who injected human volunteers with adrenocorticotropic hormone (ACTH), a hormone released from the anterior pituitary and which stimulates steroid secretion from the adrenal cortex. They found a 73% reduction in circulating eosinophils 4 hours after ACTH injection in normal individuals, but only a 4% reduction in patients with adrenal atrophy. However, injection of the latter with corticosteroids resulted in a 63% reduction in the number of circulating eosinophils.

Since the environment clearly influences the number of circulating eosinophils in otherwise unstimulated animals, the object of these experiments was to determine the fluctuations which occur in NIH mice kept under standard conditions and to establish a framework for future experimental design.

Results

Longterm observations on the number of circulating eosinophils

Blood eosinophils and total white blood cells were counted in mice bled intermittently over a three month period. The results are presented in Figs. 3 and 4. They show that the number of circulating eosinophils rose progressively during this period. Not only did the number of eosinophils increase in absolute terms (Fig.3), they also increased relative to the total circulating leukocyte population (Fig.4). Two possibilities can be considered to explain this increase. It could be an age-related phenomenon, or it could be due to the effect of repeated blood sampling of the mice.

The effect of age on circulating eosinophils

To investigate these possibilities a group of mice were kept until they reached the age of nine months. Half were bled on six occasions at regular 3 day intervals and the other half bled only at the beginning and the end of the experiment. Regular bleeding by this regime did not induce an increase in the number of circulating eosinophils (Fig.5). However it can be seen that the average number of circulating eosinophils is greater in these nine month old mice than the three month old mice.

Diurnal fluctuation of eosinophils

Four groups of mice were bled simultaneously at the start of the experiment to determine baseline values between 0900 and 1000 hours. Each group was then bled on four occasions, three days apart, but at a

different time on each day: 0900 hours, 1300 hours, 1700 hours and 2100 hours. The results of these group rotations are shown in Fig.6 (a, b, c, d). The pooled data of four groups for each time are shown in Fig.7. The pattern is clearly established in each case. The number of circulating eosinophils peaks in the early afternoon at a value which is approximately three times greater than the value observed just after the onset of darkness.

Figure 3. Total number of circulating eosinophils in NIH mice.

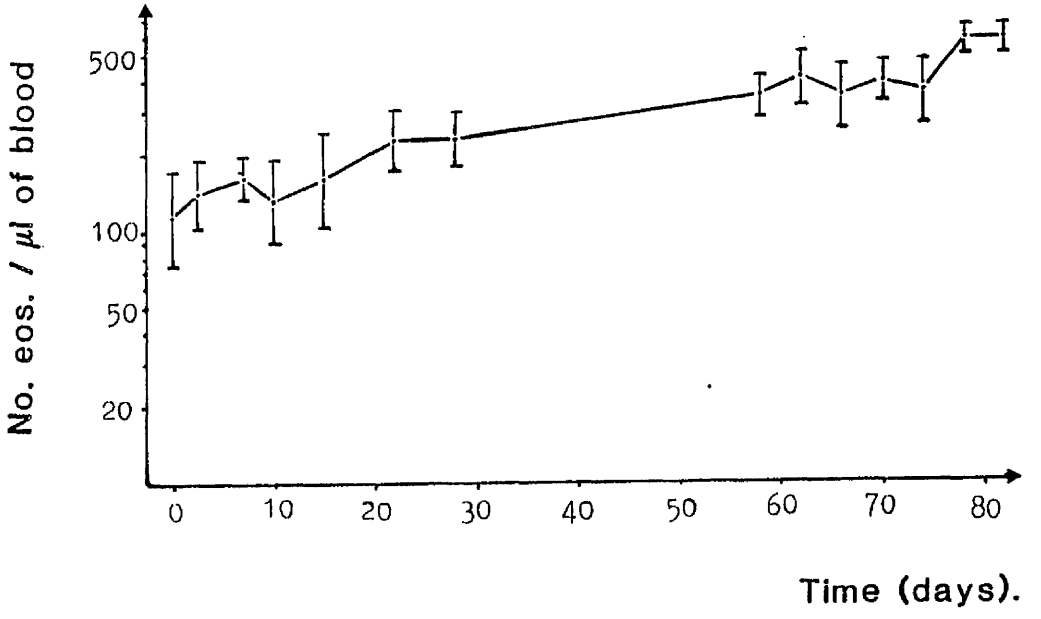


Figure 4. Percentage of circulating eosinophils in NIH mice.

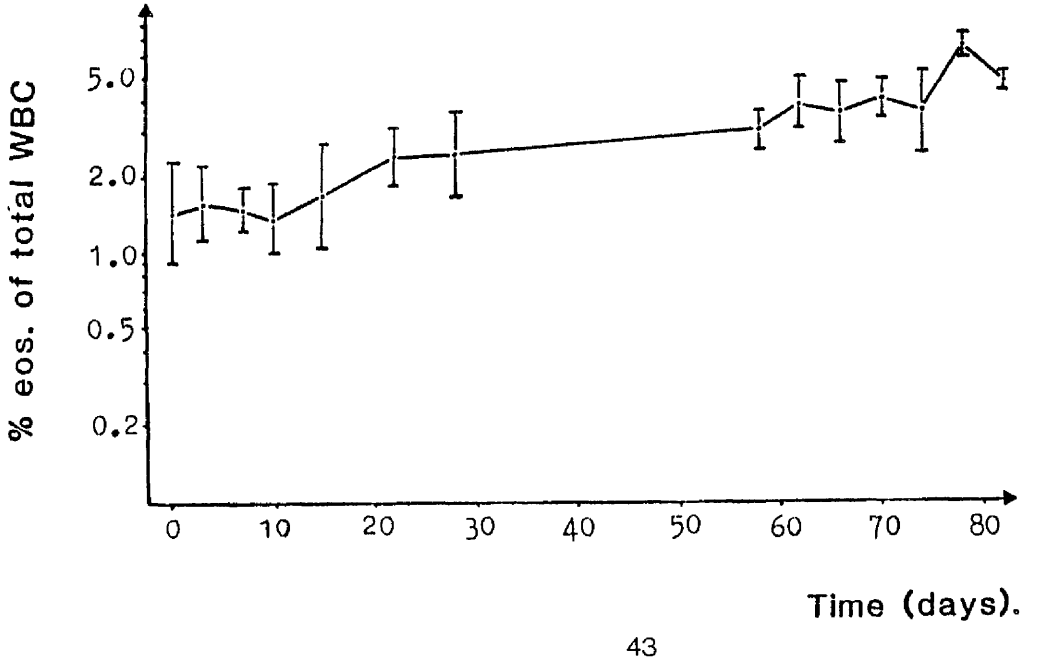


Figure 5. Number of circulating eosinophils in 9 – month – old NIH mice.

Mice bled at regular 3 day intervals.

Mice bled at beginning and end of experiment.

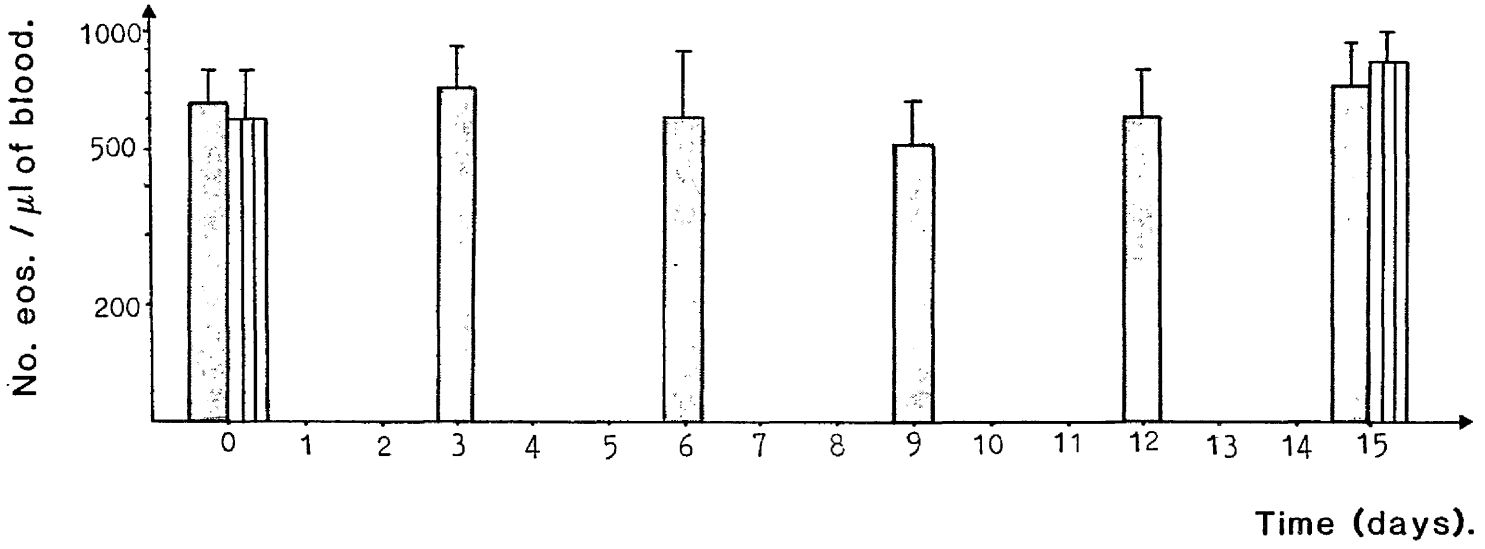


Figure 6. Diurnal fluctuations in the number of circulating eosinophils in NIH mice.

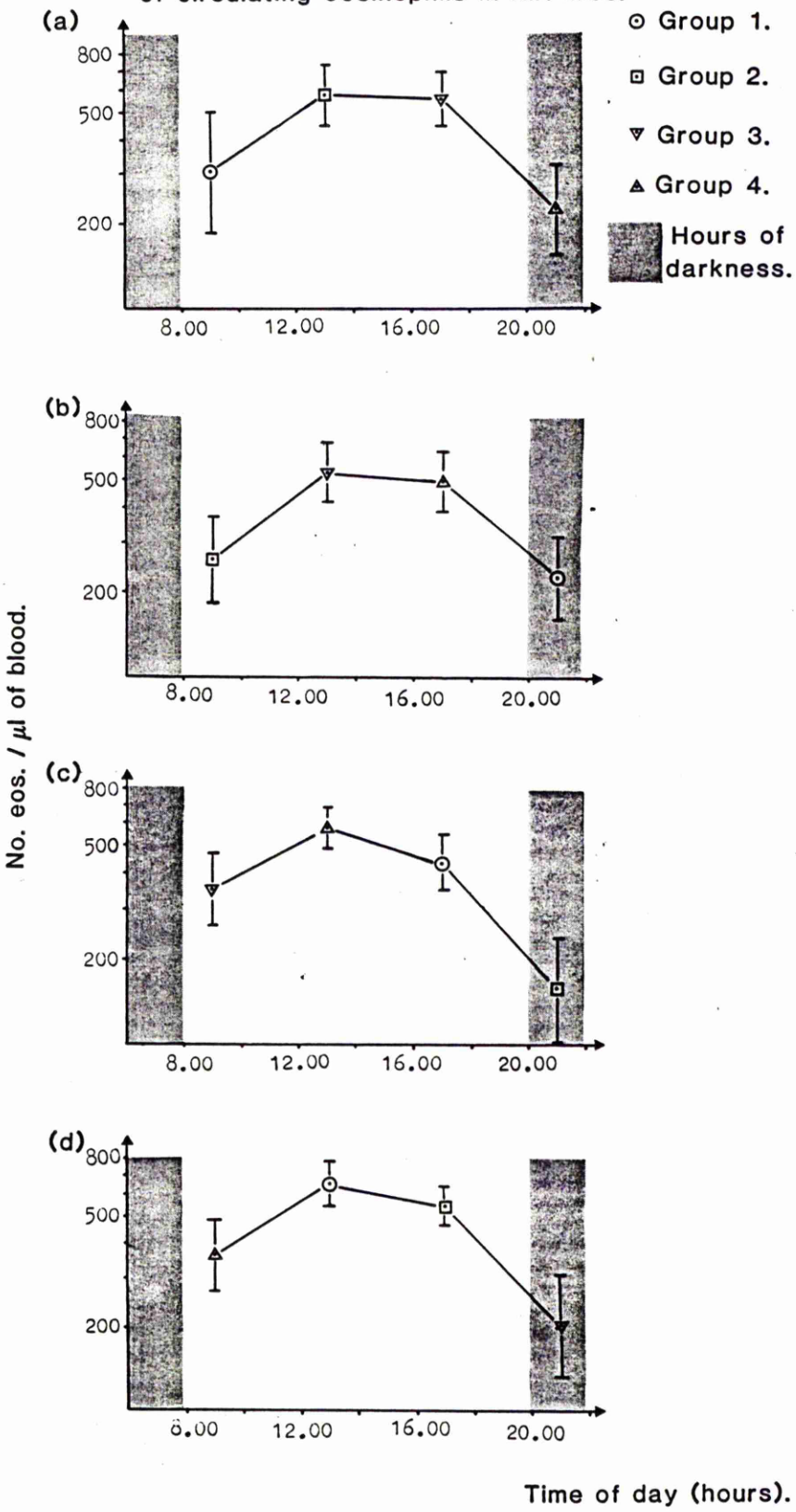
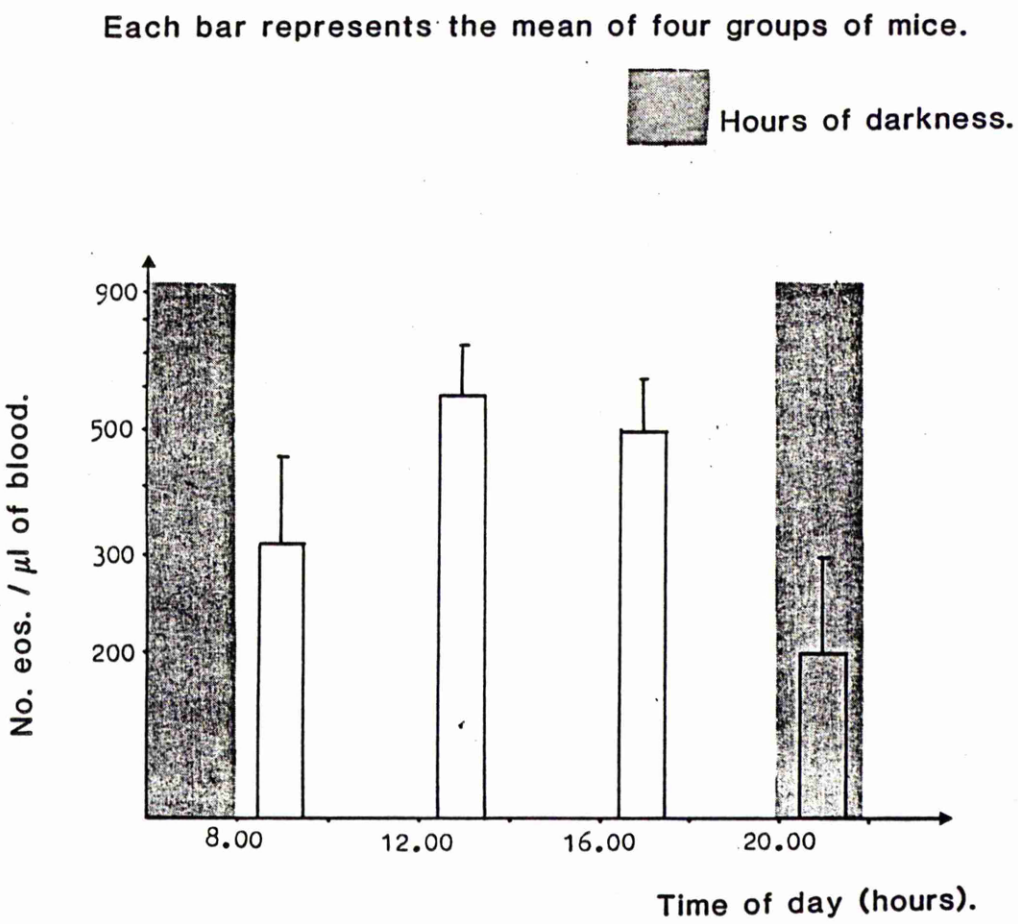


Figure 7. Diurnal fluctuations in the number of circulating eosinophils in NIH mice : pooled data from fig. 6.



Discussion

The results presented here have shown the inherent variations in the circulating eosinophil pool in NIH mice. Previous workers have attributed eosinophil fluctuations to the hormonal state of the animal, in particular to steroids. Adrenocorticotropic hormone (ACTH) is released from the anterior pituitary and stimulates steroid secretion from the cells of the adrenal cortex. The release of ACTH is controlled by CRH which is released from the hypothalamus in response to a variety of stressful stimuli, such as cold, fright, or pain (Eckert, 1978). In 1939, Dalton and Selye produced an alarm reaction in rats by subcutaneous injection of formaldehyde solution and found that a blood eosinopoenia occurred 8 hours later. An eosinopoenia has also been reported in humans subjected to stressful situations such as surgery (Roche, Thorn and Hills, 1950) or a combination of physical and emotional trauma (Renold, Quigley, Kennard and Thorn, 1951) and attributed to the level of circulating steroids.

Fig.7 shows that in NIH mice kept in a daily cycle of 12 hours light from 0800 hours to 2000 hours, the number of circulating eosinophils at 1300 hours is approximately twice the 0900 hours value and three times the 2100 hours value. If the number of circulating eosinophils is inversely proportional to the corticosteroid level, then one might expect the number of eosinophils to peak 5 or 6 hours earlier than it actually does, since the minimum level of circulating corticosteroids in the mouse has been reported to occur prior to the onset of daylight and to rise throughout the day to a maximum prior to the onset of darkness (Ungar, 1964). However, there does appear to be a lag period for the effects of corticosteroids to manifest themselves

on the eosinophil population. Hills et al (1948) demonstrated that a blood eosinopoenia occurred 4 hours after intramuscular injection of ACTH into normal volunteers, while Sabag, Castrillon and Tchernitchin (1978) reported a blood eosinopoenia in rats 6 hours after the intravenous injection of cortisol. Indirect elevation of serum corticosteroid levels in rats by exposure to an alarm stimulus resulted in an ensuing eosinopoenia 8 hours later (Dalton and Selye, 1939).

A possible age variation in the level of circulating eosinophils has also been observed in these animals (Figs. 3 and 4). The increase in the number of circulating eosinophils shown in Fig.3 does not appear to be the result of a general leukocytosis, since the percentage of eosinophils in the circulating leukocyte population increases (Fig.4). It is possible that this gradual increase was due to the repeated collection of blood samples over a long period of time. However, if this were the case, the number of eosinophils would be expected to return to the initial level during the 30-day rest period. Other workers have found an increase in the number of circulating eosinophils in mice bled for several weeks. Ruitenberg et al (1977) found that during a 28 day bleeding period the number of circulating eosinophils doubled in otherwise unstimulated nu/+ mice. Colley (1976) reported that there was an increase in the number of circulating eosinophils in mice bled every day for 6 days, but there was no increase in mice bled every second day. In these experiments mice were never bled more frequently than once every 3 days, but they were bled for a much longer period than those in the experiments presented by Colley.

The other possibility is that this is an age-related phenomenon.

Repeated bleeding of a group of 9 month old mice did not induce an increase in the numbers of circulating eosinophils over a 15 day period. The number of eosinophils was, however, slightly greater in 9 month old than in 3 month old mice (Fig.5). This possible age variation in the number of circulating eosinophils could be due to inherent hormonal changes in the animal.

These results, in conjunction with current literature, indicate that precautions should be taken to minimise possible variations when designing experiments to investigate eosinophil functions. These are:

i. experimental procedures such as blood sampling or tissue recovery should be performed on a regular basis each day

ii. animals should be maintained under constant conditions with minimal variations in the light/dark cycle or temperature

iii. animals should be subjected to the minimum amount of stress when being handled or experimented upon

iv. animals should be age-matched rather than weight-matched for experiments.

CHAPTER 2

CHANGES IN EOSINOPHIL NUMBERS AND DISTRIBUTION FOLLOWING T. SPIRALIS INFECTION OF MICE

Introduction

Animals differ in their ability to mount an immune response to a T. spiralis infection. NIH mice can expel intestinal worms more quickly than B.10 mice. They also harbour fewer muscle larvae following infection (Wakelin, 1980). It has been found that the resistance to T. spiralis in mice is influenced by a gene present in the H-2 complex (Wasson, Brooks, Babish and David, 1983).

The eosinophil response to T. spiralis in mice is also genetically determined. NIH mice have been shown to be high responders while B.10 mice are low responders, exhibiting a reduced eosinophilia following infection (Wakelin and Donachie, 1983).

The principal object of these experiments was to observe the blood and tissue eosinophilia in NIH following T. spiralis infection, and to record the point at which the blood eosinophil population stabilized.

Results

Haematological changes in T. spiralis-infected mice

The course of the blood eosinophilia was monitored in mice following infection with T. spiralis. Infection produces an eosinopoenia around day 6, which precedes a rapid increase to around 2400 eos/µl on day 13 (Fig.8). Although there is a slight reduction on day 16, the peak eosinophilia of over 6000 eos/µl of blood occurs on day 20. The number of circulating eosinophils diminished to around 500/µl by day 30 and maintains this baseline level with fluctuating 'waves' for the following eight weeks. This eosinophilic response to T. spiralis is specific, i.e. it is not simply due to a general

leukocytosis. Fig.9 shows that when eosinophils are analysed as a percentage of the total circulating leukocyte population, the pattern is very similar to that of the absolute eosinophil counts, indicating that the changes are occurring in the eosinophil population independent of general leukocyte variations. Eosinophils constitute approximately 2% of the total circulating leukocytes prior to infection, and at the peak of the eosinophilic response can account for as much as 30% of the leukocytes in the blood. Following the peak eosinophilia, the eosinophils remain at 4-6% of the leukocyte population which is 2-3 times the pre-infection level.

Tissue eosinophil changes in T. spiralis-infected mice

From the results shown in Figs. 10-13 it appears that the blood cannot always be used as an indicator of eosinophil variation since it does not reflect tissue infiltration by these cells. The number of eosinophils in the spleen increases gradually over the first three weeks of infection but decreases by week four (Fig.10). The number of eosinophils in the mesenteric lymph nodes showed this same pattern, with the exception of a decrease on day 17 prior to the peak on day 22 (Fig.11). The number of circulating eosinophils also peaks at approximately three weeks post infection, but the number of circulating eosinophils is reduced at one week post infection compared to pre-infection levels. This decrease is not observed in the numbers of MLN or splenic eosinophils. The number of eosinophils in the lung reflect the pattern of the blood eosinophila, reflecting the fact that it is most likely to be blood rather than tissue eosinophils which are being counted in this case (Fig.12). Fig.13 shows that few

eosinophils were found in the diaphragm until day 12, but their numbers were still increasing on day 27 at a time when the numbers of eosinophils in the other organs, including blood, were diminishing. The extent of the skeletal muscle infiltration can be seen in Plates 1 and 2. Sections of small intestine showed no change, most probably due to the fact that they were taken from a portion of gut too close to the duodenum, where the worms will not establish.

Figure 8.

Eosinophil response of NIH mice to oral infection with T. spiralis.

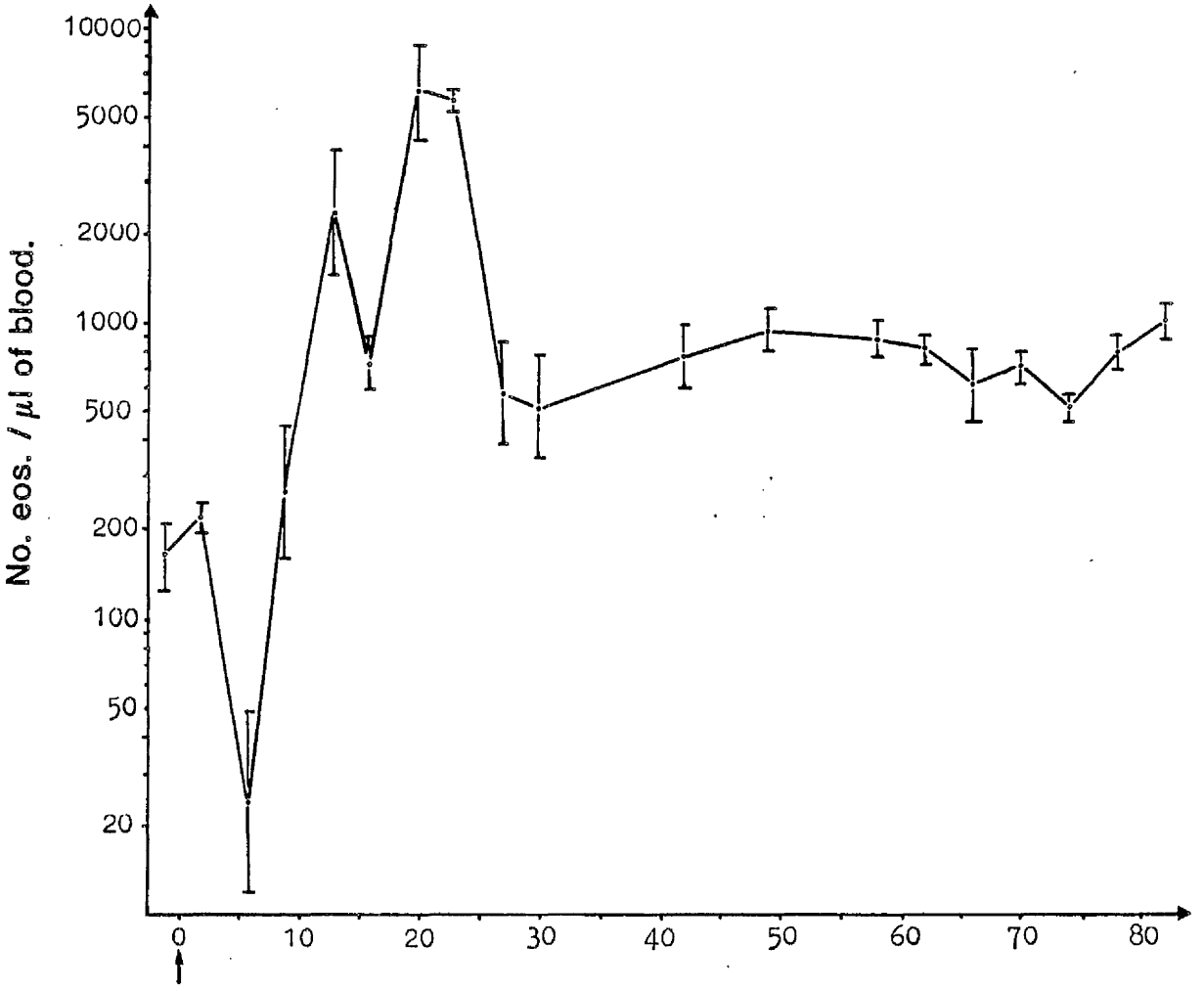


Figure 9.

Eosinophil response of NIH mice to oral infection with T. spiralis.

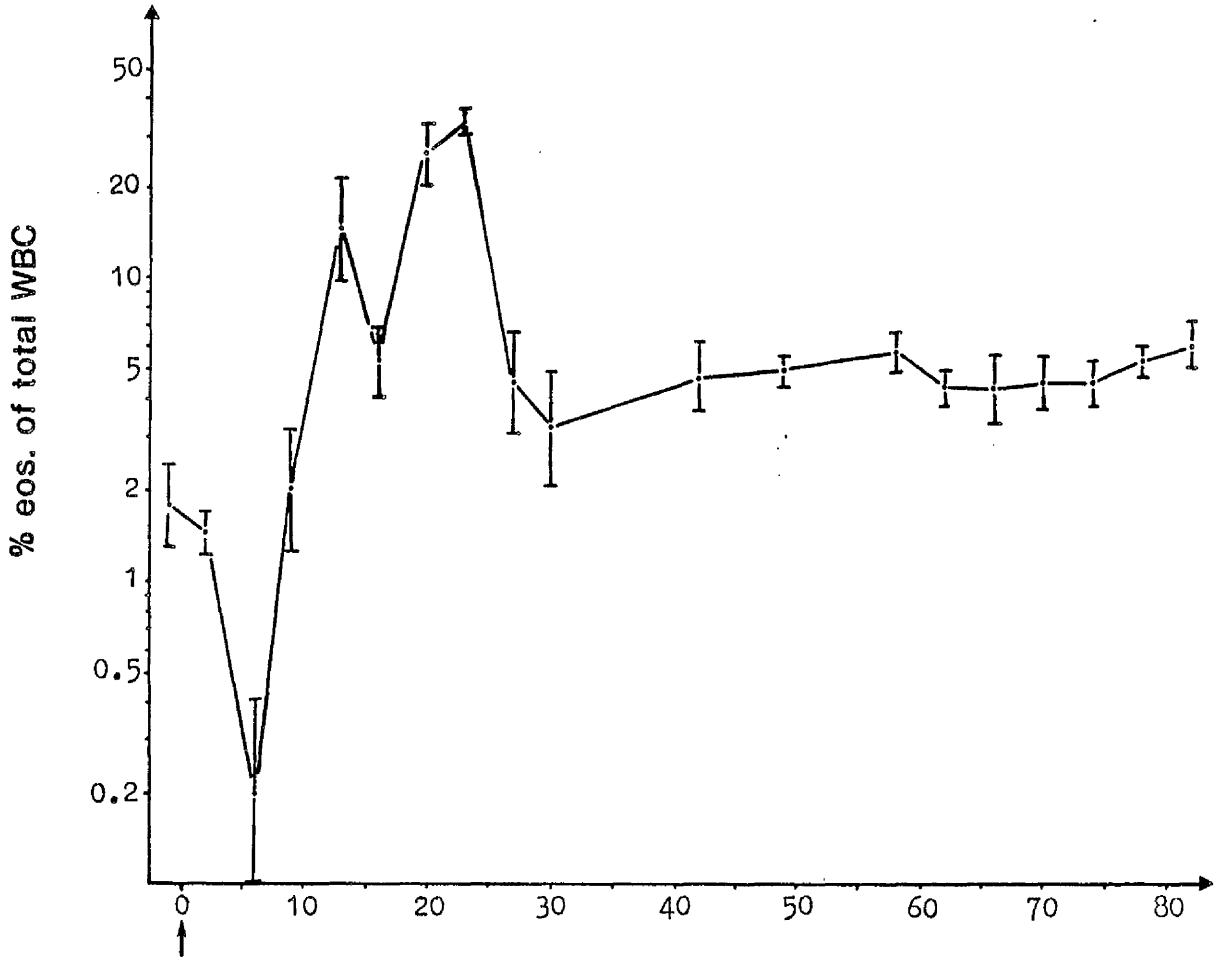


Figure 10. Splenic eosinophil response following T. spiralis infection.

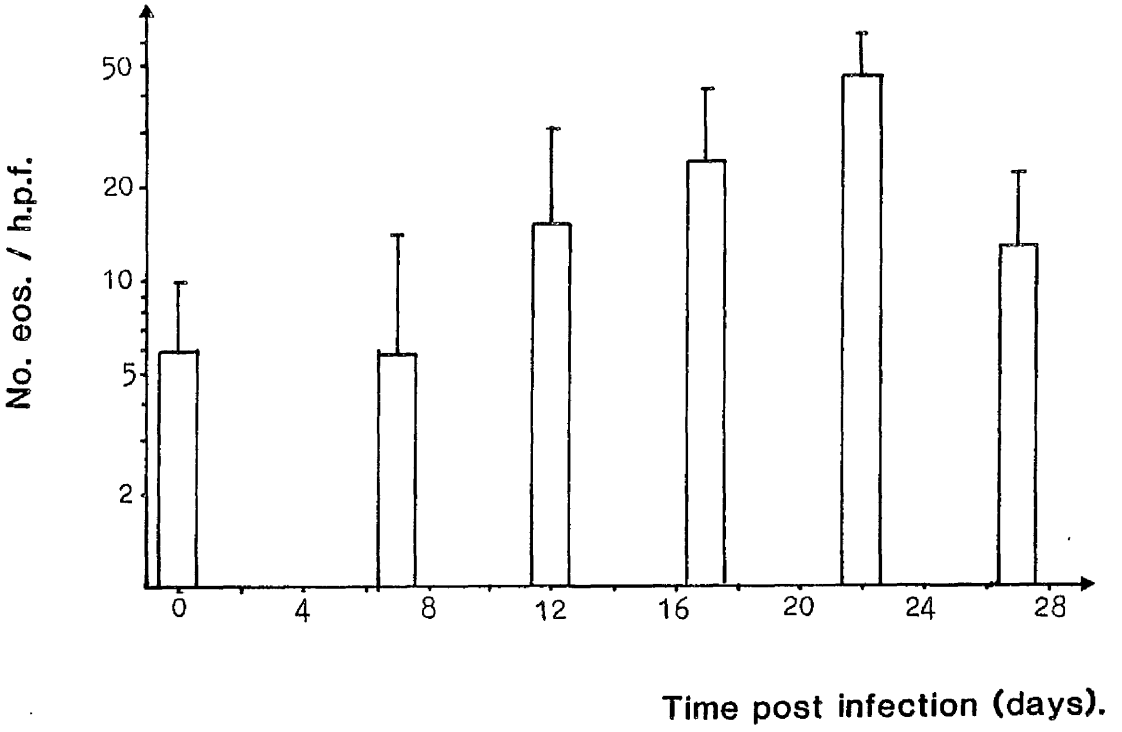


Figure 11. MLN eosinophil response following T. spiralis infection.

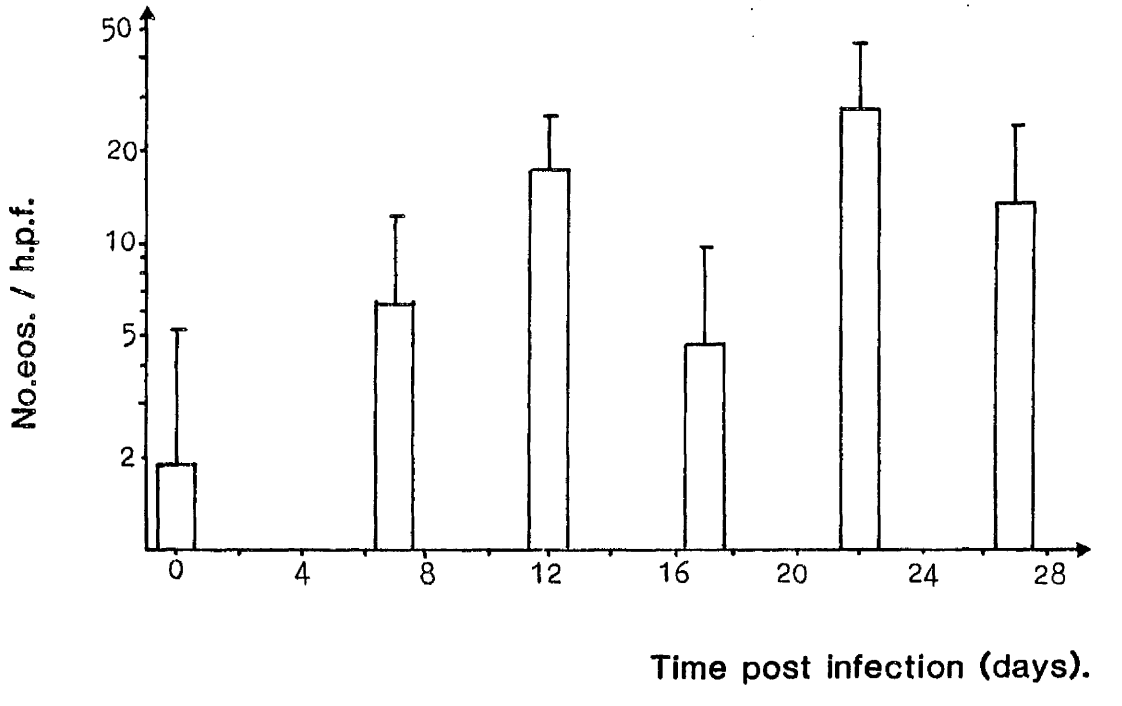


Figure 12. Pulmonary eosinophil response following T. spiralis infection.

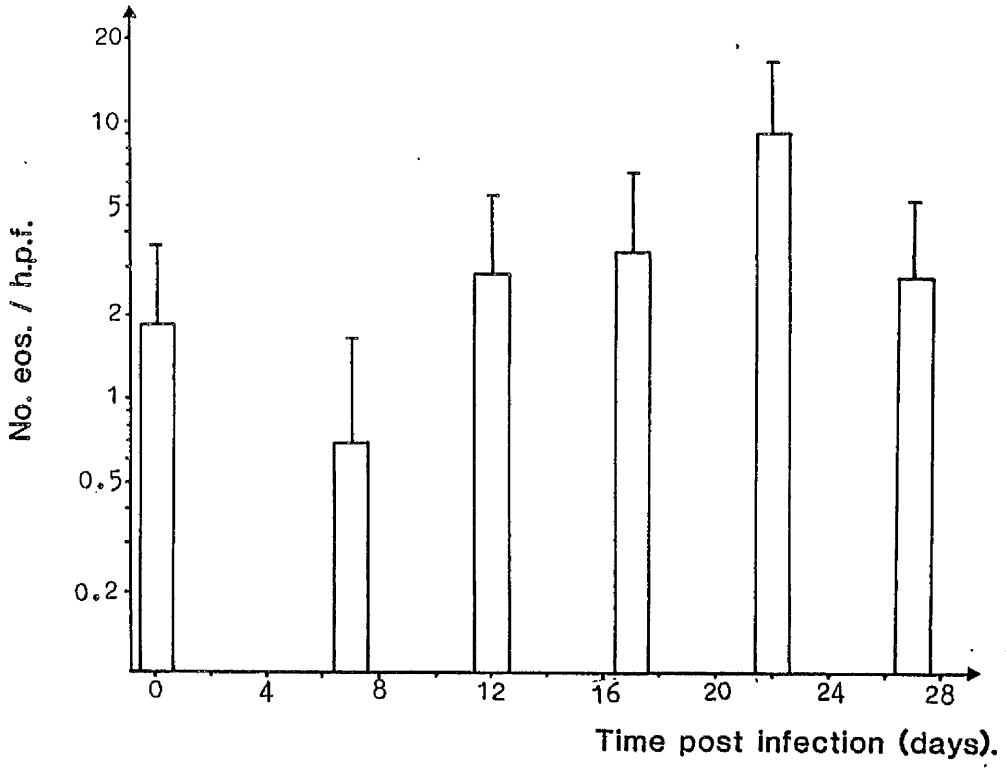
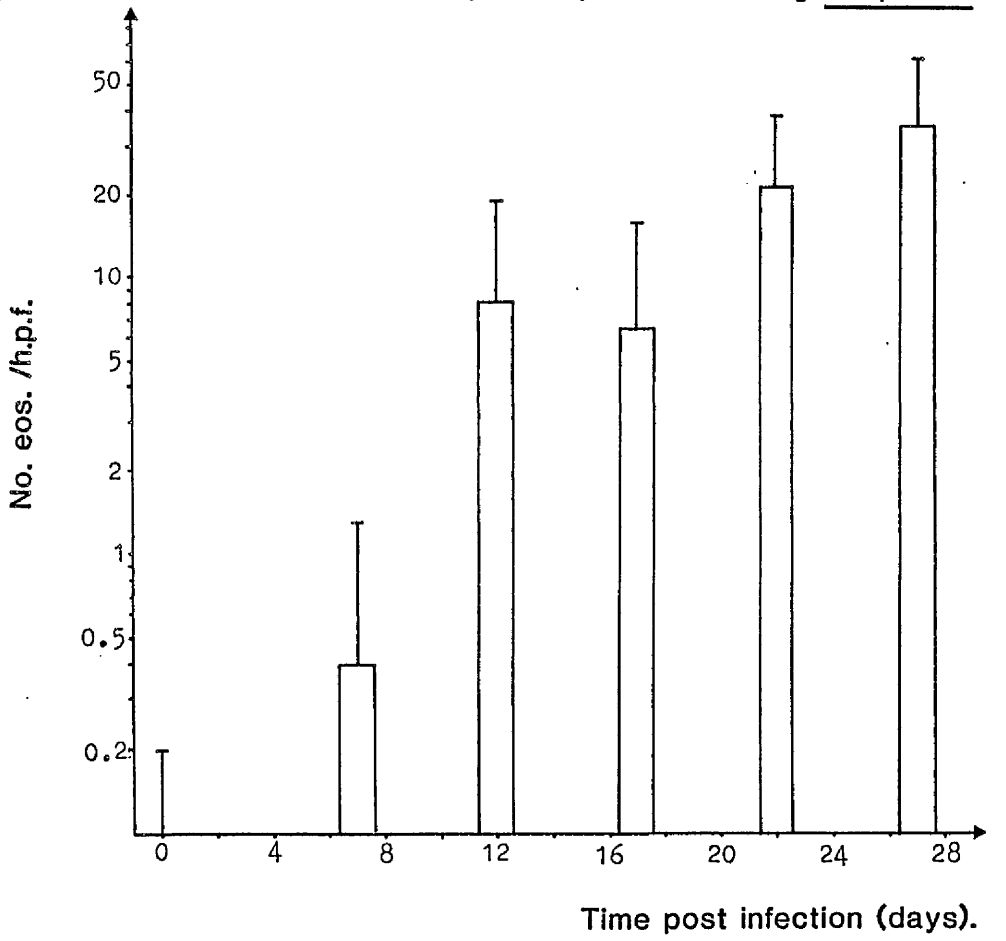


Figure 13. Skeletal muscle eosinophil response following T. spiralis infection.



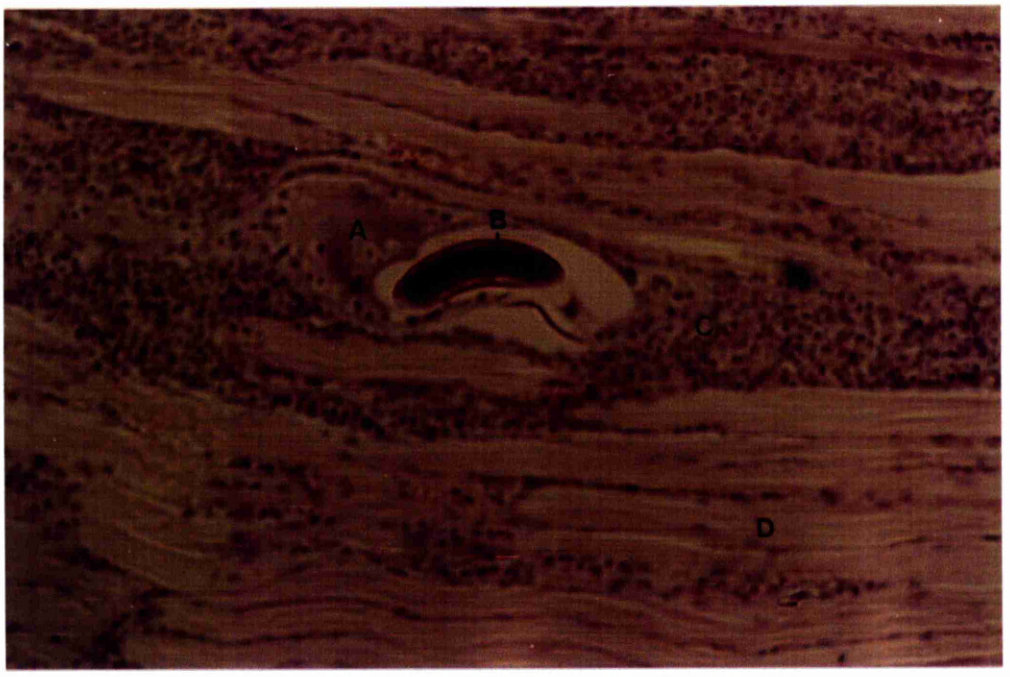


PLATE 1. Mouse diaphragm with encysted T. spiralis larva, 27 days post infection. A: Larval Cyst; B: Section through larva; C: Cellular infiltrate; D: Skeletal muscle fibres.
Carbol chromotrope/Ehrlichs haematoxylin x 325

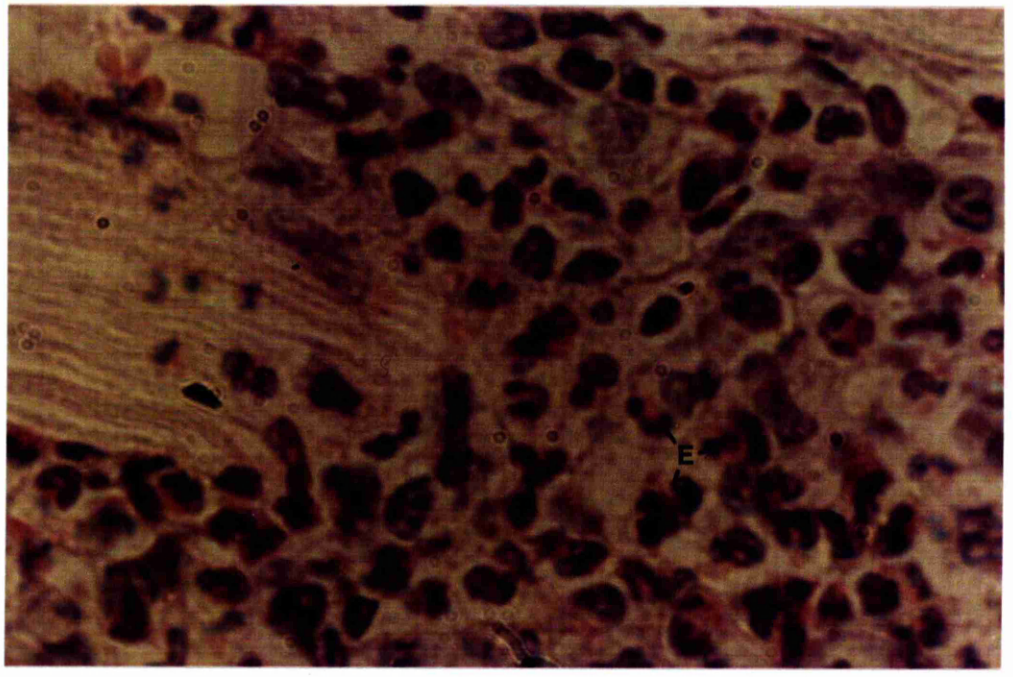


PLATE 2. Higher magnification of Plate 1, showing the dense cellular infiltrate around the encysted larva. Eosinophils (E) are characterized by their prominent pink/red staining granules
Carbol chromotrope and Ehrlichs haematoxylin x 1280

Discussion

From the work of Mahmoud et al (1977) it is clear that in a normal situation, bone marrow eosinophil production equals tissue demand. The blood eosinophil is a transient stage, having a half life of less than 7 hours in normal rats (Spry, 1971b). Changes in the number of blood eosinophils will reflect this supply and demand situation. When the bone marrow supply increases, the number of blood eosinophils increases. Spry (1971b) has shown that the blood half-life of eosinophils in rats injected with T. spiralis is similar to the half-life in normal animals. If tissue demand exceeds bone marrow supply, then this will obviously be reflected in the blood by a reduction in the number of eosinophils. With this in mind, the eosinopoenia occurring around 6 days after infection with T. spiralis is probably due to an increased tissue demand for eosinophils which is not matched by output (Figs.8 and 9). This coincides with newborn larval production and intestinal migration (Gardiner, 1976). Mahmoud (1980) has shown that EPP production in response to a T. spiralis infection takes 48-72 hours to produce an increase in blood eosinophils, and the eosinopoenia on day 6 is followed by an eosinophilia, peaking on day 13 (Fig.8). There is a slight drop again by day 16 which may be due to the fact that the eosinophilic 'overshoot' is being suppressed by a reduction in EPP production, or it may be due to an increased tissue demand exceeding eosinophil production since this coincides with larval penetration of skeletal muscle cells (Harley and Gallichio, 1971). The peak eosinophilia occurs on day 21 post infection, from which the numbers of circulating eosinophils drops rapidly but maintains a level greater than the pre-

infection level for several weeks. This coincides with adult worm rejection and the end of the migratory phase of infection (Bell, McGregor and Despommier, 1980).

Histological examination of tissues was performed at the times of blood eosinophilia to complement these observations. There are several possible mechanisms for tissue eosinophil recruitment. Kay (1970) demonstrated eosinophil chemotaxis towards complement components, and the observations of Mackenzie et al (1978) that the surfaces of infective larvae and adult T. spiralis worms can activate complement could account for an eosinophilic infiltration of the gut during the early stages of infection with a concomitant peripheral blood eosinopoenia. Jensen and Castro (1981) have shown that T. spiralis larvae incubated in both normal and immune sera will induce chemotaxis of eosinophils, but the effect is greater with immune serum. Lymphocyte products, such as ESP (Colley, 1973) are capable of inducing eosinophil migration and to be produced in vivo during T. spiralis infection (Rand and Colley, 1982).

When sections of spleen, MLN, lungs and skeletal muscle were examined for eosinophils, all, with the exception of the muscle, showed that the peak numbers of eosinophils occurred around day 22 (Figs.10-13). In the case of the lungs this almost certainly reflects the number of eosinophils in the blood, i.e. it is blood, rather than tissue, eosinophils which are being counted. This is reinforced by the fact that there is a drop in pulmonary eosinophils around day 7, coinciding with the blood eosinopoenia (Fig.12).

Eosinophils have been shown to accumulate in the lymphoid tissue draining the site of antigen challenge within hours of exposure to the antigen (Litt, 1964). The spleen may be responding to circulating

antigen and the MLN to the concomitant deposition of newborn larvae and rejection of adult worms (Figs.10 and 11).

The diaphragm was analysed to give an indication of skeletal muscle infiltration of eosinophils. The diaphragm, being constantly in use, is one of the most heavily infected muscles in mice, and it is therefore a good indicator of events (Stewart and Charniga, 1980). The number of muscle eosinophils was still increasing on day 27 post infection at a time when the peripheral eosinophilia was declining (Fig.8 and 13), underlining the fact that using the blood as a monitor does not always give an accurate reflection of events.

The results have shown the major blood and tissue eosinophilic responses following infection of NIH mice with T. spiralis larvae and established a baseline useful in future manipulations of T. spiralis-infected mice.

CHAPTER 3

PRODUCTION AND ANALYSIS OF AN ANTIGENIC PREPARATION DERIVED FROM A T. SPIRALIS HOMOGENATE

Introduction

Both somatic and excretory/secretory antigenic components are present in T. spiralis larval homogenates. In 1941, Oliver-Gonzalez recovered serum from rabbits immunized with powdered larvae, and found that it contained antibodies which could precipitate substances orally extruded by larvae in vitro. Both types of antigen are also expressed during a whole infection. Tanner and Gregory (1961) tested serum from rabbits infected with T. spiralis against a preparation of lyophilized infective larvae by immunoelectrophoresis (IEP). They found eleven distinct antigens in the larval preparation and described four of them as being metabolic.

Larval preparations are capable of inducing protection against a subsequent oral challenge infection (Despommier, Campbell and Blair, 1977). Despommier and Laccetti (1980) prepared a fraction from homogenized larvae which they designated S_3. This fraction was shown to be very heterogeneous by SDS-PAGE. IEP with hyperimmune rabbit serum revealed 19 precipitation arcs. Subsequent immunoaffinity chromatography of the S_3 fraction with monoclonal antibodies has revealed the presence of antigens found in the α and β stichocytes of the larvae (Silberstein and Despommier, 1984).

The object of the work presented here was to employ the isolation procedure described by Despommier and Laccetti to find an antigenic preparation suitable for coupling to latex particles for subsequent injection into mice.

Results

Analysis of TSAG by SDS-PAGE

Plate 3 shows the band pattern of two separate batches of TSAG when run on SDS-polyacrylamide gels. The heterogeneity of the preparation is evident, which is only to be expected considering the simplicity of the procedure. The bands range in MW from 107kD to 27kD, with the most ubiquitous component banding at 45kD. However, the SDS-PAGE demonstrates that TSAG prepared on separate occasions will contain the same components, i.e. the technique is reproducible.

Immunoprecipitation of TSAG by OID

The anti-TSAG and anti-TS WI sera were tested for antibody activity to various dilutions of TSAG (Plate 4). The anti-TS WI serum produced the strongest precipitation bands when tested against TSAG at 1 mg/ml. This band became fainter as the TSAG was diluted indicating antibody excess. In contrast, the strongest precipitation bands with the anti-TSAG sera occurred when tested against 0.1 mg/ml TSAG. The most important conclusions that can be drawn from these results are:

i. the TSAG is proven to be antigenic in that it will induce a humoral immune response when injected into an animal;

ii. the TSAGis precipitated by anti-TS WI serum, indicating the expression of some of the components of TSAG during the course of a *T. spiralis* infection;

iii. the absence of cross-reactivity between the precipitin bands produced by the anti-TSAG and anti-TS WI sera indicates that the TSAG contains antigenic components not expressed during an infection.

These results indicated that some sera contained higher antibody titres to TSAG than others. A direct comparison of the titres was performed by haemagglutination of TSAG-coated SRBC.

Measurement of anti-TSAG antibodies

The relative anti-TSAG titres in the various serum samples are shown in Table 1. The highest titre was found in the anti-TS WI serum taken 16 days after the final challenge with larvae. This was also the serum sample which produced the heaviest precipitin band in OID with 1 mg/ml TSAG. It was this serum which was selected for use in the IFAT studies. This assay showed that the TSAG could be used to coat SRBC's while retaining its antigenic properties, but it also had to be shown to coat latex particles.

Agglutination of latex particles

Latex particles were incubated in various concentrations of TSAG and assayed for the presence of bound antigen by agglutination with anti-TSAG serum. The results are shown in Table 2. Sufficient amounts of antigenic material bound to the particles to result in agglutination when incubated in specific antiserum. This effect could be titrated out by diluting the antiserum. TSAG solutions of 1 mg/ml and 0.5 mg/ml were sufficient to cause agglutination, but no agglutination was observed with uncoated particles or with coated particles incubated in NRS.

IFAT studies on antigen-coated latex particles

Particles incubated in concentrations of BGG ranging from 3.0 mg/ml to 0.125 mg/ml all showed a similar level of fluorescence with specific anti-BGG serum (Table 3). Similar particles incubated in NRS showed no detectable fluorescence. However, uncoated particles incubated in anti-BGG serum also fluoresced. To test if the anti-BGG serum contained antibodies which reacted with BSA, it was tested against both BGG and BSA by OID. The BGG produced strong precipitin lines but no reaction could be detected with BSA (Plate 5). The observed effect was not due to rabbit serum proteins binding to the latex, since non-specific staining was not observed in particles incubated with NRS.

Particles incubated in TSAG were compared to particles incubated in BGG to observe the relative levels of fluorescence. The brightest fluorescence occurred with BGG-coated particles incubated with specific antiserum. Anti-TS WI serum produced a stronger fluorescence with TSAG-coated particles than anti-TSAG serum. Again, uncoated particles incubated in anti-BGG, but not anti-TSAG, anti-TS WI or NRS, fluoresced. These results served to confirm the presence of parasite-derived antigenic material on the latex particles.

What was unknown was the 'saturation point' of the particles with TSAG. Particles were incubated in various concentrations of TSAG to observe the effect on the intensity of fluorescence. This was essentially a repeat of the latex agglutination experiment (Table 2), but using a different technique to detect antigen on the particles. The results, presented in Table 5, show that no detectable difference was observed between particles incubated in 1.0 mg/ml TSAG and those

incubated in 0.5 mg/ml TSAG. The fluorescence was dimmer with particles incubated in 0.2 mg/ml TSAG. This implied that the particles were 'saturated' with TSAG at a concentration of 0.5 mg/ml. No non-specific fluorescence was observed. Particles incubated in 1.0 mg/ml TSAG are shown in Plate 6.

As previously mentioned, the preparation of the TSAG is slightly different from that described by Despommier and Laccetti (1980) in that the larvae were homogenized in PBS rather than sucrose (S). This is because the sucrose, apart from not being buffered, inhibits the binding of TSAG to latex particles (Table 6).

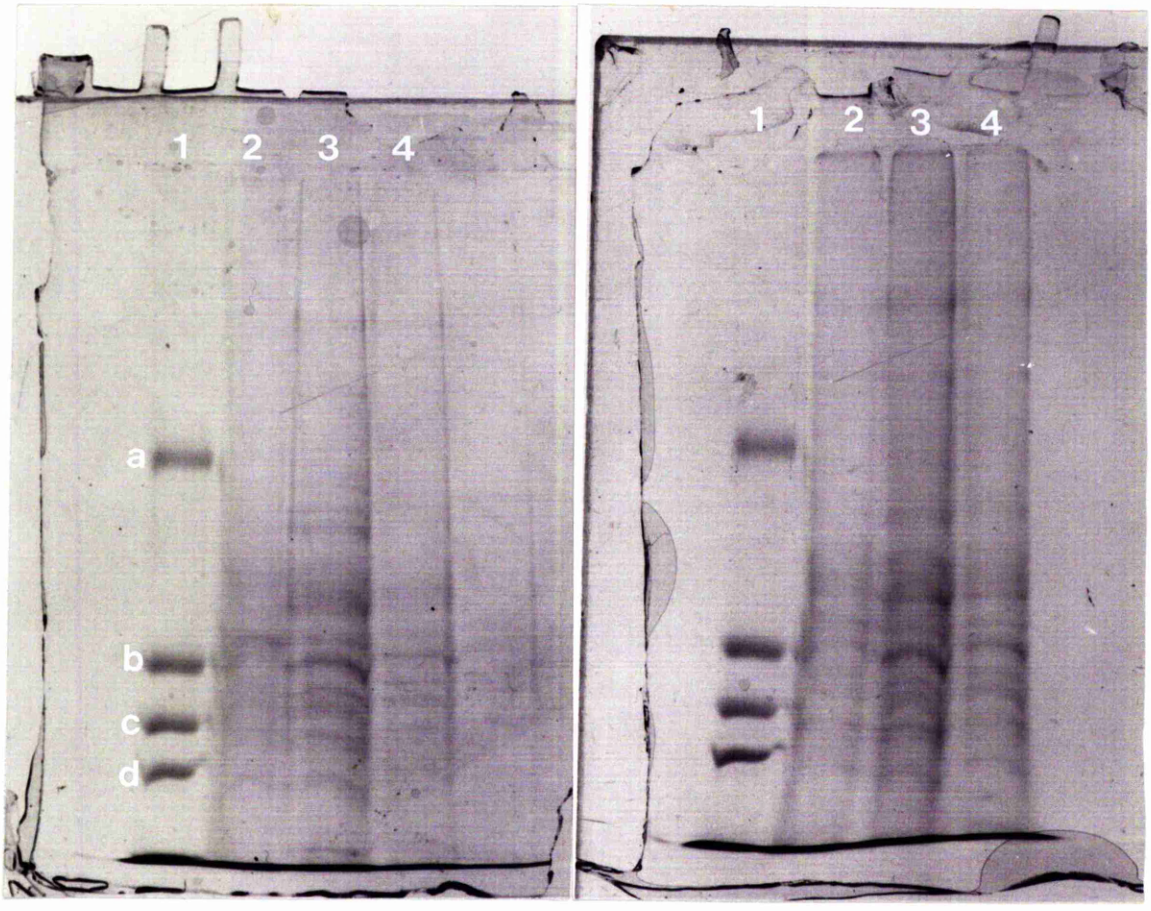


PLATE 3. SDS-PAGE analysis of two separately prepared batches of TSAG. 1: Protein standards; 2: 20 mg TSAG; 3: 80 mg TSAG; 4: 40 mg TSAG. Protein standards: a) 66kD, b) 45kD, c) 36kD, d) 29kD

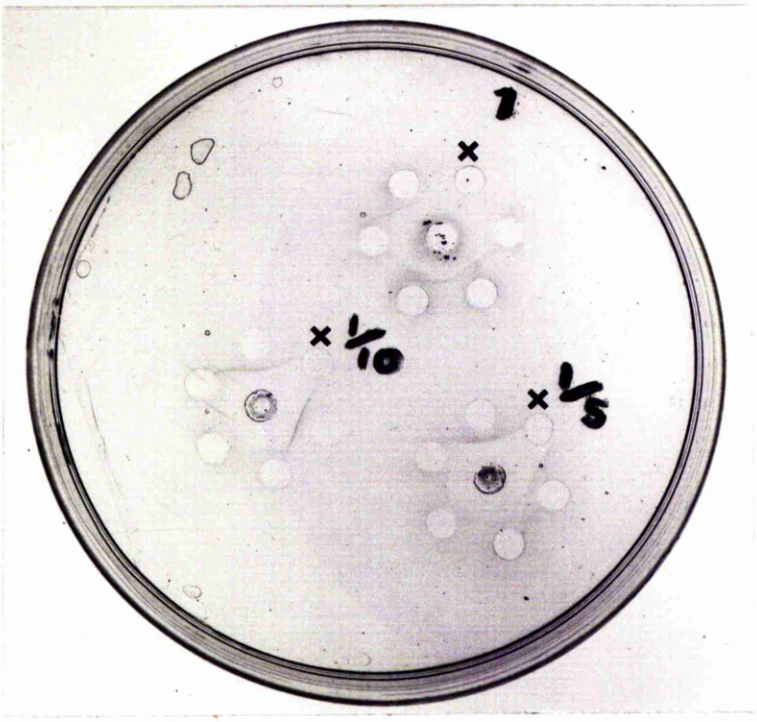
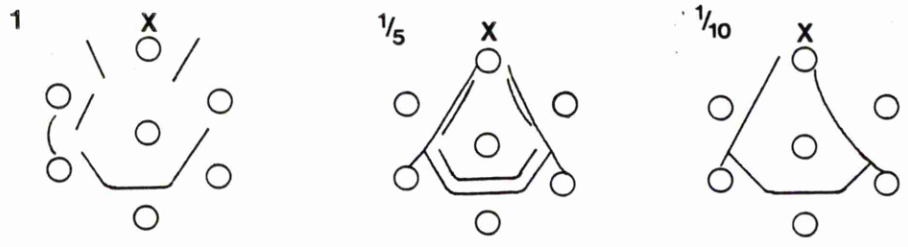


PLATE 4. OID analysis of TSAG with serum from an immunized or infected rabbit

DIAGRAM OF PLATE 4



CENTRE WELLS

1. 1 mg/ml TSAG
1/5 0.2 mg/ml TSAG
1/10 0.1 mg/ml TSAG

OUTER WELLS (CLOCKWISE FROM X)

NRS
Anti-TSAG, 7 weeks post injection
Anti-TS WI, 4 weeks post 3° infection
Anti-TS WI, 16 days post 4° infection
Anti-TS WI, 22 days post 4° infection
Anti-TSAG, 7 1/2 weeks post injection

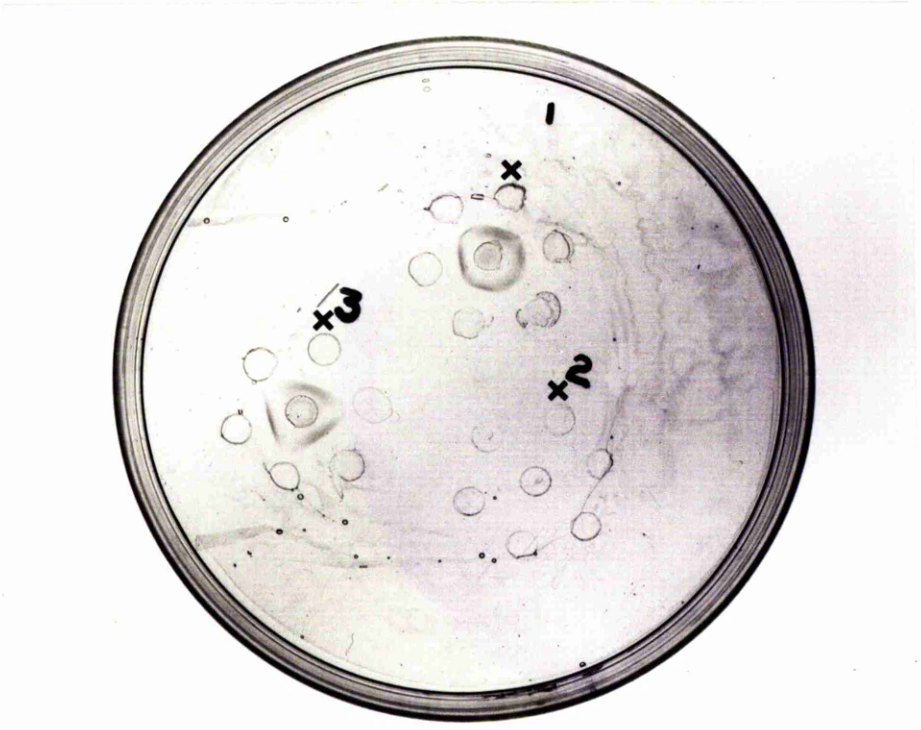


PLATE 5. OID analysis of BGG and BSA with anti-BGG serum. 1. Serial doubling dilutions of 1 mg/ml BGG solution, clockwise from X. 2. Serial doubling dilutions of 1 mg/ml BSA solution, clockwise from X. 3. Alternate serial dilutions of 1 mg/ml BGG and 1 mg/ml BSA clockwise from X. Anti-BGG in centre wells.

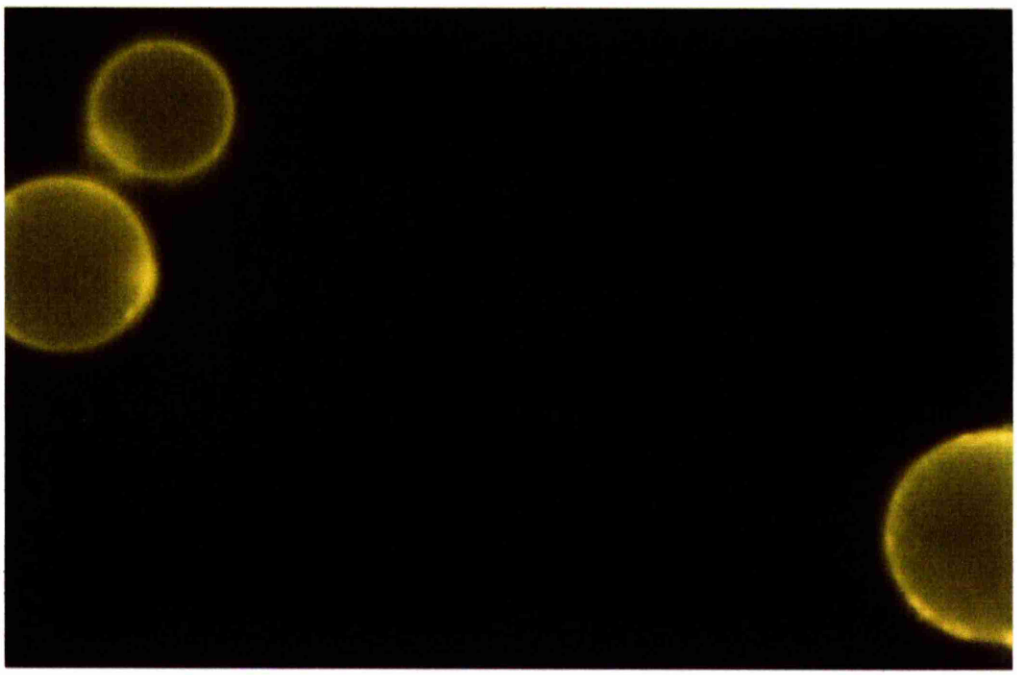


PLATE 6. Latex-TSAG particles incubated in rabbit anti-TS WI serum and goat anti-rabbit Immunoglobulin FITC. x 112.

Table 1. Anti-TSAG titres measured by haemagglutination.

Serum sample	Titre	Uncoated control cells
Anti-TSAG, 7 weeks post injection	1:1280	<1:10
Anti-TSAG, 7½ weeks post injection	1:1280	<1:10
Anti-TS WI, 4 weeks post 3° infection	1:1280	<1:10
Anti-TS WI, 4 days post 4° infection	1:2560	<1:10
Anti-TS WI, 9 days post 4° infection	1:2560	<1:10
Anti-TS WI, 16 days post 4° infection	1:5120	<1:10
Anti-TS WI, 22 days post 4° infection	1:2560	<1:10
NRS	<1:10	<1:10

Table 2. Agglutination of latex particles incubated in TSAG.

Antigen concentration	Serum	Serum dilution			
		1:2	1:4	1:8	1:16
Latex / 1mg/ml TSAG	Anti-TSAG	+	+	−	−
" 0.5mg/ml "	"	+	−	−	−
" 0.2mg/ml "	"	±	−	−	−
Latex / PBS	"	−	−	−	−
Latex / 1mg/ml TSAG	NRS	−	−	−	−
" 0.5mg/ml "	"	−	−	−	−
" 0.2mg/ml "	"	−	−	−	−
Latex / PBS	"	−	−	−	−

+: Agglutination, −: No agglutination

Table 3. IFAT analysis of BGG-coated particles.

Antigen concentration	Serum	Conjugate	Result
Latex / 3.0mg/ml BGG	1:10 dilution anti-BGG	1:40 dilution GAR/Ig/FITC	++++
" 1.0 mg/ml "	"	"	++++
" 0.5 mg/ml "	"	"	++++
" 0.25mg/ml "	"	"	++++
" 0.125mg/ml "	"	"	++++
Latex / PBS	"	"	++
Latex / 3.0mg/ml BGG	1:10 dilution NRS	"	-
" 1.0mg/ml "	"	"	-
" 0.5mg/ml "	"	"	-
" 0.25mg/ml "	"	"	-
" 0.125mg/ml "	"	"	-
Latex / PBS	"	"	-

++++:Strong fluorescence

-:Negligible fluorescence

Table 4. Comparison of antigen-coated particles by IFAT.

Antigen concentration	Serum (diluted 1:10)	Conjugate (diluted 1:40)	Result
Latex / 1.0mg/ml TSAG	Anti-TS WI	GAR/Ig/FITC	+++
"	Anti-TSAG	"	++
"	NRS	"	-
Latex / 3.0mg/ml BGG	Anti-BGG	"	++++
"	NRS	"	-
Latex / PBS	Anti-TS WI	"	-
"	Anti-TSAG	"	-
"	Anti-BGG	"	++
"	NRS	"	-

++++ :Strong fluorescence

- :Negligible fluorescence

Table 5. IFAT analysis of TSAG-coated particles.

Antigen concentration	Serum (diluted 1:10)	Conjugate (diluted 1:40)	Result
Latex / 1.0mg/ml TSAG	Anti-TS WI	GAR/Ig/FITC	+++
" 0.5mg/ml "	"	"	+++
" 0.2mg/ml "	"	"	++
Latex / PBS	"	"	-
Latex / 1.0mg/ml TSAG	NRS	"	-
" 0.5mg/ml "	"	"	-
" 0.2mg/ml "	"	"	-
Latex / PBS	"	"	-

Table 6. A comparison of TSAG preparations by IFAT.

Antigen preparation	Serum (diluted 1:10)	Conjugate (diluted 1:40)	Result
Latex / 1.0mg/ml TSAG (S)	Anti-TS WI	GAR/Ig/FITC	-
" " "	"	"	+++
" " " (S)	NRS	"	-
" " "	"	"	-
Latex / PBS	Anti-TS WI	"	-
"	NRS	"	-

++++: Strong fluorescence, -: Negligible fluorescence

Discussion

There are various methods for the recovery of antigenic material from T. spiralis larvae. ES larval products can confer a degree of protective immunity to a challenge infection (Campbell, 1955). Oral administration of metabolic antigens has also been found to produce a more rapid and pronounced mast cell and a longer lasting eosinophil response in the intestine when followed by infection (Tronchin et al, 1979). Recent work has demonstrated the presence of such antigens in larval homogenates (Silberstein and Despommier, 1984).

Antigen preparation by homogenization seems to be a more favourable method than the collection of ES products released in culture. Firstly, it is more quickly prepared and can be prepared at low temperatures, so the likelihood of protein denaturation is reduced. Secondly, it is more likely to yield greater amounts of antigen since both somatic and metabolic larval fractions are antigenic (Tanner and Gregory, 1961), and thirdly, it is less open to variability than ES preparations, since ES production and contamination with somatic material is obviously dependent on worm viability which can vary between cultures.

Initial attempts at production of the S_3 antigen described by Despommier and Laccetti (1980) proved unsatisfactory. Total protein yield was low and the final product contained Triton X-100, a detergent which could not be totally removed even after dialysis. This made it impractical for coupling to latex particles for injection into animals. Also, the S_3 fraction cannot be said to have the advantage over other preparations of being pure. It contains non-antigenic components which are not functional, at least in the sense

of conferring immunity (Despommier and Laccetti, 1984). For these reasons the S_1 fraction was employed as an antigenic preparation. It also contains all of the antigens present in subsequent fractions.

The preparation of the S_1 fraction was modified from the method described by Despommier and Laccetti (1980) in that larvae were homogenized in a buffered salt solution as opposed to sucrose, which was found to inhibit the binding of antigen to latex particles (Table 6).

Barringa (1981) compared five methods of extracting antigenic fractions of T. spiralis larvae: homogenization, sonication, extrusion, lyophilization and repeated freeze-thawing. He found that homogenization produced the most bands on SDS-PAGE and the greatest number of antigenic components by IEP, and recommended it as the most appropriate procedure for general purposes.

The TSAG fraction is heterogeneous, but it is reproducible (Plate 3). It is immunogenic in that it can elicit a humoral response when injected into a rabbit and contains antigenic components expressed during a whole infection (Plate 4). Anti-TSAG and anti-TS WI sera show different activities indicating that different antigens are being expressed in the TSAG not normally expressed during an infection. This corresponds to the findings of Ortega-Pierres, Chayen, Clark and Parkhouse (1984) who prepared monoclonal antibodies to infective larvae, adult worms and newborn larvae. In each case two antibodies against a defined single surface antigen were isolated, both of which precipitated the same component from detergent solubilized surface antigen preparations. However, only one bound to the surface of the living worm, indicating that the other must be directed against an antigenic epitope not expressed in the intact worm

surface.

The TSAG was shown to be a suitable preparation for the coating of tanned SRBC's (Table 1). It should be noted that tannic acid was used as the coupling agent because the TSAG had been prepared in PBS. An alternative coupling agent such as chromium chloride cannot be used in this case because its action is inhibited by PBS (Parish and McKenzie, 1978). When anti-TSAG titres were measured by haemagglutination, the highest titre was found in hyper-infected rabbit serum. Since this sample gave the strongest reaction with TSAG-coated SRBC, it was used for the majority of IFAT studies.

TSAG was initially shown to be present on latex particles by producing an agglutination reaction when latex-TSAG particles were incubated in immune, but not normal, serum (Table 2). The development of this technique showed that it was necessary to include BSA in the washing buffer.

IFAT was used as a more sensitive method for identification of TSAG on the particles, and confirmed the presence of antigenic material on the latex (Tables 4 and 5). No detectable difference in the level of fluorescence was found between particles incubated in 1.0 mg/ml TSAG and 0.5 mg/ml TSAG. Schriber and Zucker-Franklin (1974) reported that 350,000 latex particles 45 μm in diameter bound approximately 200 μg of antigen from a 3 mg/ml solution. The surface area of spheres 45 μm in diameter is approximately 4 times greater than the surface area of spheres 27.5 μm in diameter, so the amount of antigen taken up by 350,000 particles would be around 50 μg. Since this number of particles is in excess of the number of particles incubated in TSAG for these studies, it implies that increasing the

concentration of TSAG would not result in a corresponding increase in the amount of antigen bound.

The level of fluorescence was greater with particles incubated in BGG than TSAG (Table 4). This could be because BGG being a relatively pure preparation, coated the particles with more specifically antigenic material than TSAG, which is a heterogeneous preparation. Alternatively, it could be a reflection of the quality of the antiserum, the anti-BGG serum containing specific antibodies of a higher affinity or titre than the anti-TS WI serum. The observed effect is likely to be due to a combination of these.

Particles incubated in varying concentrations of BGG did not show a concomitant reduction in fluorescence (Table 2). Uncoated particles also fluoresced in the presence of anti-BGG, but not normal, serum. The obvious explanation for this is that the anti-BGG serum contained antibodies reacting with BSA. This did not show up on OID (Plate 5) but it is possible that the reaction is picked up by fluorescence because it is a much more sensitive technique. BSA is needed to absorb out any free sites on the latex particles, particularly those incubated in low concentrations of antigen or in PBS alone. Otherwise, rabbit serum proteins could bind to the particles which would then be picked up by the goat anti-rabbit immunoglobulin conjugate. Bovine (Eady, Trigg and Orr, 1978) and human (Schriber and Zucker-Franklin, 1974) gamma globulins have been shown to bind to the surface of latex particles. However, the anti-TSAG serum does not show a similar cross-reactivity and these results have shown that an antigen preparation produced by homogenization of T. spiralis larvae can be used to coat inert latex particles thereby rendering them antigenic.

CHAPTER 4

INDUCTION OF EOSINOPHILIA IN MICE BY PULMONARY EMBOLIZATION OF ANTIGEN-COATED LATEX PARTICLES

Introduction

The pulmonary embolization of T. spiralis described by Basten et al (1970) was possible because the larvae were large enough to lodge in the capillaries of the lung following intravenous injection. In 1974, Schriber and Zucker-Franklin developed a method for localising non-parasitic antigenic material in the pulmonary capillary bed. They coated inert latex particles with HGG and injected them intravenously into rats on two occasions 10-14 days apart. The primary injection produced a limited but significant increase in the number of blood eosinophils, maximum at 5-6 days. A more pronounced eosinophilia developed after the secondary injection, peaking 5 days post injection. This effect was not observed with uncoated particles or antigen alone, but could be reproduced with ovalbumin-coated particles, implying that it is the tissue localisation of antigen which is the critical factor in the induction of eosinophilia. The anatomical site of antigen localisation also appears to be critical in eliciting an eosinophilic response. Since attempts at inducing a blood eosinophilia by subcutaneous, intracutaneous or intraperitoneal injection of latex-HGG particles have been unsuccessful (Schriber and Zucker-Franklin, 1975).

These experiments have been repeated with BGG-coated latex particles (Eady, Trigg and Orr, 1978). These workers demonstrated both a peripheral blood and pulmonary eosinophilia. The peak blood eosinophilia was found to occur 5 days after the secondary injection of latex-BGG. However, cell counts of bronchoalveolar lavage fluid revealed that the peak pulmonary eosinophilia occurred 48 hours after the secondary injection of latex-BGG. This enhanced response was

shown to be antigenically specific in that rats injected with latex-BGG only developed a secondary response when challenged with latex particles coated with homologous (BGG) but not heterologous (BSA) antigen.

The experiments presented here were designed to investigate the effect of injecting latex particles coated with parasite antigens into naive and T. spiralis-infected mice, and to compare the response to particles coated with non-parasitic antigen.

Results

Response to injection of latex-BGG in mice

The blood eosinophil counts in NIH mice following injection of latex-BGG are shown in Fig.14. A small increase in the number of circulating eosinophils occurred 6-9 days after the primary injection of latex-BGG. This response was significantly enhanced 6 days later after the secondary injection of latex-BGG. A small increase in circulating eosinophils occurred in mice injected with uncoated latex particles, peaking around day 9 post secondary injection.

Response to injection of latex-TSAG in uninfected mice

The response to injection of latex-TSAG was similar to that of latex-BGG in time-course and magnitude (Fig.15). An increase in circulating eosinophils followed the primary injection of latex-TSAG, peaking around 9 days post injection. An accelerated, enhanced response was observed following the secondary injection of latex-TSAG, peaking around day 6 post injection. Although injection of latex particles alone induced an increase in eosinophil numbers, the response was not accelerated or enhanced following the secondary injection. This implies that the increase following injection of uncoated particles is a non-specific effect.

Response to injection of latex-TSAG in *T. spiralis* infected mice

Primary injection of latex-TSAG induced a 2-3 fold increase in the number of circulating eosinophils, peaking around day 9 post injection (Fig.16). The response to the secondary injection of latex-

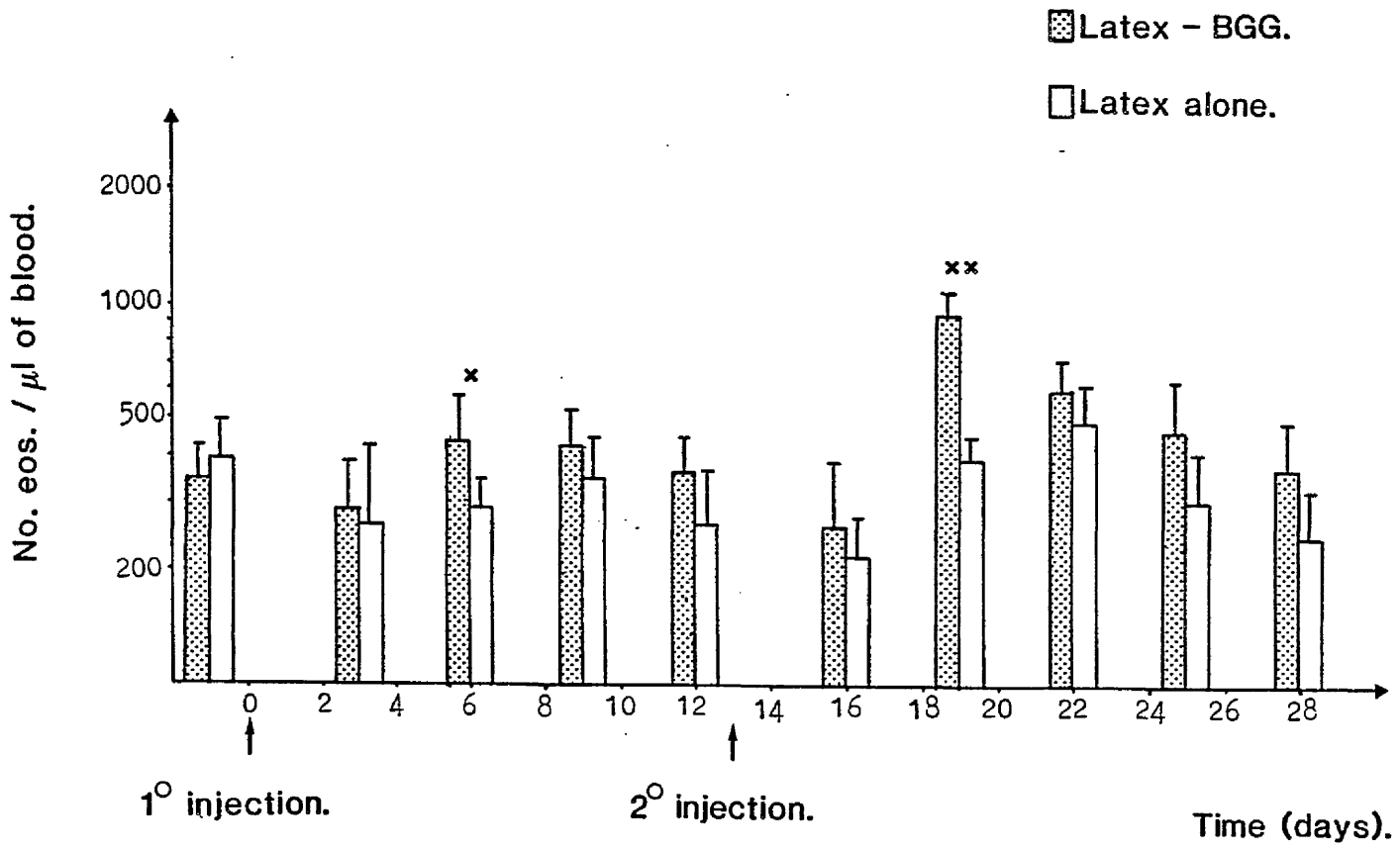
TSAG was accelerated and enhanced, peaking 6 days post injection. The primary and secondary responses were greater in infected mice compared to uninfected mice. Injection of latex alone also increased the number of circulating eosinophils in these mice, but again the response appeared to be non-specific.

Measurement of the pulmonary lesions

Injection of latex resulted in a cellular accumulation around the particles following pulmonary embolization (Table 7). The responses were slightly greater in mice injected with antigen-coated particles, but no differences were observed between infected and uninfected mice in the first 72 hours post injection. Measurements made after the secondary injection will include particles embolized after the primary injection, since it is impossible to say with certainty which particles belong to each injection. This makes interpretation of the secondary response difficult, i.e. although the mean lesion diameter is larger it is impossible to say whether this is due to a more rapid cell accumulation in sensitized animals or due to cell accumulations around primary-injected particles. Examples of these lesions are shown in Plates 7-11. Mast cells may account for a large proportion of these cells, but eosinophils are present.

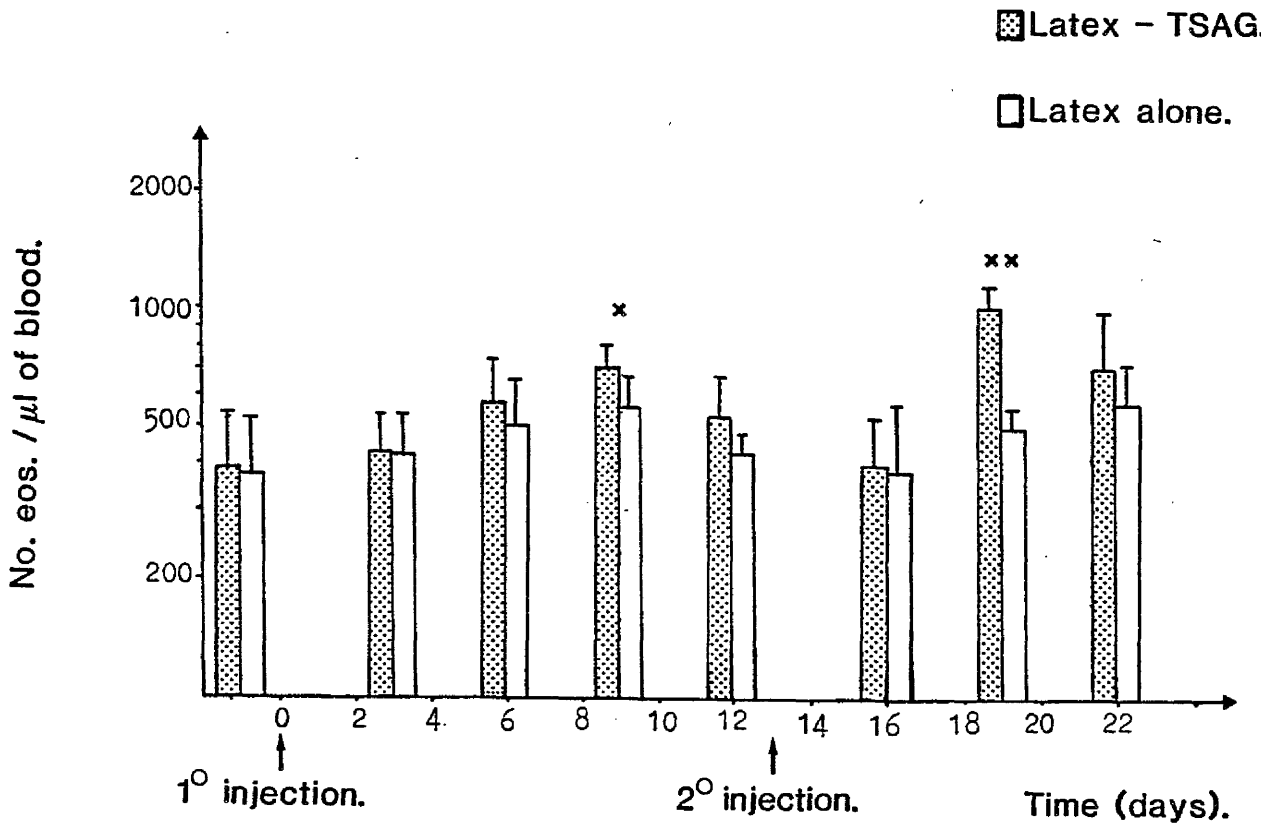
Similar frozen sections were screened by IFAT to attempt to investigate antigen retention in sensitized and non-sensitized animals. These experiments proved fruitless, probably because the amounts of antigen on each particle in section are too small to be detected.

Figure 14. Blood eosinophil response to injection of latex –BGG and latex particles alone in NIH mice.



× significant, ×× highly significant difference between groups.

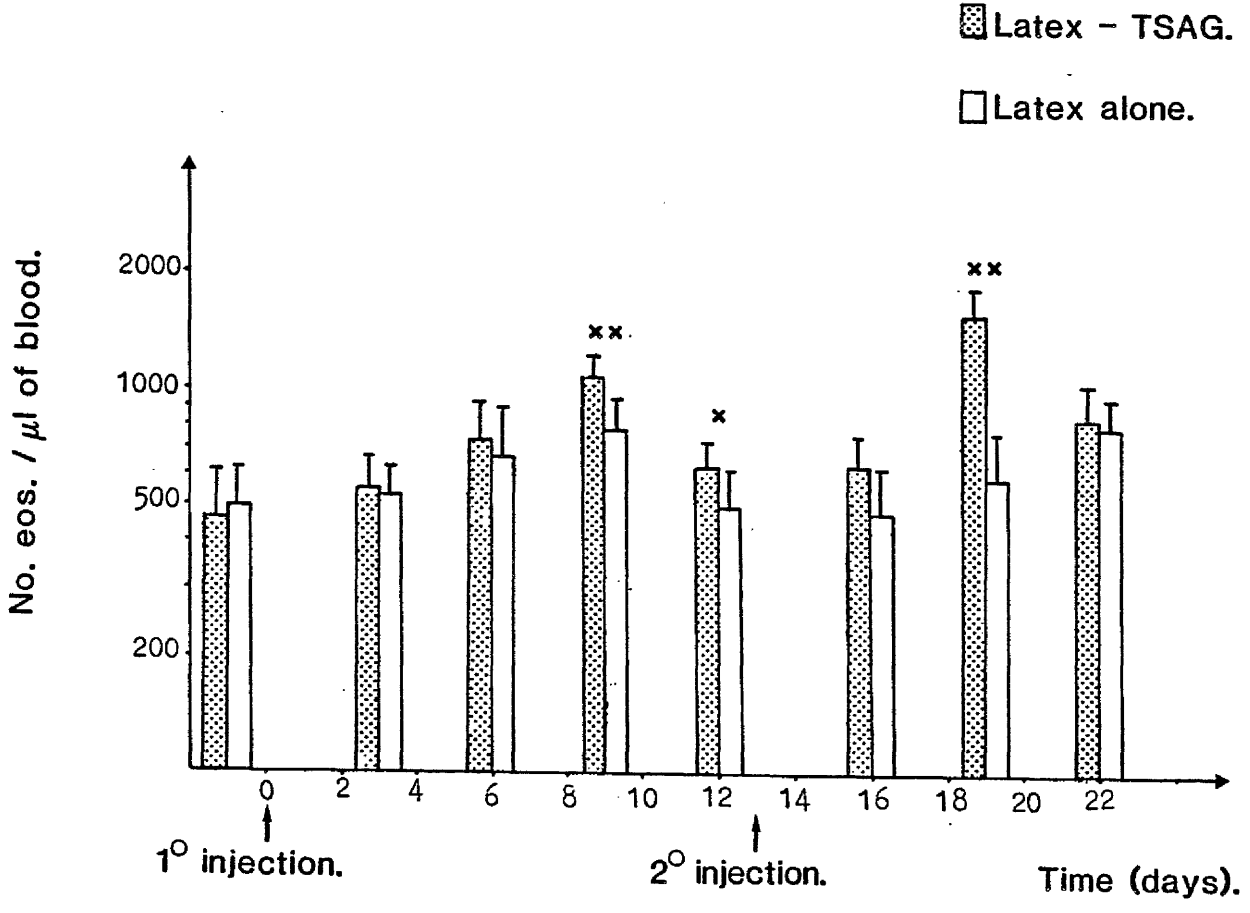
Figure 15. Blood eosinophil response to injection of latex – TSAG and latex particles alone in uninfected NIH mice.



× significant, ×× highly significant difference between groups.

Figure 16.

Blood eosinophil response to injection of latex - TSAG and latex particles alone in T. spiralis - infected NIH mice.



× significant, ×× highly significant difference between groups.

Table 7. Mean diameters (in μm) of cell accumulations around embolized particles in the lungs of mice.

Treatment	Time post injection (hours)					
	1	4	8	24	48	72
1° injection of uncoated latex	31.5 ± 5.8	36.7 ± 7.5	39.0 ± 8.5	41.3 ± 8.2	43.6 ± 9.1	46.7 ± 9.6
1° injection of latex–BGG	31.0 ± 5.9	34.1 ± 6.9	37.3 ± 8.2	41.7 ± 9.2	52.1 ± 9.2	53.7 ± 13.5
2° injection of latex–BGG	44.1 ± 15.2	46.3 ± 18.2	49.5 ± 16.0	53.6 ± 14.7	56.5 ± 14.5	62.3 ± 16.9
1° injection of latex–TSAG	30.0 ± 5.5	31.5 ± 6.2	33.7 ± 6.2	39.3 ± 7.0	47.4 ± 12.8	51.4 ± 11.2
2° injection of latex–TSAG	41.3 ± 13.7	41.3 ± 14.1	42.3 ± 15.0	47.7 ± 13.0	54.1 ± 16.0	59.5 ± 20.1
1° injection of latex–TSAG after T. spiralis infection	31.6 ± 7.7	33.4 ± 7.9	32.1 ± 6.4	37.6 ± 8.0	43.9 ± 10.1	50.7 ± 14.3
2° injection of latex–TSAG after T. spiralis infection	40.5 ± 14.0	44.6 ± 14.6	41.9 ± 12.1	54.9 ± 16.5	56.6 ± 15.0	60.9 ± 17.4

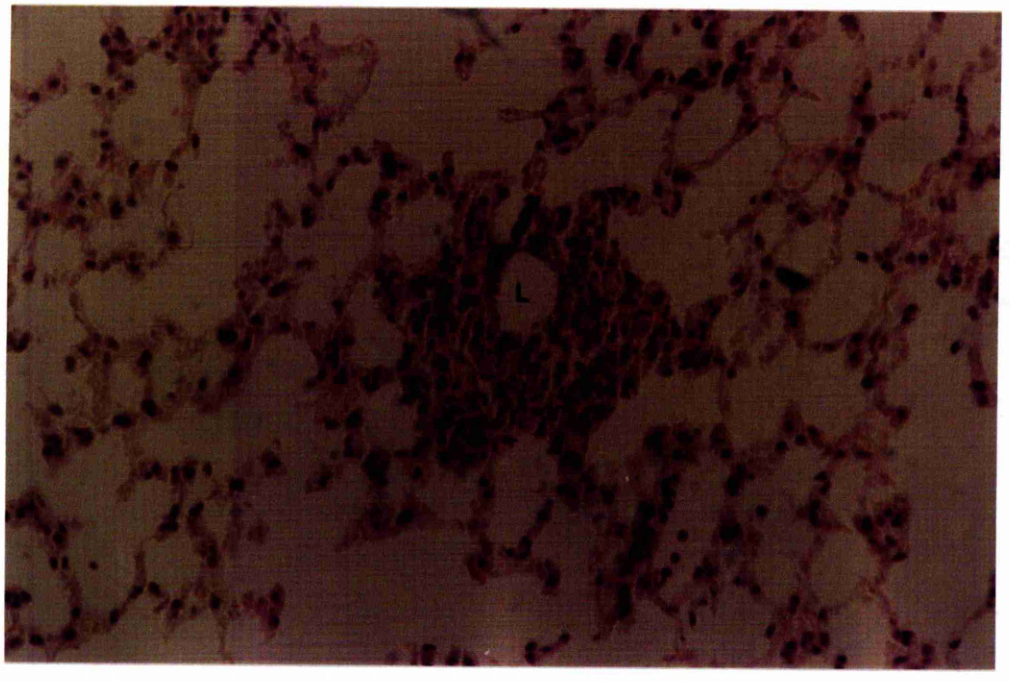


PLATE 7. Latex-TSAG particle embolized in mouse lung, 72 hours post secondary injection, showing the extent of the cell accumulation around the particle (L).
Modified haemalum and eosin x 208.

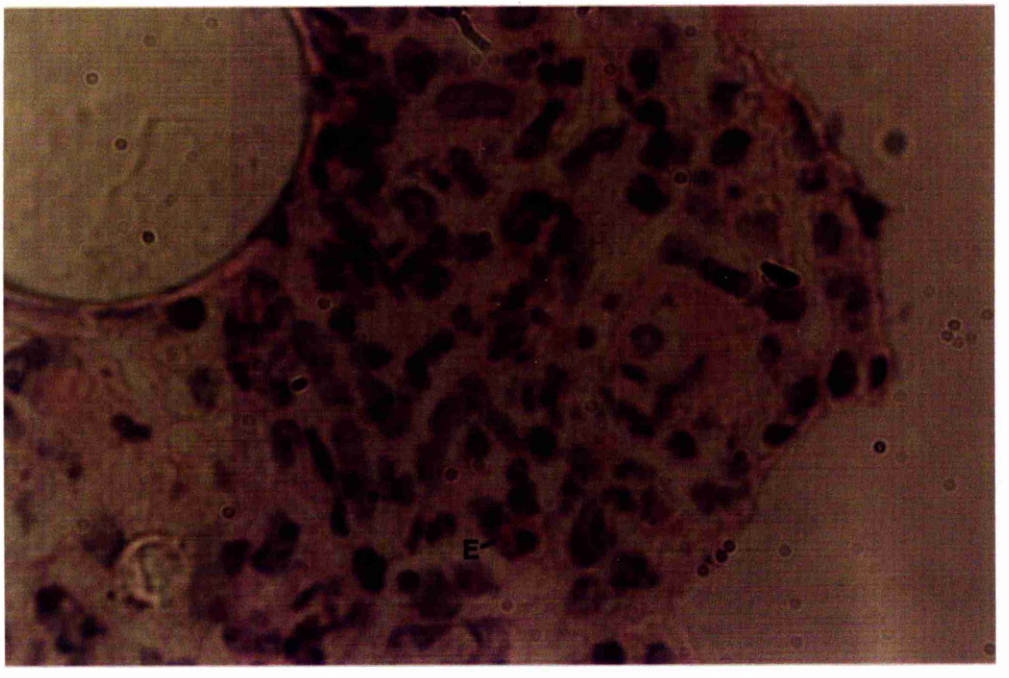


PLATE 8. Higher magnification of Plate 7, showing eosinophils (E) in the cell accumulation around the latex-TSAG particle.
Modified haemalum and eosin x 1280

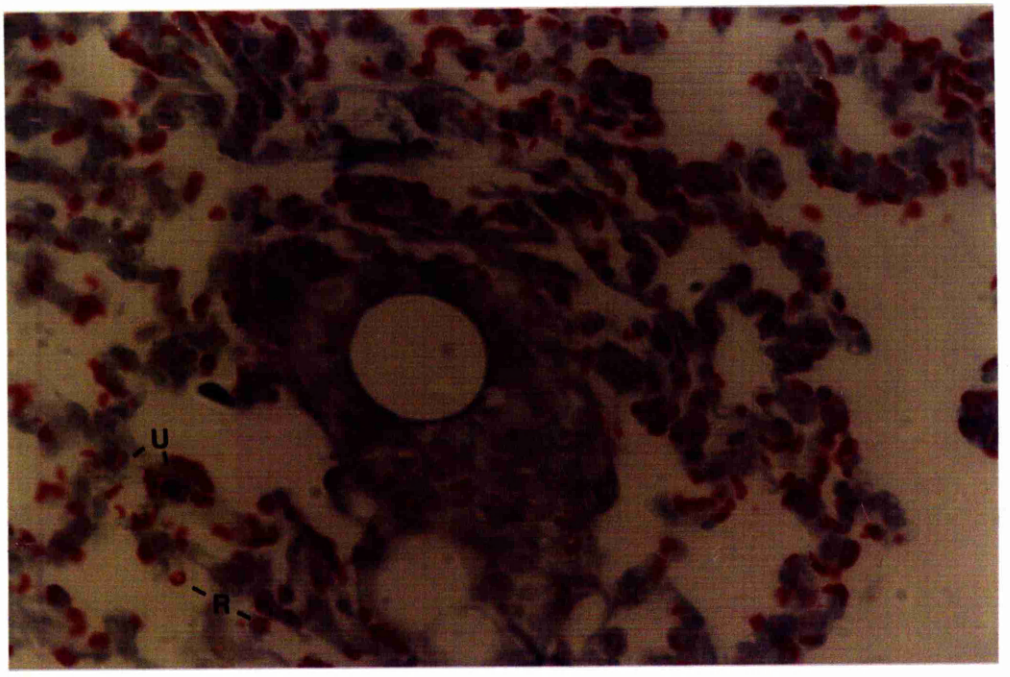


PLATE 9. Latex-BGG embolized in mouse lung, showing a large number of mononuclear granular staining cells (U). Red blood cells (R) are also intensely stained.
Modified dominici x 512

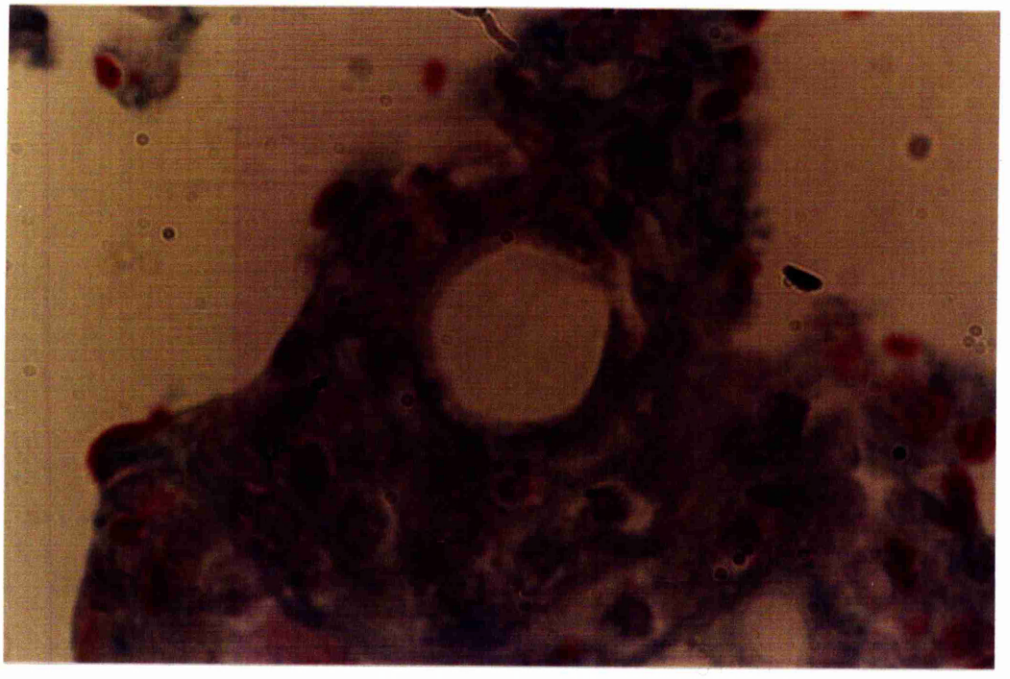


PLATE 10. Higher magnification of Plate 9. The mononuclear granular cells can be seen to be a major component of the cell accumulation around the latex particle.
Modified dominici x 1280

Discussion

A peripheral blood eosinophilia following pulmonary embolization of antigen-coated latex particles has previously been reported in rats (Schriber and Zucker-Franklin, 1974, 1975; Eady et al, 1978). These experiments have shown that a similar effect can be produced in mice, by both parasite-derived and non-parasite-derived antigens. Injection of latex-BGG particles into mice induced a 3-fold increase in the number of circulating eosinophils, peaking around 6 days after the secondary injection (Fig.14). BGG has previously been used as a coating antigen for latex particles injected into rats (Eady et al, 1978), but parasite-derived antigens have never previously been used in this fashion to induce eosinophilia.

The effect of injection of latex-TSAG was studied in naive and T. spiralis-infected mice. Injection of latex-TSAG into naive mice produced a response similar to that observed in mice injected with latex-BGG (Fig.15). There appears to be two types of response. The eosinophilia following the secondary injection of latex-BGG or latex-TSAG was accelerated and enhanced compared to the response to the primary injection. However, injection of uncoated particles also induced a slight elevation in the number of peripheral blood eosinophils, but this response was not significantly elevated following the secondary injection, and also peaked later at 9 days after both primary and secondary injections. This implies an antigen-specific and a non-specific mechanism in the induction of eosinophilia.

The infected mice developed a greater eosinophilia following the injection of latex-TSAG than uninfected mice (Fig.16). However, this

does not seem to be a specific antigen-driven response. The primary response to latex-TSAG peaked around day 9, while the secondary response was enhanced and accelerated, peaking around day 6 post injection. In addition, the response to latex alone was greater in infected than in naive mice, but still peaked on day 9 post injection. The infected mice had been given 400 T. spiralis larvae 12 weeks before the injection of the latex particles, and it is possible that these mice were in a condition of heightened responsiveness to an eosinophilic stimulus, for example due to increased EPP production (Mahmoud et al, 1977) or ERF (Spry, 1971b), both of which are produced upon exposure to T. spiralis larvae. The kinetics of the response suggest that the eosinophilia is due to EPP rather than ERF, since EPP takes effect later than ERF.

Lung samples were taken from mice over the first 72 hours after the primary and secondary injections of latex particles, with a view to investigating the effect of sensitization or pre-infection on the rate of antigen disappearance from the particle surface. However, although antigen could be detected on the surface of free particles in vitro by IFAT, this technique could not detect antigen on particles in situ. This is most likely due to the fact that whole particles were viewed over the entire surface whereas sectioned particles would have only a small halo of antigen around the periphery. This would give a halo of fluorescence but it was found that all particles reflected the non-specific tissue background fluoresence thus giving the impression of a halo.

Histological examination of lung sections showed large cellular accumulations around the latex-TSAG particles, containing eosinophils (Plates 7 and 8). Histological examination of the pulmonary lesions

following the embolization of latex-HGG particles in rats showed that they resembled a granulomatous response with a dense mononuclear cell infiltrate 24 hours after injection, with eosinophils increasing in number by 48 hours (Schriber and Zucker-Franklin, 1975). The extent of the mononuclear cell infiltrate around latex-TSAG particles in mice can be seen in Plates 9 and 10.

The granulomas which form around S. mansoni eggs have been found to be smaller in animals infected for a long period of time (Domingo and Warren, 1968). This effect was not observed in the cell accumulations around latex-TSAG particles injected into T. spiralis-infected mice (Fig.16). The cellular accumulations were similar in magnitude in both infected and naive mice, and also in mice injected with latex-BGG. Substantial accumulations also occurred around uncoated latex particles which correlates with their ability to induce an increase in the number of peripheral blood eosinophils (Table 7).

These experiments serve to show that a parasite antigen preparation can be used to induce a peripheral blood eosinophilia without exposure to whole parasites.

CHAPTER 5

AN INVESTIGATION OF A POSSIBLE THYMUS-INDEPENDENT MECHANISM OF EOSINOPHILIA

Introduction

A wealth of information exists suggesting that eosinophilic responses are thymus-dependent, but apparent thymus-independent eosinophilic responses have been reported. Rothwell and Love (1975) found that both thymectomized and sham-operated control guinea pigs developed an intestinal eosinophil response following infection with Trichostrongylus colubriformis. A peripheral blood eosinophilia has been reported in Rnu/Rnu rats after a Fasciola hepatica infection (Doy and Hughes, 1982). These rats also produce a peripheral blood eosinophilia in response to an Ascaris suum infection (Pritchard and Eady, 1981). This response is specific in that it is enhanced following a secondary A. suum infection, and moreover the peak eosinophilia was found to be greater in the athymic Rnu/Rnu rats than in heterozygous Rnu/+ controls.

An eosinophilia following parasite infection has also been reported in nude mice. Although small between-group differences are observed, the peripheral blood eosinophil counts in nu/+, nu/nu and nu/nu thymus-reconstituted rose sharply to peak at a similar value following infection with Toxocara canis eggs. Polymorphonuclear cells, mainly eosinophils, accumulate around T. canis larvae in the skeletal muscles of both nu/+ and nu/nu mice, but the granulomas in nu/nu mice are smaller and contain fewer eosinophils than those in nu/+ and nu/nu thymus reconstituted mice (Sugane and Oshima, 1982).

Parasite infections are potent inducers of eosinophilic responses, but the object of these experiments was to investigate the responses of Rnu/Rnu and Rnu/+ rats to a non-parasite stimulus of eosinophilia.

Results

Testing ATS activity in vitro

ATS activity was tested in vitro for its ability to mediate a complement-dependent lytic reaction. The viabilities of thymocyte and mesenteric lymph node cell suspensions were measured at the beginning of the experiment and 40-50 minutes after incubation in ATS/NRS or NRS alone. The final counts were corrected to take into account the initial viabilities of the cell suspensions. Following incubation, only 4% of the thymocytes incubated in ATS/NRS remained viable, while 90% of the thymocytes incubated in NRS alone were viable. 36% of the mesenteric lymph node cells incubated with ATS/NRS remained viable but ATS alone had virtually no effect on cell viability. These results imply that the ATS was acting mainly on T cells. However, the ATS had to be shown to have in vivo activity by being capable of abrogating a T cell dependent response.

Testing ATS activity in vivo

The system used to investigate ATS activity in vivo was the rat contact sensitivity response to oxazolone. The effect of primary and secondary challenge with oxazolone on ear thickness are shown in Fig.17 (a) and (b). The ATS-treated rats did not develop a response following the primary challenge, but did show an increase in ear thickness following the secondary challenge, which peaked around 72 hours post challenge. This response was similar in time-course, but smaller in magnitude, to the primary response in controls. The controls developed an accelerated, enhanced response to the secondary challenge which peaked around 24 hours. The response to secondary

challenge in the ATS-treated rats resembled a primary response, indicating T cell depletion at the time of sensitization, but reconstitution by primary challenge. These results showed that the ATS could be used effectively *in vivo*. This procedure was therefore employed in rats prior to injection with latex particles, with a secondary dose of ATS prior to the secondary injection of latex.

Response of Rnu/Rnu and Rnu/+ rats to latex injection

The effect of injection of Rnu/Rnu rats with uncoated or BGG-coated latex particles are shown in Fig.18. Injection of uncoated particles produced an increase in the number of circulating eosinophils. This response is similar following both the primary and secondary injections of latex. The secondary response to latex-BGG in these animals is slightly greater than the primary response. All responses peak around 7 days after each injection. The response of Rnu/+ rats to an identical immunization procedure is shown in Fig.19. These rats developed an enhanced eosinophilic response following the secondary injection of latex-BGG. A slightly enhanced response also developed following the secondary injection of latex alone, implying a non-specific mechanism for the induction of eosinophilia. There is also an antigen-specific mechanism, since the secondary response to latex-BGG is greater than that to latex alone. It was this apparent antigen-specific response which was investigated by ATS.

The effects of ATS on the latex-BGG response

There was an increase in the number of circulating eosinophils in both Rnu/Rnu and Rnu/+ rats following the primary and secondary injections of latex-BGG (Fig.20). The peak response occurred earlier in rats treated with ATS than in untreated rats, and there is a 50% reduction in the number of circulating eosinophils on day 20 in the ATS treated rats. However, the ATS did not abrogate the eosinophilic response to the injection of latex particles.

ATS treatment resulted in a reduction in the number of eosinophils recovered by lung lavage 48 hours after secondary injection of latex-BGG in Rnu/Rnu, but not Rnu/+, rats (Fig.21). Latex-BGG appeared to induce a greater pulmonary eosinophilia than injection of latex alone, but the standard deviations deters one from drawing too many conclusions from these results.

Assaying lung lavage supernatants for IL-1 activity

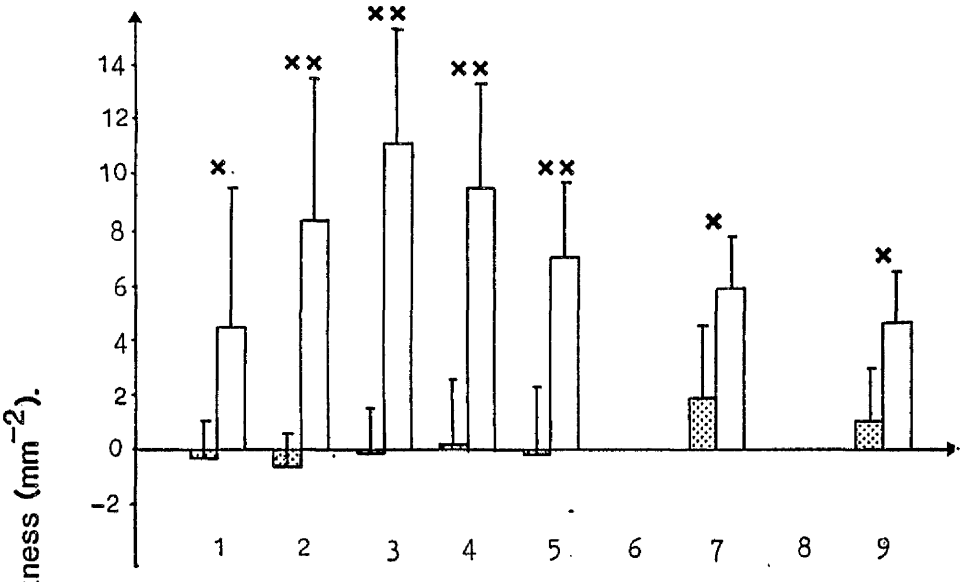
The ability of lung lavage supernatants from Rnu/Rnu and Rnu/+ rats to stimulate thymocyte proliferation *in vitro* is shown in Fig.22. These lavages were recovered from rats injected with latex-BGG with and without previous ATS treatment. No group greatly enhanced ^{3}H-thymidine uptake, but all groups did stimulate significant ^{3}H-thymidine uptake at a 1:40 dilution when compared to the PHA control. Pre-injection of rats with ATS did not influence the response, nor was there any difference between lavages recovered from Rnu/Rnu and Rnu/+ rats. All groups showed a reduced ^{3}H-thymidine uptake at a 1:4 dilution of the lavage supernatants. This could have been due to the presence of a substance inhibiting IL-1 activity. Fig.23 (a,b) shows

the effect of NAG on three dilutions of lung lavage supernatants. NAG is a monosaccharide which will inhibit the action of a soluble suppressor of T cell proliferation (SISS-T; Greene, Fleisher and Waldmann, 1981). The uptake of ^{3}H-thymidine was still inhibited at a 1:4 dilution of lung lavage supernatants, showing that the observed effect is not due to SISS-T. These results also reinforce the data presented in Fig.21, i.e. there is little or no difference between lavages recovered from animals injected with latex alone, latex-BGG, or latex-BGG preceded by ATS treatment, and little difference between lavages recovered from Rnu/Rnu and Rnu/+ rats.

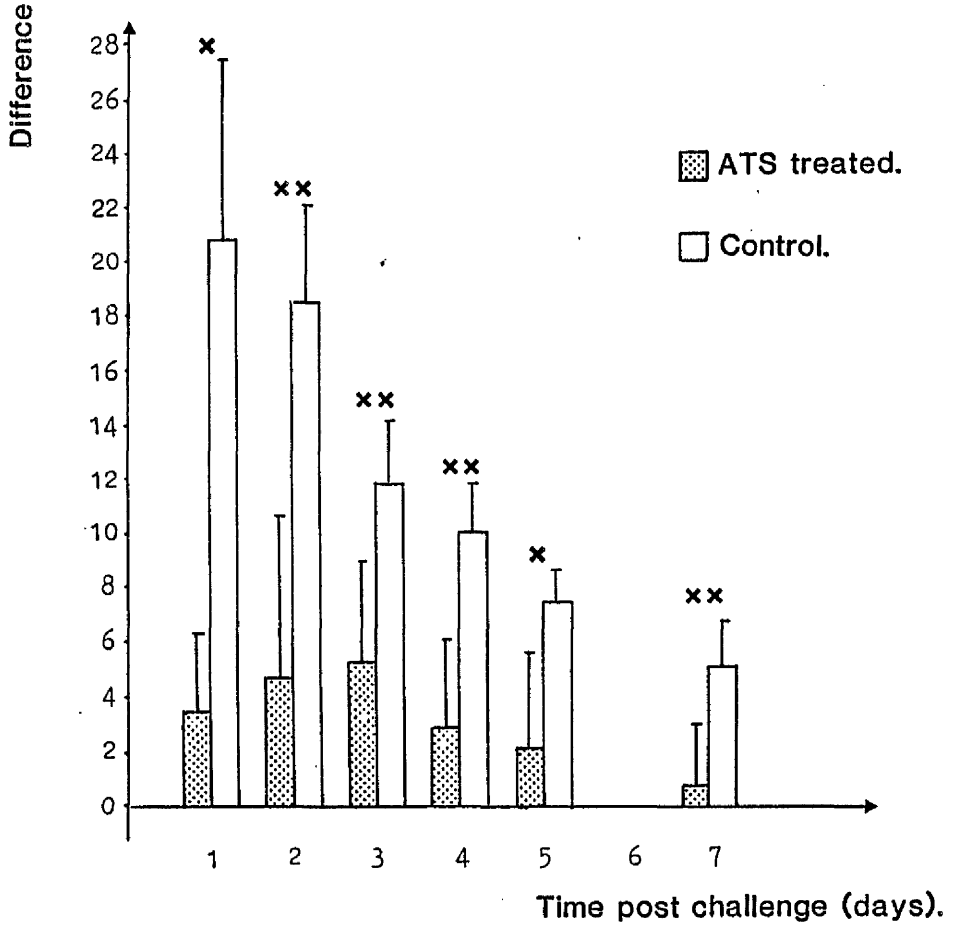
Histological examination of lung tissues

Plates 11-16 show sections of lung taken from Rnu/Rnu and Rnu/+ rats. Cell accumulations around latex-BGG particles 15 days after the secondary injection are not larger than those around uncoated particles in Rnu/Rnu rats (Plates 12 and 13). Similarly, the cell accumulations around uncoated and BGG-coated latex particles 7 days after the secondary injection in Rnu/+ rats do not show a clear difference (Plates 13 and 14). A direct comparison between Rnu/Rnu and Rnu/+ rats 9 days after the secondary injection of latex-BGG and ATS treatment does not reveal any obvious differences between the groups (Plates 15 and 16).

(a). Response to primary challenge.

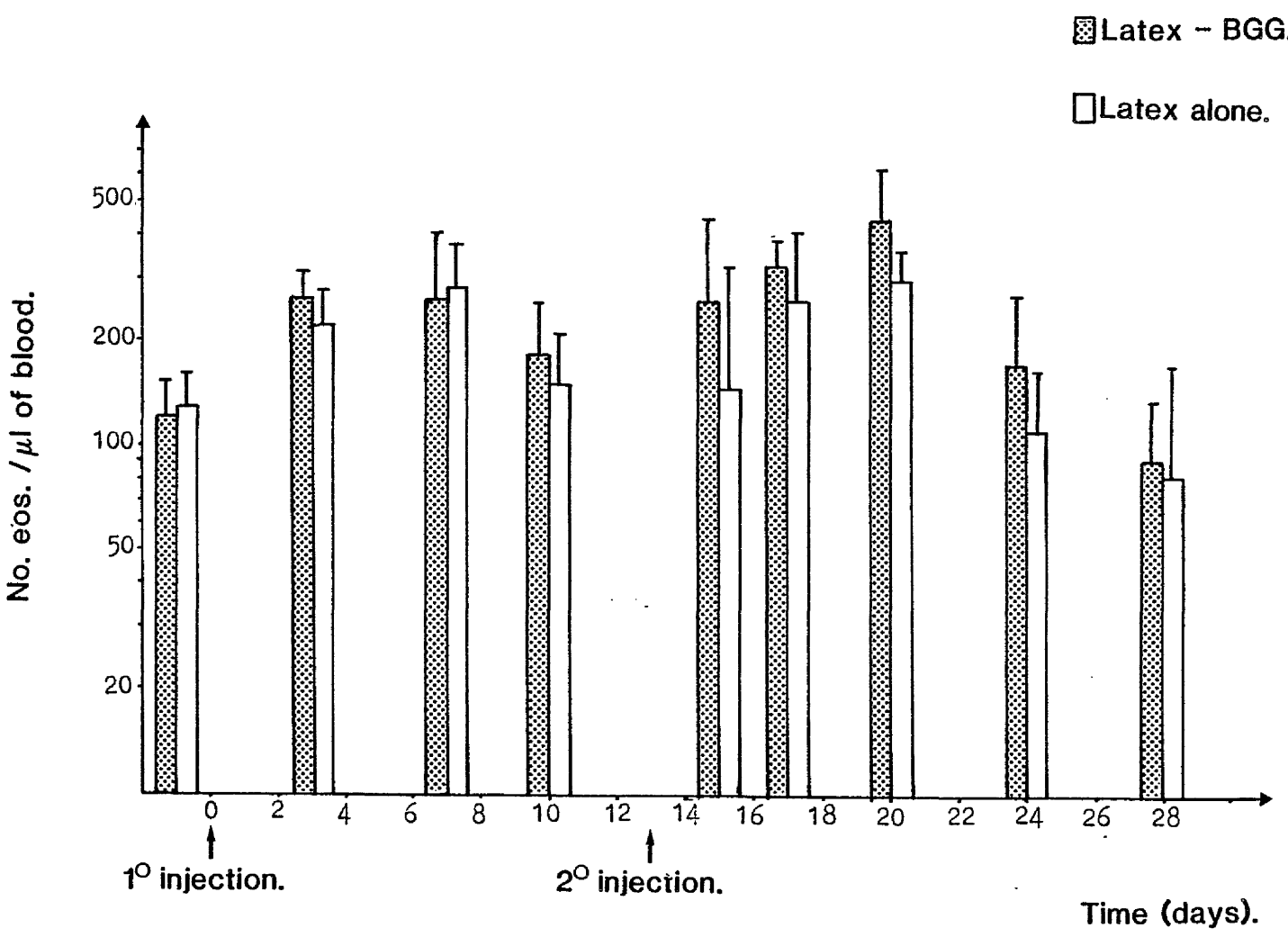


(b). Response to secondary challenge.



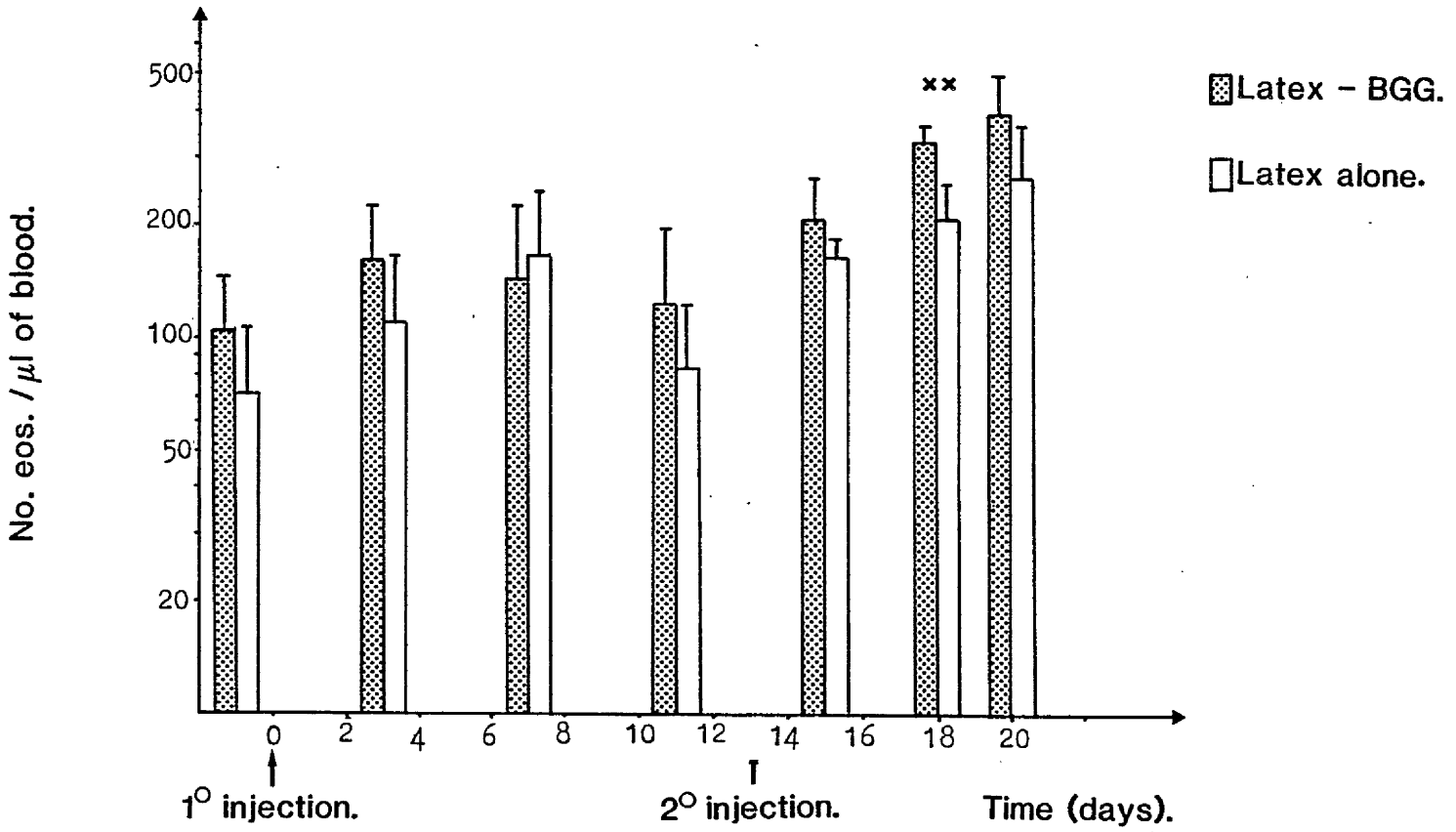
× significant, ×× highly significant difference between groups.

Figure 18. Blood eosinophil response to injection of latex – BGG and latex particles alone in Rnu/Rnu rats.



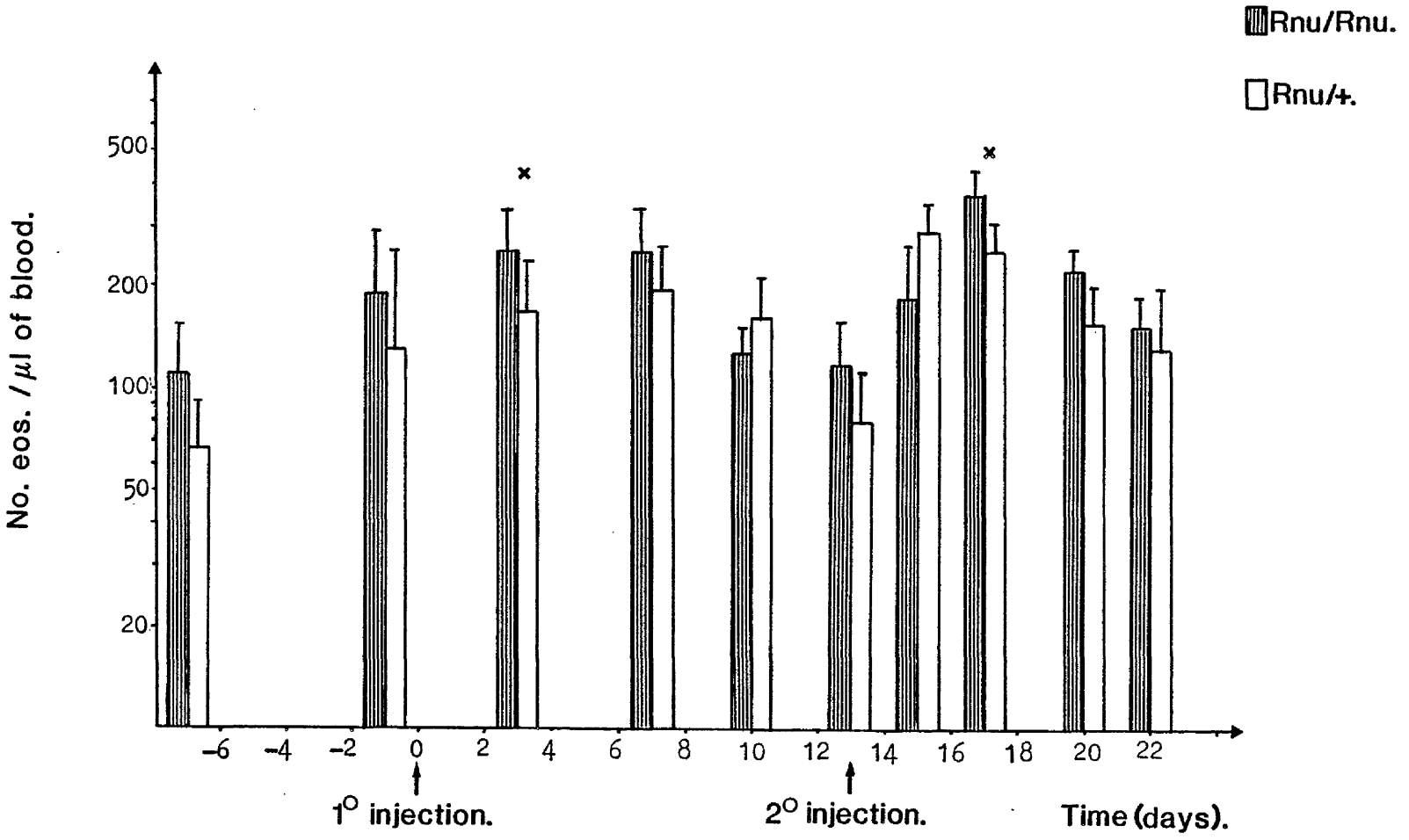
No significant difference was observed between the groups on any given day.

Figure 19. Blood eosinophil response to injection of latex – BGG and latex particles alone in Rnu/+ rats.



×× highly significant difference between groups.

Figure 20. Blood eosinophil response to injection of latex – BGG in Rnu/Rnu and Rnu/+ rats treated with ATS.



× significant difference between groups.

Figure 21. No. of eosinophils in lung lavages of rats.

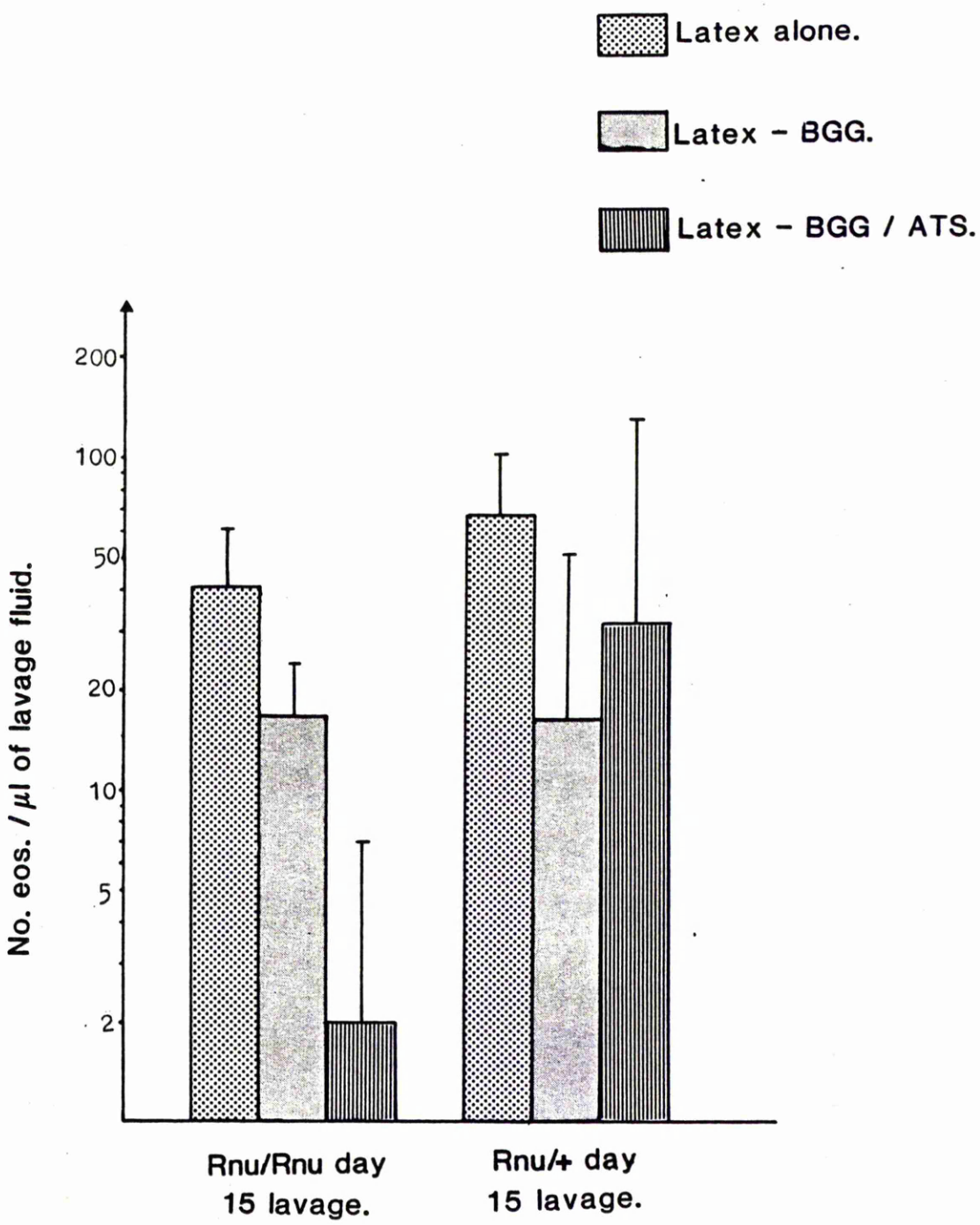
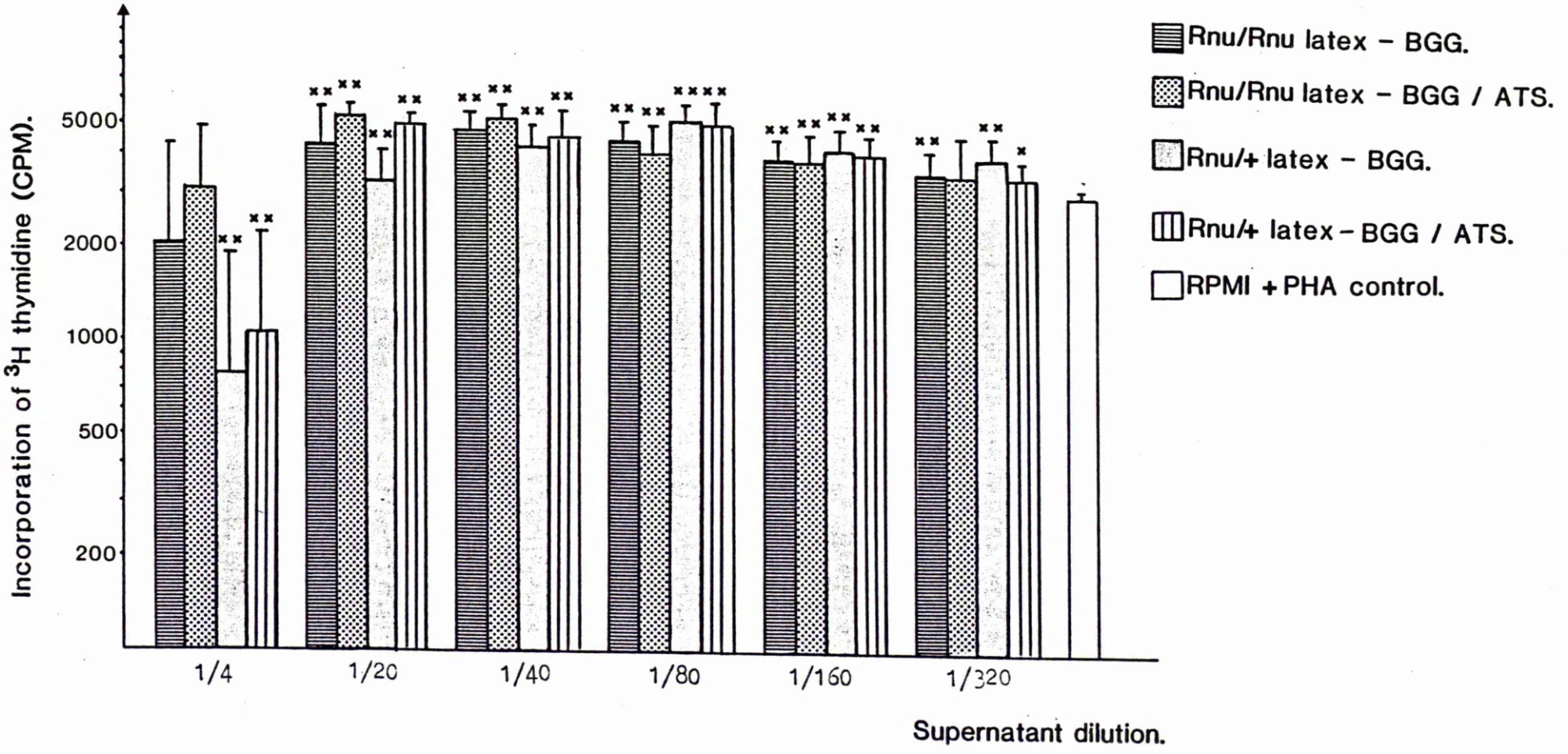
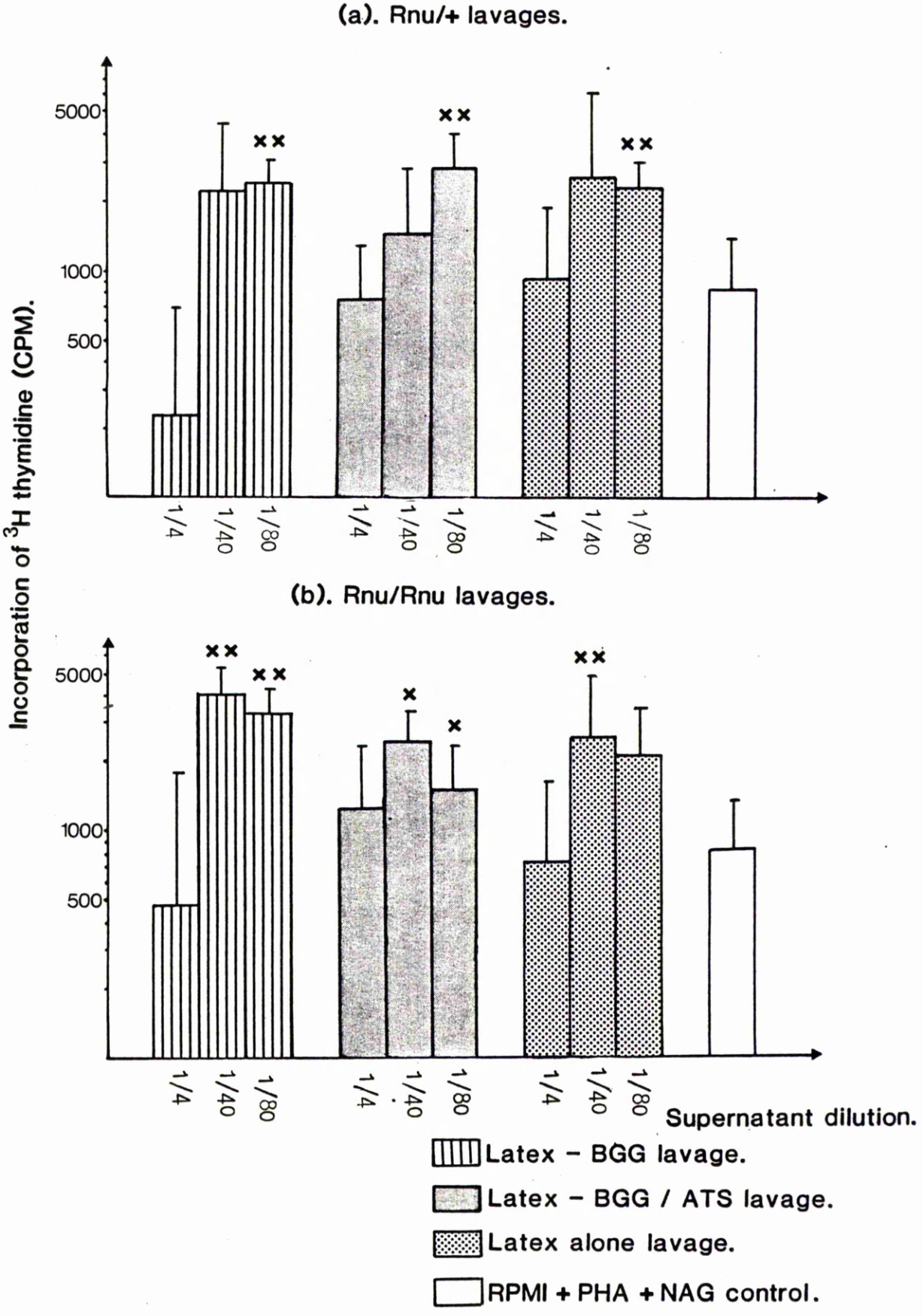


Figure 22. Effect of rat day 15 lung lavage supernatants on mouse thymocyte proliferation in vitro.



*significantly, **highly significantly different from control.

Figure 23. Effect of day 15 rat lung lavage supernatants on mouse thymocyte proliferation in vitro.



× significantly, ×× highly significantly different from control.

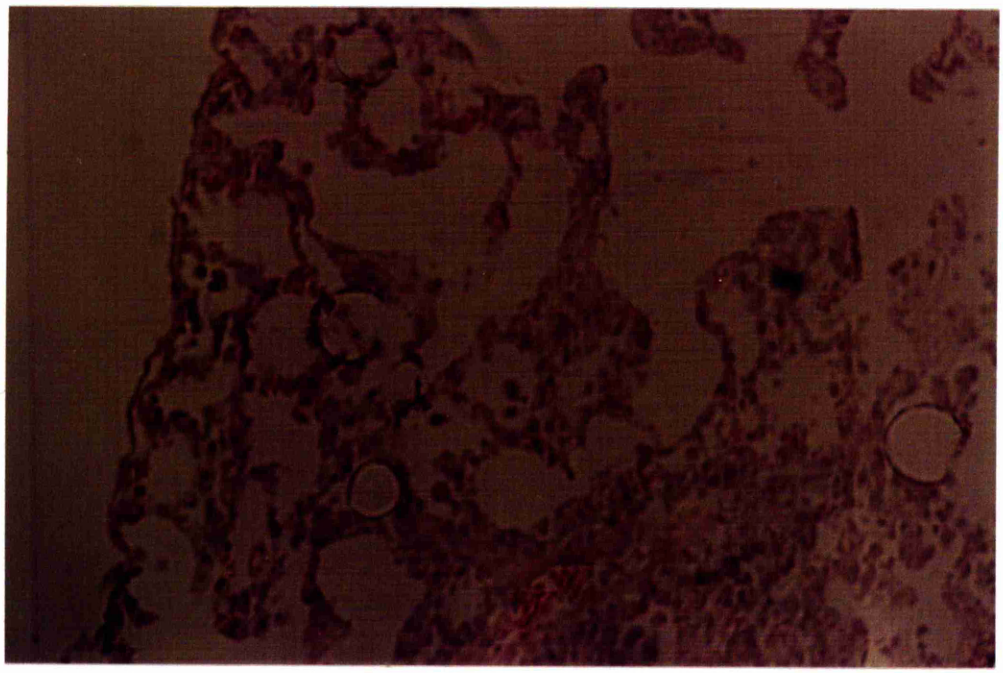


PLATE 11. Rat lung (Rnu/Rnu), 15 days following the secondary injection of latex-BGG, showing embolized particles.
Haemalum and Eosin x 208.

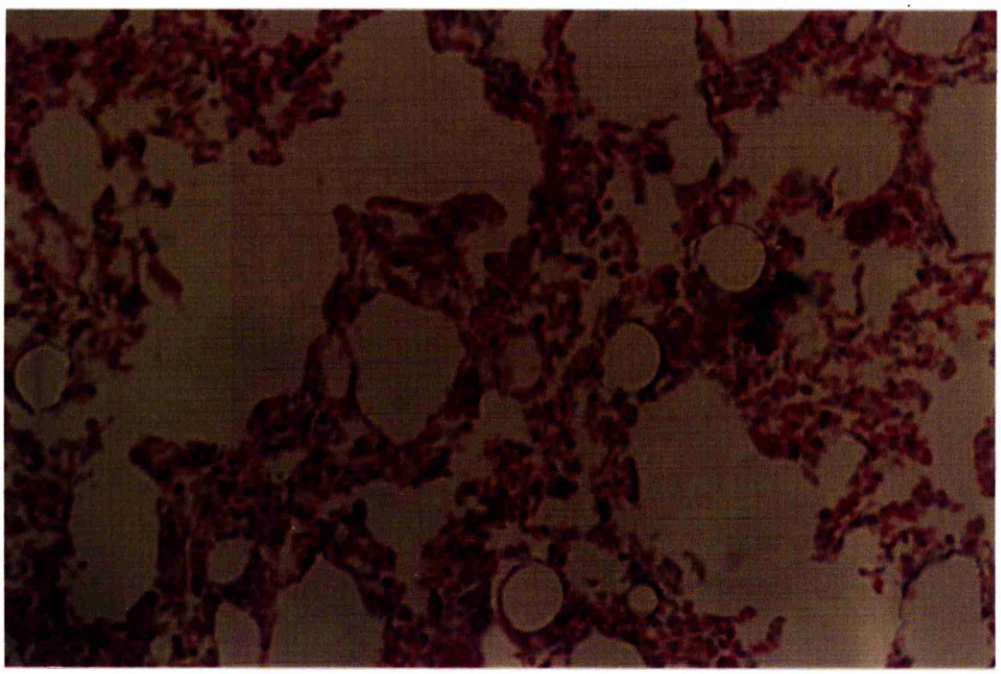


PLATE 12. Rat lung (Rnu/Rnu), 15 days following the secondary injection of uncoated latex particles.
Haemalum and eosin x 208.

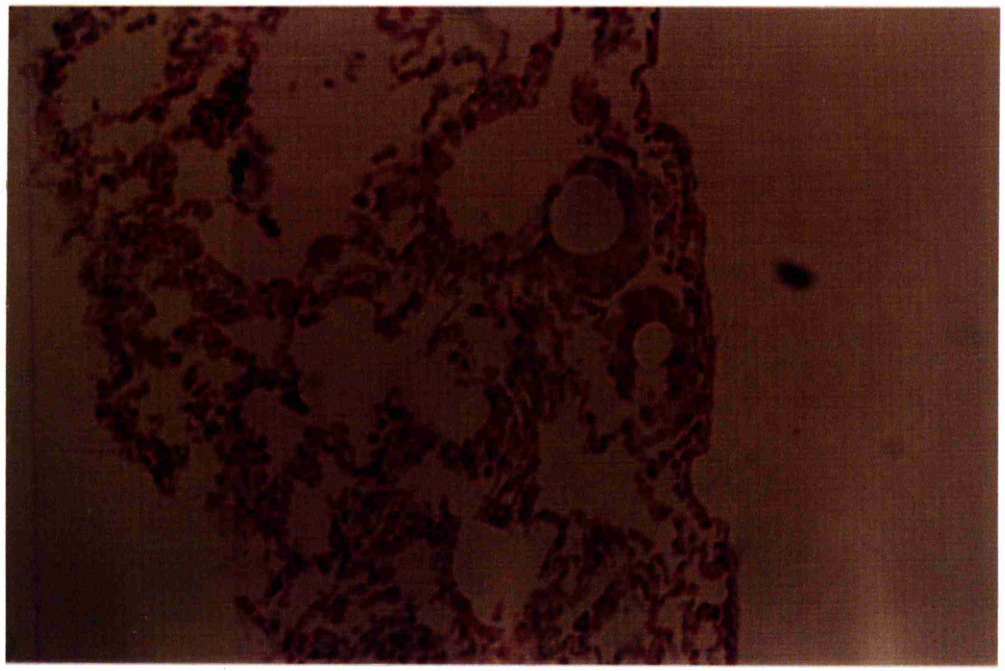


PLATE 13. Rat lung (Rnu/+), 9 days following the secondary injection of latex-BGG, showing embolized particles. Haemalum and eosin x 208.

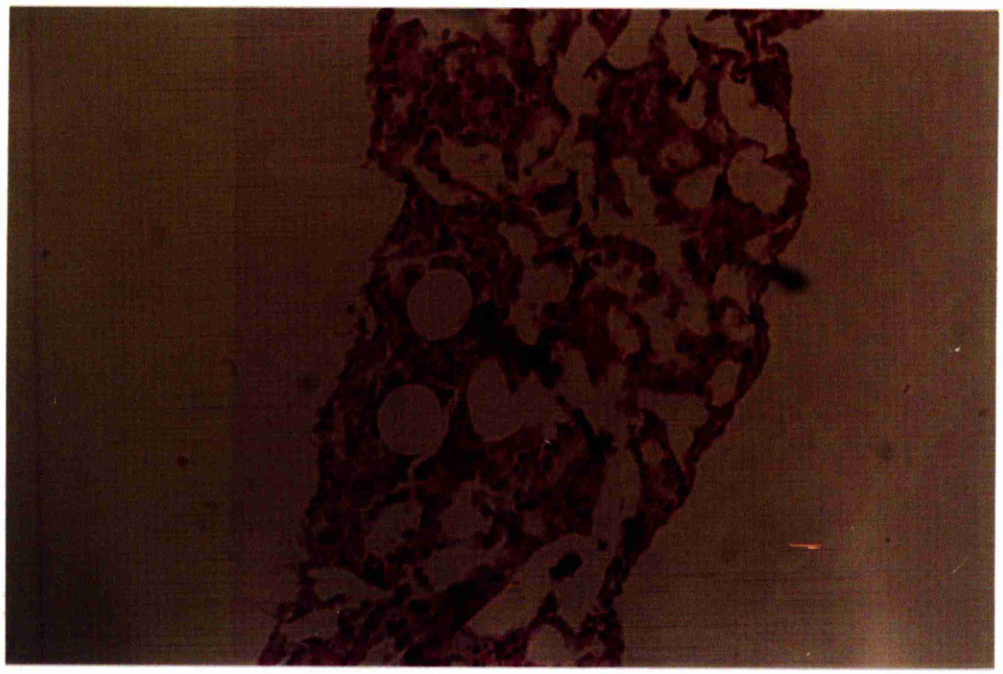


PLATE 14. Rat lung (Rnu/+), 9 days following the secondary injection of uncoated latex particles. Haemalum and eosin x 208.

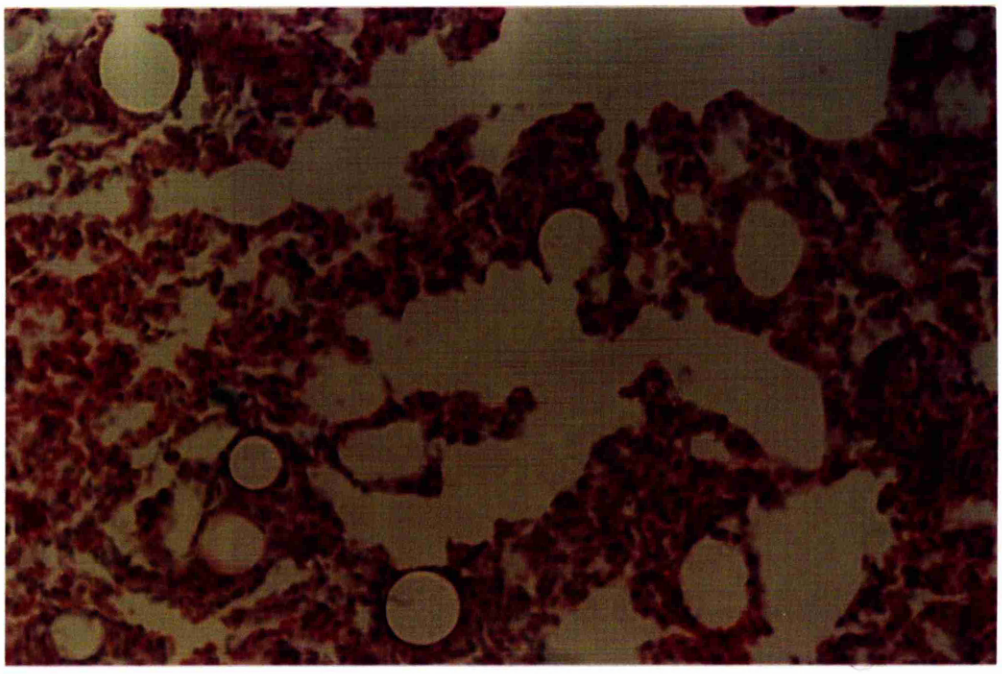


PLATE 15. Rat lung (Rnu/Rnu), 9 days following the secondary injection of latex-BGG after pre-treatment with ATS.
Haemalum and eosin x 208.

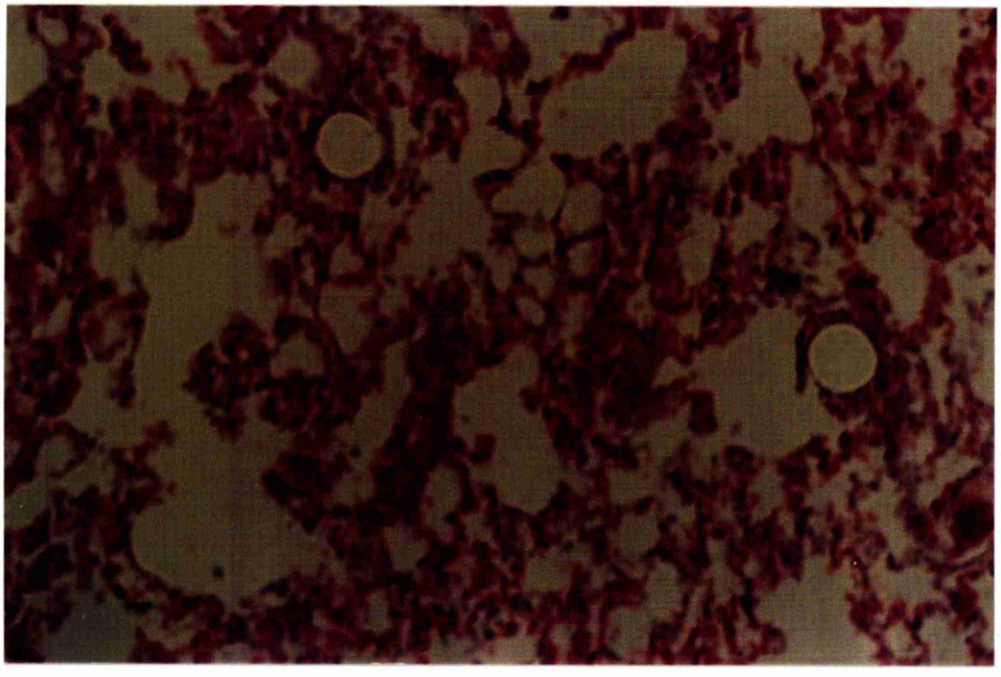


PLATE 16. Rat lung (Rnu/+), 9 days following the secondary injection of latex-BGG after pre-treatment with ATS.
Haemalum and Eosin x 208

Discussion

It has previously been reported that neither Rnu/Rnu nor Rnu/+ rats develop a peripheral blood eosinophilia following the injection of latex particles, either alone or coated with BGG (Pritchard and Eady, 1981). In contrast to those observations, Figs.18 and 19 show that an increase in circulating eosinophils can occur in these animals following the pulmonary embolization of latex particles. The peak response, which occurred 7 days after the secondary injection of latex-BGG was of similar magnitude in both Rnu/Rnu and Rnu/+ rats. Both groups also developed an increased number of circulating eosinophils following the injection of uncoated latex particles, implying the existence of at least one non-antigen specific mechanism for the induction of an eosinophilic response in these animals. This lack of specificity could also, theoretically, circumnavigate the necessity for T cells. Why these rats should have developed an eosinophilia following this treatment while those of Pritchard and Eady did not is difficult to explain since the experiments were performed under identical conditions. The response of the athymic Rnu/Rnu rats was unexpected, but equally difficult to explain is the failure of the Rnu/+ rats to develop an eosinophilic response following latex-BGG injection in Pritchard and Eady's experiments.

Despite their failure to develop a peripheral blood eosinophilia, Pritchard and Eady did report a local pulmonary eosinophilia in both Rnu/Rnu and Rnu/+ rats following pulmonary embolization of latex-BGG particles, maximum at 48 hours after the secondary injection. They found that embolization of latex particles alone failed to induce a comparable lung eosinophilia in either groups. Fig.21 confirms these

observations, and infer that the presence of antigenic material on the particles induces a greater local recruitment of eosinophils. In the experiments described here, this also appears to result in increased eosinophil production, producing a peripheral blood eosinophilia several days later.

In order to determine if the antigen-specific response was under T cell control, the animals were injected with ATS prior to the injection of latex particles. The contact sensitivity response to oxazolone has been shown to be thymus dependent (Parrott, 1967), so this was used as an indicator of T cell depletion in vivo by ATS. The immunization procedure which successfully diminished this response in Rnu/+ rats (Fig.17) was employed in Rnu/Rnu and Rnu/+ rats prior to latex-BGG injection. ATS altered the time course, but did not totally abrogate, the peripheral blood eosinophilic response to latex-BGG (Fig.20), and reduced the number of eosinophils recovered by lung lavage of Rnu/+ rats (Fig.21). The inconsistencies in this technique, exemplified by the large deviations from the mean, are due to uncontrollable parameters, such as cells being trapped in mucus produced in the lungs (which can be substantial, especially in Rnu/Rnu rats) or the rupture of blood vessels.

Clearly, the ATS did have some effect on the response. If it was due to T cell depletion then it implies the existence of a residual T cell population in Rnu/Rnu rats. However, Vos, Ruitenberg, Van Basten, Buys, Elgersma and Kruizinga (1983) failed to find T cells in the spleen, MLN or Peyer's patches of Rnu/Rnu rats using monoclonal antibodies. The alternative is that the ATS could have had a more direct effect, by killing eosinophils themselves, since it was not a monoclonal antibody.

As an indirect indication of T cell activity, the lung lavage supernatants were assayed for IL-1 activity. IL-1 is a macrophage product which stimulates thymocyte proliferation (Aarden, Brunner and Cerottini, 1979). Mononuclear cell products have been shown to influence eosinophil function (Veith and Butterworth, 1983; Vadas, Nicola, Lopez, Metcalf, Johnson and Pereira, 1984), and recent studies have revealed that macrophage activity may be under T cell control. Macrophages from nude mice were found to produce greater amounts of H_2O_2 than those from euthymic or thymus-reconstituted nude mice and to be more effective at killing Listeria monocytogenes, suggesting that this elevated macrophage activity was due to a lack of suppressor T cells (Sharp and Colston, 1984).

IL-1 production by alveolar macrophages was assayed in Rnu/Rnu and Rnu/+ rats following injection of latex-BGG, latex alone, or latex-BGG following ATS treatment (Figs.22 and 23). All groups showed some thymocyte proliferation enhancing activity in their lavage supernatants, but on the basis of Sharp and Colston's observations, one would expect IL-1 production to be greater in Rnu/Rnu than Rnu/+ rats, and for ATS treatment to result in increased production in Rnu/+, but not Rnu/Rnu rats. Neither of these phenomena were observed. However, the presence of a residual T cell population in Rnu/Rnu rats cannot be ruled out, since it is possible that the T cell population was not sufficiently depleted by ATS treatment to affect macrophage activity. What these experiments do show is that Rnu/Rnu and Rnu/+ rats can respond in a similar fashion to injection of latex particles. These responses include peripheral

blood and tissue eosinophilia and IL-1 production which raises doubts about the immunological status of these animals.

GENERAL DISCUSSION

Although certain physical manipulations can be employed to increase or decrease the number of circulating eosinophils in an animal, inherent changes also occur which have been attributed to the level of circulating steroids. This relationship between eosinophils and steroids is an inverse one - the higher the level of circulating steroids, the lower the number of circulating eosinophils (Sabag et al, 1978). In addition to affecting eosinophil function, glucocorticoids also affect eosinophil function, by inhibiting adherence (Clarke, Gallin and Fauci, 1979), chemotaxis (Altman, Hill, Hairfield and Mullarkey, 1981) and eosinophil-mediated antibody-dependent cellular cytotoxicity reactions (Hallam, Pritchard, Trigg and Eady, 1982). Why a relationship should exist between steroids and eosinophils was partly explained by Peterson, Altman, Hills, Gosney and Kadin (1981) who demonstrated receptor sites specific for glucocorticoids on eosinophils.

The inherent variation in circulating eosinophils in NIH mice raises a crucial question. Where do the eosinophils which leave the circulation go to? An indication of their fate was provided by Sabag et al (1978). Histological analysis of rats injected with cortisol showed an increase in several lymphoid organs, but not other tissues, 24 hours post injection. Conversely, they found a decrease in lymphoid eosinophils 7 days after adrenalectomy. The functional significance of these observations is uncertain, but they further emphasize the conclusions of Chapter 1, i.e. that experimental procedures investigating eosinophil numbers or function should be performed on a strict programme.

There also appears to be a physiological link between corticosteroids and sex hormones, since peak glucocorticoid levels

during the circadian rhythm are generally higher in the human female than male (Asfeldt, 1971) and oestrogen administration will increase the peak cortisol levels (Daly and Elstein, 1972). It has also been established that oestrogen can affect eosinophil distribution, implying an eosinophil oestrogen receptor.

The first indication of such a receptor came during investigations on the retention of oestrogens in the rat uterus following systemic injection of tritiated oestradiol (Tchernitchin, 1967). Autoradiographs revealed cells which incorporated the label in a considerably greater proportion than the rest of the uterus. The labelled cells were identified as eosinophils and further studies showed that the quantity of oestradiol bound by uterine eosinophils depended on the hormonal state of the animal and was inversely proportional to the oestrogen level (Tchernitchin, 1970). In addition to uterine eosinophils, oestrogen receptors have been found in eosinophil-rich human blood leukocyte preparations (Tchernitchin and Tchernitchin, 1976). Eosinophils will migrate to the uterine luminal epithelium then undergo autolysis when the animal enter oestrus (Lee, 1982). Eosinophil migration has been shown to be dependent on the systemic, and not the uterine, level of oestrogen (Tchernitchin and Garland, 1983). For these reasons, only male animals were used in the studies in this thesis. Since eosinophil distribution is clearly affected by the oestrus cycle, the use of female animals introduces another parameter of variability into the system.

Having standardized the conditions as far as possible, mice were infected with T. spiralis, a potent inducer of eosinophilia. How much of this eosinophilia is due to migration of larvae and how much is due

to antigenic expression? There are several difficulties involved in trying to answer this question, since the tissue damage caused in the intestine and muscle by invading larvae cannot be reproduced without live worms, nor can total antigen expression be mimicked.

However, the antigen will induce an eosinophilic response in mice when introduced in the appropriate manner. Although the primary response was greater in previously-infected mice than in naive mice, the secondary response in infected mice was greater than the primary response, implying that the mice are being exposed to novel antigens in the TSAG preparation. This correlates with the observations in rabbits infected with T. spiralis or injected with TSAG, that the TSAG contains antigenic components not expressed during an infection. These results indicate that the antigens are at least in part responsible for the eosinophilic response to T. spiralis. However, are they more potent than non-parasite antigens at inducing eosinophilia?

The secondary response to latex-TSAG in uninfected mice peaked at around 1000 eosinophils/ul of blood, while the secondary response to latex-BGG peaked at approximately 950 eosinophils/ul. These two antigen preparations are unrelated, but the conformation or composition of an antigen may be important in the induction of an eosinophilic response. Campbell (1942) reported that the addition of cysteine residues enhances its 'eosinotactic' potential. There may be such a physical similarity between BGG and the components of TSAG.

These mice developed a 2-3 fold increase in the numbers of circulating eosinophils following the pulmonary embolization of antigen-coated particles. Schriber and Zucker-Franklin (1974, 1975) have reported a 10-fold increase in the number of blood eosinophils in

rats injected with latex-HGG, while Eady et al (1978) found that injection of latex-BGG induced a 5-fold increase in circulating eosinophils. Since both these groups used Sprague-Dawley rats in their experiments, it indicates that HGG may be a more potent inducer of eosinophilic responses than BGG. Strain variation has been reported in the magnitude of the eosinophil response to Ascaris suum (Vadas, 1982) and T. spiralis (Wakelin and Donachie, 1983) infection in mice, so it is possible that Sprague-Dawley rats may be predisposed to producing a greater eosinophilic response than NIH mice. It is interesting to note that in Schriber and Zucker-Franklin's and Eady et al's experiments, the rats developed a 3-4 fold increase in circulating eosinophils following injection of uncoated latex particles, i.e. the antigen-specific response was greater to latex-HGG than to latex-BGG, but the non-specific response to uncoated particles was similar in both groups. NIH mice, although having higher levels of circulating eosinophils prior to injection, never showed as much as a 2-fold increase in the number of circulating eosinophils following the injection of uncoated particles.

However, there are other factors which must be taken into consideration which could affect both the specific and non-specific responses. The rats were each injected with 3.5×10^5 particles 45 /um in diameter on two occasions, while the mice were each injected with 3.5×10^4 particles 27.5 /um in diameter on two occasions. These dose and particle-size differences could account for the differences between rats and mice. These factors combined also mean that mice were exposed to a smaller amount of antigen than rats. Mice, however, are much smaller animals, which balances out these differences to some

extent.

Another factor which appears to influence the extent of an eosinophilic response is the tissue sequestration of antigen. TSAG and BGG were coupled to latex particles for pulmonary embolization since it has been shown that HGG (Schriber and Zucker-Franklin, 1974) and BGG (Eady et al, 1978) will not induce an eosinophilia in rats when injected alone.

However, recent reports have shown that tissue localization of antigen is not a necessary prerequisite for blood eosinophilia induced by parasite or non-parasite antigens. Vadas (1981) showed that cyclophosphamide pretreatment of mice injected with KLH emulsified in FCA resulted in the development of a peripheral blood eosinophilia. This has been repeated with an antigenic preparation extracted from T. spiralis larvae (Wakelin and Donachie, 1983). Cyclophosphamide-sensitive T suppressor cells have been implicated in the control of granulomatous responses to S. mansoni (Chensue, Wellhausen and Boros, 1981; Greene and Colley, 1981) so it is possible that a similar mechanism exists for the suppression of eosinophilic responses induced by latex particles.

Since the observations of Basten and Beeson (1970), T cells have been implicated in the induction of eosinophilic responses. However, Pritchard and Eady (1981) reported that nude rats developed a lung, but not a peripheral blood, eosinophilia following pulmonary embolization of latex-BGG particles. The results in Chapter 5 show that both Rnu/Rnu and Rnu/+ rats can develop a blood eosinophilia following injection of latex particles. ATS treatment altered, but did not abrogate, the peripheral blood eosinophilia following injection of latex-BGG and reduced the pulmonary eosinophilia in

Rnu/Rnu rats. This implies that there is a residual T cell population in Rnu/Rnu rats or that the ATS also acts on another cell population, i.e. eosinophils. In addition, Rnu/Rnu and Rnu/+ rats showed similar levels of IL-1 production by alveolar macrophages but Sharp and Colston (1984) have reported that macrophages from nude mice are more active than those from normal mice due to a lack of suppressor T cells.

IL-1 production and release from mononuclear cells has been demonstrated by non-antigenic stimuli both in vitro and in vivo. In vitro exposure of macrophages to latex particles has been shown to increase both IL-1 production and release (Gery, Davies, Derr, Krett and Barranger, 1981). Enhanced IL-1 activity by alveolar macrophages in vivo has been demonstrated in rats following asbestos inhalation (Hartmann, Georgian, Oghiso and Kagan, 1984). This is a non-specific effect and may account for the similarity in the IL-1 responses between rats injected with latex-BGG and uncoated particles, i.e. it is the physical embolization of the particles which induces the response. If the response is non-specific, it would also explain the lack of effect of pre-treatment of the rats with ATS. However, this theory is not consistent with the idea that macrophages are in some way under T cell suppression, which should be removed by ATS. It is possible that the effect of ATS given prior to injection of latex-BGG to inhibit sensitization had diminished by the time the lavages were taken (day 15).

These conflicting results may be due to peculiarities in this particular strain of rat. A breeding colony of Rowett nude rats, from which the rats used in these experiments were derived, was re-

established in 1975 following their initial discovery 20 years previously. They were found to have an abnormal thymus rudiment, show lymphocyte depletion of T cell-dependent areas of lymph nodes and Peyer's patches, and their lymphocytes showed a failure to respond to T cell mitogens such as PHA and concanavalin A *in vitro* (Festing, May, Connors, Lovell and Sparrow, 1978). However, despite failing to develop a peripheral blood eosinophilia following infection with *N. brasiliensis* (Ogilvie, Askenase and Rose, 1980) or *T. spiralis* (Perrudet-Badoux, Boussac-Aron, Ruitenberg and Elgersma, 1980), Rnu/Rnu rats were found to develop a peripheral blood eosiniphilia following infection with *Fasciola hepatica* (Doy and Hughes, 1982) or *A. suum* (Pritchard and Eady, 1981). Moreover, the eosinophilias following these latter infections were found to be greater in Rnu/Rnu than in Rnu/+ rats. These various observations may be due to inherent properties of the parasites or their antigens in their ability to elicit eosinophilic responses. However, as they stand, they do nothing to clarify the degree of T cell control over eosinophil production and mobilization. The number of circulating eosinophils in unstimulated Rnu/Rnu and Rnu/+ rats are similar. This is consistent with previous reports (Ogilvie *et al*, 1980; Day and Hughes, 1982; Pritchard and Eady, 1982). This has also been found to be true for thymectomized (Walls *et al*, 1971) and athymic (Ruitenberg *et al*, 1977) mice when compared to normal controls. The homeostatic mechanisms regulating the number of circulating eosinophils would appear to be T cell-independent in these animals, since a substance such as EPP would not be produced (Mahmoud *et al*, 1977).

However, not all eosinophil-stimulating factors are produced by T cells. Supernatants of mononuclear cell cultures from both

eosinophilic and normal individuals have been shown to significantly enhance antibody-dependent, complement-independent eosinophil-mediated killing of S. mansoni schistosomulae in vitro (Veith and Butterworth, 1983). These mononuclear cell cultures were mainly monocytes and lymphocytes, and when separated into adherent and non-adherent populations, it was found that greater enhancing activity was present in the adherent population. Treatment of the adherent cell population with a monoclonal antilymphocyte antibody and complement resulted in a 76% drop in cell viability, but the eosinophil-enhancing activity of the remaining cells was unaltered. This activity could not be attributed to ESP, EPP or ECF-A. Also, human mononuclear cell populations consisting of greater than 95% monocytes have been found to stimulate antibody-dependent killing of tumour cells by eosinophils. If eosinophil-stimulating factors have non-T cell origins, eosinophilopoietic factors may have similar alternative origins.

Another alternative to explain the control of the eosinophil population is that the animals are not truly athymic. It now appears that even congenitally athymic animals may not be devoid of cells with T lymphocyte properties. Lymphoid cells from nude mice have been found with Thy-1, Lyt-1 and Lyt-2 surface markers (MacDonald, Lees, Sordat, Zaech, Maryanski and Bron, 1980) and to produce IL-2, a T cell product (MacDonald, Lees, Glasebrook and Sordat, 1982). The frequency of IL-2 producing cells in nude mice appears to increase with age and can eventually reach levels 5-10 times lower than those found in euthymic littermates (MacDonald and Less, 1984). If the responses to different stimuli require different amounts of T cell cooperation, a

small population of T cells may be sufficient to mount an eosinophil response to F. hepatica, A. suum or latex-BGG particles, but not to T. spiralis or N. brasiliensis.

It is interesting to note that the mast cell response to T. spiralis in mice is also T cell-dependent, since it does not occur in athymic mice, but does occur in thymus-reconstituted mice (Ruitenberg and Elgersma, 1976). The other component of the immediate hypersensitivity response, IgE, is also under the control of antigen-specific helper and suppressor T cells (Ishizaka, Yodoi, Suemara and Hirashima, 1983). Suppression of the IgE response in rats infected with T. spiralis by anti-IgE globulin has been shown to result in a marked depletion of the tissue eosinophils and a concomitant increase in the numbers of encysted larvae. However, although the peripheral blood eosinophilia was reduced, there was still a considerable increase following infection of the treated rats (Dessein, Parker, James and David, 1981). Therefore, although IgE may play a role in the induction of eosinophilic responses, it is not essential. The remaining question is: could mast cell mediators be involved in the response to latex particles, particularly in the non-specific response to uncoated particles?

Mast cell products, such as histamine, ECF-A, PGD_2 and HETE's (Gallin et al, 1980; Goetzl and Austen, 1980; Goetzl, 1980) will attract and localise eosinophils. Similar to the eosinophil population in nude animals, mast cells have been found to be present in normal numbers in some tissues of athymic mice (Keller, Hess and Riley, 1976). Histological analyses have shown an abundance of mononuclear cells which may be connective tissue mast cells surrounding latex particles in normal mice. Whether or not these

cells are mast cells is debatable. They are certainly not mucosal mast cells, which do not retain their granular integrity following tissue fixation in formalin (Stroebl, Miller and Ferguson, 1981) and attempts to demonstrate mast cells by staining the sections with astra blue and safranin were unsuccessful. These cells have the appearance of mast cells and are not eosinophils which stain up with a slightly more orange appearance in these sections. However, ultrastructural studies of latex-HGG lesions in rats have revealed very few mast cells (Schriber and Zucker-Franklin, 1975). It is interesting to note the presence of these cells in the accumulations around latex particles, but since they have been stained rather unconventionally by the variability in a technique designed to stain eosinophil granules, it is impossible to say for certain if they are mast cells.

If mast cell mediators were responsible for the eosinophilia, there would have to be slow release of the mediators to produce an eosinophilia 6 days after latex embolization. The fact that response to uncoated particles peaks later than the response to antigen coated particles in mice suggests the involvement of at least two mechanisms in the recruitment of eosinophils.

Cell accumulations, which can be considerable (Plate 7) develop around both uncoated and antigen-coated particles, further emphasizing the involvement of non-specific responses which may be responsible, at least in part, for the phenomena observed in Rnu/Rnu rats. It is difficult to say how long the antigen remains on the particles <u>in situ</u>, since attempts to clarify this by fluorescence were unsuccessful. However, the lung sections were taken for the first 72 hours following injection of the latex particles and Rand, Chanton,

Runge, English and Colley (1983) have reported that eosinophil accumulation occurs for 36 hours after injection of SEA in S. mansoni-infected mice.

Alveolar macrophages may be involved in the tissue recruitment of eosinophils via an antigen-specific mechanism. Alveolar macrophages possess Fc receptors for IgE (Boltz-Nitulescu and Spiegelberg, 1981) and SRS-A has been shown to be released by alveolar macrophages via an IgE-dependent mechanism (Rankin, Hitchcock, Merrill, Huang, Brashler, Back and Askenase, 1984). Although this is a mast cell-independent response, it is still thymus-dependent due to IgE involvement.

These results have shown that eosinophilic responses can be elicited by many means and further emphasize how poorly understood the factors which control these responses are. The inherent fluctuations in eosinophil numbers and distribution complicate the situation, particularly on occasions when information is recorded at various times over a 24 hour period. Although these fluctuations can be minimised by careful experimental design and controlled laboratory conditions, a question mark remains over results recorded in clinical situations where factors such as stress and hormonal variations are virtually impossible to standardise for a study.

APPENDIX

AN EVALUATION OF HISTOLOGICAL TECHNIQUES FOR VISUALIZING EOSINOPHILS

Analysis of Blood Cells

Before blood cells can be counted, the blood must first be collected into a suitable anticoagulant. The calcium chelater EDTA has been used throughout this work because it has been reported to have a less detrimental effect on blood cells than heparin. A comparison of the two revealed that EDTA produced better leukocyte stainability and preserved the integrity of the cells better when used at the correct concentration. The dipotassium salt of EDTA was used because it is more soluble than the disodium salt (Schalm, Jain and Carroll, 1975).

Staining methods for tissue eosinophils

Eosinophils are usually identified by their characteristic granules, but certain staining procedures do not always reveal the granules, particularly in rodents. As recently as 35 years ago many haematologists still believed that eosinophils did not exist in the mouse because the staining procedures developed for human eosinophils did not stain the granules of mouse eosinophils so intensely (Speirs, 1980). In these studies it was identification of tissue eosinophils which was more of a problem, since all blood eosinophil counts were performed on cell suspensions using Discombes fluid rather than by analysis of blood smears, which are much more liable to lend to misinterpretation.

Initially, a variety of fixatives were tested on tissue samples recovered from mice infected with *T. spiralis* which were guaranteed to contain a large number of eosinophils. The fixatives included 10% formalin, Hellys, St Marie and Carnoys. The effect of these fixatives

on the ease of identification of eosinophils in sections stained with haemalum and eosin revealed that formalin-based fixatives were the best fixatives for eosinophil preservation, the simplest being 10% formalin which was used on all subsequent tissue samples.

After fixation, the tissues were embedded in paraffin wax for sectioning. All traces of unbound formalin were washed out of the tissues by placing the tissues (always less than 1 cm thick) in running tap water for 2-3 hours. Tissues were then dehydrated by passage through a series of alcohols: 30%, 50%, 70%, 90% and two changes of absolute alcohol, spending an hour in each solution. Tissue specimens were cleared in two changes of xylene, for an hour in each, transferred to a bath of 50% xylene/50% wax for 3 hours, then passed through two baths of 100% wax for 3 hours each and finally embedded in wax.

Sections 6 µm thick were cut from each specimen and transferred to 3" x 1" glass slides coated with a little egg albumin and allowed to dry before staining.

As can be seen from Plate 17, although H & E stains up eosinophil granules, it also produces a lot of background staining, and although eosinophils can be identified on a morphological basis by their nuclei, cytoplasmic staining is not distinct. For this reason, a variety of specialist stains were tested.

The first of these is a modification of the standard H & E stain, using an aqueous-based eosin which is then washed out from the background leaving eosinophil granules staining bright pink. An example of this stain can be seen in Plate 8, with eosinophils clearly visible.

The second technique is a method for the selective staining of eosinophil granules by alkaline orcein in a concentrated urea solution (Goldstein, 1963). Of all the methods tried this was the most unsatisfactory. Despite using the recommended fixative and counterstain, eosinophils around latex particles could not be demonstrated with confidence. Eosinophil granules should stain blue/purple.

The third method is a modified Dominici stain, described by Litt (1963). This method is shown in Plate 19. Although Bouin-Hollande fixative is recommended, formalin gives good results, probably because Bouin-Hollande is a formalin-based fixative. Litt reported that eosinophil granules should stain up orange. However, one of the problems of this technique is reproducibility. It was found that the stain varied markedly for different batches of slides due to loss of stain in the dehydration procedure (compare Plates 10 and 19).

The final method is a modification of the technique described by Lendrum (1944). Lendrum suggested a decolorizing step in 1% acid alcohol following the nuclear counterstain. However, it was found that better contrast was obtained if this step was omitted and the sections stained in Ehrlichs haematoxylin. This method can be seen in Plate 20, demonstrating a pulmonary eosinophilic infiltration in a T. spiralis-infected mouse, with eosinophils clearly visible. Of all the methods tried, this was the most satisfactory, since it could be reproduced in separate batches of slides.

The variability in aqueous-based stains is due to their differentiation by passage through alcohols during dehydration. The success of carbon chromotrope and modified H & E is probably due to the fact that they are used at high temperatures. Caution should be

exercised when using these techniques, since over-exposure to heat can result in detachment of the sections from the slides.

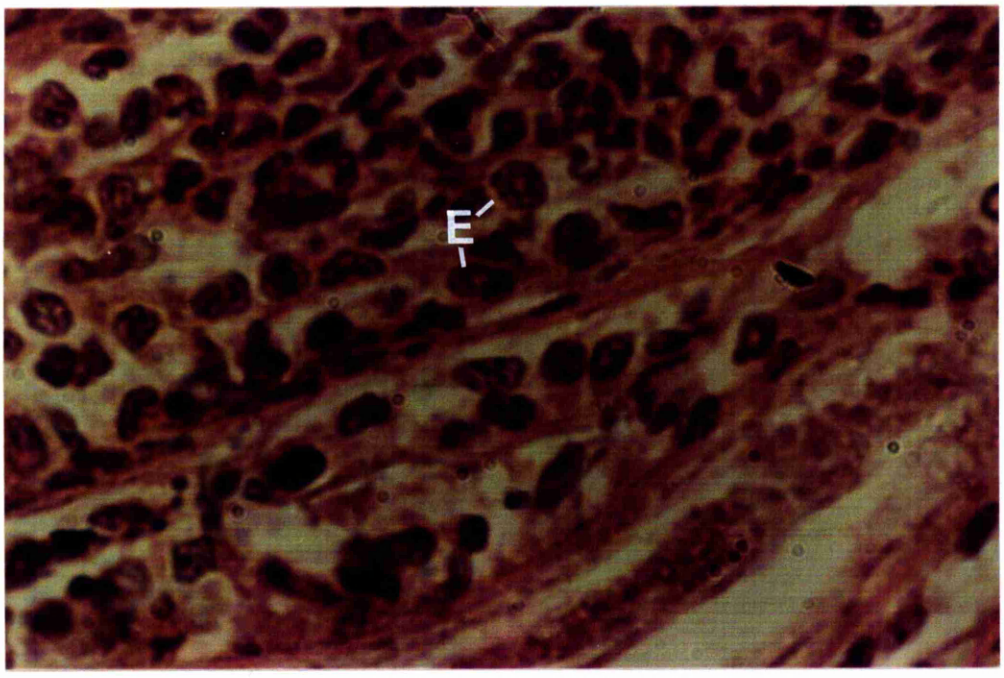


PLATE 17. Dense cellular infiltrate of skeletal muscle from a mouse infected with T. spiralis. Eosinophils mainly evident by nuclear morphology.
Haemalum and eosin x 1280.

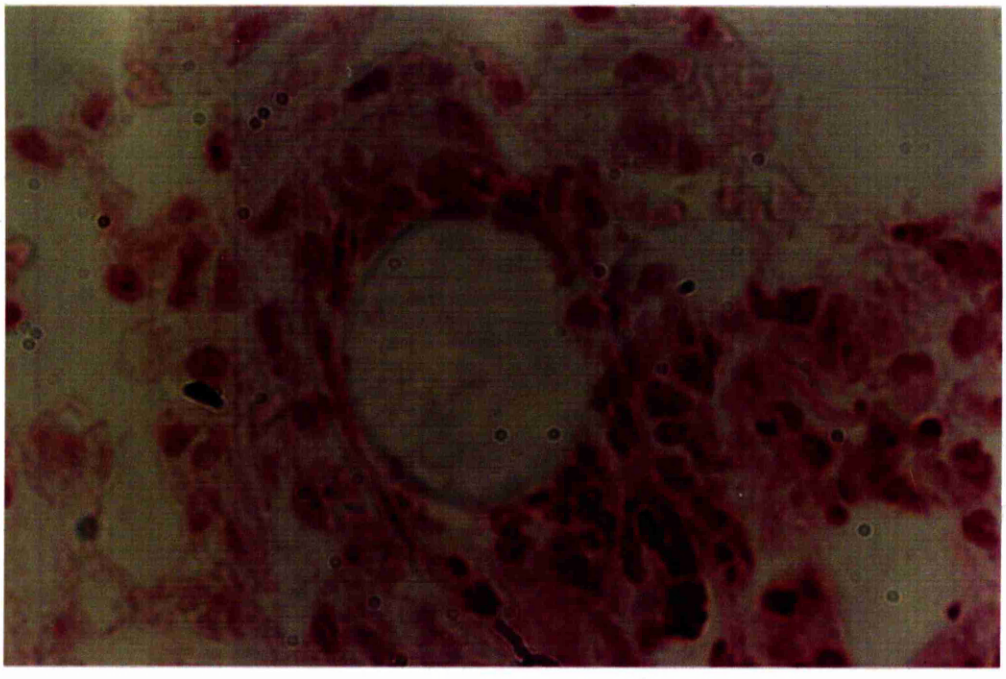


PLATE 18. Accumulation around a latex-BGG particle embolized in mouse lung. Eosinophils, visualized by other stains, are not easily identified.
Alkaline orcein and basic fuchsin x 1280

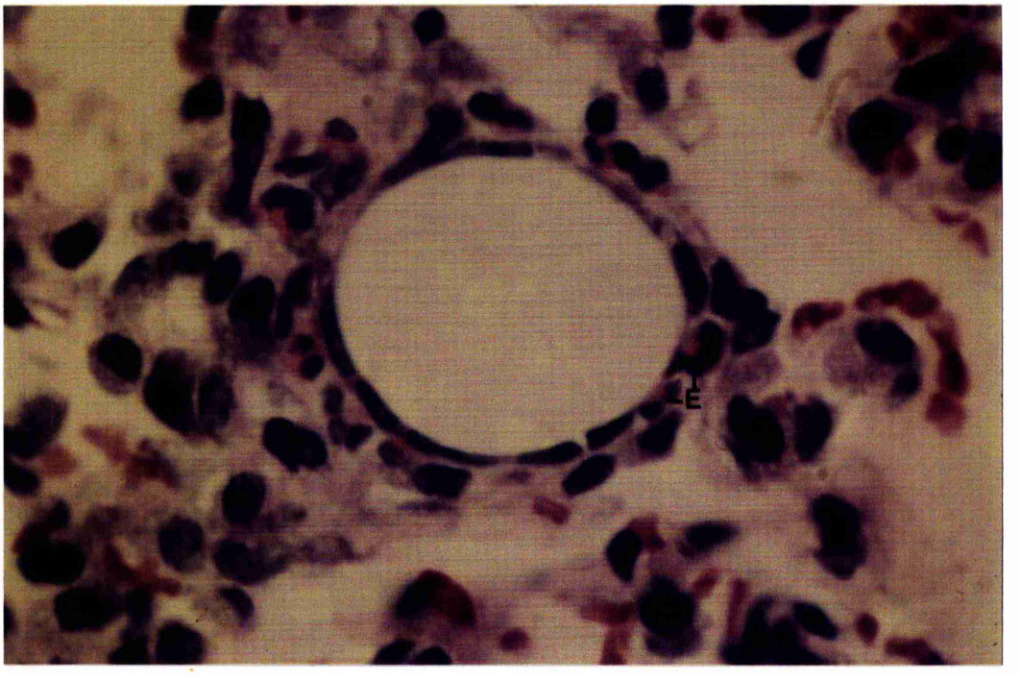


PLATE 19. Cell accumulation around a latex-BGG particle embolized in mouse lung. Eosinophils identified by their granules.
Modified Dominici x 1280

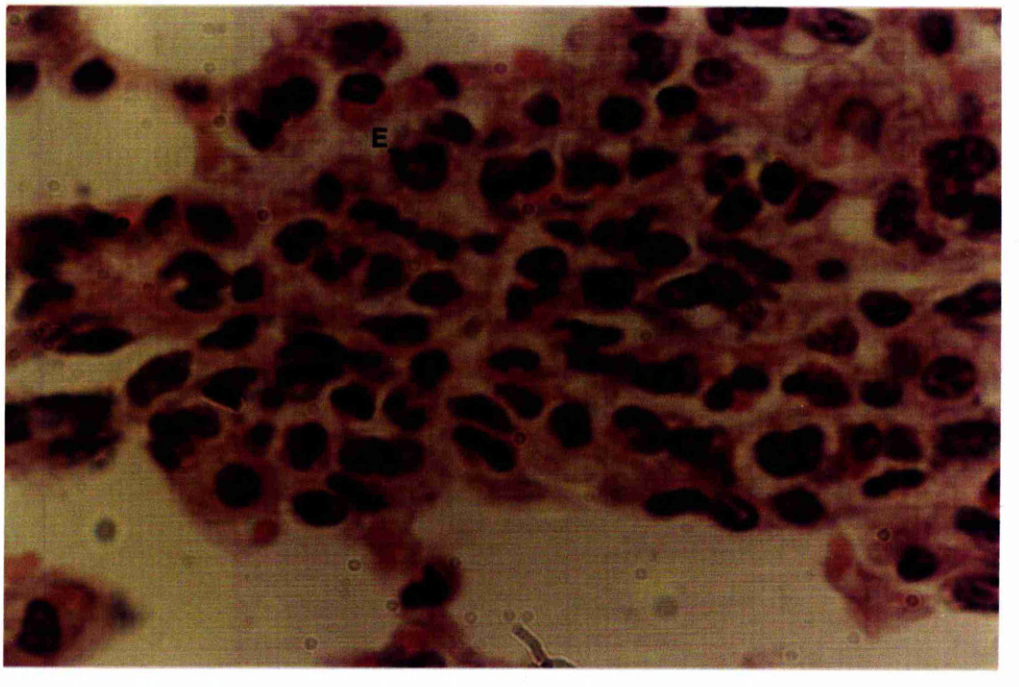


PLATE 20. Eosinophilic infiltrate of lung from a mouse infectd with T. spiralis. Eosinophils easily identified by granular staining.
Carbol chromotrope and Ehrlichs haematoxylin x 1280

Haemalum and Eosin

Xylene 1	5 min
Absolute alcohol 1	5 min
90% alcohol	3 min
70% alcohol	3 min
50% alcohol	3 min
30% alcohol	3 min
Distilled water	Rinse
Mayers haemalum	4 min
Scotts tap water	1 min
Distilled water	Rinse
30% alcohol	3 min
50% alcohol	3 min
70% alcohol	3 min
90% alcohol	3 min
0.5% eosin in absolute alcohol	1 min
Absolute alcohol 2	1 min
Xylene 2	2 min
Xylene 3	3 min

Modified Haemalum and Eosin

Xylene 1	5 min
Absolute alcohol 1	5 min
90% alcohol	3 min
70% alcohol	3 min
50% alcohol	3 min
30% alcohol	3 min
Distilled water	Rinse
Mayers haemalum	4 min
Scotts tap water	1 min
Distilled water	Rinse
1.5% aqueous eosin at 60°C	15 min
Wash in running tap water	30-45 min
30% alcohol	3 min
50% alcohol	3 min
70% alcohol	3 min
90% alcohol	3 min
Absolute alcohol 2	2 min
Xylene 2	2 min
Xylene 3	3 min

Alkaline Orcein in Urea

Xylene 1	5 min
Absolute alcohol 1	5 min
90% alcohol	3 min
70% alcohol	3 min
Orcein in saturated urea	15 min
0.5% aqueous basic fuchsin	2 min
95% alcohol	30 sec
Absolute alcohol 2	30 sec
Xylene 2	2 min
Xylene 3	3 min

The orcein solution was made by dissolving 2 g of sodium diethylbarbiturate in 100 ml of 70% alcohol. The pH was brought to 9 by the cautious addition of dilute HCl, then 1 g of orcein was added. Finally, urea was added to saturation.

Modified Dominici

Xylene 1	5 min
Absolute alcohol 1	5 min
90% alcohol	3 min
70% alcohol	3 min
50% alcohol	3 min
30% alcohol	3 min
Distilled water	Rinse
0.5% orange G/0.5% eosin Y	5 min
0.1M PBS, pH 6.2	Rinse
0.3% toluidine blue O	15 sec
0.1M PBS, pH 6.2	Rinse
70% alcohol	30 sec
90% alcohol	30 sec
Absolute alcohol	2 min
Xylene 2	2 min
Xylene 3	3 min

The staining solutions were prepared in 0.1M PBS, pH 6.2.

Carbol Chromotrope and Haematoxylin

Xylene 1	5 min
Absolute alcohol	5 min
90% alcohol	3 min
70% alcohol	3 min
50% alcohol	3 min
30% alcohol	3 min
Distilled water	Rinse
Ehrlichs haematoxylin	3 min
Distilled water	Rinse
Carbol chromotrope at 60°C	30 min
Tap water	Rinse
70% alcohol	1 min
90% alcohol	1 min
Absolute alcohol 2	2 min
Xylene 2	2 min
Xylene 3	3 min

The carbol chromotrope solution was made by dissolving 1g of pure phenol crystals in a flask by gently heating the outside of the flask with hot tap water. 0.5 g of carbol chromotrope 2R was added to the phenol and mixed well. This mixture was then dissolved in 100 ml of distilled water and filtered. The stain keeps for at least 3 months.

REFERENCES

AARDEN, L.A., BRUNNER, T.K., CEROTTINI, J-C., DAYER, J-M., DE WECK, A.L., DINARELLO, C.A., DI SABATO, G., FARRAR, J.J., GERY, I., GILLIS, S., HANDSCHUMACHER, R.E., HENNEY, C.S., HOFFMANN, M.K., KOOPMAN, W.J., KRANE, S.M., LACHMAN, L.B., LEFKOWITS, I., MISHELL, R.I., MIZEL, S.B., OPPENHEIM, J.J., PAETKAU, V., PLATE, J., ROLLINGHOFF, M., ROSENTREICH, D., ROSENTHAL, A.S., ROSENWASSER, L.J., SCHIMPL, A., SHIN, H.S., SIMON, P.L., SMITH, K.A., WAGNER, H., WATSON, J.D., WECKER, E. AND WOOD, D.D. (1979). Revised nomenclature for antigen-nonspecific T cell proliferation and helper factors. J. Immunol., 123, 2929.

ALI KHAN, A. (1966). The post-embryonic development of Trichinella spiralis with special reference to ecdysis. J. Parasitol., 52, 248-259.

ALTMAN, L.C., HILL, J.S., HAIRFIELD, W.M. and MULLARKEY, M.F. (1981). Effects of corticosteroids on eosinophil chemotaxis and adherence. J. Clin. Invest., 67, 28-36.

ARCHER, G.T. and HIRSCH, J.G. (1963). Isolation of granules from eosinophil leukocytes and study of their enzyme content. J. Exp. Med., 118, 277-286.

ASFELDT, V.H. (1971). Plasma corticosteroids in normal individuals with reference to the circadian rhythm, ACTH test, insulin test and dexamethasone suppression test. Scand. J. Clin. Lab. Invest., 28, 61-70.

BAEHNER, R.L. and JOHNSTONE, R.B. (1971). Metabolic and bactericidal activities of human eosinophils. Br. J. Haematol., 20, 277-285.

BARRINGA, O.O. (1981). Influence of routine extraction procedures in the composition of Trichinella spiralis extracts. J. Parasitol., 67, 120-123.

BARTLEMETZ, S.H., DODGE, W.H. and BASS, D.A. (1980). Differential regulation of spleen cell-mediated eosinophil and neutrophil-macrophage production. Blood, 55, 489-493.

BASS, D.A. and SZEJDA, P. (1979). Eosinophils versus neutrophils in host defence. Killing of newborn larvae of Trichinella spiralis by human granulocytes in vitro. J. Clin. Invest., 64, 1415-1422.

BASTEN, A. and BEESON, P.B. (1970). Mechanism of eosinophilia. II. Role of the lymphocyte. J. Exp. Med., 131, 1288-1305.

BASTEN, A., BOYER, M.H. and BEESON, P.B. (1970). Mechanism of eosinophilia. I. Factors affecting the eosinophil response of rats to Trichinella spiralis. J. Exp. Med., 131, 1271-1287.

BELL, R.G., McGREGOR, D.D. and ADAMS, L.S. (1982). Studies on the inhibition of rapid expulsion of Trichinella spiralis in rats. Int. Arch. Allergy Appl. Immunol., 69, 73-80.

BELL, R.G., McGREGOR, D.D. and DESPOMMIER, D.D. (1980). Trichinella spiralis: Mediation of the intestinal component of protective immunity in the rat by multiple, phase specific, antiparasitic responses. Exp. Parasitol., 47, 140-157.

BOLTZ-NITULESCU, G. and SPIEGELBERG, H.L. (1981). Receptors specific for IgE on rat alveolar macrophages. Cell. Immunol., 59, 106-114.

BROWN, T.R. (1898). Studies on trichinosis with especial reference to the increase of the eosinophilic cells in the blood and muscle, the origin of these cells and their diagnostic importance. J. Exp. Med., iii, 315-348.

BUYS, J., WEVER, R., VAN STIGT, R. and RUITENBERG, E.J. (1981). The killing of newborn larvae of Trichinella spiralis by eosinophil peroxidase in vitro. Eur. J. Immunol., 11, 843-845.

CAMPBELL, C.H. (1955). The antigenic role of the excretions and secretions of Trichinella spiralis in the production of immunity in mice. J. Parasitol., 41, 483-491.

CAMPBELL, D.H. (1943). Relationship of the eosinophil response to factors involved in anaphylaxis. J. Infect. Dis., 72, 42-48.

CHANDLER, A.C. and READ, C.P. (eds) (1961). P403-413 in Introduction to Parasitology (10th edition). John Wiley and Sons (New York).

CHEEVER, A.W. (1968). A quantitative post-mortem study of Schistosomiasis mansoni in man. Am. J. Trop. Med. Hyg., 17, 38-64.

CHENSUE, S.W., WELLHAUSEN, S.R. and BOROS, D.L. (1981). Modulation of granulomatous hypersensitivity. II. Participation of Ly1 and Ly2 T lymphocytes in the suppression of granuloma formation and lymphokine production in Schistosoma mansoni-infected mice. J. Immunol., 127, 363-367.

CLARKE, R.A.F., GALLIN, J.I. AND FAUCI, A.S. (1979). Effects of in vivo prednisone on in vitro eosinophil and neutrophil adherence and chemotaxis. Blood, 53, 633-641.

COHEN, S. and WARD, P.A. (1971). In vitro and in vivo activity of a lymphocyte and immune complex-dependent chemotactic factor for eosinophils. J. Exp. Med., 133, 133-146.

COLLEY, D.G. (1973). Eosinophils and immune-mechanisms. I. Eosinophil Stimulation Promoter (ESP): A lymphokine induced by specific antigen or phytohaemagglutinin. J. Immunol., 110, 1419-1423.

COLLEY, D.G. (1975). Immune responses to a soluble schistosomal egg antigen preparation during chronic primary infection with Schistosoma mansoni. J. Immunol., 115, 150-156.

COLLEY, D.G. (1976). Variations in peripheral blood eosinophil levels in normal and Schistosoma mansoni-infected mice. J. Lab. Clin. Med., 83, 871-876.

CROFTON, J. and DOUGLAS, A. (eds) (1981). Chapter 28 in Respiratory Diseases (3rd edition). Blackwell Scientific Publications (Oxford).

DALTON, A.J. and SELYE, H. (1939). The blood picture during the alarm reaction. Folia. Haematol., 62, 397-407.

DALY, J.R. and ELSTEIN, M. (1972). Hypothalamo-pituitary-adrenal function during oral contraceptive therapy with combined oestrogen-progestogen and progestogen-only preparations. J. Obstet. Gynaec. Br. Common W., 79, 544-549.

DE CHATELET, L.R., MIGLER, R.A., SHIRLEY, P.S., MUSS, H.B., SZEJDA, P. and BASS, D.A. (1978). Comparison of intracellular bactericidal activities of human neutrophils and eosionophils. Blood, 52, 609-617.

DESPOMMIER, D.D. (1974). P187-198. in Trichinellosis. C.W. Kim (ed). Intext Educational Publishers (New York).

DESPOMMIER, D.D., CAMPBELL, W.C. and BLAIR, L.S. (1977). The in vivo and in vitro analysis of immunity to Trichinella spiralis in mice and rats. Parasitology, 74, 109-119.

DESPOMMIER, D.D. and LACCETTI, A. (1980). Trichinella spiralis: Proteins and antigens isolated from a large-particle fraction derived from the muscle larva. Exp. Parasitol., 51, 279-295.

DESPOMMIER, D.D. and LACCETTI, A. (1981). Trichinella spiralis: Partial characterization of antigens isolated by immuno-affinity chromatography from the large-particle fraction of the muscle larvae. J. Parasitol., 67, 322-339.

DESPOMMIER, D.D. and MULLER, M. (1976). The stichosome and its secretion granules in the mature muscle larva of Trichinella spiralis. J. Parasitol., 62, 775-785.

DESSEIN, A.J., PARKER, W.L., JAMES, S.L. and DAVID, J.R. (1981). IgE antibody and resistance to infection. I. Selective suppression of the IgE antibody response in rats diminishes the resistance and the eosinophil response to Trichinella spiralis infection. J. Exp. Med., 153, 423-436.

DISCOMBE, G. (1946). Criteria of eosinophilia. Lancet i, 195-196.

DOMINGO, E.O. and WARREN, K.S. (1968). Endogenous desensitization: Changing host granulomatous response to schistosome eggs at different stages of infection with Schistosoma mansoni. Am. J. Pathol., 52, 369-377.

DOY, T.G. and HUGHES, D.L. (1982). The role of the thymus in the eosinophil response of rats infected with Fasciola hepatica. Clin. Exp. Immunol., 47, 74-76.

EADY, R.P., TRIGG, S. and ORR, T.S.C. (1978). Anaphylactic bronchoconstriction in rats immunized with antigen-coated latex particles. Clin. Exp. Immunol., 32, 283-289.

ECKERT, R. (1978). Chapter 11 in Animal Physiology. Eckert, R. and Randall, D. (eds). W.H. Freeman and Co. (San Fransisco).

FINE, D.P., BUCHANAN, R.D. and COLLEY, D.G. (1973). Schistosoma mansoni infection in mice depleted of thymus dependent lymphocytes. I. Eosinophilia and immunologic responses to a schistosomal egg preparation. Am. J. Pathol., 71, 193-206.

FOOT, E.C. (1963). Eosinophil turnover in the rat. Nature, 198, 297-298.

GALLIN, J.I., WEINSTEIN, A.M., CRAMER, E.B. and KAPLAN, A.P. (1980). Chapter 11 in The Eosinophil in Health and Disease. Mahmoud, A.A.F. and Austen, K.F. (eds). Grune and Stratton (New York).

GARDINER, C.H. (1976). Habitat and reproductive behaviour of Trichinella spiralis. J. Parasitol., 62, 865-870.

GERY, I., DAVIES, P., DERR, J., KRETT, N. and BARRANGER, J.A. (1981). Relationship between production and release of lymphocyte-activating factor (interleukin 1) by murine macrophages. I. Effects of various agents. Cell. Immunol., 64, 293-303.

GLEICH, G.J., LOEGERING, D.A. and MALDONADO, J. (1973). Identification of a major basic protein in guinea pig eosinophil granules. J. Exp. Med., 137, 1459-1471.

GOETZL, E.J. (1980). Chapter 10 in The Eosinophil in Health and Disease. Mahmoud, A.A.F. and Austen, K.F. (eds). Grune and Stratton (New York).

GOETZL, E.J. and AUSTEN, K.F. (1980). Chapter 9 in The Eosinophil in Health and Disease. Mahmoud, A.A.F. and Austen, K.F. (eds) Grune and Stratton (New York).

GOLDSTEIN, D.J. (1963). Selective staining of eosinophil granules in sections by alkaline orcein in a concentrated urea solution. Stain. Technol., 38, 49-51.

GREENE, B.M. and COLLEY, D.G. (1976). Eosinophils and immune mechanisms. III. Production of the lymphokine eosinophil stimulation promoter by mouse T lymphocytes. J. Immunol., 116, 1078-1083.

GREENE, W.C., FLEISHER, T.A. and WALDMANN, T.A. (1981). Soluble suppressor supernatants elaborated by concanavalin A-activated human mononuclear cells. I. Characterization of a soluble suppressor of T cell proliferation. J. Immunol., 126, 1185-1191.

GROVE, D.I., MAHMOUD, A.A.F. and WARREN, K.S. (1977). Eosinophils and resistance to Trichinella spiralis. J. Exp. Med., 145, 755-759.

HALLAM, C., PRITCHARD, D.I., TRIGG, S. and EADY, R.P. (1982). Rat eosinophil-mediated antibody-dependent cellular cytotoxicity: Investigations of the mechanisms of target cell lysis and inhibition by glucocorticoids. Clin. Exp. Immunol., 48, 641-648.

HARLEY, J.P. AND GALLICHIO, V. (1971). Trichinella spiralis: Migration of larvae in the rat. Exp. Parasitol., 30, 11-21.

HARTMANN, D.P., GEORGIAN, M.M., OGHISO, Y. and KAGAN, E. (1984). Enhanced interleukin activity following asbestos inhalation. Clin. Exp. Immunol., 55, 643-650.

HILLS, A.G., FORSHAM, P.H. and FINCH, C.A. (1948). Changes in circulating leukocytes induced by the administration of pituitary adrenocorticotropic hormone (ACTH) in man. Blood, 3, 755-768.

HIRASHIMA, M., TASHIRO, K., SAKATA, K. and HIRASHIMA, M. (1984). Isolation of an eosinophil chemotactic lymphokine as a natural mediator for eosinophil chemotaxis from concanavalin A-induced reaction sites in guinea pigs. Clin. Exp. Immunol., 57, 211-219.

HIRSCH, J.G. AND HIRSCH, B.I. (1980). Chapter 1 in The Eosinophil in Health and Disease. Mahmoud, A.A.F. and Austen, K.F. (eds). Grune and Stratton (New York).

ISHIZAKA, K., YODOI, J., SUEMURA, M. and HIRASHIMA, M. (1983). Isotype-specific regulation of the IgE response by IgE-binding factors. Immunology Today, 4, 192-196.

JACKSON, G.J. (1959). Fluorescent antibody studies of Trichinella spiralis infections. J. Infect. Dis., 105, 97-117.

JAMES, S.L. and COLLEY, D.G. (1978). Eosinophil-mediated destruction of Schistosoma mansoni eggs. III. Lymphokine involvement in the induction of eosinophil functional abilities. Cell. Immunol., 38, 48-58.

JENSEN, D.L. and CASTRO, G.A. (1981). Trichinella spiralis: Generation in the presence of rat serum of factors chemotactic for rat cells. Exp. Parasitol., 52, 53-61.

JUNGERY, M., CLARK, N.W.T. and PARKHOUSE, R.M.E. (1983). A major change in surface antigens during the maturation of newborn larvae of Trichinella spiralis. Mol. Biochem. Parasitol., 7, 101-109.

KATZ, D.H. (1978). Immunoglobulin E. The allergic phenotype: Manifestation of 'allergic breakthrough' and imbalance in normal damping of IgE antibody production. Immunol. Rev., 41, 77-108.

KAY, A.B. (1970). Studies on eosinophil leukocyte migration. II. Factors specifically chemotactic for eosinophils and neutrophils generated from guinea pig serum by antigen-antibody complexes. Clin. Exp. Immunol., 7, 723-737.

KAY, A.B. (1979). The role of the eosinophil. J. Allergy Clin. Immunol., 64, 90-104.

KAZURA, J.W. and AIKAWA, M. (1980). Host defence mechanisms against Trichinella spiralis infection in the mouse: Eosinophil-mediated destruction of newborn larvae in vitro. J. Immunol., 124, 355-361.

KAZURA, J.W. and GROVE, D.I. (1978). Stage-specific antibody-dependent eosinophil-mediated destruction of Trichinella spiralis. Nature, 274, 588-590.

KAZURA, J.W., MAHMOUD, A.A.F., KARB, K.S. and WARREN, K.S. (1975). The lymphokine eosinophil stimulation promoter and human Schistosomiasis mansoni. J. Infect. Dis., 132, 702-706.

KELLER, R., HESS, M.W. and RILEY, J.F. (1976). Mast cells in the skin of normal hairless and athymic mice. Experentia, 32, 171-172.

KIMURA, I., MORITANI, Y. and TANIZAKI, Y. (1973). Basophils in bronchial asthma with reference to reagin-type allergy. Clin. Allergy, 3, 195-202.

KLEBANOFF, S.J., JONG, E.C. and HENDERSON, W.R. (1980). Chapter 6 in The Eosinophil in Health and Disease. Mahmoud, A.A.F. and Austen, K.F. (eds). Grune and Stratton (New York).

LACHMAN, L.B., HACKER, M.P. and HANDSCHUMACHER, R.E. (1977). Partial purification of human lymphocyte-activating factor (LAF) by ultrafiltration and electrophoretic techniques. J. Immunol., 119, 2019-2023.

LEE, S.H. (1982). Uterine epithelial and eosinophil oestrogen receptors in rats during the oestrus cycle. Histochemistry, 74, 443-452.

LENDRUM, A.C. (1944). The staining of eosinophil polymorphs and entero-chromaffin cells in histological sections. J. Path. Bact., 56, 441.

von LICHTENBERG, F. (1962). Host response to eggs of S. mansoni. I. Granuloma formation in the unsensitized laboratory mouse. Am. J. Path., 41, 711-731.

LITT, M. (1963). Studies in experimental eosinophilia. V. Eosinophils in lymph nodes of guinea pigs following primary antigenic stimulation. Am. J. Path., 42, 529-547.

LITT, M. (1964). Eosinophils and antigen-antibody reactions. Ann. N.Y. Acad. Sci., 116, 964-985.

LOVE, R.J., OGILVIE, B.M. and McLAREN, D.J. (1976). The immune mechanism which expels the intestinal stage of Trichinella spiralis from rats. Immunology, 30, 7-15.

MACDONALD, H.R. and LEES, R.K. (1984). Frequency and specificity of precursors of IL-2 producing cells in nude mice. J. Immunol., 132, 605-610.

MACDONALD, H.R., LEES, R.K., SORDAT, B., ZAECH, P., MARYANSKI, J.L. and BRON, C. (1981). Age-associated increase in expression of the T cell surface markers Thy-1, Lyt-1 and Lyt-2 in congenitally athymic (nu/nu) mice: Analysis by flow microfluorometry. J. Immunol., 126, 865-870.

MACKENZIE, C.D., PRESTON, P.M. and OGILVIE, B.M. (1978). Immunological properties of the surface of parasitic nematodes. Nature, 276, 826-828.

MAHMOUD, A.A.F. (1980). Chapter 4 in The Eosinophil in Health and Disease. Mahmoud, A.A.F. and Austen, K.F. (eds). Grune and Stratton (New York).

MAHMOUD, A.A.F., STONE, M.K. and KELLERMEYER, R.W. (1977). Eosinophilopoietin: A circulating low molecular weight peptide-like substance which stimulates the production of eosinophils in mice. J. Clin. Invest., 60, 675-682.

MANSON BAHR, P.H. (ed) (1966). P963-966 in Mansons Tropical Diseases (16th edition). Balliere, Tindall and Cassel (London).

MILLER, A.M., COLLEY, D.G. and McGARRY, M.P. (1976). Spleen cells from Schistosoma mansoni-infected mice produce diffusible stimulator of eosinophilopoiesis in vivo. Nature, 262, 586-587.

MILLER, F., DE HARVEN, E. and PALADE, G.E. (1966). The structure of the eosinophil leukocyte granules in rodents and man. J. Cell. Biol., 31, 349-362.

MOORE, D.V. and SANDGROUND, J.H. (1956). The relative egg producing capacity of Schistosoma mansoni and Schistosoma japonicum. Am. J. Trop. Med. Hyg., 5, 831-840.

McCOY, O.R. (1940). Rapid loss of Trichinella larvae fed to immune rats and its bearing on the mechanism of immunity. Am. J. Hyg., 32 (Sect D), 105-116.

OGILVIE, B.M. (1967). Reagin-like antibodies in rats infected with the nematode parasite Nippostrongylus brasiliensis. Immunology, 12, 113-131.

OGILVIE, B.M., ASKENASE, P.W. and ROSE, M.E. (1980). Basophils and eosinophils in three strains of rats and athymic (nude) rats following infection with the nematodes Nippostrongylus brasiliensis or Trichinella spiralis. Immunology, 39, 385-389.

OLDS, G.R. and MAHMOUD, A.A.F. (1980). Role of host granulomatous response in murine Schistosomiasis mansoni: Eosinophil mediated destruction of eggs. J. Clin. Invest., 66, 1191-1199.

OLIVER-GONZALEZ, J. (1940). The in vitro action of immune serum on the larvae and adults of Trichinella spiralis. J. Infect. Dis., 67, 292-300.

OLIVER-GONZALEZ, J. (1941). The dual antibody basis of acquired immunity in trichinosis. J. Infect. Dis., 69, 254-270.

OPIE, E.L. (1904a). The occurrence of cells with eosinophile granulation and their relation to nutrition. Am. J. Med. Sci., 127, 217-239.

OPIE, E.L. (1904b). An experimental study of the relation of cells with eosinophile granulation to infection with an animal parasite (Trichina spiralis). Am. J. Med. Sci., 127, 477-493.

ORR, T.S.C. (1973). Mast cells and allergic asthma. Brit. J. Dis. Chest., 67, 87-106.

ORTEGA-PIERRES, G., CHAYEN, A., CLARK, N.W.T. and PARKHOUSE, R.M.E. (1984). The occurrence of antibodies to hidden and exposed determinants of surface antigens of Trichinella spiralis. Parasitology, 88, 359-369.

PARISH, C.R. and McKENZIE, I.F.C. (1978). A sensitive rosetting method for detecting subpopulations of lymphocytes which react with alloantisera. J. Immunol. Methods., 20, 173-183.

PARKHOUSE, R.M.E. and CLARK, N.W.T. (1983). Stage specific secreted and somatic antigens of Trichinella spiralis. Mol. Biochem. Parasitol., 9, 319-327.

PARROTT, D.M.V. (1967). The response of draining lymph nodes to immunological stimulation in intact and thymectomized animals. J. Clin. Pathol., 20, 456-465.

PERRUDET-BADOUX, A., BOUSSAC-ARON, Y., RUITENBERG, E.J. and ELGERSMA, A. (1980). Preliminary studies on the course of a Trichinella spiralis infection in athymic nude rats. J. Parasitol., 66, 671-673.

PETERSON, A.P., ALTMAN, L.C., HILLS, J.S., GOSNEY, K. and KADIN, M.E. (1981). Glucocorticoid receptors in normal human eosinophils: comparison with neutrophils. J. Allergy Clin. Immunol., 68, 212-217.

PHILIPP, M., PARKHOUSE, R.M.E. and OGILVIE, B.M. (1980). Changing proteins on the surface of a parasitic nematode. Nature, 287, 538-540.

PHILIPP, M., TAYLOR, P.M., PARKHOUSE, R.M.E. and OGILVIE, B.M. (1981). Immune response to stage-specific surface antigens of the parasitic nematode Trichinella spiralis. J. Exp. Med., 154, 210-215.

PHILLIPS, S.M., Di CONZA, J.J., GOLD, J.A. and REID, W.A. (1977). Schistosomiasis in the congenitally athymic (nude) mouse. I. Thymic dependency of eosinophilia, granuloma formation, and host morbidity. J. Immunol., 118, 594-599.

PRITCHARD, D.I. and EADY, R.P. (1981). Eosinophilia in athymic nude (rnu/rnu) rats - thymus independent eosinophilia. Immunology, 43, 409-416.

RAND, T.H., CLANTON, J.A., RUNGE, V., ENGLISH, D. and COLLEY, D.G. (1983). Murine eosinophilia labelled with Indium-III Oxine: Localisation to delayed hypersensitivity reactions against a Schistosomal antigen and to lymphokine in vivo. Blood, 61, 732-739.

RAND, T.H. and COLLEY, D.G. (1982). Influence of a lymphokine fraction containing eosinophil stimulation promoter (ESP) on oxidative and degranulation responses of murine eosinophils. Cell. Immunol., 71, 334-345.

RANKIN, J.A., HITCHCOCK, M., MERRILL, W.W., HUANG, S.S., BRASHLER, J.R., BACK, M.K. and ASKENASE, P.W. (1984). IgE immune complexes induce immediate and prolonged release of leukotriene C_4 (LTC_4) from rat alveolar macrophages. J. Immunol., 132, 1993-1999.

RENOLD, A.E., QUIGLEY, T.B., KENNARD, H.E. and THORN, G.W. (1951). Reaction of the adrenal cortex to physical and emotional stress in college oarsmen. New Eng. J. Med., 244, 754-757.

ROCHE, M., THORN, G.W. and HILLS, A.G. (1950). The levels of circulating eosinophils and their response to ACTH in surgery. (Their use as an index of adrenocortical function). New Eng. J. Med., 242, 307-314.

ROITT, I.M. (1977). Chapter 6 in Essential Immunology (3rd edition). Blackwell Scientific Publications (Oxford).

ROTHWELL, T.L.W. and LOVE, R.J. (1975). Studies on the responses of basophil and eosinophil leukocytes and mast cells to the nematode Trichostrongylus colubriformis. II. Changes in cell numbers following infection of thymectomized and adoptively or passively immunized guinea pigs. J. Pathol., 116, 183-194.

RUITENBERG, E.J. and ELGERSMA, A. (1976). Absence of intestinal mast cell response in congenitally athymic mice during Trichinella spiralis infection. Nature, 264, 258-260.

RUITENBERG, E.J., ELGERSMA, A., KRUIZINGA, W. and LEENSTRA, F. (1977). Trichinella spiralis infection in congenitally athymic (nude) mice: Parasitological, serological and haematological studies with observations on intestinal pathology. Immunology, 33, 581-587.

RYTOMAA, T. (1960). Organ distribution and histochemical properties of eosinphil granulocytes in the rat. Acta. Pathol. Microbiol. Scand., 50, (Suppl. 140), 1856-1865.

SABAG, N., CASTRILLON, M.A. and TCHERNITCHIN, A. (1978). Cortisol-induced migration of eosinophil leukocytes to lymphoid organs. Experentia, 34, 666-667.

SARLES, M.P. (1938). The in vitro action of immune rat serum on the nematode Nippostrongylus muris. J. Infect. Dis., 62, 337-348.

SCHALM, O.W., JAIN, N.C. and CARROLL, E.J. (eds) (1975). Chapter 2 in Veterinary Haematology (3rd edition). Lea and Febiger (Philadelphia).

SCHRIBER, R.A. and ZUCKER-FRANKLIN, D. (1974). A method for the induction of blood eosinophilia with simple protein antigens. Cell. Immunol., 14, 470-474.

SCHRIBER, R.A. and ZUCKER-FRANKLIN, D. (1975). Induction of blood eosinophilia by pulmonary embolization of antigen-coated particles: The relationship to cell-mediated immunity. J. Immunol., 114, 1348-1353.

SHARP, A.K. and COLSTON, M.J. (1984). The regulation of macrophage activity in congenitally athymic mice. Eur. J. Immunol., 14, 102-105.

SHER, R., WADEE, A. and JOFFE, M. (1983). The enhancement of eosinophil function by lymphocyte supernatants. Clin. Exp. Immunol., 51, 525-534.

SILBERSTEIN, D.S. and DESPOMMIER, D.D. (1984). Antigens from Trichinella spiralis that induce a protective response in the mouse. J. Immunol., 132, 898-904.

SIN, Y.M. and SAINTE-MARIE, G. (1965). Granulocytopoiesis in the rat thymus. I. Description of the cells of the neutrophilic and eosinophilic series. Br. J. Haematol., 11, 613-623.

SOULSBY, E.J.L (ed) (1965). P824 in Helminths, Arthropods and Protozoa of Domesticated Animals. Balliere, Tindall and Cassel (London).

SPIERS, R.S. (1980). Chapter 4 in The Eosinophil in Health and Disease. Mahmoud, A.A.F. and Austen, K.F. (eds). Grune and Stratton (New York).

SPITZNAGEL, J.K., DALLDORF, F.G., LEFELL, M.S., FOLDS, J.D., WELSH, I.R.H., COONEY, M.H. and MARTIN, L.E. (1974). Character of azurophil and specific granules purified from human polymorphonuclear leukocytes. Lab. Invest., 30, 774-785.

SPRY, C.J.F. (1971a). Mechanism of eosinophilia. V. Kinetics of normal and accelerated eosinopoiesis. Cell Tissue Kinet., 4, 351-364.

SPRY, C.J.F. (1971b). Mechanism of eosinophilia. VI. Eosinophil mobilization. Cell Tissue Kinet., 4, 365-374.

STEWART, G.L. and CHARNIGA, L.M. (1980). Distribution of Trichinella spiralis in muscles of the mouse. J. Parasitol., 66, 688-689.

STROBEL, S., MILLER, H.R.P. and FERGUSON, A. (1981). Human intestinal mucosal mast cells: Evaluation of fixation and staining techniques. J. Clin. Pathol., 34, 851-858.

SUGANE, K. and OSHIMA, T. (1982). Eosinophilia, granuloma formation and migratory behaviour of larvae in the congenitally athymic mouse infected with Toxocara canis. Parasite Immunol., 4, 307-318.

TANNER, C.E. and GREGORY, J. (1961). Immunochemical study of the antigens of Trichinella spiralis. I. Identification and enumeration of antigens. Canad. J. Microbiol., 7, 473-481.

TCHERNITCHIN, A.V. (1967). Autoradiographic study of (6,7-^{3}H) oestradiol-17ß incorporation into rat uterus. Steroids, 10, 661-668.

TCHERNITCHIN, A.V. (1970). Radioautographic analysis of 6,7-^{3}H oestradiol-17 ß uptake in rat uterus following extraction of endogenous oestrogen. Steroids, 15, 799-808.

TCHERNITCHIN, A.V. and GARLAND, P. (1983). Oestrogen levels in the blood, not in the uterus, determine uterine eosinophilia and oedema. J. Endocrinol., 99, 123-130.

TCHERNITCHIN, A.V. and TCHERNITCHIN, X. (1976). Characterization of the oestrogen receptors in the uterine and blood eosinophil leukocytes. Experentia, 32, 1240-1242.

TRONCHIN, G., DUTOIT, E., VERNES, A. and BIGUET, J. (1979). Immunization of mice with metabolic antigens of Trichinella spiralis larvae: Effects on the kinetics of intestinal cell response including mast cells and polymorphonuclear eosinophils. J. Parasitol., 65, 685-691.

UNGAR, F. (1964). In vitro studies of adrenal-pituitary circadian rhythms in the mouse. Ann. N.Y. Acad. Sci., 117, 374-385.

VADAS, M.A. (1981). Cyclophosphamide pretreatment induces eosinophilia to nonparasite antigens. J. Immunol., 127, 2083-2086.

VADAS, M.A. (1982). Genetic control of eosinophilia in mice: Gene(s) expressed in bone marrow-derived cells control high responsiveness. J. Immunol., 128, 691-695.

VADAS, M.A., NICOLA, N., LOPEZ, A.F., METCALF, D., JOHNSON, G. and PEREIRA, A. (1984). Mononuclear cell-mediated enhancement of granulocyte function in man. J. Immunol., 133, 202-207.

VAITUKAITIS, J.L. (1981). Production of antisera with small doses of immunogen: Multiple intradermal injections. Methods Enzymology, 73B, 46-52.

VEITH, M.C. and BUTTERWORTH, A.E. (1983). Enhancement of human eosinophil-mediated killing of Schistosoma mansoni larvae by mononuclear cell products in vitro. J. Exp. Med., 157, 1828-1843.

VENGE, P., DAHL, R., HALLGREN, R. and OLSSON, I. (1980). Chapter 8 in The Eosinophil in Health and Disease. Mahmoud, A.A.F. and Austen, K.F. (eds). Grune and Stratton (New York).

VOS, J.G., RUITENBERG, E.J., VAN BASTEN, N., BUYS, J., ELGERSMA, A. and KRUIZINGA, W. (1983). The athymic nude rat. IV. Immunocytochemical study to detect T cells and immunological and histopathological reactions against Trichinella spiralis. Parasite Immunol., 5, 195-215.

WAKELIN, D. (ed) (1984). Chapters 7 and 8 in Immunity to Parasites. How Animals Control Parasite Infections. Edward Arnold Ltd (London).

WAKELIN, D. and DONACHIE, A.M. (1983). Genetic control of eosinophilia. Mouse strain variation in response to antigens of parasite origin. Clin. Exp. Immunol., 51, 239-246.

WALLS, R.S., BASTEN, A., LEUCHARS, E. and DAVIES, A.J.S. (1971). Mechanisms for eosinophilic and neutrophilic leucocytoses. Br. Med. J., 3, 157-159.

WARREN, K.S. (1964). Experimental pulmonary schistosomiasis. Trans. Roy. Soc. Trop. Med. Hyg., 58, 228-233.

WARREN, K.S. (1972). The immunopathogenesis of schistosomiasis: A multidiciplinary approach. Trans. Roy. Soc. Trop. Med. Hyg., 66, 417-434.

WARREN, K.S., DOMINGO, E.O. and COWAN, R.B.T. (1967). Granuloma formation around schistosome eggs as a manifestation of delayed hypersensitivity. Am. J. Pathol., 51, 735-756.

WARREN, K.S., KARP, R., PELLEY, R.P. and MAHMOUD, A.A.F. (1976). The eosinophil stimulation promoter test in murine and human Trichinella spiralis infection. J. Infect. Dis., 134, 277-280.

WASSOM, D.L., BROOKS, B.O., BABISH, J.G. and DAVID, C.S. (1983). A gene mapping between the S and D regions of the H-2 complex influences resistance to Trichinella spiralis infections of mice. J. Immunogenet., 10, 371-378.

WASSOM, D.L. and GLEICH, G.J. (1979). Damage to Trichinella spiralis newborn larvae by eosinophil major basic protein. Am. J. Trop. Med. Hyg., 28, 860-863.

WEINSTOCK, J.V. and BOROS, D.L. (1981). Heterogeneity of the granulomatous response in the liver, colon, ileum and ileal Payers patches to schistosome eggs in murine Schistosomiasis mansoni. J. Immunol., 127, 1906-1909.

WEINSTOCK, J.V. and BOROS, D.L. (1983). Organ-dependent differences in composition and function observed in hepatic and intestinal granulomas isolated from mice with Schistosomiasis mansoni. J. Immunol., 130, 418-422.

WELLER, P.F. and GOETZL, E.J. (1979). The regulatory and effector roles of eosinophils. Adv. Immunol., 27, 339-371.

WELLER, P.F., WASSERMAN, S.I. and AUSTEN, K.F. (1980). Chapter 7 in The Eosinophil in Health and Disease. Mahmoud, A.A.F. and Austen, K.F. (eds). Grune and Stratton (New York).

VOFFEY, J.M. and BURGHAN, R.K. (1964). Myeloid metaplasia in the cervical lymph gland of the normal guine pig. J. Anat., 93, 681.

ZEIGER, R.S., YURDIN, D.L. and COLTEN, H.R. (1976). Histamine metabolism. II. Cellular and subcellular localization of the catabolic enzymes, histaminase, and histamine methyl transferase in human leukocytes. J. Allergy Clin. Immunol., 58, 172-179.

ZUCKER-FRANKLIN, D. (1980). Chapter 3 in The Eosinophil in Health and Disease. Mahmoud, A.A.F. and Austen, K.F. (eds). Grune and Stratton (New York).

SUPPLEMENT

The information presented here expands on some of the material presented in the thesis.

MATERIALS AND METHODS

Animals

All experiments with mice contained 6 animals per group. Experiments with rats were performed using 5 animals per group. Animals were maintained under conventional conditions, and were not specific germ free. The animals were allowed to settle to their conditions prior to use in the experiments. With the exception of counts on diurnal fluctuations, the animals were always routinely bled between 9.00 and 10.30 hours.

Latex Particles

Uncoated latex beads were prepared for injection into animals according to the method described for coated particles (page 32), with PBS alone as the incubation medium.

Histology

When lung samples were analysed for embolized particles, 15 particles were measured per lung. The variation in diameter size of the beads due to sectioning should be the same in each sample, and should therefore balance out over a number of sections.

With regard to Figs. 10-13, the fields were selected so as not to include part of a larva or capsule, but they did include cellular infiltrates around the parasites.

Results

The total WBC's for Figs. 3 and 4 are given below for a direct comparison with the percentage of eosinophils.

Day Number	No. WBC/ul ($x10^3$)	%Eosinophils
0	8.76	1.60
3	8.98	1.67
7	10.93	1.53
10	9.60	1.44
15	9.48	1.86
22	9.40	2.52
28	9.28	2.64
58	11.03	3.14
62	10.09	4.07
66	9.36	3.75
70	9.24	4.24
74	9.58	3.88
78	8.47	6.76
82	11.74	4.91

These results show that there are variations in the number of circulating WBC's over a long period of time, but there is not the consistent upward trend which is observed in the eosinophil population.

Discussion

The technique of using latex particles to localise antigenic material in the lungs of mice was a novel application at the time that this work was performed. This technique could be exploited to investigate a variety of *in vivo* responses to foreign particulate material. A major modification of the method presented here would be the use of a single purified parasite-derived antigen to coat the beads. In this way it may be possible to determine the importance of specific antigens in the development of a host immune response to

parasites, since the antigen could be localized in tissue in a similar fashion to that which occurs during a parasite infestation.

Uncoated latex beads were shown to elicit an eosinophilic response. This may be due to certain surface properties of the beads, for example, hydrophobicity. Alternatively, there is the possibility that they cause damage to the lung vessel endothelium which in turn acts as stimulus for eosinophil release. If so, this could be correlated with the tissue damage which occurs with parasites which have a lung stage in their life cycle. The serum from animals injected with latex beads could be analysed for the products of tissue damage to test this hypothesis.

Another possible reason for the response to uncoated beads could be the presence of pyrogen, since the washing buffers were not checked for its presence.

The results following injection of latex beads into Rnu/Rnu rats differed from those of Pritchard and Eady (1981). They did not observe a peripheral blood eosinophilia in Rnu/Rnu or Rnu/+ rats. This could be due to differences in the conditions of animal maintainence, since it appears that the immunological status of the animals at the time of injection of the beads is a critical factor in the development of an immune response. For example, intratracheal injection of agarose beads into BALB/c mice previously immunized with methylated BSA in FCA produces larger granulomas than agarose beads injected into unimmunized mice. This is true for both uncoated beads or beads conjugated to specific antigen (Kobayashi, Allred and Yoshida, 1985). Dextran beads are also capable of inducing pulmonary granulomas in mice, and are more effective in this respect than latex

beads (Allred, Kobayashi and Yoshida, 1985). Since dextran is a bacterial product, this further emphasises a possible role for pyrogen in this response.

References

Allred, D.C., Kobayashi, K and Yoshida, T. (1985). Anergy-like immunosuppression in mice bearing pulmonary foreign-body granulomatous inflammation. Am. J. Pathol., 121, 466-473.

Kobayashi, K., Allred, D.C. and Yoshida, T. (1985). Mechanisms of suppressed cell mediated immunity and impaired antigen-induced interleukin 2 production in granuloma-bearing mice. J. Immunol., 135, 2996-3003.