IMMUNITY TO *TRICHINELLA SPIRALIS*

THESIS

FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY

BY

RICHARD K GRENCIS

DEPARTMENT OF ZOOLOGY

UNIVERSITY OF GLASGOW

DECEMBER 1982

TABLE OF CONTENTS

TITLE PAGE i

CONTENTS ii

DEDICATION vi

ACKNOWLEDGEMENTS vii

PREFACE viii

STATEMENT BY AUTHORS ix

ABBREVIATIONS x

SUMMARY xii

GENERAL INTRODUCTION 1

GENERAL MATERIALS AND METHODS 12

A. General Techniques

Animals 13
Maintenance of *T. spiralis* 13
Worm recovery techniques 14
Anthelmintic 15
Antibiotic 16
Irradiation 16
Protein Estimation 16

B. Cell Techniques

Preparation of cell suspensions 17
Assays for blast cell activity 18
Cell enrichment techniques 19

C. Preparation of Antisera 22

Immunodiffusion 23

D. Statistics 23

E. Solutions 24

SECTION 1

The immune response of mice to vaccination with soluble worm antigens 27

Chapter 1

Immunization of mice with soluble worm antigen. An examination of the parameters required for effective protection 36

Summary Points: Chapter 1 52

Chapter 2

Adoptive transfer of immunity with cells taken from mice immunized with soluble worm antigen 53

Summary Points: Chapter 2 67

Chapter 3

Immunization of mice against T. spiralis with a soluble crude worm antigen. A brief examination and correlation with the responses known to occur after a natural infection 68

Summary Points: Chapter 3 78

SECTION 1 Discussion 79

SECTION 2

The cellular responses to infection of mice with T. spiralis 86

Chapter 4

The cellular response in mice to infection with T. spiralis: A preliminary analysis .. 92

Summary Points: Chapter 4 109

Chapter 5

Short lived dividing cells mediate adoptive transfer of immunity to T. spiralis in mice. I. Availability of cells in relation to cellular changes in the mesenteric lymph node .. 110

Summary Points: Chapter 5 118

Chapter 6

Analysis of the cells mediating immunity in mice to infection with T. spiralis. Positive and negative selection of MLNC 119

Summary Points: Chapter 6 137

Chapter 7

Short lived dividing cells mediate adoptive transfer of immunity to T. spiralis in mice. Characteristics of the cells involved .. 138

Summary Points: Chapter 7 163

Chapter 8

Memory to T. spiralis infections in mice, possible mechanisms involved 164

Summary Points: Chapter 8 181

Chapter 9

Generation of cells in MLN with the capacity to adoptively transfer immunity against T. spiralis in mice 182

Summary Points: Chapter 9 197

SECTION 2 Discussion 198

SECTION 3

Chapter 10

Immunity to T. spiralis in mice. Factors involved in direct anti-worm effects 208

Discussion 226

Summary Points: Chapter 10 230

SECTION 4

Chapter 11

Specific cross-immunity between T. spiralis and T. muris: immunization with heterologous infections and antigens and transfer of immunity with heterologous immune mesenteric lymph node cells 232

Discussion 237

Summary Points: Chapter 11 240

SUMMARY DISCUSSION 241

REFERENCES 249

To Pauline, with love.

ACKNOWLEDGEMENTS

This thesis would not have been completed without the guidance, help and encouragement of Professor Derek Wakelin. A great many thanks are due to him, and also to Professor C A Hopkins especially for the continued interest he has shown in my work since my departure from Glasgow.

Special thanks go to Paul, Tim, Redwan, Hassan, Anne and Walter for their encouragement, friendship and skilled assistance. I would also like to thank Melody, Iona, Carrie, Delia and David for all their help.

Thanks are also due to Dr J M Behnke and all the members of the Nottingham Experimental Parasitology Unit who spent valuable time in making the transition from Glasgow to Nottingham a smooth and happy one.

Thanks go to Helen and Wendy for skilled secretarial assistance, and to Jane for typing this thesis with accuracy and speed.

Finally, I owe much to my family and would like to thank them for their support and encouragement throughout the last three years.

PREFACE

A proportion of the work conducted for this thesis has been published and, is therefore presented in published form. This has resulted in some variation in the presentation of data, between and within the major sections of this thesis. However, in order to minimize the effects of the variation and ease comprehension of section discussions, each chapter is followed by a list of summary points stating the main findings from the experiments described in each chapter.

This is to certify and confirm that R K Grencis made a substantial contribution to the scientific content and composition of all joint papers, published and accepted for publication which have been included as an integral part of this thesis.

....

Professor D Wakelin

....

Dr T D G Lee

Abbreviations

In addition to the standard chemical and numerical abbreviations, the following were used in this thesis:-

Ag	Antigen.
BM	Bone marrow.
C'	Complement.
cpm	Counts per minute.
^{51}Cr	Sodium (^{51}Cr) chromate.
DTH	Delayed Type Hypersensitivity.
F_1	First filial generation.
FACS	Fluorescence activated cell sorter.
FCA	Freunds complete adjuvant.
FCS	Foetal calf serum.
FIA	Freunds incomplete adjuvant.
FITC	Fluorocein isothyocyanate.
GPC	Guinea pig complement.
H-2	Major histocompatability region in mice.
^3H	Tritium.
HBSS	Hanks' balanced salt solution.
Ig	Immunoglobulin.
IMLNC	Immune mesenteric lymph node cells.
[^{125}I] IUDR	(^{125}I) - iodo deoxyuridine.
i.p.	Intra peritoneal (ly).
i.v.	Intravenous (ly).
LIF	Lymphocyte inhibitory factor.
MIF	Macrophage inhibitory factor.
MLN	Mesenteric lymph node.
MLNC	Mesenteric lymph node cells.

NBS	Newborn calf serum.
n.d.	Not done.
P	Probability.
PBS	Phosphate buffered saline.
PEC	Peritoneal exudate cells.
PGE_2	Prostaglandin E_2.
PI	Post Infection.
RLP	Recirculating lymphocyte pool.
s.c.	Subcutaneously.
S.D.	Standard deviation.
S.E.	Standard Error.
TDL	Thoracic duct lymphocytes.
VBL	Vinblastine sulphate.
W/V	Weight/Volume.

Summary

The work presented in this thesis aimed to provide new information concerning the immune response mounted by mice against infection with the nematode *Trichinella spiralis*.

The first section examined the capacity of a soluble crude worm (muscle larva) antigen to protect mice against homologous infection. Vaccination of mice with this antigen was shown to confer protection against a challenge infection. The antigen was most effective when administered subcutaneously in conjunction with Freunds incomplete adjuvant, and exhibited dose dependent effects. Low doses of antigen (10μg - 100μg total protein) were effective in reducing worm fecundity, with higher doses of antigen (up to 500μg total protein) causing an accelerated expulsion of the adult worm burden from the gastro intestinal tract. The ability of vaccination with antigen to protect against infection was inherited in a simple dominant manner.

Adoptive transfer of immunity with lymphocytes from vaccinated mice was possible, but dependent upon the route of antigen administration and adjuvant used. Upon challenge, vaccinated mice exhibited an elevated *in vitro* blast cell activity in the MLN two days post infection, a situation analagous to that observed in the MLN of mice after a secondary infection. Vaccination with antigen via the footpad was also successful in protecting mice against a challenge infection. After vaccination via this route,

the draining node (popliteal) exhibited an elevated *in vitro* blast cell activity.

The second section of this thesis is concerned with the cellular immune response of NIH mice to natural infection with *T. spiralis*. In NIH mice after a primary infection, there was an increase in cellularity and *in vitro* blast cell activity of the MLN, predominately due to Ig-ve (T) cells. These increases peaked on day 8 post infection, after which there was a decline. The capacity of MLNC to adoptively transfer immunity against *T. spiralis* was coincident with populations exhibiting high *in vitro* blast cell activity. After a secondary infection given on day 21, similar increases in cellularity, *in vitro* blast cell activity and T cell numbers of the MLN were evident, although accelerated in comparison to the series of events after a primary infection. Peak levels were observed on day 4 post challenge after which there was a decline. Again, the capacity of MLNC to adoptively transfer immunity was correlated with a population of rapidly dividing Ig-ve (T) cells.

Positive selection of Thy 1.2 bearing MLNC using a FACS, confirmed the T cell nature of the MLNC which mediate expulsion of *T. spiralis* from the gut. Negative selection of an Ly 1+ population of MLNC from infected mice using cytotoxic anti Ly 2+ monoclonal antibody demonstrated that the T cell subpopulation which mediates worm expulsion does not bear the Ly 2+ antigen, and is presumably Ly 1+.

The rapidly dividing nature of the mediator MLNC was confirmed using positive selection techniques to separate large lymphocytes of low density (isopycnic centrifugation and velocity sedimentation) coupled with the techniques of radiolabelling with DNA precursors and adoptive transfer. Treatment of cell donors with the mitotic inhibitor vinblastine sulphate completely abrogated the capacity of MLNC/T enriched populations of MLNC to adoptively transfer immunity, and the ability of these cells to incorporate a radiolabelled DNA precursor.

The expulsion of a secondary infection of T. spiralis in a manner indicative of a classical secondary response, implies the existence of immunological memory. Successful adoptive transfer of immunity with T cell enriched fractions of spleen cells taken from donors long after worm expulsion suggested the existence of memory T cells either within the spleen or recirculating lymphocyte pool. After infection with T. spiralis NIH mice exhibited long lived responsiveness to parenterally administered T. spiralis antigens, evidenced by DTH responses. Evidence was also presented for a cell trapping response, occurring in the MLN early after both primary and secondary infections, a mechanism which promotes naive/ immune cell-antigen interactions in the node draining the site of infection.

Using the techniques of chemical abbreviation of infection and surgical transplantation of adult T. spiralis worms into the intestine, experiments demonstrated the poor immunogenicity of the adult stage.

The third section presents an examination of the direct anti-worm effects of serum antibody raised by a single infection of *T. spiralis*, and demonstrated, that upon passive transfer, serum antibody was capable of reducing worm fecundity and length, but not of accelerating expulsion of the worm burden from the gut. Adoptive transfer of B cell enriched fractions or T cell enriched fractions of MLNC into irradiated recipients suggested that antibody producing (B) cells, probably through T cell help were capable of direct anti-worm effects, but not of mediating worm expulsion. An examination of the bone marrow as the site of anti-*T. spiralis* antibody producing proved negative.

The final section of the thesis presents a brief investigation of the cross protection observed between *T. spiralis* and *T. muris*. A soluble crude antigen preparation from either parasite was shown to protect against a challenge worm infection. The immunological basis of the cross protection was confirmed by the successful adoptive transfer of MLNC from donors previously infected with either *T. spiralis* or *T.muris*, upon challenge by either parasite.

GENERAL INTRODUCTION

Parasite immunology is a dynamic and rapidly developing discipline. The last fifteen years has seen a dramatic increase in research into the immune responses to parasites, generated in part by an enhanced appreciation of the medical and economic problems associated with parasitic infection in the developing countries. Coupled with modern immunological techniques, research in this discipline, in addition to providing much new information concerning the varied immune responses to parasitic infection, has contributed much to the understanding of immune effector mechanisms. For example, study of the eosinophilia often associated with parasitic infections, particularly helminths, has greatly increased our knowledge of the role of the eosinophil in disease processes (See Willmott, 1980).

As a result of this intensive research, a number of excellent reviews of parasite immunology have been written (Ogilvie and Jones, 1971; Soulsby, 1972; Cohen and Sadun, 1976; Askanase, 1979; Mitchell, 1979; Wesley-Leid and Williams, 1979; Mansfield, 1981; Befus and Bienenstock, 1982; Keusch, 1982; Möller, 1982) and as such a lengthy review of the literature is unnecessary and out with the scope of this general introduction. Rather, a brief survey of some of the recent trends in immunoparasitological research will serve to highlight the exciting and informative areas of research into which parasite immunology is expanding.

The notion of a simple division of the immune response into humoral and cellular components is no longer tenable when the many cell populations, subpopulations and soluble

factors involved in regulation and modulation of the immune response are considered. Together with the complexity of parasite life cycles and parasite antigens, the study of the complex immune response to parasitic infection presents a daunting challenge. In consequence much of the research into parasite immunology necessarily relies heavily upon the adaptation and modification of approaches tried and tested in traditional immunology.

The availability of hypothymic nude mice and rats has allowed the use of a variety of approaches in immunological research that has greatly facilitated the examination of the control of immune responses to various antigens (Sparrow, 1980). The impact of this approach to study has been no less in immunoparasitology. Many host responses to parasite infection exhibit a strong T cell dependence (See Mitchell, 1980) and T lymphocytes have been shown to participate in the protective immune response to a number of parasites including Leishmania tropica (Louis, Zubler, Coutinho, Lima, Behin, Mauel and Engers, 1982), Plasmodium chabaudi (McDonald, and Phillips, 1978), Schistosoma mansoni (Doughty and Phillips, 1982), Nippostrongylus brasiliensis (Nawa, Parish and Miller, 1978) and Trichinella spiralis (Wakelin and Wilson, 1979a). Recent work upon the L. tropica/mouse system (Louis et al, 1982) has utilized the new technique of T cell cloning (Schwartz, 1982) to produce parasite specific T cells capable of mediating a variety of immunological functions in order to investigate the roles of different T cell lines in immunity to the parasite.

Application of immunochemical and radiolabelling techniques originally used to identify cell surface proteins has demonstrated the limited number of major parasite antigens on the surface of parasites, including T. spiralis, N. brasiliensis and Brugia sp. (Maizels, Philipp and Ogilvie, 1982). In the case of the African trypanosomes, the surface consists entirely of a single gylcoprotein moiety that is subject to antigenic variation (Bridgen, Cross and Bridgen, 1976).

A common feature of many parasite infections is chronicity, and the study of immune depression associated with parasitic infection is an active area of research. Although the term immune depression can include a variety of suppressive mechanisms a great deal of recent work has been concentrated upon suppressor cell involvement in parasitic diseases (See Playfair, 1982). Scott (1981) has shown that mice infected with T. cruzi develop suppressor T cells which act upon the development of delayed type hypersensitivity (DTH) reactions against the parasite. Work on L. tropica in BALB/c mice has shown that this strain of mice fails to control an infection because of the development of a specific suppressor cell of the phenotype Thy 1 +, Ly $^{1+}$, 1J $^{-}$ (Howard, Hale and Liew, 1981). The modulation of granuloma formation around S. mansoni eggs in mice is mediated by a suppressor T cell of the phenotype Ly2+, 1J+ which can inhibit the Ly1+ cell responsible for DTH (Chensue, Wellhausen and Boros, 1981). From an immunological point of view parasite/host models show an enormous potential for the study of suppressor cell mediated

phenomena.

Variations in host susceptibility and/or resistance to parasites are readily demonstrated in natural host populations (Wakelin, 1978b) and exploitation of genetically based variations in host resistance to parasites using inbred strains of animal hosts can be a useful approach to the analysis of immune mechanisms operating against parasites (See Mitchell, 1981a). It has been proposed that the protozoan parasite L. tropica becomes well established in BALB/c mice because of the defective recognition of H-2 antigens on infected macrophages by Ly1+ T cells (Handman, Cereding and Mitchell, 1979; Mitchell, Curtis, Scollay and Handman, 1981). Differences in intensity of granuloma formation around S. japonicum eggs and differences in anti-egg antibody responses in inbred strains of mice are being used to identify egg antigens responsible for granulomatous hypersensitivity against S japonicum. Using anti-egg hybridoma-derived antibodies and anti-idiotypic antibodies against these hybridoma antibodies, studies are in progress examining the modulation of the granuloma response (Mitchell, Garcia, Cruise,Tiu and Hocking, 1982). Work on the T. spiralis/mouse system has shown that both H-2 and non H-2 linked genes influence immunity to the parasite (Wassom, David and Gleich, 1979; Wakelin, 1980) and a recent study (Wakelin and Donachie, 1983) has shown a strong influence of alleles at the H-2K and I loci upon immunity to T. spiralis, and a modulating effect of H-2D alleles. These studies emphasize the importance and complexity of response patterns to parasite infections.

The development of monoclonal antibodies has revolutionized the study of immunology and their application to parasite immunology is, and will not be, any less profound. Monoclonal antibodies to parasite antigens can be of use in diagnosis, in examination of the mechanisms of immunity to parasites and particularly in investigations of the parasite antigens involved in the generation of protective responses (see Mitchell, 1981b). It has proven possible to produce a monoclonal antibody against a membrane antigen of Plasmodium berghei sporozoites which can protect against infection (Yoshida, Nussenzweig, Potocnjak, Nussenzweig and Aikawa, 1980) and Capron and co-workers have obtained a rat IgG2a monoclonal which can facilitate eosinophil mediated killing of schistosomula (Capron, Dessaint, Capron, Joseph and Pestel, 1980). Monoclonal antibodies have also revolutionized lymphocyte membrane studies (Williams, 1980) enabling precise identification of the characteristics of lymphocyte subsets and their efficient collection by negative and positive selection techniques. The use of monoclonals in this way has been used to identify the lymphocytes involved in immunity to a number of parasites including L. tropica (Louis et al, 1982) S.mansoni (Chensue et al, 1981) and T. spiralis (Krco, David and Wassom, 1982).

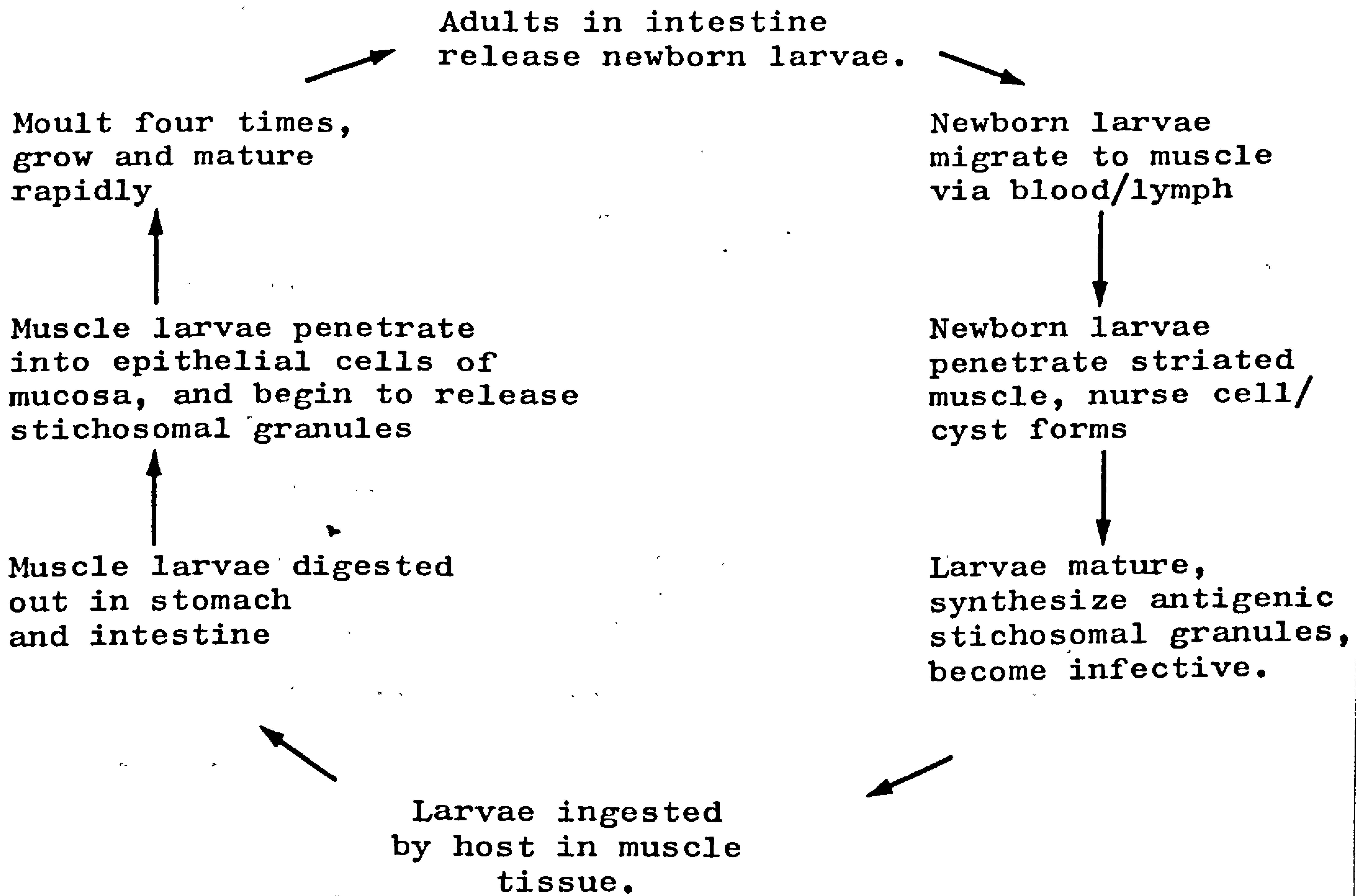
As can be observed from the above brief survey, parasite immunology is going through a dynamic phase in its development, and laboratory models of parasitic infection have provided the majority of information contributing to our present day understanding of the immune mechanisms operating

against parasites. The study of gastrointestinal nematode/host systems has been an active area of research for many years and many laboratory models have been examined including N. brasiliensis, Trichostrongylus coloubriformis, Nematospiroides dubius, Trichuris muris, and Ascaris suum (see Cohen and Sadun, 1976). In addition to providing much information concerning the role of the immune mechanisms involved in interactions with these parasites, such laboratory models can also provide convenient systems for the study of a variety of immunopathological disorders, eg, clinical allergy, N. brasiliensis in rats (Pritchard and Eady, 1980) and A. suum in monkeys (Pritchard, Eady, Jackson, Orr, Richards, Trigg and Wells, 1982). Despite the volume of research carried out upon these and other systems and the variety of immune mechanisms shown to be operating against parasites, much of the information is fragmented and this precludes synthesis or meaningful comparison.

The work presented in this thesis is conducted upon a well researched parasite/host model; that of T. spiralis infections in the mouse. This parasite is easily maintained in the laboratory and will establish in a wide spectrum of laboratory animals. Detailed studies have been made of parasite morphology, behaviour, epidemiology, host pathology and the immunology of infection with T. spiralis, and research has generated enough interest to warrant the establishment of an International Commission responsible, periodically, for examination and collation of recent research into this parasitic disease (see Kim, Ruitenberg and Teppema, 1981).

T. spiralis passes through all its life cycle stages (Fig A) in one host, and the immune response of the host has shown to be generated by a combination of all the life cycle stages (See Wakelin and Denham, 1982). T. spiralis, in common with many other gastrointestinal helminths, exhibits a spontaneous cure phenomenon in the host and re-infection induces a greatly accelerated worm loss, sometimes rapid enough to prevent significant establishment. Early work showed that the loss of worms from the small intestine was immunologically mediated (Coker, 1955; Markell, 1958), and over the years a great deal of research has been directed towards elucidating the mechanisms involved. As a result of this research, a number of excellent reviews have been written about responses mounted by the host against the worm (Kagan, 1960; Larsh, 1963; Larsh, 1970; Castro, 1976; Despommier, 1977; Wakelin and Denham, 1982). Much information has been derived from work in murine hosts, particularly mice. One strain of inbred mouse which mounts a pronounced immune response against T. spiralis, and has been used extensively, is the NIH strain (Kennedy, Wakelin and Wilson, 1979; Manson-Smith, Bruce and Parrott, 1979; Rose, Parrott and Bruce, 1976a; Wakelin and Lloyd, 1976a; Wakelin and Wilson, 1977a; Wakelin and Wilson, 1979a; Wakelin, 1980). The work presented in this thesis examines the immune responses generated in NIH mice by T. spiralis, using a number of approaches extensively used in traditional immunological studies. The T. spiralis NIH mouse model provides a convenient and manipulable system for gaining a detailed and comprehensive understanding of some of the immune mechanisms operating during parasitic

Figure A. Life Cycle of *Trichinella spiralis*



infection, and may provide a sound base for comparison with other gastroinestinal nematode/host systems.

The first section of the thesis is an investigation of the immunity generated by immunization (vaccination) of mice using a crude worm antigen. Although vaccination *per se* is not a novel approach (See Clegg and Smith, 1978), it does afford a method of presenting antigen to the host in a more defined and regulated manner than by natural infection, and by direct comparison allows a more precise identification of those responses generated by a natural infection. A thorough knowledge of the nature of protective immunity resulting from "artificial" immunization and its correlation with that arising from a natural infection is an important and often neglected component in the study of vaccination against nematode infections.

The second section of the thesis examines a number of aspects involved in the spontaneous cure response. From the early studies of Landsteiner and Chase (1942) on delayed type hypersensitivity, adoptive transfer of immunity with lymphocytes between genetically similar animals has been a powerful tool for the study of the immune response. This type of information has yielded much information concerning the immune response mounted against *T. spiralis* in both rats and mice (See Wakelin and Denham, 1982). The second section examines; in detail, the generation, characteristics and recruitment of the immuno competent cells involved in mediating expulsion of *T. spiralis* from the gastrointestinal tract.

Although adoptive transfer of immunity against T. spiralis with lymphocytes is a consistent and informative technique, the passive transfer of serum antibodies as an approach to examining the immune mechanisms operating against gastro intestinal helminths has been, and still is, an active area of research (Ogilvie and Love, 1974; Moqbel, 1977; Behnke and Parish, 1979) and the concept of antibody-mediated damage to worms is a well established theme (Ogilvie and Jones, 1971). A definite protective role for antibody involvement in immunity to T. spiralis has been described by Moloney and Denham (1979) and shown to operate against new born larvae, although no definite protective role for antibody in mediating expulsion of the worm from the small intestine has been agreed upon. The third section of the thesis investigates the possible role of serum antibody in mediating immunity to T. spiralis, using both adoptive transfer of immunity with cells, and passive transfer of immunity with serum.

Another laboratory model of intestinal nematode parasitism is the mouse whipworm, Trichuris muris/mouse system. A trichuroid nematode, like T. spiralis, T. muris generates an immune response in the host capable of removing the worm from the colon, the site of infection (See Lee, 1982). The fourth and final part of this thesis is an investigation into the possible cross-reactivity between T. spiralis and T. muris, which provides an interesting approach to the study of shared antigens, and the responses stimulated by similar antigens presented in different areas of the gastrointestinal tract.

GENERAL MATERIALS AND METHODS

Animals

Male mice were used throughout and were 7 - 8 weeks old at the start of the experiments unless otherwise stated. Inbred NIH mice were obtained from Hacking and Churchill Ltd or Olac 1976 Ltd. Inbred B10G congenic mice were obtained from Olac 1976 Ltd, and outbred CFLP mice were obtained from Hacking and Churchill Ltd. C57BL/10 Sc/Sn and (C57BL/10 x NIH) F_1 hybrids were bred at the Wellcome Laboratories for Experimental Parasitology.

Wistar rats (outbred) were bred at the Wellcome Laboratories for Experimental Parasitology.

All animals were caged in groups of six animals unless otherwise stated, and housed under conventional animal house conditions.

Parasite

Trichinella spiralis

The strain of *Trichinella spiralis* used originated from the London School of Hygiene and Tropical Medicine, and was maintained by passage through outbred CFLP stock mice. Infective muscle larvae were obtained by artificial digestion of the skinned and eviscerated carcasses of stock mice which had been infected for at least 35 days. The carcass was minced and digested for approximately two hours in 0.5% pepsin/HCl in tap-water

at 37°C with continuous agitation. Undigested remains were filtered off on a coarse mesh and the larvae collected by repeated washing and sedimentation in warm 0.9% NaCl. Finally, the larvae were suspended in 0.2% agar and the total volume adjusted to give the required number in 0.2 ml. Larvae were injected per os into the stomach using a syringe and blunted needle.

Adult worm recovery

Adult worms were recovered from the intestines of animals using the modified Baermann technique of Wakelin and Wilson (1977a). Animals were killed by chloroform overdose and the entire small intestine removed and cut open along its length. The intestine was placed on a nylon gauze and submerged in approximately 40 ml of pre-warmed Hanks' balanced salt solution (HBSS) in a 50 ml beaker. The beakers were incubated for 2-3 hours during which time the worms migrated through the gauze. After this time, the worms were transferred to a 10 cm petri dish for counting.

In vitro Assay for female fecundity

The Assay used was as described by Wakelin and Wilson (1977a). Female worms were taken from two mice from each experimental group at the end of each experiment when the animals were killed for estimation of adult worm number. The worms were collected after approximately 45 min incubation and transferred into 25 ml conical flasks containing 9 ml of HBSS and 1 ml of heat inactivated

foetal calf serum (FCS, Gibco Europe) or heat inactivated newborn calf serum (NBS, Gibco Europe). Twenty five worms were taken from each mouse and the 50 worms per group incubated in one flask for 4 hrs at 37°C. After this time the female worms were removed by filtration through gauze, the medium centrifuged and the larvae resuspended in 1 ml of HBSS. Four samples, each of 50μl were examined under the microscope and the larvae counted. The total number of larvae originally present was determined and the female fecundity expressed as the number of larvae produced/female/hour.

Worm length

Twenty five adult female worms taken from worm burdens recovered from each experimental group were measured from camera lucida drawings using a map measurer or a bit-pad digitizer linked to a D.E.C.P.D.P. 11/34 computer.

Anthelmintic

Methyridine (Mintic, I.C.I.) was used to remove *T. spiralis* from the intestine. The drug was given *per os* at a dose level of 1000 mg/Kg body weight. Due to the toxic nature of the drug, animals that had undergone surgery received the required dose in two diluted injections given approximately seven hours apart. The efficacy of the drug was determined in infected animals which had received a dose of mintic and were killed

one day later; no worms were observed in the gut at this time.

Antibiotic

Oxytetracycline hydrochloride (Terramycin, Pfizer Ltd.) at a concentration of 165 mg/litre was given in drinking water to mice which had undergone surgery as well as to their controls.

Irradiation of animals

Mice were irradiated using a ^{60}Cobalt source in the Chemistry Department of Nottingham University. The exposure rate was calculated from ^{60}Cobalt decay tables and a calibration curve of the exposure rate.

Protein Estimation

A standard laboratory method used for estimating protein is that of Lowry, Rosenbrough, Farr and Randall (1951). However, this method is laborious and therefore, the modified Lowry technique of Schacterle and Pollack (1973) has been used. Maximum accuracy with this assay is obtained in the range of 8-40μg protein per ml of final volume, and therefore, various dilutions of test samples were assayed in order to obtain an accurate estimate. All samples were assayed in triplicate and the mean value used for calculation of protein concentration.

One ml of the alkaline copper reagent (see solutions)

was mixed with 1 ml of the sample in a glass test tube and allowed to stand undisturbed for 10 minutes at room temperature. 4 ml of the phenol working solution (see solutions) were added forcibly and rapidly. The test tube was incubated at 55°C for 5 minutes and then read in a spectrophotometer at 650 n m.

The standard used for calibration was Bovine Serum Albumin, Fraction V (Sigma).

Preparation of cell suspensions and cell transfer

The appropriate lymphoid organ (mesenteric lymph node, spleen, thymus or popliteal node) was stripped of connective tissue and fat *in situ*, excised and placed in cold (4°C) medium 199 (Gibco, Europe) with L-glutamine and Hepes buffer (25mM). The medium was supplemented with 5% FCS or NBS and 5 i.u./ml heparin (B.D.H. Chemicals Ltd). Any remaining fat was removed, the tissue cut into small pieces and crushed through a nylon or zinc sieve into a sterile petri dish containing medium using a rubber tipped syringe plunger. The suspension was left to stand for 5 minutes to allow cell clumps and debris to sink to the bottom of the dish. The supernatant was collected and centrifuged at 200 g for 5 minutes and the cells resuspended in a known volume of fresh medium. The ability of live cells to exclude Trypan blue was used as a test of viability. 50µl of a cell suspension was mixed (1 in 20) with a 2% w/v solution of trypan blue in HBSS, and the number of live cells counted in a

haemocytometer. Viability was usually greater than 90%. The concentration of cells was adjusted to give the required number in a volume not greater than 0.5 ml. Cells were injected intravenously (i.v.) into recipient mice via a lateral tail vein.

Assay for Blast cell activity

(a) Macroculture

Lymphocytes were prepared as above except that the cells were suspended in culture medium (see solutions). 4 ml of culture medium were placed in sterile plastic tissue culture grade tubes (Sterilin) and a further 1 ml of medium containing 1 - 2 x 10^7 cells were added. The tubes were gassed with 5% CO_2, and 2μCi of 5- $[^{125}I]$ -iodo-2'-deoxyuridine, $[^{125}I]$ -UdR specific activity 5Ci/mg (Amersham International) in 0.9% NaCl was added to each tube. Triplicate samples were assayed. The cells were incubated for two hours at 37°C. After incubation the cells were washed three times by centrifuging at 200 g and resuspending in fresh medium 199. The activity in each tube was measured by counting for one minute in a Packard Tricarb liquid scintillation spectrometer. $[^{125}I]$ -UdR, a thymidine analogue is incorporated by cells undergoing DNA synthesis and therefore is a convenient label for dividing (blast) cells in lymphocyte suspensions.

(b) Microculture

Lymphocytes were prepared in culture medium as above. 150μl of culture medium were added to each of the wells of a sterile flat bottomed microtitre plate (Flow Laboratories Ltd). 1μCi [methyl - ^{3}H] - Thymidine, (specific activity 2 Ci/mmol; Amersham International) in a volume of 50μl of culture medium were added to each well. A further 50μl of medium containing 3-5 x 10^5 cells were added to each well. Each sample was assayed, at least in triplicate. The microtitre plate was covered and incubated for 2 hrs at 37°C in an atmosphere of 5% CO_2 in air and 95% relative humidity. After incubation, the cells were harvested onto glass fibre filter paper discs using a Titretek cell harvester (Flow Laboratories Ltd). The discs were dried for 35 minutes at 60°C and each one placed into a vial containing 5 ml of a scintillation cocktail (Fisofluor '1'; Fisons Scientific Instruments). The vials were chilled overnight in the dark and the activity of each vial counted for one minute using a Packard Tricarb liquid scintillation spectrometer.

[Methyl-^{3}H] - Thymidine is an analogue of thymidine and is incorporated into newly synthesized DNA, and therefore gives an indication of cell division.

Enrichment of cell populations for T and B lymphocytes

Separation of cell suspensions into non-adherent (T-cell enriched) and adherent (B-cell enriched) fractions was carried out by passage of the cells through nylon

wool columns similar to the methods of Julius, Simpson and Herzenberg (1973), Handwerger and Swartz (1974) and Trizio and Cudkowicz (1974).

2.5 g of nylon wool (Fenwel Laboratories) were evenly packed into a 30 ml disposable syringe to the 25 ml level. After autoclaving, the column was washed through with medium 199 containing L-glutamine and Hepes buffer, with the addition of 5 i.u. heparin/ml and 5% FCS. The column was then saturated with this medium and pre-incubated for 1½ hours at 37°C. The cells were prepared in the normal way and prior to loading onto the column were filtered through glass wool to remove any debris and cell clumps. A maximum of 5 x 10^8 cells in a volume of between 10 and 15 ml were layered onto the nylon wool dropwise to ensure even penetration of the column. After the cell suspension had been loaded, the column was incubated for 45 minutes at 37°C. Cells were collected from the column by two methods. The non adherent cells were gently washed through the column with warm (37°C) medium 199. The adherent cells were collected by gently teasing apart the nylon wool in a 100 ml crystallizing dish containing cool medium 199. Both suspensions were washed by centrifugation and resuspending in fresh medium before counting and assessment of Immunoglobulin (Ig) positive or Thy 1.2 positive cells. This technique allowed the recovery of approximately 50% of the cells applied to column.

Fluorescent labelling of cells

(a) Immunoglobulin bearing cells

Samples of 1-5 x 10^6 cells were suspended in 500µl medium 199 containing 0.01% NaN_3 and a 1/50 dilution (HBSS) of fluorocein conjugated rabbit anti-mouse immunoglobulin (Nordic Laboratories). After 30 minutes at 4°C in the dark the cells were washed twice in HBSS and resuspended in 0.25 ml 50% glycerine in HBSS. The labelled cells were examined under water immersion with a Leitz Ortholux 1 microscope fitted with a Ploem Incident light fluorescent system.

(b) Thy 1.2 bearing cells

Samples of cells were treated in the same manner as above except that fluoroscein conjugated anti-Thy 1.2. serum (AKR anti-C3H, Searle Laboratories) was used at a dilution of 1/10.

Absorption of Guinea Pig Complement

Guinea pig serum tends to be cytotoxic for mouse cells and consequently often needs to be absorbed. Agarose has been found to be suitable for this purpose (Cohen and Schlesinger, 1970).

A $\frac{1}{3}$ dilution (Medium 199) of neat Guinea Pig Complement (GPC, Wellcome reagents) was absorbed by the addition of 80 mg agarose (BDH Chemicals Ltd). This

mixture was kept on ice with frequent agitation for 40 minutes. After centrifugation for 15 minutes at 2000 g the supernatant was collected for use.

Percoll

Percoll (Pharmacia Fine Chemicals) is a medium of heterogeneously sized colloidal silica particles coated with polyvinylpyrolidone. It is non-toxic, has a low osmolarity, low viscosity, will not penetrate biological membranes, is autoclavable, and exhibits spontaneous gradient formation during centrifugation. The combination of these properties makes it an ideal material for use in certain experiments involving the separation of lymphocytes.

To produce a solution of percoll having an osmolarity of approximately 300 mos/kg H_2O, 9 parts of percoll were mixed with 1 part of a 10 fold concentrated solution of Hanks balanced salt solution (Flow laboratories). This stock solution is taken as the neat standard, dilutions of percoll being made with reference to this stock solution.

Production of Antisera

Blood was collected from mice by cardiac puncture using a 19 gauge needle and a 2 ml syringe. The blood was allowed to clot in a centrifuge tube at 4°C overnight and after ringing, centrifuged for 15 minutes at 1800g. The serum was removed and stored in 1 ml aliquots at

$^{-}40^{o}C$ until use.

Immodiffusion: Standard Ouchterlony test

Microscope slides (50 mm x 75 mm) were each pre-coated with 3 ml 0.3% purified agar (B.D.H. Chemicals Ltd) in distilled water and dried at 80°C - 100°C. After cooling, 5 ml of hot (85°C) purified agar containing 3% polyethylene glycol 6000 (B.D.H. Chemicals Ltd) in PBS pH 7.2 were pipetted onto each slide. The slides were allowed to cool and wells cut using the appropriate tool. The agar was removed from the wells using a pasteur pipette and the wells filled with the appropriate antigen or antibody solution. The slides were placed in a humid chamber and left undisturbed at 4°C. After the reaction was complete, the slides were washed in 0.3 M NaCl at 4°C for 24-48 hours. The slides were pressed and dried with filter paper and then stained with Coomasie blue (see solutions) for 10 minutes. The slides were destained (see solutions) for 15-20 minutes.

Statistics

The student's t-test was used to assess the significance of differences between mean values in results. P values of less than 0.05 were considered significant.

Solutions

Modified Hank's Balanced Salt Solution (HBSS). (Hopkins and Stallard, 1974)

Hank's solution was modified by excluding glucose and Na HCO_3 and increasing the remaining salts to an osmotic pressure of 300 m-Osmoles.

Solution 1.

NaCl	168 g
KCl	8 g
KH_2PO_4	2 g
Na_2HPO_4	4 g
0.02% Phenol red	200 ml

Made up to 2 litres with de-ionized water.

Solution 2.

$CaCl_2.2H_2O$	3.92 g
$MgCl_2.6H_2O$	2.00 g

Made up to 2 litres with de-ionized water.

105 ml of solutions 1 and 2 were mixed and made up to litre with de-ionized water (Final pH 7.2).

Phosphate buffered saline (PBS, pH 7.2)

Na_2HPO_4	2.84 g
$NaH_2 PO_4.2H_2O$	2.76 g
NaCl	9.00 g

Made up to 1 litre with distilled water.

Trypan blue

The stock solution, 2 g trypan blue (B.D.H. Chemicals Ltd) made up to 100 ml with distilled water was diluted 1:20 in HBSS for use in the dye exclusion test for cell viability.

Alkaline copper reagent (For Protein estimation)

A solution of sodium hydroxide (0.5N) containing 10% sodium carbonate, 0.1% potassium hydrogen tartrate, and 0.05% copper sulphate. This reagent was stable for 30 days at room temperature.

Phenol reagent (For Protein estimation)

2N Folin-Ciocalteau reagent (B.D.H. Chemicals Ltd) diluted to 1N with distilled water. A working solution was prepared by making a 1/9 dilution (distilled water) of the 1N solution.

Medium for cell culture

This medium was used for both macroculture and microculture. Sterile technique was observed in preparation.

RPM1 1640 medium without L-glutamine Without Na HCO_3	100.0 ml
Sodium bicarbonate 7.5% solution	0.8 ml
L-glutamine 200 mM	1.0 ml

Penicillin/streptomycin 10,000 units/ml	1.0 ml
Heat inactivated foetal calf serum	5.0 ml

All constituents were obtained from Gibco Europe.

Solutions for Immunodiffusion

(a) Stain

A solution of 0.1% Coomasie brilliant blue in 10% glacial acetic acid, 45% absolute ethanol and 45% distilled water.

(b) Destain

A solution of 10% glacial acetic acid, 25% absolute ethanol and 65% distilled water.

SECTION 1

THE IMMUNE RESPONSE OF MICE TO VACCINATION WITH SOLUBLE WORM ANTIGENS.

Vaccination of man and his domestic animals is primarily aimed at prophylactic immunization against infectious disease. Due to the fact that helminth diseases remain among the major health problems of today (W.H.O., 1981) vaccination agains helminth parasites has been the subject of much research over the last fifty years (See reviews by Stoll, 1961; Silverman, 1970; Clegg and Smith, 1978; Urquhart, 1980; Lloyd, 1981). The mainstream of research into vaccination against helminth infection in laboratory animals has also been directed towards protection against infection, utilizing antigen preparations presented to the host in a number of different forms (See Clegg and Smith, 1978).

However, in addition to information obtained on the efficacy of a vaccine to protect, vaccination studies may provide valuable information concerning the immune response of the host to infection. One advantage of vaccination over natural infection as a means of stimulating the immune response, is that this method of immunization results from the controlled stimulation of the immune system by administration of a relatively harmless antigen preparation, rather than from the uncontrolled stimulation by natural infection. This is particularly important in studies of helminth infection. Being metazoan organisms, and often displaying intricate life cycles, helminths present the host immune system with a considerable and complex antigenic challenge (See Pery and Luffau, 1979). Vaccination studies carried out in well defined host/

parasite systems proffer the opportunity to study the immune response to helminth antigens under conditions in which many variables can be controlled.

One group of helminths which has received much attention in this respect are the nematodes and, with notable exception of the filarial worms, most studies have been carried out on nematodes of vertebrates living in or associated with the gastrointestinal tract. There have been many studies of immunization against a variety of species of gastrointestinal nematodes using killed parasite antigens or metabolic products derived from *in vitro* culture of the parasites. These species include *Trichostrongylus colu briformis* (Rothwell and Love, 1974; Rothwell, 1978), *Nippostrongylus brasiliensis* (Murray, Robinson, Grierson and Crawford, 1979), *Nematospiroides dubius* (Cypess, 1970; Day, Howard, Prowse, Chapman and Mitchell, 1979), *Strongyloides ratti* (Sheldon, 1937) and *Trichuris muris* (Wakelin and Selby, 1973; Jenkins and Wakelin, 1977). Early attempts to vaccinate against *Trichinella spiralis* yielded variable results. McCoy (1935) attained a considerable degree of protection using a powdered larval preparation, but Bachman and Molina (1933) failed to show protection using similar methods. Campbell (1955) successfully protected mice using antigen preparations of material secreted into culture media. Protection using this type of antigen was also observed by Ewart and Olson (1961). More recent work by Despommier and colleagues has shown that very good protection can be

achieved using extracts of muscle larvae homogenate (Despommier and Muller, 1970; Despommier, Campbell and Blair, 1977a). Despommier and Muller (1970) have shown that protection is associated with an antigen prepared from the stichosome of muscle larvae. The stichosome is a large organ filling most of the anterior part of the worm and consists of a series of stichocyte cells which surround the oesophagus. The stichocytes are ducted into the oesophagus and it is thought that granular material secreted into the ducts is involved in extracorporeal digestion (Bruce, 1974; Wright, 1979). Despommier and Muller, (1976) have shown the granules to be antigenic and recent work (Despommier, 1981; Despommier and Lacetti, 1981) has concentrated upon purifying and characterizing these protection inducing antigens.

The immune response of the host to natural infection with T. spiralis in mice and rats has also received a considerable amount of attention (See review by Wakelin and Denham, 1982). Thus, the T. spiralis/mouse system, about which there is a wealth of information concerning the immune response to infection, provides an ideal model for a comparative and complementary study of immunization by artificial means, ie, vaccination.

This section of work aims to provide such a study using the T. spiralis/NIH mouse system as a model. The study comprises three chapters namely, Chapter 1, the establishment of baseline parameters of infection and a

vaccination regime which affords good protection against infection; Chapter 2, an examination of the adoptive transfer of the immune response generated by vaccination, and finally Chapter 3, a short examination and correlation of the host responses induced by vaccination with those induced by infection.

MATERIALS AND METHODS

ANTIGENS

The antigens used in the present study were prepared following two protocols similar to those described by Jenkins (1977) and Despommier, Muller, Jenks and Fruitstone (1974). The two antigenic preparations will be referred to as the soluble crude antigen and the soluble particle associated antigen, respectively. Both have been shown to be effective in immunizing mice against challenge nematode infections. Jenkins and Wakelin (1977) protected mice against a challenge *T. muris* infection using a soluble crude *T. muris* antigen, and Despommier *et al* (1977a) protected rats against a challenge infection of *T. spiralis* using a soluble particle associated *T. spiralis* antigen. *T. spiralis* muscle larvae were obtained by pepsin digestion of muscle taken from Wistar rats infected for at least 35 days. The protocols for the preparation of the antigens are shown in Figures A and B. Antigens were assayed for total protein using the method of Schacterle and Pollack (1973) which is described in detail in the general materials and methods. The antigens were adjusted to the appropriate final concentrations using an Amicon ultrafiltration unit (Model 8MC, Amicon Corporation) using a PM 10 membrane to retain molecules of molecular weights $\geq 10,000$. The unit was operated under a pressure of ≤ 25 lb/in^2.

Figure A. Protocol for preparation of Soluble Crude Antigen

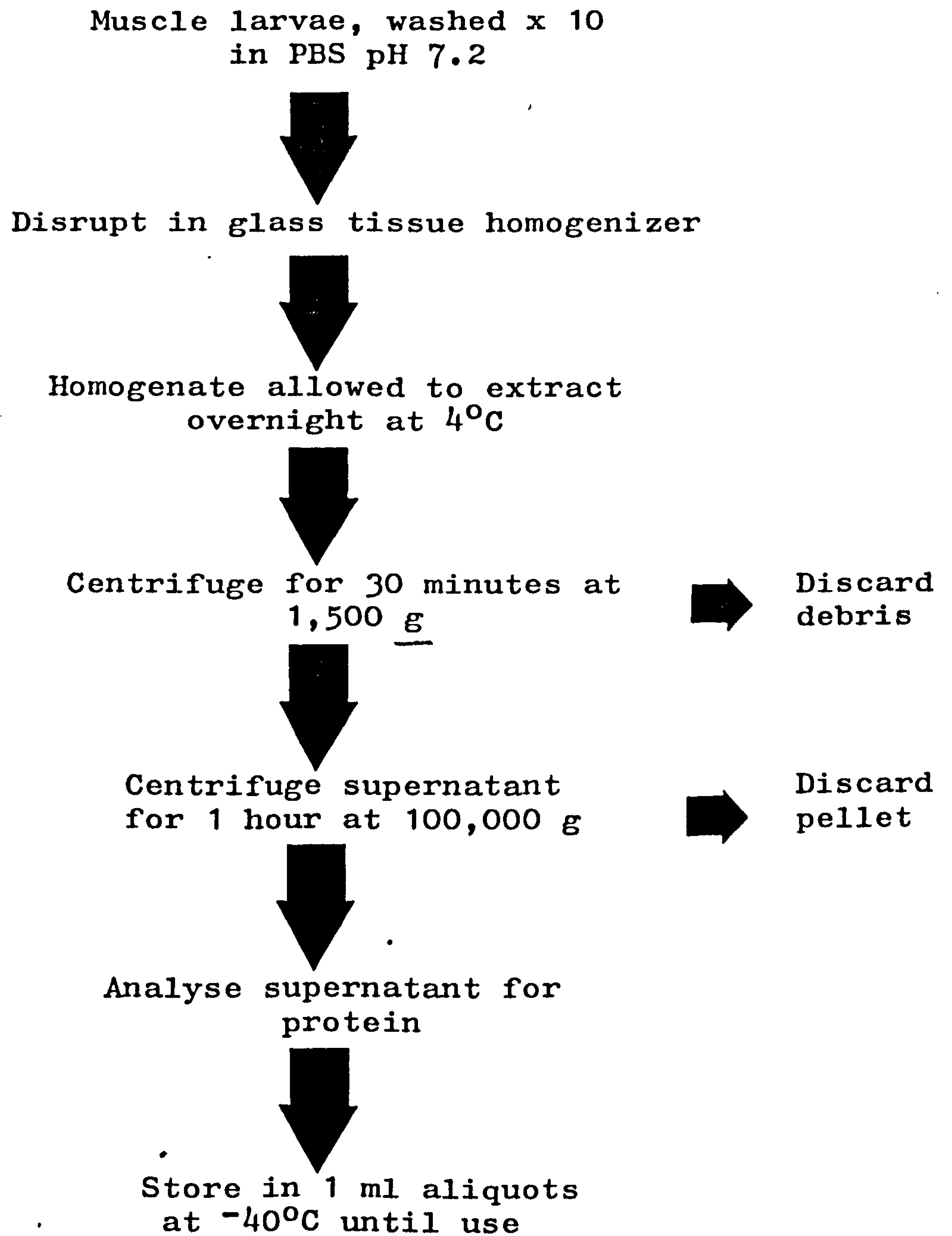
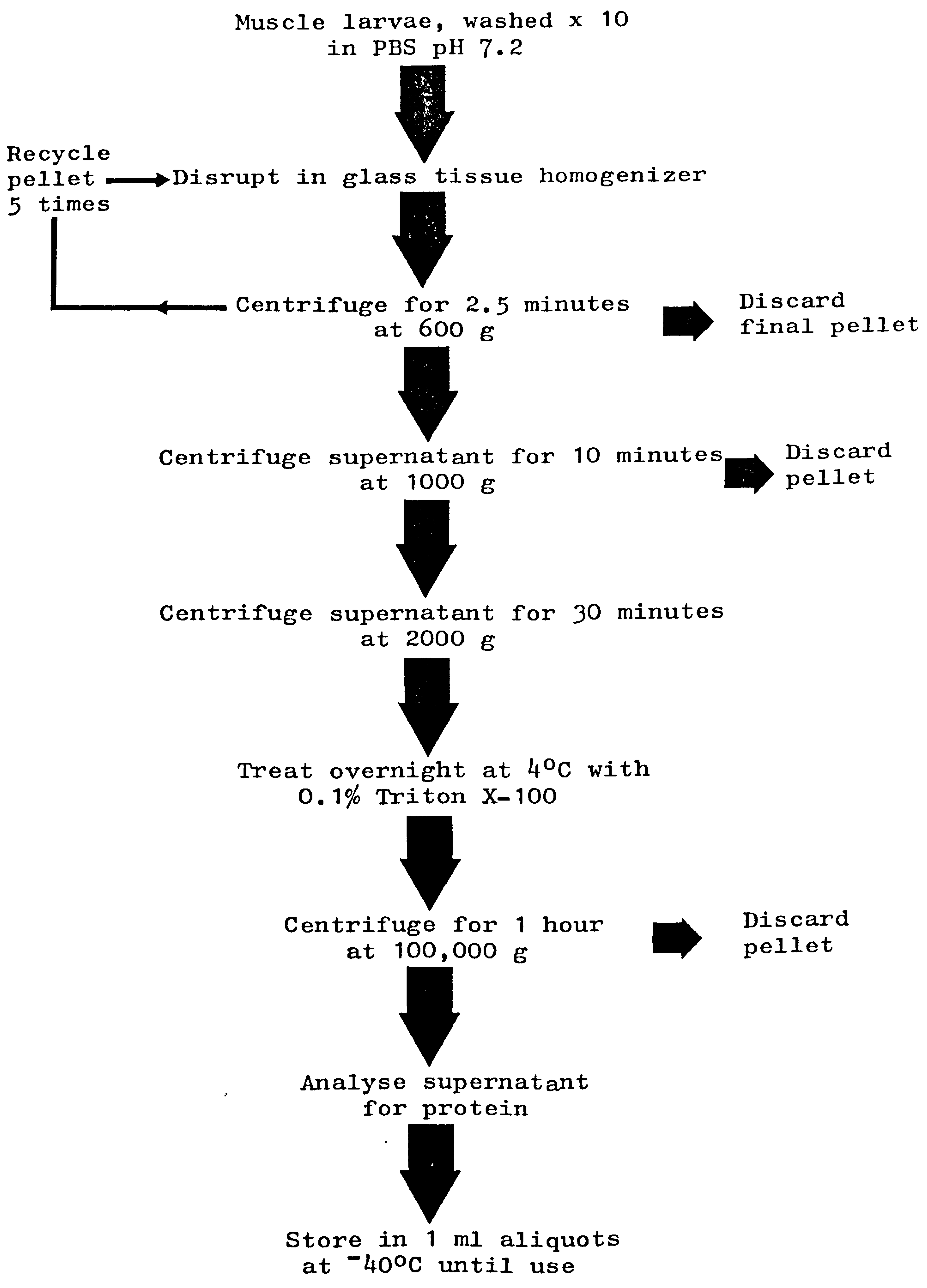


Figure B. Protocol for preparation of Soluble Particle Associated Antigen.



Adjuvants

Both Freund's Complete adjuvant (FCA) and Freund's Incomplete adjuvant (FIA) were used. To produce an adjuvant-antigen emulsion, adjuvant was placed in a small beaker and an equal volume of antigen added dropwise followed by rapid passage into and out of a 1 ml gillette sabre syringe. The emulsion was tested by dropping into cold water. When the drops remained discrete the emulsion was ready for injection.

Cell Suspensions

Details of the preparation, adoptive transfer, fluorescent and isotopic labelling of cells are described in the general materials and methods.

Worm Recovery

Adult worm recovery and female fecundity were measured using the methods of Wakelin and Wilson (1977a) and are described fully in the general materials and methods.

CHAPTER 1

Immunization of mice with soluble worm antigen.

An examination of the parameters
required for effective protection.

Studies of protection against nematode infection using worm antigens as immunizing agents have met with both failure and success (See Clegg and Smith, 1978). Recent evidence from studies of artificial immunization against trichuroid nematodes using antigen from dead worms have shown a consistency in protection lacking in early studies (Wakelin and Selby, 1973; Jenkins and Wakelin, 1977; Despommier et al, 1977a; Despommier and Lacetti, 1981). Therefore, immunization protocols similar to those used in such studies have been taken as guidelines for the present investigation.

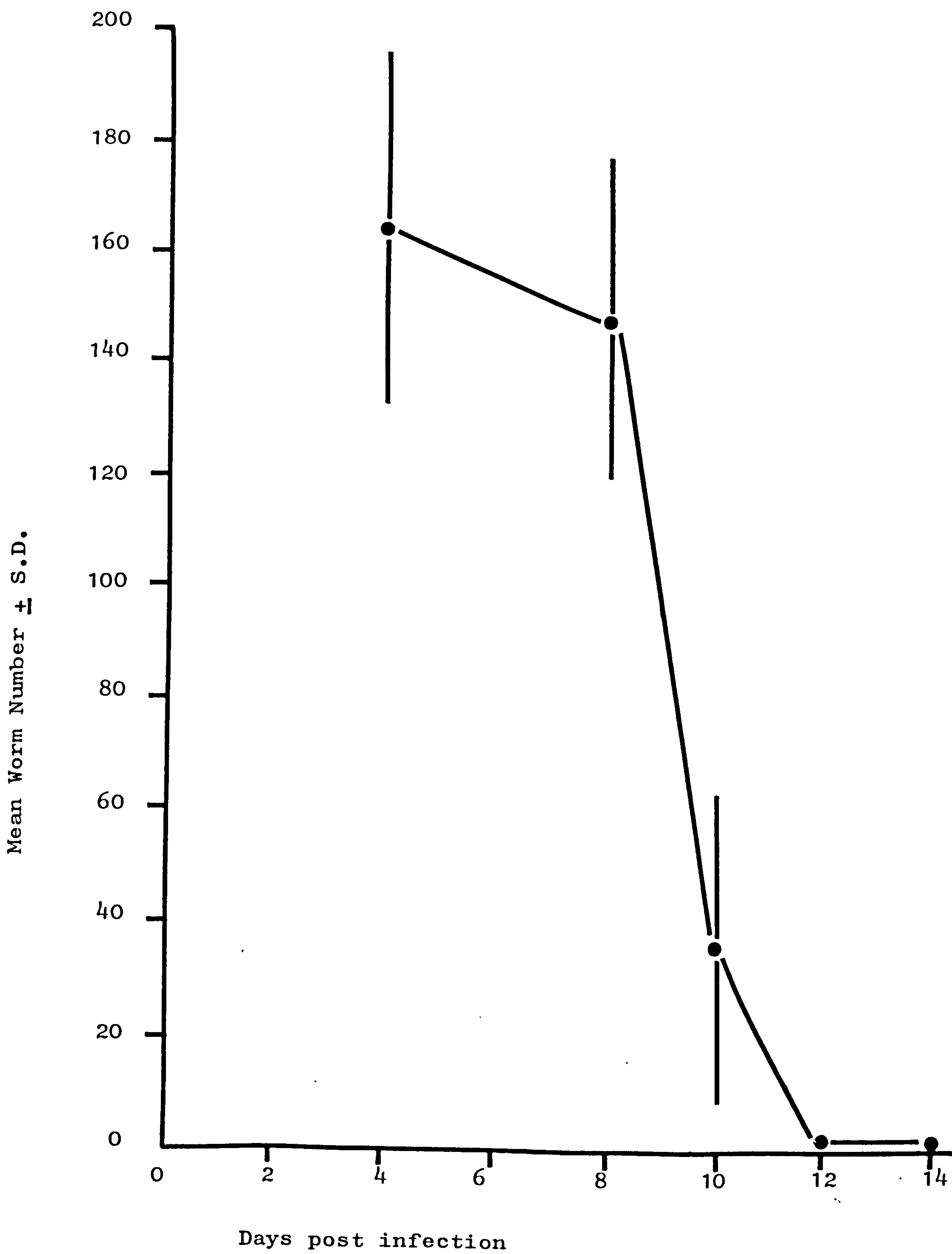
Kinetics of a Primary infection of *T.spiralis* in mice

An initial experiment was carried out to define the course of a primary infection in NIH mice. Thirty 8-week old male NIH mice were infected with 300 *T. spiralis* muscle larvae on day 0. Groups of six mice were killed at various times post infection to assess the total number of worms in the small intestine. The kinetics of a primary infection are shown in Fig 1.1. Approximately 55% of the inoculum established in the small intestine. Worm expulsion began after day 8 post infection, and was essentially complete by day 12.

Kinetics of a Secondary infection of *T. spiralis* in mice

An experiment was designed to examine the course of a secondary infection given 21 days after a primary infection. Twenty-four 8-week old male NIH mice were

Figure 1.1. Kinetics of a primary infection in NIH mice of 300 *T. spiralis* muscle larvae.



infected with 300 T. spiralis muscle larvae on day 0, and challenged on day 21 with 300 T. spiralis muscle larvae. Groups of six mice were killed on various days post challenge, and the total number of worms in the intestine counted. The results demonstrate that after establishment of the worms in the gut expulsion started after day 4, being almost complete by day 8 post challenge (Fig 1.2). Thus, expulsion of worms in a secondary infection is accelerated compared with that of a primary infection.

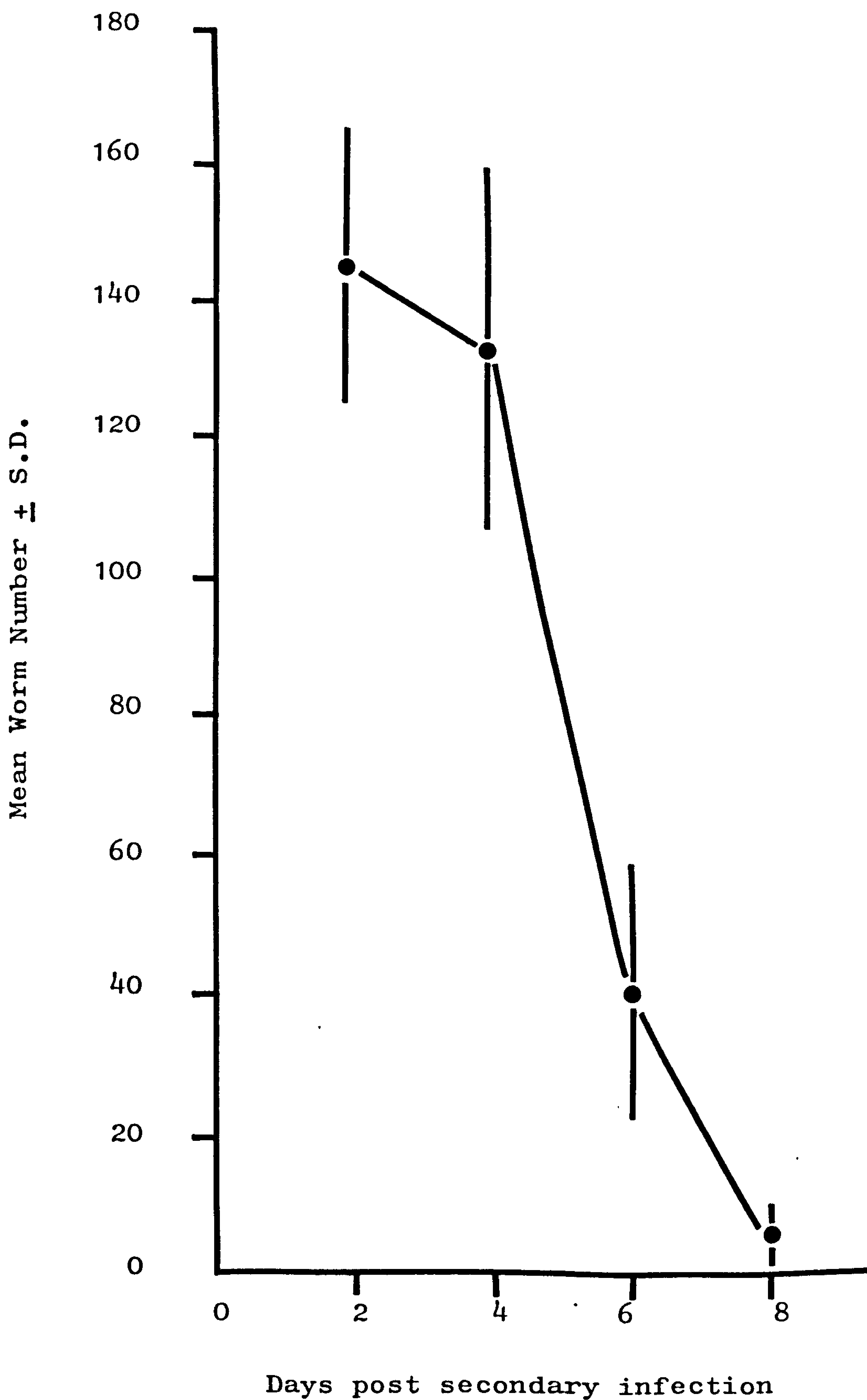
Immunization of mice with soluble crude antigen

An initial experiment was designed to examine the efficacy of the soluble crude antigen preparation to protect against re-infection. Seventy-two 8-week old male NIH mice were divided into four experimental groups of eighteen mice each. The immunization protocol is shown below.

GROUP	IMMUNIZATION	
	Day -14	Day -7
I	----	----
II	PBS + FIA	PBS
III	250μg Ag + FIA	----
IV	125μg Ag + FIA	125μg Ag

Immunized mice each received a total of 250μg (total protein) of the soluble crude antigen, administered subcutaneously. All groups were challenged with 300 T. spiralis muscle larvae on day 0. Six mice from each experimental group were killed on days 4, 8 and 12 post

Figure 1.2. Kinetics of a secondary infection in NIH mice of 300 T. spiralis muscle larvae. Primary infection of 300 T. spiralis muscle larvae given on day - 21.



infection to assess total worm numbers in the small intestine.

The results show that Freunds Incomplete adjuvant administered with PBS had no effect upon worm numbers when compared to controls. Immunization with antigen had no effect upon establishment of worms on the gut as assessed by worm numbers in the small intestine on day 4 post infection. However, by day 8 post infection worm numbers in the gut of immunized groups were significantly lower than the controls (Table 1.1). Thus, immunization with the soluble crude antigen does afford a measurable degree of protection against infection, indicated by an accelerated expulsion of the worm population from the small intestine.

Immunization of NIH mice with soluble particle associated antigen

An experiment was designed to confirm earlier reports (Despommier et al, 1977a) that a soluble particle associated antigen was efficient in protecting against infection with T. spiralis.

Two groups of animals received a total of 250 μg protein antigen divided into two equal doses. The first dose was given subcutaneously with FIA on day-14. The second dose was given without FIA on day-7. Two groups of animals received control immunizations of PBS + FIA. Together with two groups of untreated control animals, all

Table 1.1. Immunization of NIH mice with 250μg (total protein) soluble crude *T. spiralis* antigen.

	Worm recoveries after infection					
	Day 4		Day 8		Day 12	
Group	Mean	S.D.	Mean	S.D.	Mean	S.D.
I (control)	137.2	26.2	148.2	15.3	0.0	
II+	149.0	17.9	152.8	21.4	0.0	
III	156.7	12.2	75.0*	14.2	0.0	
IV	162.3	14.5	65.6*	22.1	0.0	

* Mean significantly different from control
\+ n = 5

the mice were challenged with 300 muscle larvae on day 0. Animals were assessed for total worm numbers in the small intestine on days 4 and 8 post infection. The results demonstrate that immunization with the soluble particle associated antigen does not affect establishment of the worms in the gut, but does accelerate expulsion of the worm population (Table 1.2). These results confirm an earlier report by Despommier et al (1977a) of protection against infection using a similar antigen.

Dose response curve of the soluble crude antigen

The previous experiments have shown that both the soluble crude antigen and the soluble particle associated antigen protect mice against infection, assessed by an accelerated expulsion of the worm burden from the gut. Because of the relative ease with which large amounts of soluble crude antigen could be prepared, when compared to the soluble particle associated antigen, the soluble crude antigen was chosen for further study.

An experiment was designed to examine the dose response for the soluble crude antigen. Groups of mice were immunized with varying amounts of antigen given subcutaneously in one or two doses, according to the standard two-week immunizing protocol. Immunized mice, together with a control group were infected on day 0 with 360 *T. spiralis* muscle larvae. Eight days post infection the worm burdens in the intestines were measured. An *in vitro* measurement of female fecundity was also taken to

Table 1.2. Immunization of NIH mice with 250μg (total protein) soluble particle associated antigen.

	Worm recoveries after infection			
	Day 4		Day 8	
Group	Mean	S.D.	Mean	S.D.
Control	130.6	24.8	127.3	29.3
PBS + FIA	129.3	13.7	139.1	27.6
Ag + FIA	141.2	33.6	60.3*	27.6

* Mean significantly different from control

give a more sensitive assay of immunity.

The results show that immunization with as little as 100μg (total protein) antigen protected animals against infection as measured by worm expulsion. However, female worm fecundity was depressed in mice that had received only 10μg (total protein) antigen (Table 1.3). It should be noted that the method used for measuring female fecundity is only semi-quantitative and serves only to provide a crude estimate, and not an absolute value of female fecundity. Best protection was achieved by immunizing animals with a total of 500 μg (total protein) antigen given subcutaneously in two doses, ie, 250μg protein antigen with FIA on day-14, and 250 μg protein antigen alone on day-7. This immunizing regime was taken as the standard for further experiments.

Route of administration of soluble crude antigen

The previous experiments have shown that protection with antigen is successful when antigen is administered to animals via the subcutaneous route (s.c). An experiment was designed to examine the protection generated by antigen given via the intraperitoneal route (i.p), and in multiple doses. This type of regime has been shown to be very successful in protecting rats against infection with N. brasiliensis (Murray et al, 1979). Immunized animals were administered a total of 500μg (total protein) antigen according to the experimental design described overleaf.

Table 1.3. Dose response curve for soluble crude antigen.

Group	Worm recoveries day 8 after infection		
	Mean	S.D.	Fecundity (larvae per female per hour)
Control	268.0	26.1	4.05
PBS/FIA + PBS	233.0	36.3	4.32
PBS/FIA	256.3	27.3	4.57
5μgAg/FIA + 5μgAg	216.0	31.4	1.07
10μgAg/FIA	219.1	42.7	0.37
50μgAg/FIA + 50μgAg	202.5	40.8	0.90
100μgAg/FIA	181.2*	42.8	1.80
125μgAg/FIA + 125μgAg	135.2*	15.9	0.70
250μgAg/FIA	110.8*	69.4	0.32
250μgAg/FIA + 250μgAg	95.5*	25.3	0.57
500μgAg/FIA	124.6*	27.4	0.50

* Mean significantly different from control.

GROUP	IMMUNIZATION REGIME		
	Day - 21	Day - 14	Day - 7
I	--------	--------	--------
II	--------	250μg Ag + FIA s.c.	250μg Ag s.c.
III	133μg Ag + FIA s.c.	133μg Ag + FIA s.c.	133μg Ag s.c.
IV	--------	250μg Ag + FIA i.p.	250μg Ag i.p.
V	133μg Ag + FIA i.p.	133μg Ag + FIA i.p.	133μg Ag i.p.
VI	PBS + FIA i.p.	PBS + FIA i.p.	PBS i.p.

All animals were challenged on day 0 with 285 muscle larvae. Eight days post infection protection was identified by accelerated expulsion of the worm population from the intestine, and by the depression of female fecundity. FIA and PBS given intraperitoneally did not have any effect on worm numbers in the gut or on female fecundity. Protection was successful when antigen was given either subcutaneously or intraperitoneally, although immunization via the subcutaneous route was marginally more effective (Table 1.4). Antigen given in three doses was no more effective in protecting animals than when given in two doses.

Immunization of fast responder and slow responder mice with soluble crude antigen.

Responses to infection with *T. spiralis* vary considerably between inbred strains of mice and can be

Table 1.4. Effect of route of administration of antigen and number of doses of antigen upon immunization against *T. spiralis*.

Group	Worm recoveries day 8 after infection		
	Mean	S.D.	Fecundity (larvae per female per hour)
I (control)	113.2	28.9	2.62
II	15.8*	9.8	n.d.
III	32.0*	14.5	0.58
IV	40.0*	11.4	0.52
V	47.2*	14.9	0.57
VI	82.7	20.9	2.70

* Mean significantly different from control.

n.d. = not done.

classified arbitrarily into fast and slow responders, dependent upon the onset of expulsion of the worms from the small intestine. NIH mice have been ascribed fast responder status and C57Bl/10 mice slow responder status. F_1 hybrids of NIH and C57Bl/10 parents exhibit the characteristic NIH fast responder pattern of expulsion (Wakelin, 1980).

An experiment was designed to examine and compare the responses of NIH, C57Bl/10 and F_1 (NIH x C57Bl/10) mice to the vaccination schedule proven to be successful in protecting NIH mice against infection. Immunized animals were given a total of 500μg (total protein) antigen in two doses according to the standard immunization regime. Together with unimmunized controls, all animals were infected with 300 muscle larvae on day 0. Animals were assessed for intestinal worm burdens on days 4, 8, and 12 post infection.

Immunization of NIH mice with antigen confirmed the results of earlier experiments. Good protection against infection was evident as shown by accelerated expulsion of the worms from the gut. Immunization of C57Bl/10 mice did not protect against a challenge infection, at least in terms of expulsion, on the days examined. However, immunization of F_1 hybrids (NIH x C57Bl/10) did protect against challenge with lower numbers of worms recovered from the immunized group compared to the control group on day 8 post infection (Table 1.5). These preliminary experiments do suggest that at least in terms of worm expulsion, the response to immunization

with soluble crude antigen is similar to the response to infection, ie, inherited in a simple dominant manner.

Table 1.5. Immunization of fast responder and slow responder strains of mice against *T. spiralis* infection with a soluble crude antigen.

Group/Strain	Worm recoveries after infection					
	Day 4		Day 8		Day 12	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
NIH control	98.4	30.6	110.6	25.9	n.d.	
NIH immunized	106.1	17.9	49.2*	17.6	n.d.	
C57Bl/10 control+	123.4	35.4	132.6	24.7	120.3	17.4
C57Bl/10 immunized+	127.3	14.4	100.5	34.4	129.4	25.0
F_1(NIH x C57Bl/10)+	119.2	23.7	112.3	29.8	0.0	
F_1 immunized+	102.1	19.6	62.1*	21.9	0.0	

* Mean significantly different from corresponding control.

\+ n = 5

n.d. = not done

Summary Points

Chapter 1

(1) In NIH mice given a primary infection of T. spiralis approximately 55% of the inoculum establishes and matures. Worm expulsion begins after day 8 and is essentially complete by day 12 post infection.

(2) During a secondary infection of T. spiralis in NIH mice, worm expulsion starts after day 4 and is almost complete by day 8 post challenge.

(3) Protection against infection with T. spiralis is possible by vaccination with soluble worm (muscle larvae) antigen given parenterally with adjuvant. In vaccinated mice worm loss begins between days 4 and 8 of infection and worm fecundity is reduced. The response to vaccination with worm antigen in rapid and slow responder mice follows the pattern of response to infection and is inherited in a simple, dominant manner.

(4) Mice can be immunized with small amounts of antigen. Worm loss is accelerated in mice given 100 - 500μg (total protein) antigen. With smaller amounts expulsion is not accelerated but fecundity is reduced.

CHAPTER 2

Adoptive transfer of immunity with cells taken from mice immunized with soluble worm antigen.

Experiments involving the adoptive transfer of immune lymphocytes have helped to clarify the nature of the responses involved in mediating immunity to infection with T. spiralis (Crum, Despommier and McGregor, 1977; Wakelin and Wilson, 1977a; Wakelin and Donachie, 1980). This chapter describes experiments designed to investigate the adoptive transfer of immunity using cells taken from animals immunized with worm antigen. Information obtained from such experiments would aid a comparison between host responses to artificial immunization with those to a natural infection with T. spiralis and lead to better defined analyses in which known amounts of identifiable antigenic moieties could be used to elicit responses.

Adoptive transfer of immunity with cells taken from vaccinated mice I. An initial study.

An initial experiment was designed to examine the adoptive transfer of immunity using spleen cells or mesenteric lymph node cells (MLNC) taken from mice immunized subcutaneously with soluble crude antigen. Mice were each immunized with a total of 500μg protein antigen according to the standard immunization regime. Seven days after the administration of the second dose of the antigen (day 0) MLNC suspensions and spleen cell suspensions were prepared. Syngeneic recipient mice each received either 2.5×10^7 spleen cells or 1.5×10^7 MLNC. As a positive control for adoptive transfer one group of mice received 2.3×10^7 immune MLNC from donors which had received an infection of 300 T. spiralis muscle

larvae 8 days previously. Controls, immunized controls and cell recipients were challenged on day 0 with 280 muscle larvae. Eight days post infection evidence for the successful adoptive transfer of immunity was assessed by counting total worm numbers in the small intestine.

The results show that adoptive transfer of 1.5×10^7 MLNC or 2.5×10^7 spleen cells from vaccinated mice did not cause accelerated expulsion of the worm population from the small intestine even though the immunized control group did show a strong degree of protection. Adoptive transfer of 2.3×10^7 MLNC from infected donors did cause an accelerated worm expulsion (Table 2.1).

Adoptive transfer of immunity with cells taken from vaccinated mice II. A re-examination using the soluble particle associated antigen.

In view of the failure to show adoptive transfer of immunity using cells taken from mice immunized with a soluble crude antigen, a repeat experiment was performed. However, in this case, the cell donors were immunized subcutaneously with the soluble particle associated antigen. Mice were each immunized with a total of 500μg protein antigen according to the standard immunization regime. MLNC and spleen cells were taken from two groups of immunized mice 7 days after receiving the second

Table 2.1. Adoptive transfer of immunity with cells taken from mice immunized with soluble crude worm antigen or by prior infection

	Worm recoveries day 8 after infection	
Group	Mean	S.D.
Controls		
No treatment	127.3	39.3
Control Immunization with 250μgAg/FIA + 250μg Ag	60.3*	26.5
Cell recipients		
2.3×10^7 MLNC from infected donors	50.2*	11.4
1.5×10^7 MLNC+ from immunized donors	106.7	15.9
2.5×10^7 spleen cells from immunized donors	116.3	40.8

* Mean significantly lower than no treatment control.
+ n = 5

antigen dose (day 0). Recipient mice received either 2.4×10^7 MLNC or 2.4×10^7 spleen cells. A positive control group of mice that had received 2.5×10^7 immune MLNC from donors which had been infected with T. spiralis 8 days previously was also included. Controls, immunized controls and cell recipients were challenged on day 0 with 280 T. spiralis muscle larvae. Eight days post infection total worm numbers in the gut and female fecundity were measured.

Again, the results demonstrate that adoptive transfer of cells from vaccinated mice was not successful. The immunized mice did show a strong degree of protection and the group given cells from infected donors did show adoptive transfer of immunity (Table 2.2).

Adoptive transfer of immunity with cells taken from vaccinated mice III. Stimulation of the intestine with soluble crude antigen.

The previous two experiments failed to demonstrate adoptive transfer of immunity using cells taken from mice vaccinated subcutaneously. Other workers have shown that the route of administration of an antigen has an important bearing on the particular immune response stimulated (Leskowitz and Waksman, 1960; W.H.O. 1976) and this has been confirmed in a host parasite system by Vernes (1976), studying vaccination against T. spiralis in mice and minipigs. Indeed, his studies demonstrated that immunization by the oral route gave superior results

Table 2.2. Adoptive transfer of immunity with cells taken from mice immunized with soluble particle associated worm antigen or by prior infection.

	Worm recoveries day 8 after infection		
Group	Mean	S.D.	Fecundity (larvae per female per hour)
Controls			
No treatment	109.0	12.3	3.72
Control immunization with 250μgAg/FIA+250μgAg	32.6*	32.7	2.08
Cell recipients			
2.5 x 10^7 MLNC from infected donors	42.9*	15.4	1.61
2.4 x 10^7 MLNC from immunized donors	104.6	41.7	3.75
2.4 x 10^7 spleen cells from immunized donors	124.0	35.4	3.65

* Mean significantly lower than no treatment control.

to immunization via the subcutaneous route. Therefore, experiments were designed in which antigen was administered orally in conjunction with antigen given subcutaneously, in an attempt to generate cells capable of transferring immunity. Groups of mice were immunized subcutaneously, orally, and both subcutaneously and orally. Mice immunized subcutaneously each received 500μg (total protein) soluble crude antigen in two doses according to the standard immunization regime. Mice immunized orally each received 2 mg (total protein) soluble crude antigen administered per os in two doses on days -14 and -7. A cell donor/recipient ratio of 3:1 was used and recipients each received between 3 and 5 x 10^7 cells on day 0. Together with the appropriate control groups, cell recipients were challenged with 300 T. spiralis muscle larvae on the day of cell transfer. Eight days post infection total worm numbers in the gut and female fecundity were assessed.

Again the results failed to show adoptive transfer of immunity as assessed by an accelerated expulsion of worms from the intestine. However, female fecundity was markedly depressed in the groups that had received cells. Administration of antigen orally did not enhance the responses elicited by antigen given subcutaneously, and when given alone, did not cause either an accelerated expulsion or a depression of female fecundity (Table 2.3).

Table 2.3. Adoptive transfer of immunity with cells taken from mice immunized with soluble crude worm antigen subcutaneously and orally.

	Worm recoveries day 8 after infection		
Group	Mean	S.D.	Fecundity (larvae per female per hour)
Controls			
No treatment	220.2	16.7	4.30
Control immunization s.c.	128.7*	27.7	1.98
Control immunization orally	201.7	19.3	3.72
Control immunization s.c. + orally	144.0*	20.5	1.25
Cell recipients			
3.6 x 10^7 MLNC from donors immunized s.c.	181.2	35.9	2.41
3.5 x 10^7 spleen cells + from donors immunized s.c.	243.6	21.8	1.77
3.6 x 10^7 MLNC from donors immunized s.c. + orally	226.2	21.6	2.64
4.2 x 10^7 spleen cells from animals immunized s.c. + orally	233.0	31.7	1.95

* Mean significantly different from no treatment control.

+ n= 5

Although the results do show that immunity can be transferred adoptively with cells from vaccinated mice, transfer is effective only to a limited degree.

Adoptive transfer of immunity with cells taken from vaccinated mice IV. Effect of adjuvant and route of immunization.

Murray et al (1979) studied immunization against N. brasiliensis in the rat and demonstrated that both the choice of adjuvant and its route of administration were important in stimulating a functional immune response. An experiment was designed to compare immunization via the intraperitoneal route with FCA as a method of stimulating cells capable of transferring immunity, with that of immunization via the subcutaneous route with FIA. FCA is a simple water-in-oil emulsion with the addition of extracts of Mycobacteria tuberculosis to the oil phase. The addition of Mycobacteria to the adjuvant has been shown to cause dramatic changes in the immune response generated by immunization. In general, cell mediated immunity and antibody synthesis are enhanced and the mononuclear phagocytic system is stimulated (See W.H.O., 1976).

Mice were immunized with soluble crude antigen (500μg total protein) given in two doses, on day -14 and day -7. A cell donor/recipient ratio of 3:1 was used and cell recipients each received approximately 5 x 10^7 cells on day 0. Together with appropriate control groups

cell recipients were infected with 300 _T. spiralis_ muscle larvae on the day of cell transfer. Eight days post infection total worm numbers in the small intestine and female fecundity were measured.

Immunization of mice by both subcutaneous and intraperitoneal routes was successful. Immunization resulted in both a reduced worm number in the intestine and a depressed female fecundity on day 8 post infection. Adoptive transfer of immunity with cells was also successful in mice that had received spleen cells and MLNC from donors immunized intraperitoneally. Recipient mice showed accelerated expulsion of the worm burden and a depression in female fecundity. However, recipients which had received spleen cells from animals immunized subcutaneously also showed evidence of accelerated expulsion and reduced female fecundity, whereas recipients of MLNC showed only a depressed female fecundity. The results from the recipients of cells taken from animals immunized with PBS and FCA did not present any evidence to suggest that adoptive transfer of immunity was due to the Mycobacteria in the adjuvant (Table 2.4).

The results confirm and extend the observations of the previous experiment, that adoptive transfer of immunity with cells from vaccinated mice is possible. They also suggest that the degree of immunity transferred is related to the number of cells transferred, and may be related to the route and adjuvant used for immunization.

Table 2.4. Adoptive transfer of immunity with cells taken from mice immunized with soluble crude antigen given subcutaneously (s.c.) with FIA or intraperitoneally (i.p.) with FCA.

Group	Worm recoveries day 8 after infection		
	Mean	S.D.	Fecundity (larvae per female per hour)
Controls			
No treatment	120.5	15.7	3.47
Control immunization given s.c.	49.6*	29.2	1.24
Control immunization given i.p.	29.3*	13.4	1.60
Cell recipients			
5.0 x 10^7 spleen cells from animals immunized with PBS/FCA i.p.	130.1	18.6	4.00
5.1 x 10^7 MLNC from animals immunized s.c.	111.5	23.0	1.76
5.1 x 10^7 spleen cells from animals immunized s.c.	73.5*	34.0	1.17
5.3 x 10^7 MLNC from animals immunized i.p.	65.4*	25.5	1.29
5.1 x 10^7 spleen cells from animals immunized i.p.	82.0*	22.6	1.46

* Mean significantly different from no treatment control.

Adoptive transfer of immunity with cells taken from vaccinated mice V. Definitive experiments.

In order to consolidate the findings of the previous experiment, a further two experiments were conducted. As previously, immunized mice were given a total of 500μg (protein) soluble crude antigen in two doses, either intraperitoneally with FCA or, subcutaneously with FIA. A cell donor recipient ratio of 3:1 was used in each experiment and cell recipients each received between 4×10^7 and 8×10^7 cells on day 0. Together with the appropriate control groups animals were challenged with muscle larvae on the day of cell transfer. Total worm numbers and female fecundity were measured eight days post infection.

The results of the experiments are shown in Tables 2.5 and 2.6 and demonstrate that adoptive transfer of immunity with cells from vaccinated mice is possible when more than 4×10^7 cells are transferred. Spleen cells are consistently effective in accelerating expulsion and depressing female fecundity. MLNC are less effective and work best when taken from donors immunized intraperitoneally.

Collectively, the results show that under certain conditions immunization via the parenteral route gives rise to cells capable of mediating immunity to T. spiralis, a situation known to occur in response to a natural enteral infection with T. spiralis (Larsh, Goulson and Weatherly, 1964a; Wakelin and Lloyd, 1976b).

Table 2.5. Adoptive transfer of immunity with cells taken from vaccinated mice. Mice infected with 350 *T. spiralis* muscle larvae on day 0.

Group	Worm recoveries day 8 after infection		
	Mean	S.D.	Fecundity (larvae per female per hour)
Controls			
No treatment	236.3	25.1	3.55
Control immunization given s.c.	121.8*	20.9	0.85
Control immunization given i.p.	88.7*	26.3	0.57
Cell recipients			
6.4×10^7 MLNC from animals immunized s.c.	187.8	27.9	1.45
4.5×10^7 spleen cells from animals immunized s.c.	157.2*	8.3	2.47
7.6×10^7 spleen cells from animals immunized s.c.	136.3*	9.9	1.47
4.5×10^7 MLNC from animals immunized i.p.	159.5*	34.5	2.50
4.4×10^7 spleen cells from animals immunized i.p.	133.5*	16.1	2.57

* Mean significantly different from no treatment control.

Table 2.6. Adoptive transfer of immunity with cells taken from vaccinated mice. Mice infected with 300 *T. spiralis* muscle larvae on day 0.

Group	Worm recoveries day 8 after infection		
	Mean	S.D.	Fecundity (larvae female per hour)
Controls			
No treatment	167.4	20.1	4.00
Control immunization given s.c.	72.9*	11.6	1.43
Control immunization given i.p.	54.3*	15.4	0.88
Cell recipients			
5.7×10^7 MLNC from animals immunized s.c.	112.6*	11.8	1.26
5.6×10^7 spleen cells from animals immunized⁺ s.c.	86.7*	13.2	0.71
4.0×10^7 MLNC from animals immunized i.p.	90.9*	16.7	1.86
5.5×10^7 spleen cells from animals immunized i.p.	72.6*	12.7	0.65

* Mean significantly different from no treatment control.

+ n = 5

Summary Points

Chapter 2

(1) Adoptive transfer of immunity against *T. spiralis* is possible with spleen and MLNC taken from vaccinated mice.

(2) Effectiveness of adoptive transfer of immunity with cells from vaccinated mice is dependent upon the route of antigen administration, type of adjuvant used, and number of cells transferred. Overall, transfer of immunity from vaccinated mice is less effective than when cells from infected mice are used.

Chapter 3: Immunization of mice against *T. spiralis* with a soluble crude worm antigen. A brief examination and correlation with the responses known to occur after a natural infection.

Immunization of animals with parasite antigens is a well researched area of immunoparasitology. Attempts to protect the host against re-infection and parasite induced pathology have been investigated using both protozoan and metazoan parasite antigens (See Mitchell and Anders, 1982). However, few studies have examined the immune response generated by vaccination with an aim to investigating the similarities with and differences from immunity generated by a natural infection. This type of study has been undertaken for protozoan parasites such as the Plasmodium spp. (Bawden, Palmer, Leef and Beaudoin, 1979; Eugui and Allison, 1979; Playfair, 1979; Taylor and Siddiqui, 1979), but neglected for gastrointestinal nematodes which is surprising considering the wealth of research conducted upon vaccination against helminths (Clegg and Smith 1978; Lloyd, 1981). The following chapter aims to provide such a study using the T. spiralis/NIH mouse model.

Grencis and Wakelin (1982) have shown in NIH mice that expulsion of T. spiralis from the gastrointestinal tract is mediated by rapidly dividing T cells (blasts) present in the mesenteric lymph node (MLN) during infection. A series of experiments were conducted in which a number of response parameters were assessed in mice immunized with a soluble crude worm antigen to provide a comparison with changes known to be associated with the development of immunity to a natural infection.

Changes in the MLN and spleen of NIH mice after immunization with a soluble crude worm *T. spiralis* antigen

An initial experiment was designed to examine the cellularity, composition in terms of Ig positive cells, and *in vitro* blast cell activity of the MLN and spleen of mice vaccinated with antigen. Grencis and Wakelin (1982) have demonstrated an increase in cellularity, total numbers of T cells and blast cell activity in the MLN during the course of active intestinal infection with *T. spiralis.*

Groups of NIH mice were immunized subcutaneously with a total of 500μg (total protein) soluble crude worm antigen according to the standard regime. Together with the appropriate controls, cellularity, % composition and *in vitro* blast cell activity of the spleen and MLN were measured on day 7, day 14, and day 28 after the first injection of antigen. Control animals received either no treatment or were given PBS and FCA on day 0 and PBS only on day 7. To confirm that the vaccination was effective, one group of vaccinated mice and one group of untreated controls were challenged with 300 *T. spiralis* muscle larvae on day 14, and worm burdens in the small intestine assessed eight days later.

The results did not demonstrate any differences between the spleen or the MLN of vaccinated and non-vaccinated mice on any of the days examined (Table 3.1). The vaccination regime was shown to be effective, vaccinated mice having a mean worm burden of 51.5 ($\pm$ 20.0) on day 8

Table 3.1. Changes in the spleen and MLN of NIH mice after vaccination with 500μg (total protein) soluble crude worm antigen.

SPLEEN	Mean No Cells Per Organ x 10^7			% Ig + ve cells per organ			Mean Labelling+ Index		
Days post infection	7	14	28	7	14	28	7	14	28
CONTROL	12.1	12.1	13.8	50.5	46.0	49.1	---	---	---
Vaccinated with PBS	11.5	10.1	12.7	49.9	44.4	48.0	96	94	104
Vaccinated with Worm Antigen	10.7	12.6	13.6	47.1	49.3	45.8	95	105	94
MLN									
CONTROL	2.7	3.8	2.3	32.4	35.3	37.4	---	---	---
Vaccinated with PBS	2.5	3.0	2.0	33.6	35.0	33.0	101	98	98
Vaccinated with Worm Antigen	2.8	2.5	2.1	31.8	33.0	30.0	97	99	103

+ [(c.p.m. for experimental cells)/(c.p.m. for control cells)] x 100

post infection, and control mice having a mean worm burden of 102.0 (± 13.6).

Immunization of NIH mice with a soluble crude worm antigen; stimulation of a discrete lymph node

It is possible that the results from the previous experiment failed to show any changes that are associated with a natural infection because the parameters were not assessed in the node or lymphoid organ draining the site of antigen injection.

In order to study the responses in a node draining the site of antigen administration experiments were designed in which mice were vaccinated with soluble crude worm antigen in the hind footpad. This area is drained solely by the popliteal lymph node (Tilney, 1971).

Two preliminary experiments were designed to assess the degree of protection generated by vaccination with worm antigen in the footpad. In each experiment one group of NIH mice were each injected with a total of 200μg soluble crude worm antigen in an emulsified form with Freunds incomplete antigen (FIA). 50μl of the antigen/FIA emulsion was injected subcutaneously into each hind footpad on day -14. Control vaccinated mice received an emulsion of FIA and PBS pH 7.2. Together with untreated controls, all animals were challenged with T. spiralis muscle larvae on day 0 and worm burdens in the small intestine measured on day 8 post infection.

Vaccination with soluble crude worm antigen was successful in protecting mice against a challenge infection as assessed by the accelerated expulsion of the worms from the small intestine (Table 3.2.)

A subsequent experiment was designed to examine the cellularity and in vitro blast cell activity of the popliteal node after vaccination. NIH mice were vaccinated with a total of 200μg soluble crude worm antigen via the hind footpads on day -14. Control mice were vaccinated with PBSpH 7.2. On days 4, 7 and 12 post injection, cellularity and in vitro blast cell activity was measured. Mice vaccinated with worm antigen showed significantly higher values than controls on all the days examined, (Table 3.3). Efficiency of vaccination in protecting mice was confirmed by the accelerated expulsion of a challenge infection of 300 *T. spiralis* muscle larvae, given 14 days after antigen injection, and assessed 8 days post infection. Vaccinated mice had a mean worm burden of 92.3 ($\pm$ 19.1) and control mice had a mean worm burden of 181.6 ($\pm$ 20.4).

In vitro blast cell activity of the MLN from vaccinated animals after a challenge infection

Work by Grencis and Wakelin (1982) has shown that the increased blast cell activity observed in the MLN of NIH mice, in response to a challenge (secondary) infection of *T. spiralis* is accelerated compared with that seen in a primary infection. Cells in the MLN exhibit an increased blast cell activity on day 2 post secondary infection, whereas cells in the MLN 2 days after a primary infection

Table 3.2. Immunization of NIH mice with 200μg (total protein) soluble crude worm antigen via the hind footpad

	Worm recoveries day 8 after infection			
	Expt 1+		Expt 2++	
Group	Mean	S.D.	Mean	S.D.
Control	242.2	17.1	146.1	16.5
PBS + FIA	237.4	21.6	156.3	20.0
Ag + FIA	156.7*	15.1	92.8*	17.0

* Mean significantly different from control

\+ Expt 1. challenge infection of 350 *T. spiralis* muscle larvae

++ Expt 2. challenge infection of 285 *T. spiralis* muscle larvae

Table 3.3. Changes in the popliteal node of NIH mice after immunization with worm antigen via the hind footpad.

	Mean No cells per node x 10^6			Mean labelling index+		
Days post injection	4	7	12	4	7	12
Controls (PBS + FIA)	1.5	1.4	1.6	---	---	---
Ag + FIA	2.5	3.0	2.4	209*	233*	231*

* Mean significantly different from control

\+ See Table 3.1.

exhibit no such increase.

An experiment was designed to examine the in vitro blast cell activity in vaccinated mice 2 days after a natural challenge infection, in order to examine whether worm antigen given parenterally induces an accelerated response similar to that evident after a natural primary infection.

NIH mice were each vaccinated with a total of 500μg (total protein) soluble crude worm antigen according to the standard vaccination regime. On day 0 together with the appropriate controls, vaccinated mice were challenged with 300 T. spiralis muscle larvae. 2 days post infection, MLNC from all groups were assessed for in vitro blast cell activity.

The results show that immunization with antigen generates an increased blast cell response in the MLN after a challenge infection, similar to that exhibited by MLNC taken from mice 2 days after a secondary infection of 300 T. spiralis muscle larvae. MLNC from mice given a primary of 2 days duration did not exhibit an increased blast cell response. (Table 3.4). Efficiency of vaccination with worm antigen was confirmed by the accelerated expulsion of a challenge infection of 300 T. spiralis assessed 8 days post infection. Vaccinated mice harboured a mean worm burden of 4.3 (± 7.6) and control untreated mice having a mean worm burden of 103.1 (± 37.5).

Table 3.4. *In vitro* blast cell activity of the MLN from vaccinated NIH mice after a challenge infection.

Group	Mean labelling index[+] Day 2 post challenge
Day 2 infection	118
Day 2 infection vaccinated mice	145*
Day 2 infection [a] infected mice	161*

* mean significantly different from control

[+] See Table 3.1.

a Primary infection given day -21

Summary Points

Chapter 3

(1) No changes in cell number, % composition or blast cell activity were observed in the spleens or MLN of mice after vaccination with worm antigen given subcutaneously in the dorsal region.

(2) Vaccination of mice against *T. spiralis* was possible when worm antigen was given subcutaneously in the hind footpad, and increased blast cell activity in the popliteal node was observed after vaccination via this route.

(3) *In vitro* blast cell activity was evident in the MLN from vaccinated mice on day 2 after a challenge infection, and was similar to the blast cell activity of the MLN from mice 2 days after a secondary infection.

Discussion

The kinetics of infection with *T. spiralis* muscle larvae in NIH mice have been described by a number of workers (Wakelin and Lloyd, 1976a; Manson-Smith, Bruce, Rose and Parrott, 1979; Kennedy, 1980a; Alizadeh and Wakelin, 1982) and the experiments described here confirm their results, namely that in a primary infection adult worms are expelled from the intestine between days 8 and 12 post infection. The capacity of a primary infection of *T. spiralis* to generate resistance to re-infection has long been known. Rappaport and Wells (1951) observed that a secondary infection of *T. spiralis* in mice was expelled from the intestine much earlier than in a primary infection. In the present study, this observation is borne out in the NIH strain of mice, challenge infections of *T. spiralis* being expelled from the intestine between days 4 and 8 post challenge.

A comparable degree of immunity to that generated by infection can be gained by vaccination of mice with worm antigen. In the present study it was found that several factors influenced the degree of protection afforded by vaccination with a soluble worm antigen including the quantity of antigen administered and the number of doses given. Soluble crude worm *T. spiralis* antigen was always administered in conjunction with Freunds incomplete adjuvant (FIA) as Jenkins (1977) has previously shown that soluble crude antigen from *Trichuris muris* is significantly more effective in protecting against infection with *T. muris*, when given in conjunction with FIA. Freunds complete adjuvant (FCA) was found to be no more

effective than FIA in the generation of protection when given in conjunction with T. spiralis antigen. Therefore it is likely that in this system, the major role of adjuvant is to provide a slow releasing repository of T. spiralis antigen. Adjuvants have been shown to exert their effects upon the immune response in a number of different and complex ways (W.H.O., 1976) and consistantly to enhance protection against reinfection in a number of helminth vaccination studies, including Dictyocaulus viviparus (Silverman, Poynter and Podger, 1962), T. colubriformis (Rothwell, 1978), N. brasiliensis (Poulain, Pery and Luffau, 1976) and T. spiralis (Denham, 1968). The present experiments demonstrated that T. spiralis antigen was consistantly effective in protecting mice against re-infection, when given in conjunction with FIA, although the degree of protection varied between immunization schedules. Protection was effective when using both the soluble crude antigen, as prepared in vaccination studies against T. muris (Jenkins, 1977), and a soluble particle associated antigen, as prepared in vaccination studies against T. spiralis (Despommier et al, 1977a), although the soluble crude antigen was shown to be more effective and more easily obtained. The soluble crude antigen was shown to have dose dependent effects. Low doses (10μg total protein) of antigen reduced female worm fecundity, whilst higher doses ($\geqslant$100μg total protein) accelerated expulsion of the worms from the intestine, in addition to reducing fecundity. These results are in accordance with the work of Despommier et al (1977a) using a T. spiralis particle associated antigen who observed that low doses of antigen were effective in reducing adult worm fecundity and muscle larvae burdens,

with high doses of antigen additionally accelerating expulsion of adult worms from the gut. Dose dependency is a common phenomenon in studies of vaccination against helminths and been shown to operate in a number of systems including *T. colubriformis* (Rothwell, 1978), *Fasciola hepatica* (Lang and Hall, 1977) and *Taenia taeniformis* (Kwa and Liew, 1977). Nakashima, Ohta, Kobayashi, Kato and Kato (1974) have shown that at least two doses of antigen, with a suitable time interval between are important in the induction of an effective and profound secondary response. Although many other studies of helminth vaccination have shown multiple doses of antigen to be more effective in protecting, than a single dose (see Murray, *et al*, 1979) no such trends were observed in the present study, and therefore the optimum time interval for effective protection was not examined. The route of administration of antigen has been shown to be critical in generating a particular immune response (Spencer, Waldman and Johnson, 1974) and in studies of vaccination against helminths, the effectiveness of one particular route varies between parasite/host systems. Murray *et al* (1979) found that antigen given via the intraperitoneal route was the most effective route in protecting against *N. brasiliensis*. Vernes (1976) found that the oral route gave superior protection over the subcutaneous route using metabolic antigens from *T. spiralis* muscle larvae, whereas Rothwell (1978) studying vaccination against *T. colubriformis* found the oral route the least effective, when compared to the subcutaneous, intradermal, intraperitoneal or intraduodenal routes. In the present study, vaccination with *T. spiralis* antigen via the subcutaneous route was consistently more

effective, than antigen given intraperitoneally. Immunization via the oral route was completely ineffective, and may have resulted from insufficient antigen, degradation of worm antigens by the digestive processes, the difficulty of establishing responsiveness to vaccines administered orally (Pierce and Koster, 1980; Swarbrick, Stokes and Soothill 1979) and the possible induction of mucosal tolerance (Bienenstock and Befus, 1980; Tomasi, 1980).

Variations in susceptibility to parasites is a well documented phenomenon in natural host populations (Wakelin, 1978b) and well researched in laboratory models of host/parasite interaction with particular respect to genetic control of immunological responses (See Rosenstreich, Weinblatt and O'Brien, 1982). The genetic control of immune responsiveness to T. spiralis in mice has been extensively examined (Bell and McGregor, 1980; Wakelin, 1980; Wassom, David and Gleich, 1980), and both H-2 linked andnon H-2 linked genes have been shown to exert effects. Wakelin (1980) has shown that the capacity of mice to respond to infection with T. spiralis quickly and expel the majority of the adult worm burden from the intestine within 12 days (NIH mice) is inherited in a dominant manner, whereas a slow response, in which worm loss occurs after 12 days (C57BL/10 mice) is inherited as a recessive characteristic.
The experiments described here show that the effective immunity generated by vaccination with worm antigen is also inherited in a dominant fashion, and although the genetic basis for this was not examined, it is reasonable to suggest that control similar to that operating upon a natural

infection, may be involved.

The observation that immunization with T. spiralis worm antigen via the parenteral route, generates an immune response operational at the intestinal level begs an investigation of the component parts of the immune response generated by antigen administered at a site distant from that of infection. Adoptive transfer of immunity with immune cells has been a useful approach in the examination of the immune response to nematode parasites and especially of T. spiralis infections in mice (See Wakelin and Denham, 1982). It is well known that MLNC from NIH mice infected with T. spiralis are capable of adoptively transferring immunity and the present study shows that vaccination with worm antigen does generate cells capable of transferring immunity as assessed by both accelerated expulsion of the worm burden from the gut and reduced fecundity of adult female worms. However, spleen cells are more effective than MLNC, in this respect and the generation of cells capable of transferring immunity is dependant upon the route of administration of antigen and the adjuvant used.

Expulsion of T. spiralis from the intestine of NIH mice has shown to be related to a population of rapidly dividing T cells in the MLN during the early stages of intestinal infection. Upon a challenge infection the production of large numbers of rapidly dividing T cells is accelerated, and the worms expelled from the gut earlier than in a primary infection (Grencis and Wakelin, 1982). Although

adoptive transfer of immunity with spleen cells from vaccinated mice was consistent, the present studies failed to detect any evidence of increased numbers of dividing T cells in the spleen or MLN of mice vaccinated S.C. in the neck region. However, the popliteal node of mice vaccinated s.c. in the hind footpad did show evidence or enhanced blast cell activity. This observation *per se* is not uncommon, as it is well known that a lymph node draining the site of antigen injection exhibits evidence of increased cell division (McConnell, Lachman and Hobart, 1974).

Comparison of immune responses generated by infection with those generated by vaccination has rarely been undertaken in helminth systems. Sher, Hieny, James and Asofsky (1982) have observed increased anti-shistosomulum antibody responses in mice vaccinated with irradiated cercariae, and from such studies have shown that resistance to a challenge infection is both thymus and B cell dependent Murray *et al* (1979) described an increase in reaginic antibody levels and intestinal mast cell numbers in rats vaccinated against *N. brasiliensis*, although the results obtained from these studies did show inconsistencies between experiments. The experiments presented in this section examined some of the components of the immune response to *T. spiralis* in vaccinated mice in the light of the responses occurring after natural infection. The presence of increased numbers of blast cells in the draining node after administration of *T. spiralis* antigen, is similar to the situation observed in the MLN, which is the draining node, after a *T. spiralis* infection. Large numbers of rapidly dividing T cells (blasts) are produced

in the MLN during active intestinal infection by the worm and upon re-infection T blasts are produced rapidly, in a manner characteristic of a classical secondary response suggesting the existence of a population of memory (T) cells. It is tempting to propose that vaccination with worm antigen also produces a population of rapidly dividing T cells in the node draining the site of antigenic injection from which memory cells are derived. Upon infection memory cells would be involved in the rapid production of T cells in the node draining the site of infection (MLN) and indeed experiments from the present study, show that the MLN of vaccinated mice does show increased blast cell activity early after a challenge infection, a characteristic only normally observed upon challenge after priming by natural infection.

Vaccination against infection is directly or indirectly the long term goal of the majority of immunoparasitological studies of today, particularly concerning medically and economically important parasites (WHO, 1981). The present study has emphasized the importance and usefulness of laboratory models in elucidating the immune mechanisms generated by vaccination and their relationship to those responses generated by infection, an important aspect in the evaluation of potential parasite vaccines.

SECTION 2:

The Cellular Response to Infection of Mice with *T. spiralis*

Parasite infections elicit host responses which may confer protective immunity and lead to a marked degree of resistance to reinfection (Mitchell, 1980). Protective immunity may involve both immunological and non-immunological mechanisms the complex nature of which make it especially difficult to separate the components of the response. A number of experimental approaches have been employed in investigations of resistance, of which the adoptive transfer of serum or immune lymphocytes has been the most common, and has provided much information concerning the immune responses to both protozoan and metazoan parasites (See Cohen and Sadun, 1976).

Protective immunity to gastrointestinal nematodes has received a large amount of attention particularly in those parasite/host systems which exhibit spontaneous cure (see Wakelin, 1978a;Ogilvie and Love, 1974). Evidence accumulated over the years shows that spontaneous cure is mediated in some way by thymus dependent immune mechanisms although the _in vivo_ effector mechanisms remain to be definitively described. Transfer of immunity with immune serum or immune cells has proved possible in a number of systems, the relative success of such transfers varying between, and within, experimental models (See Wakelin, 1978a; Bartlett and Ball, 1974; Behnke and Parish, 1981). In the _T. spiralis_ mouse/rat system immunity transferred with immune cells has shown to be consistently effective in transferring immunity when the cells are taken from lymphoid tissue draining the site of infection, ie, the mesenteric lymph node (Love, Ogilvie and McLaren, 1976; Wakelin and Wilson, 1977a)

or from the thoracic duct (Crum, Despommier and McGregor, 1977). It is known that cells expressing T cell markers mediate adoptive transfer of immunity (Wakelin and Wilson, 1979a; Crum et al, 1977) but the contribution of Ig positive cells or Thy 1 negative cells is still open to debate, and in general the characteristics of the cells mediating adoptive transfer have received little attention.

The long term resistance to re-infection exhibited in many gastrointestinal nematode host systems (Mitchell, 1980) implies the persistence of memory, and therefore, the existence of a population of memory cells. The nature of these memory cells and their anatomical location remains to be elucidated. Long term resistance to re-infection with T. spiralis has been observed in both the mouse and rat systems and two types of "secondary" response have been identified. In the rat, resistance to re-infection is expressed primarily as a rapid expulsion, a phenomenon in which the majority of the challenge worm burden is expelled within hours of re-infection. In mice rapid expulsion is a transitory phenomenon (Bell and McGregor, 1980; Alizadeh and Wakelin, 1981a) and immunity is expressed as an accelerated version of the primary response. The mechanisms mediating the latter are probably different from those mediating rapid expulsion (Alizadeh and Wakelin, 1981b; Lee and Ogilvie, 1981) and indeed the involvement of true immunological memory in rapid expulsion is still á moot point.

Analysis of memory requires both the identification of memory cell populations and the ways in which these cells are activated. Recruitment of lymphocytes to the site of antigenic challenge has been extensively examined for certain defined antigens (Hall and Morris, 1965; Hay, Lachmann and Trnka, 1973; Cahill, Frost and Trnka, 1976). It has been demonstrated both in mice and rats that after localization of antigen in the spleen or lymph node, most lymphocytes specific for the antigen are selectively depleted from the recirculating lymphocyte pool. The depletion is correlated with an increase in cell numbers at the site of antigen localization (Ford and Atkins, 1972; Sprent and Miller, 1973). Experiments have also shown that administration of antigen to the afferent lymphatic of a lymph node induces a "cell shutdown" effect in the node preventing the exit of circulating lymphocytes from the node for a short period of time (Frost and Lance, 1974; McConnell and Hopkins, 1981). Cell shutdown is followed by an increase in numbers of circulating lymphocytes flowing through the node (Cahill et al, 1976). Distinct but related observations have been described for animals infected with gastro-intestinal helminths. Experiments have shown that there is an enhanced homing of lymphocytes to the intestinal tissue early after infection. This has been observed in animals infected with N. brasiliensis (Love and Ogilvie, 1977), and Trichuris muris (Lee, 1982). In T. spiralis infected animals lymphoblast homing has been shown to be antigen independent (Rose, Parrott and Bruce, 1976a) and unrelated to increased

regional blood flow (Ottaway, Manson-Smith, Bruce and Parrott, 1980). Clearly the mechanisms involved in sequestration of both naive and primed lymphocytes, and their interaction with antigen are areas of immunoparasitology requiring investigation.

The array of protective immune responses generated by a complete infection of T. spiralis are stimulated by all stages of the life cycle, that is, pre-adults, adults (Bell, McGregor and Despommier, 1979) and migrating new born larvae (Moloney and Denham, 1979). Recent studies have now formally demonstrated marked differences in surface antigens present on each life cycle stage (See Maizels, Philipp and Ogilvie 1982) although studies on the importance of particular life cycle stages in the generation of immune responses mediated by lymphocytes are scarce.

In summary, although there is a wealth of knowledge concerning the cellular mechanisms involved in mediating immunity to gastrointestinal nematodes, much of it is fragmentary and therefore, precludes a comprehensive understanding of the immune mechanisms operating.

This section of work aims to examine some of the points discussed above, utilizing the T. spiralis/NIH mouse model. This model can be manipulated in a number of useful ways, which together with experimental approaches recently introduced to the field of immunology provide a

suitable system for the detailed study of the cellular responses to infection.

CHAPTER 4: The cellular response in mice to infection with *T. spiralis*. A Preliminary Analysis

Immunity to *Trichinella spiralis* in mice can be transferred adoptively with mesenteric lymph node cells (MLNC),(Wakelin and Wilson, 1977a). Wakelin and Wilson, (1979a) examined the capacity of MLNC suspensions enriched in T or B cells to adoptively transfer immunity, and concluded that T cells are involved in the generation of changes in the small intestine that are responsible for worm expulsion.

Several workers have observed cellular changes in the mesenteric lymph node (MLN) of animals infected with *T. spiralis*. For example, Tanner, Lim and Faubert (1978) noted an increase in number of leucocytes during the intestinal stages of infection in mice. Ottessen, Smith and Kirkpatrick (1975) observed an enhanced *in vitro* stimulation of MLNC taken from rats three days post infection and Rose, Parrott and Bruce (1976a) noted an increase in lymphoblast activity in the mesenteric lymph node during the course of intestinal infection with *T. spiralis* in mice. However, no studies have correl ated the cellular changes in the MLN of animals infected with *T. spiralis* with the capacity of MLNC to transfer immunity adoptively. This chapter provides a preliminary study of the changes which occur in the MLN of infected mice, and established techniques necessary for a more detailed analysis.

Materials and Methods

Cell Suspensions and Adoptive Transfer

Cell suspensions were prepared, enriched for T or B cells, labelled with fluoroscein conjugated antibodies, isotopically labelled, and transferred according to the techniques described in the general materials and methods.

Complement mediated cytotoxicity

To ensure elimination of the contaminating T cells from MLNC populations enriched for B cells, a complement mediated cytotoxicity test was used. The Thy 1.2 monoclonal antibody (Clone F7D5, IgM) was obtained from Olac 1976 Ltd and a preliminary experiment was conducted using unabsorbed guinea pig complement (GPC) to assess its cytotoxic titre. Thymocyte suspensions prepared from NIH mice were treated as follows. Cells were suspended in various concentrations (in Medium 199) of the anti-Thy 1.2 antibody at a cell concentration of 1×10^7 cells/ml. The suspension was incubated at room temperature for 30 mins and centrifuged at 200 g for 5 mins. The cell suspension was resuspended in a 1/10 dilution of GPC and incubated at 37^oC for 40 mins. After washing twice in fresh medium 199, the cells were counted. Appropriate controls were prepared and treated accordingly. All samples were assayed in duplicate. The cytotoxic titre of the antibody was expressed as a cytotoxic index:

$$\text{Cytotoxic Index} = \frac{\%\ \text{dead (test)} - \%\ \text{dead (control)}}{100 - \%\ \text{dead (control)}} \times 100$$

The results from the complement mediated cytotoxicity tests show that under the conditions employed, antibody at a concentration of 1/1000 killed approximately 95% of Thymocytes (Table 4.1), and this dilution was therefore used in all experiments.

Worm Recovery

Worm recovery, worm fecundity and worm length were measured using the techniques of Wakelin and Wilson (1977a). These are described fully in the general materials and methods.

Table 4.1. Complement - dependent cytotoxic activity of anti-Thy 1.2 antibody against thymocytes of NIH mice. A total of 1×10^7 thymocytes were assayed for each sample. Complement concentration = 1/10.

Antibody concentration	Cytotoxic Index	
	Sample 1	Sample 2
No treatment	11.7	6.4
Antibody (1/100) only	13.6	8.2
Complement only	9.8	8.6
1/100	92.7	94.3
1/250	89.6	95.6
1/500	93.2	96.6
1/1000	92.9	98.0
1/10,000	87.6	90.2

RESULTS:

Adoptive transfer of immunity with mesenteric lymph node cells in NIH mice: Establishment of technique

An initial experiment was designed to confirm earlier work by Wakelin and Wilson (1977a) which demonstrated the successful transfer of immunity with mesenteric lymph node cells taken from mice eight days after infection with T. spiralis. Donor NIH mice were infected with 300 muscle larvae. Cell suspensions were prepared and approximately 3.5×10^7 MLNC were injected into recipient mice in a lateral tail vein. Together with control mice which had not received cells, recipients were challenged with 300 T. spiralis muscle larvae on the day of cell transfer. Worm numbers in the intestines of both control and experimental mice were assessed on day 4 and day 8 post infection.

The results clearly demonstrate that mesenteric lymph node cells taken from infected donors are capable of transferring immunity as expressed by the accelerated expulsion of the worm burden from the intestine. Adoptive transfer of cells does not affect establishment of the worms in the gut (day 4), but does reduce their longevity in the small intestine (day 8) (Table 4.2).

Adoptive Transfer of immunity against T. spiralis in NIH mice with MLNC enriched for T or B cells

To confirm the work of Wakelin and Wilson (1979a)

Table 4.2. Adoptive transfer of immunity against *T. spiralis* infection in NIH mice with MLNC. Cells taken from donors 8 days post infection.

	Worm recoveries after infection			
	Day 4		Day 8	
Group	Mean	S.D.	Mean	S.D.
Control - no cells	147.8	19.6	156.2	12.7
3.5×10^7 MLNC	149.1	17.2	64.3*	18.5

* significantly different from control

experiments were designed to assess the adoptive transfer of immunity against T. spiralis with T cell (non-adherent) or B cell (adherent) enriched populations of mesenteric lymph node cells, using adherence to nylon wool as a method of enrichment. Cell donors were infected with 300 T. spiralis muscle larvae on day 0 and MLNC taken for transfer on day 4 and day 8 post infection. A cell donor/recipient ratio of 3:1 was used. After separation on nylon wool columns the T cell fractions (non-adherent) were 13% Ig positive (day 4); 22% Ig positive (day 8), and the B cell fractions (adherent) were 66% Ig positive (day 4); 75% positive (day 8) as assessed by direct anti-immunoglobulin fluorescence. Cells were given to recipient mice on day 0, and together with controls, all animals infected with 300 T. spiralis muscle larvae on the day of cell transfer. Worm numbers, worm fecundity and worm length were measured on day 8 post infection.

T cell enriched populations of MLNC were most successful in adoptively transferring immunity as assessed by a reduction worm number, and were more effective than unseparated populations of MLNC. B cell enriched populations of MLNC were much less effective in reducing worm numbers, although worm length and worm fecundity were markedly depressed (Table 4.3).

The results present evidence for a definite involvement of Ig negative (T cells) in mediating expulsion of the adult worm from the gastrointestinal tract. The results also suggest that an Ig positive (B cells) fraction of

Table 4.3. Adoptive transfer of immunity against T. spiralis in NIH mice with T cell and B cell enriched populations of MLNC.

Group	Day 8 post infection				
	Worm Recovery		Fecundity larvae/♀/hr	Size length m.m.	
	Mean	S.D.	Mean	Mean	S.D.
Control - no cells	209.8	25.6	6.1	2.70	0.28
Day 4 cells					
3.0×10^7 unseparated MLNC	72.6*	22.6	0.6	1.78*	0.24
2.1×10^7 T cell enriched MLNC	32.5*	13.6	5.1	2.96	0.46
1.90×10^7 B cell enriched MLNC	155.5*	23.6	2.0	2.48	0.43
Day 8 cells					
2.7×10^7 unseparated MLNC	50.6*	61.0	0.9	1.78*	0.17
2.1×10^7 T cell enriched MLNC	14.6*	5.7	n.d.	2.72	0.31
2.0×10^7 B cell enriched MLNC	155.4*	14.7	2.0	1.88*	0.27

* significantly different from control.

MLNC may contribute to worm expulsion, but does play a role in reducing worm length and fecundity. No evidence was presented to suggest that the relative roles which T and B cells in immunity to T. spiralis, change between days 4 and 8 of infection.

Adoptive transfer of immunity against T. spiralis in NIH mice using T cell enriched and B cell enriched populations of MLNC. Treatment of B cell enriched fraction with anti Thy 1.2 antibody and complement

The previous experiment has shown that a T cell enriched population of MLNC taken from donors on day 8 post infection with T. spiralis transfers a strong degree of immunity as assessed by accelerated expulsion of the worm burden from the gut. It is possible that the limited degree of immunity transferred with the B cell enriched population of MLNC was due to contaminating Ig-ve or T cells.

An experiment was therefore designed in which a B cell enriched fraction of MLNC were treated with a monoclonal IgM anti-Thy 1.2 antibody and complement to remove contaminating T cells by lysis. The capacity of this treated fraction to adoptively transfer immunity was then assessed. Cell donors were infected with 300 T. spiralis muscle larvae 8 days previous to cell transfer. A cell donor/recipient ratio of 4:1 was used. MLNC cell suspensions were prepared, and after passage through nylon wool columns in the usual manner, the B cell enriched

fraction treated with anti Thy 1.2 antibody and complement. Recipient mice received the appropriate number of cells, and together with controls, all mice were infected with 300 _T. spiralis_ on day 0. Eight days post infection total worm numbers in the small intestine were measured.

The results are presented in Table 4.4 and show that a relatively pure B cell population of MLNC does not transfer immunity as assessed by accelerated worm expulsion, and confirms that a T cell enriched population of MLNC does cause an acceleration of the expulsion of _T. spiralis_ from the gut.

Cellular changes in the MLN and spleen during the course of a primary infection of _T. spiralis_ in NIH mice

As a prelude to experiments examing any correlation between cellular changes in the MLN and the capacity of MLNC to adoptively transfer immunity (see Chapter 5), a preliminary analysis of certain cellular changes occurring in the MLN and spleen of animals which had received a primary infection of _T. spiralis_ was undertaken. Five groups of NIH mice were infected with 300 _T. spiralis_ on day 0, and a further 5 groups were used as controls. On each of days 2, 4 8, 10 and 18 post infection one group of controls and one group of infected mice were assessed for total worm numbers in the small intestine. MLNC suspensions and spleen cell suspensions were also prepared on these days and cell numbers per animal and percentage Ig and Thy 1.2 bearing cells observed. _In vitro_

Table 4.4. Adoptive transfer of immunity against T. spiralis in mice with T cell enriched and B cell enriched populations of MLNC.

	Day 8 post infection		
	Donor Cells	Worm recovery	
	% Ig + ve	Mean	S.D.
Control - no cells	----	168.8	17.7
4.0×10^7 unseparated MLNC	24.0	68.5	32.3
4.3×10^7 T cell enriched MLNC	7.6	33.3*	21.2
4.4×10^7 B cell + enriched MLNC treated with anti Thy 1.2 and complement	89.2	173.8	17.8

* significantly different from control

+ n = 5

blast activity of MLNC and spleen cells was also measured using the macroculture method.

Approximately 50% of the inoculum established in the small intestine. Expulsion began after day 8 and was almost complete by day 10 post infection (Fig 4.1). The numbers of MLNC per mouse increased during the early stages of infection, declining after day 8 to control levels. Spleen cell numbers remained near control levels until day 4 post infection, after which time there was an increase until the end of the experiment on day 18 (Table 4.5). Similar trends in the in vitro blast cell activity of MLNC suspensions and spleen cell suspensions was also observed (Table 4.6). The results of labelling studies to assess cell composition were variable. However, in the MLN, there was a slight increase in the% Thy 1.2 bearing cells on days 4 and 8 over controls. No differences were observed in cell composition of the spleen during the experimental period (Table 4.7).

The results show that profound changes in terms of cell numbers and cell division occur in the MLN during the period of intestinal infection, and that similar changes occur in the spleen towards the latter stages of intestinal infection and after expulsion of the worm from the gut.

Figure 4.1. The course of a primary infection of NIH mice with 300 *T. spiralis*.

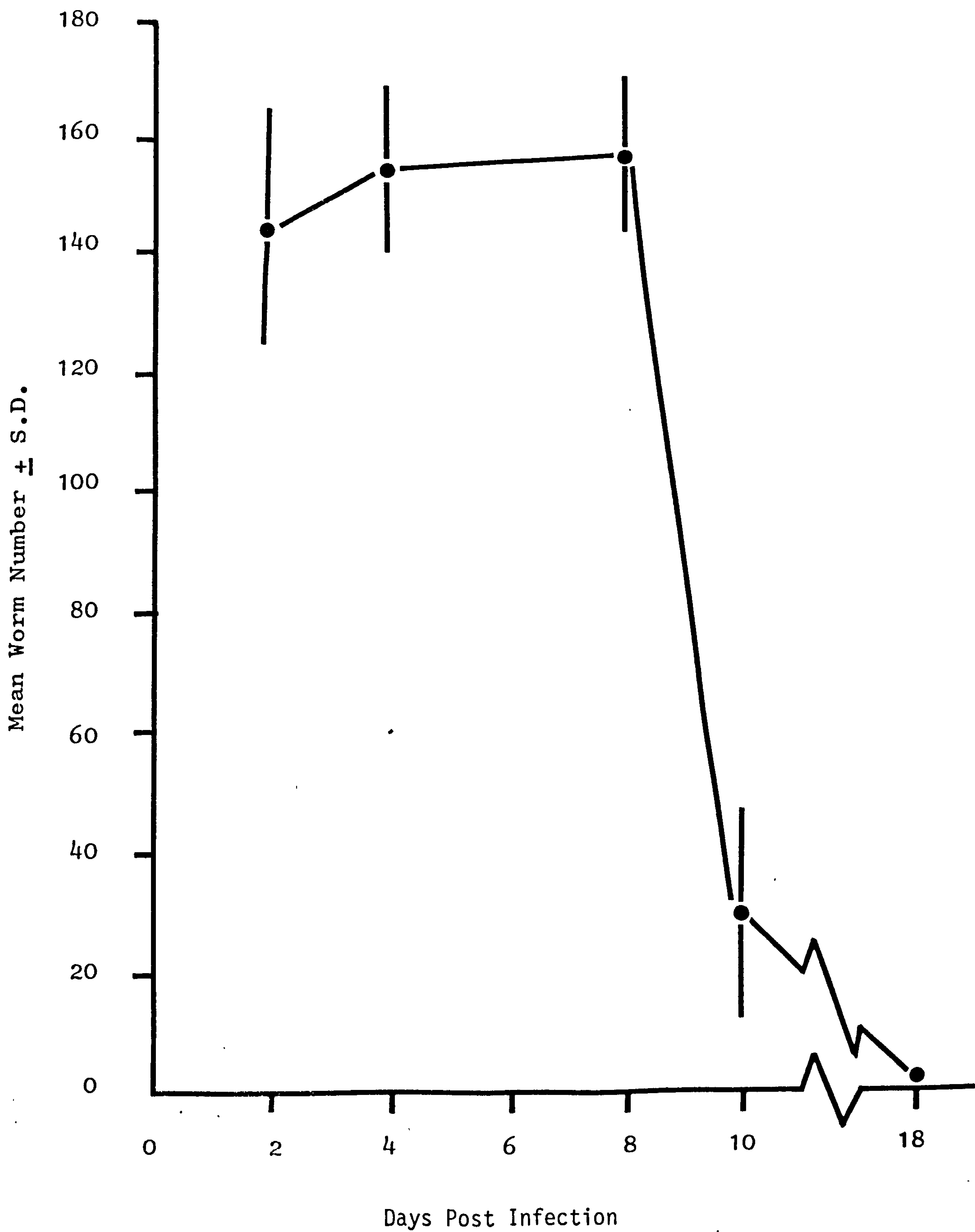


Table 4.5. Changes in cellularity of the mesenteric lymph node and spleen in NIH mice after a primary infection of 300 *T. spiralis* muscle larvae.

	Mean number of cells per organ x 10^7			
	Mesenteric lymph node		spleen	
Day post infection	Control	Infected	Control	Infected
2	3.08	3.69	7.34	7.69
4	3.90	6.68	7.84	7.76
8	3.90	6.88	7.80	8.06
10	2.79	2.49	7.01	8.88
18	2.74	2.78	7.00	11.07

Mean values based on six mice/group.

Table 4.6. *In vitro* blast cell activity of the mesenteric lymph node and spleen in NIH mice after a primary infection of 300 *T. spiralis* in mice.

	In vitro blast cell activity							
	Mesenteric lymph node				Spleen			
	Control		Infected		Control		Infected	
Days post infection	c.p.m.	(S.D.)	c.p.m.	(S.D.)	c.p.m.	(S.D.)	c.p.m.	(S.D.)
2	19743	(1833)	18365	(3188)	31045	(6640)	28649	(4522)
4	15384	(4396)	21624	(2326)	27502	(3784)	23209	(2406)
8	15884	(4486)	28438	(1664)	29751	(4012)	47774	(4669)
10	20465	(4113)	21439	(2251)	26220	(2587)	60650	(4516)
18	21965	(4231)	19917	(2450)	28437	(3016)	63669	(6245)

Mean values based on triplicate assays for each cell sample.

Table 4.7. Changes in cell composition of the mesenteric lymph node and spleen in NIH mice after a primary infection of 300 T. spiralis muscle larvae.

	% Fluorescence							
	Mesenteric lymph node				Spleen			
	Control		Infected		Control		Infected	
Days post infection	Ig	Thy 1.2	Ig	Thy 1.2	Ig	Thy 1.2	Ig	Thy 1.2
2	25.0	57.0	36.9	40.9	48.2	39.0	33.6	57.4
4	28.0	51.4	19.0	70.5	39.2	51.6	34.6	55.2
8	29.0	51.6	30.5	67.8	39.8	50.0	36.9	57.6
10	34.0	57.0	51.2	48.8	52.0	47.0	48.8	47.0
18	42.0	48.0	40.2	59.0	49.6	47.0	49.0	51.8

Mean values based on six mice/group.

Summary Points

Chapter 4

(1) MLNC taken from donors 8 days after infection are capable of transferring immunity expressed by an accelerated expulsion of the worm burden from the intestine.

(2) Techniques for separation of MLNC into populations enriched for T or B cells were established. Adoptive transfer of immunity with a T cell-enriched population of MLNC does transfer immunity as assessed by an accelerated expulsion of the worm burden. A B cell enriched population of MLNC does not cause an accelerated expulsion of the worm burden, but does depress worm fecundity and reduce worm length.

(3) Preliminary observations showed that there was an increase in cellularity, numbers of Thy 1.2 + ve cells, and *in vitro* blast cell activity of the MLN of infected mice, until day 8 after infection, after which there was a decline. In the spleen of infected mice there was an increase in cellularity and *in vitro* blast cell activity from day 8 post infection until the end of the experiment (day 18),

(5) The cells effective in mediating adoptive transfer of immunity were shown to be T cells.

Chapter 5. Short lived dividing cells mediate adoptive transfer of immunity to *T. spiralis* in mice. I Availability of cells in primary and secondary infections in relation to cellular changes in the mesenteric lymph node.

Published in Immunology Vol 46, 443 - 450 1982.

Immunology 1982 46 443

Short lived, dividing cells mediate adoptive transfer of immunity to *Trichinella spiralis* in mice

I. AVAILABILITY OF CELLS IN PRIMARY AND SECONDARY INFECTIONS IN RELATION TO CELLULAR CHANGES IN THE MESENTERIC LYMPH NODE

R. K. GRENCIS & D. WAKELIN *Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Bearsden Road, Bearsden, Glasgow*

Accepted for publication 17 December 1981

Summary. After a primary infection with the parasitic nematode *Trichinella spiralis* NIH mice showed a short lived increase in cellularity of the mesenteric lymph node (MLN), which began between days 2 and 4, peaked at day 8 and had declined by day 12. The majority of cells contributing to this increase were Ig−ve and presumed to be T cells. Coincident with the increase in cell number there was an increase in lymphoblast activity, again largely in the T-cell fraction. MLN cells taken at intervals from mice during a primary infection successfully transferred immunity, i.e. accelerated worm expulsion in recipients, on days 4 and 8, but not on day 12. It was shown that the effective cells in transferring immunity were present in the T-enriched fraction. When mice were given a second infection 21 days after a primary infection the same sequence of changes was apparent in the MLN, but the time course was accelerated, i.e. peak cellularity and lymphoblast activity occurred on day 4 post challenge. Cells capable of transferring immunity were present in the MLN on days 2 and 4 post challenge but not thereafter. As in the primary infection the effective cells, and those responsible for the cellular changes in the MLN, were T cells.

Present address and correspondence: R. K. Grencis, Department of Zoology, University of Nottingham, University Park, Nottingham NG7 2RD.

0019-2805/82/0600-0443$02.00

© 1982 Blackwell Scientific Publications

INTRODUCTION

Primary infections with the intestinal parasitic nematode *Trichinella spiralis* in mice lead to a strong immunity to reinfection and this immunity can be transferred adoptively with mesenteric node lymphocytes (Wakelin & Wilson, 1977). Whereas immunity to reinfection is long lasting (several months), adoptive transfer is possible only with cells taken from donors within a defined period post infection. Cells taken after an interval, or cells taken from multiple infected donors, are ineffective. One reason for this may be that the cells concerned are short lived effectors, present for only a limited period during the active response to infection. It is known that the cells that mediate transfer are T lymphocytes (Wakelin & Wilson, 1979) but their characteristics are not well defined. This paper defines in detail the availability of such cells in primary and secondary infections in relation to the concurrent changes in cell number and lymphoblast activity. In the accompanying paper (Wakelin, Grencis & Donachie, 1982) the cells mediating transfer have been characterized in terms of their *in vivo* activity.

MATERIALS AND METHODS

Mice

Male mice of the inbred NIH strain were obtained from Hacking & Churchill Ltd, Huntingdon. They were used at 8 weeks of age in groups of six.

Trichinella

The strain of parasite and the methods used in maintenance, infection and recovery have been described previously (Wakelin & Wilson, 1977). Mice were routinely infected with 300 larvae.

Cell transfer

Suspensions of mesenteric lymph node cells (MLNC) in medium 199 (Gibco Europe Ltd) were prepared from infected donors by standard methods (Wakelin & Wilson, 1979). The required number of cells was injected via a lateral tail vein in a volume not exceeding 0·5 ml.

Cell labelling

MLNC were prepared and suspended in RPMI 1640 medium (Flow Laboratories Ltd) supplemented with $NaHCO_3$, glutamine, foetal calf serum, heparin and antibiotics. Aliquots of 1×10^7 cells were placed in sterile plastic tissue culture grade tubes and to each was added 2 μCi of 5-[^{125}I] Iodo-2′-deoxyuridine, [^{125}I]-UdR, specific activity 5 Ci/Mg; Amersham International Ltd) in 0·9% NaCl. The tubes were gassed with 2% CO_2 and incubated for 2 hr at 37°. At the end of this time the cells were washed three times in medium 199 (Flow Laboratories Ltd) and the activity in each tube measured by counting for 1 min in a Packard Tricarb liquid scintillation spectrometer. [^{125}I]-UdR, a thymidine analogue, is incorporated by cells undergoing DNA synthesis and is therefore a convenient label for dividing (blast) cells in lymphocyte suspensions. Blast cell activity was expressed in terms of a labelling index as follows: labelling index = [(counts per minute/10^7 experimental MLNC)/(counts per minute/10^7 control MLNC)] $\times$ 100.

T- and B-cell separation

MLNC suspensions were separated into non-adherent (T-cell-enriched) and adherent (B-cell-enriched) fractions by passage through a nylon wool column (Wakelin & Wilson, 1979). Removal of contaminating T cells in the B-cell-enriched fraction was carried out by the use of Thy 1·2. F7D5 monoclonal IgM cytotoxic antibody (Olac 1970 Ltd) in conjunction with unadsorbed guinea-pig complement (Wellcome). The purity of each fraction, as assessed by staining with fluorescein-conjugated rabbit anti-mouse immunoglobulin (Nordic Immunological Laboratories) was: adherent fraction 61% Ig+ve (B) cells; non-adherent fraction 7% Ig+ve cells; adherent anti-Thy-1·2 and complement-treated fraction 90% Ig+ve cells.

Statistics

Student's *t* test was used to assess the significance of differences between mean values in results. *P* values <0.05 were considered significant.

RESULTS

Following a primary infection with muscle larvae 50%–60% of the inoculum establish in the small intestine. After approximately 8 days, expulsion of the adult worm begins and is virtually complete by day 12. If a secondary infection is given on day 21 the worms in the intestine undergo an accelerated expulsion and worm loss is almost complete by day 8.

Adoptive transfer of immunity and changes in the MLN during the course of a primary infection

To investigate the times post infection at which cells capable of mediating immunity were present in the MLN, lymphocyte suspensions were prepared from donor mice 2, 4, 8, 12 and 22 days after a primary infection. In the adoptive transfer experiments cell recipients each received 4×10^7 MLNC on the day of infection and the effectiveness of transfer was assessed by counting the numbers of worms present in recipients and controls 8 days later.

Total cell numbers and *in vitro* blast cell activity in the node exhibited similar trends over the course of a primary infection. Both were elevated above control levels by day 4 post infection, attained peak levels on day 8 and declined to near control values after day 12. Adoptive transfer of immunity with MLNC was only successful using cells taken from donors on day 4 or 8 post infection (Fig. 1).

Immunofluorescence showed that the increase in total cell number in the mesenteric lymph node (MLN) was accompanied by a substantial increase in numbers of Ig−ve cells (T cells) in the node reaching peak levels on day 8 post infection after which there was a decline (Table 1).

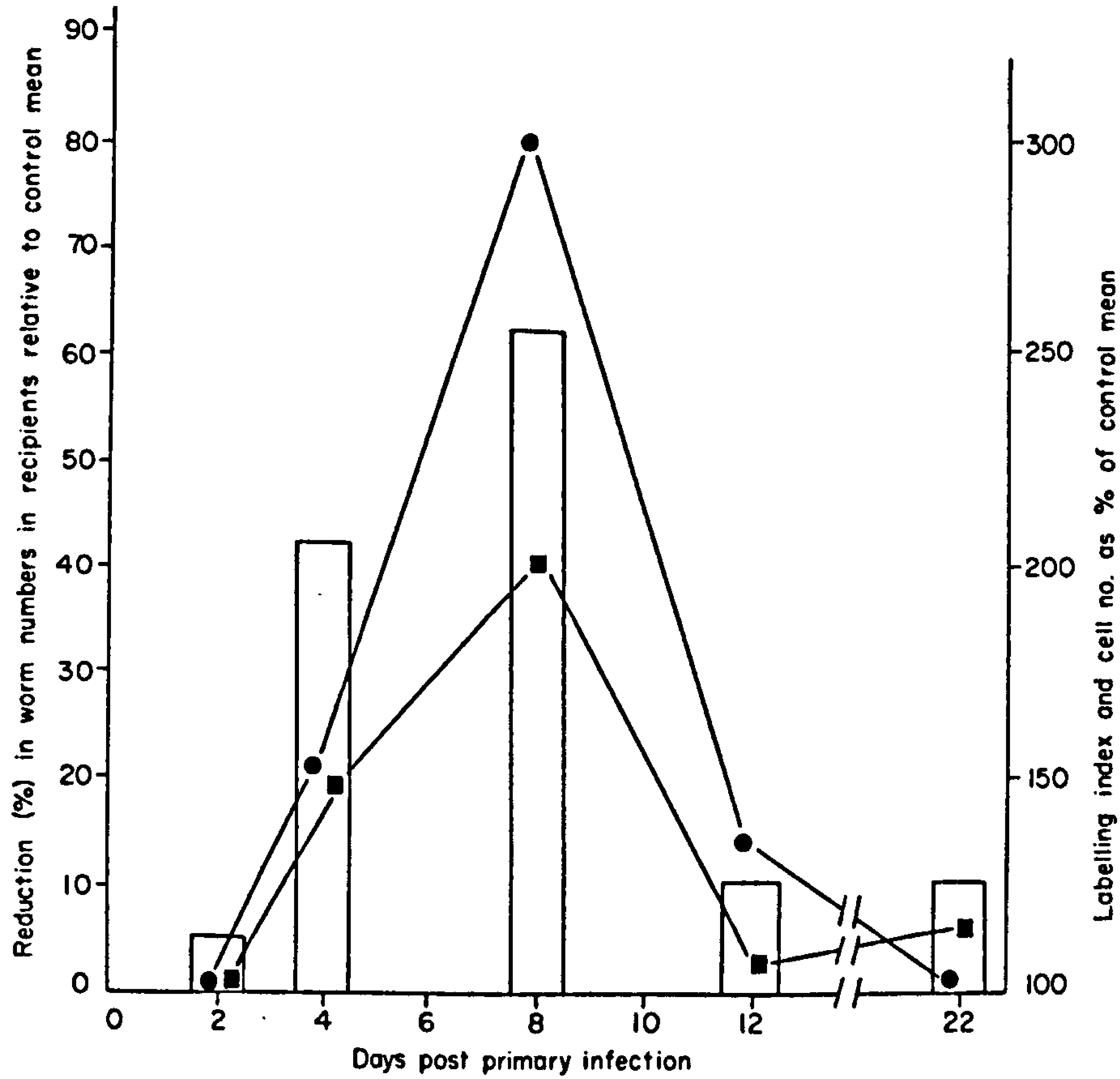


Figure 1. Adoptive transfer of immunity to *T. spiralis* in NIH mice with mesenteric lymph node cells (histogram) and related changes in total cell number (●——●) and labelling index* (■——■) of the MLN at various times post primary infection. *[(c.p.m./10^7 experimental MLNC)/(c.p.m./10^7 control MLNC)] × 100.

Table 1. Changes in cellularity of the mesenteric lymph node in NIH mice after a primary infection of 300 *T. spiralis* muscle larvae

	Mean no. of cells per node × 10^7					
	Control uninfected mice			Infected mice		
Days post infection	Total	Ig−ve	Ig+ve	Total	Ig−ve	Ig+ve
2	4·19	2·61	1·58	4·23	2·63	1·60
4	3·94	2·43	1·51	6·00	3·87	2·13
8	3·04	1·77	1·27	9·30	6·74	2·56
12	4·47	2·85	1·62	6·15	4·31	1·84
22	3·63	2·27	1·36	3·28	2·18	1·10

Mean values based on six mice/group.

Adoptive transfer of immunity and changes in the MLN following a secondary infection

Donor mice were infected with 300 *T. spiralis* on day 0 and challenged with 300 *T. spiralis* on day 21. MLNC suspensions were prepared and examined on days 2, 4, 8 and 10 post secondary infection. Cell recipients each received 4 × 10^7 cells and were challenged the same day with muscle larvae. Uninfected mice and mice given only the primary infection on day 0 served as controls.

The capacity to transfer immunity with cells was already present in the MLN on day 2 post secondary infection and was accompanied by an increase in total cell numbers and blast cell activity in the node at this time. Peak numbers and activity were attained on day 4 post secondary infection and levels were declining by day 8 (Fig. 2). The rise in total cell numbers in the

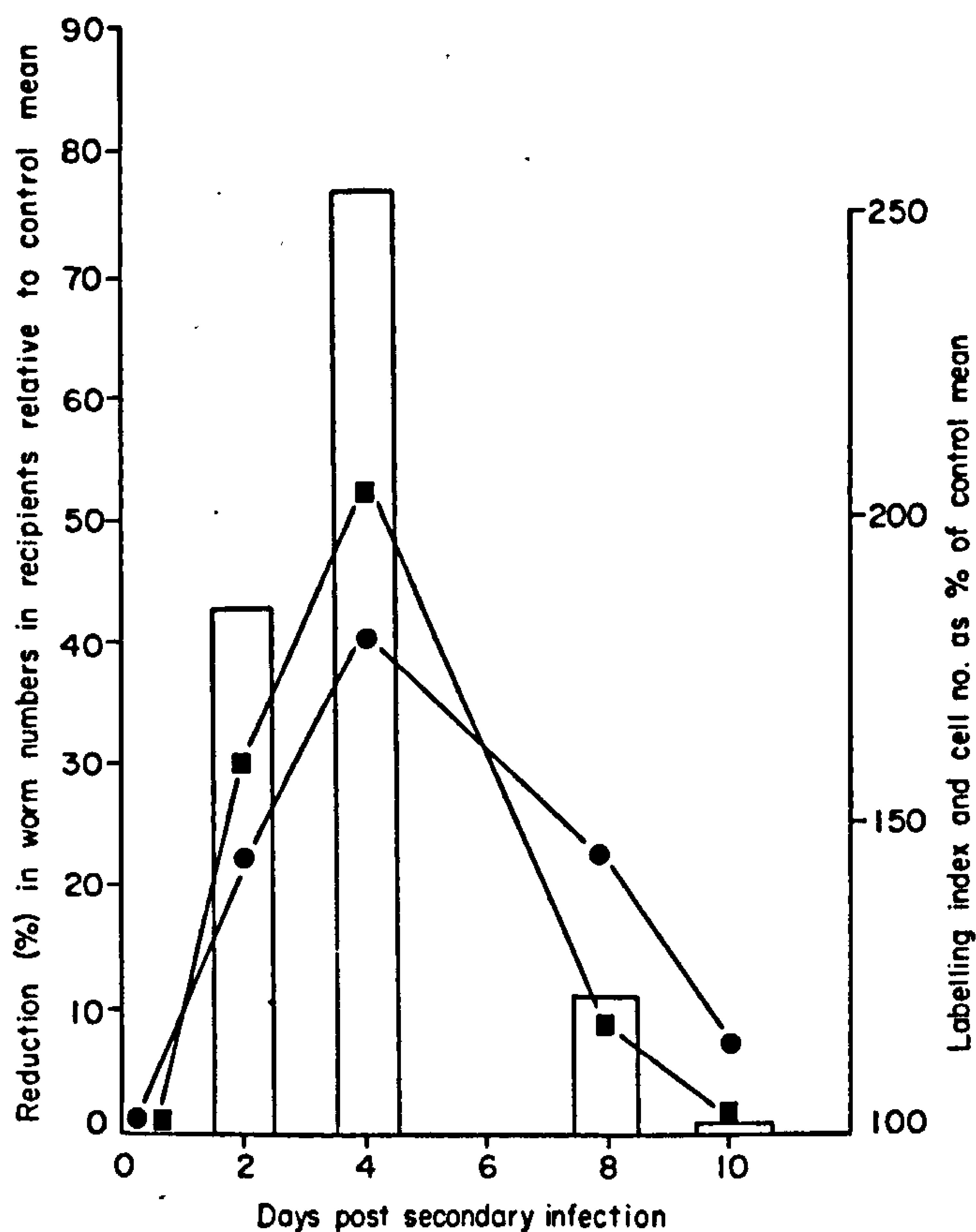


Figure 2. Adoptive transfer of immunity to *T. spiralis* in NIH mice with mesenteric lymph node cells (histogram) and related changes in total cell number (●——●) and labelling index* (■——■) of the MLN at various times post secondary infection. Primary infection given on day −21.

* See Fig. 1.

MLN was concomitant with a rise in total number of Ig−ve cells (T cells) in the node reaching peak levels on day 4 (Table 2). There were no significant differences between uninfected control mice and mice given only a primary infection at any time and, therefore, the data from the latter have been omitted.

Blast cell activity and transfer of immunity with enriched fractions of MLNC

The previous experiments show that the capacity to transfer immunity with MLNC is greatest in both primary and secondary infections at times when the MLN contains peak numbers of T cells and lymphoblasts. Therefore, a series of experiments was designed to examine the blast cell activity of enriched fractions of MLNC taken at prime times post infection and the capacity of these fractions to transfer immunity.

In the first set of experiments MLNC were taken from donors 8 days post primary infection. Cell recipients each received 4×10^7 cells of either unseparated or enriched fractions of MLNC and were challenged with muscle larvae the same day. Lymphoblast activities of the enriched fractions were examined and the results are presented in Table 3 together with the results of the adoptive cell transfer experiments. Only the unseparated population and the T-cell-enriched fraction of MLNC were capable of adoptively transferring immunity and expressing increased blast cell activity. A second series of experiments examined blast cell activity and adoptive transfer with enriched fractions of MLNC taken from donors on day 4 post secondary infection. Cell recipients each received 4×10^7 cells from either unseparated or enriched fractions of MLNC. Again, only the unseparated and T-cell-enriched fraction of MLNC expressed pronounced blast cell activity and capacity to transfer immunity (Table 4).

Table 2. Changes in cellularity of the mesenteric lymph node in NIH mice after a secondary infection of 300 *T. spiralis* on day 0. Infected mice received a primary infection on day −21

Days post secondary infection	Mean no. of cells per node $\times 10^7$					
	Control uninfected mice			Infected mice		
	Total	Ig−ve	Ig+ve	Total	Ig−ve	Ig+ve
2	2·80	1·87	0·93	3·98	2·85	1·13
4	2·35	1·56	0·79	4·17	2·89	1·28
8	2·24	1·42	0·82	3·34	2·39	0·95
10	2·40	1·61	0·79	2·75	1·80	0·95

Mean values based on six mice/group.

Table 3. *In vitro* lymphoblast activity and adoptive transfer of immunity using enriched fractions of mesenteric lymph node cells (MLNC) taken from donors 8 days after primary infection with *T. spiralis*

Group	Day 8 Mean worm number ±SD	Mean labelling index†
Control—no cells	139·3±22·1	—
Unseparated MLNC	39·6±14·8*	188*
'T' enriched	29·0±19·7*	261*
'B' enriched	127·2±36·9	117

Cell recipients each received 4×10^7 cells and were challenged with approximately 300 larvae.
* Mean significantly different from control group.
† See Fig. 1.

Table 4. *In vitro* lymphoblast activity and adoptive transfer of immunity using enriched fractions of mesenteric lymph node cells (MLNC) taken from donors 4 days after secondary infection with *T. spiralis*

Group	Day 8 Mean worm number ±SD	Mean labelling index†
Control—no cells	103·1±37·5	—
Unseparated MLNC day 4 primary infection	41·0*±17·2	135*
Unseparated MLNC	0*	197*
'T' enriched	0*	242*
'B' enriched	84·2±21·6	110

Cell recipients each received 4×10^7 cells and were challenged with approximately 300 larvae.
* Mean significantly different from control group.
† See Fig. 1.

DISCUSSION

Adoptive transfer of immunity has been used extensively in the analysis of resistance to helminth infection in laboratory models. Thus the expulsion of *Nippostrongylus brasiliensis* from the rat intestine can be accelerated by the adoptive transfer of immune MLNC (Kelly & Dineen, 1972; Dineen, Kelly & Love, 1973) or immune thoracic duct lymphocytes (TDL; Ogilvie, Love, Jarra & Brown, 1977; Nawa & Miller, 1978). Resistance to infection with *Trichostrongylus colubriformis* can be transferred between syngeneic guinea-pigs by lymphoid cell suspensions from immune donors (Wagland & Dineen, 1965; Dineen & Wagland, 1966) and in rats and mice, immunity to *T. spiralis* can be transferred with MLNC (Love, Ogilvie & McLaren, 1976; Wakelin & Wilson, 1977) or TDL (Crum, Despommier & McGregor, 1977).

In many of these systems it has been observed that adoptive transfer is most successful when cells are taken at particular times after infection of the donors. MLNC taken from rats infected for 13–15 days previously or TDL from donors infected for 10–11 days, were the most effective in transferring immunity against *N. brasiliensis* (Kelly & Dineen, 1972; Ogilvie *et al.*, 1977; Nawa & Miller, 1978). Adams & Rothwell (1977) showed that transfer of immunity against *T. colubriformis* was greatest when MLNC were taken 7 days after reinfection and that this capacity was gradually lost after 10 days. Availability of effective cells coincided with pronounced histological changes in the node (Rothwell & Dineen, 1973).

The experiments presented in this paper show that cells capable of transferring immunity to *T. spiralis* in NIH mice were present in the MLN for only a limited period of time after infection. If it is assumed that the level of protection transferred is a function of the number of cellular mediators in the MLN, then the results show that these mediators were first present in appreciable numbers between days 2 and 4 post primary infection, reached peak levels on day 8 and were declining by day 12. These changes were paralleled by those in cellularity of the MLN, the increased numbers between days 4 and 12 being predominantly of Ig−ve or T cells (Table 1). Lymphoblast activity also followed the same pattern, with peak values attained on day 8 post primary infection.

During a secondary infection similar trends were evident, but were accelerated in comparison with the primary infection. The ability to transfer immunity with MLNC was already present on day 2 post challenge and was coincident with a rise in cell numbers and blast cell activity. These responses rose to peak values by day 4 and were declining by day 8. Again, the increase in cellularity of the MLN was largely due to an increase in numbers of Ig−ve cells (Table 2).

Cellular changes in lymph nodes draining sites of *T. spiralis* infection have been described by several workers and it is clear that there is a close correlation between these changes and the course of infection. Ottesen, Smith & Kirkpatrick (1975) showed in rats that MLNC responsiveness to parasite antigen was increased within 3 days of infection, but declined in parallel with the expulsion of the parasite from the intestine. Tanner, Lim & Faubert (1978) found that the number of leucocytes present in the MLN of mice increased during the intestinal phase of infection, and although they found no major change in percentage of cells bearing T-cell-surface markers, their data imply that the total number of such cells did increase substantially. A rapid increase in MLN lymphoblast activity in infected mice was noted by Manson-Smith, Bruce, Rose & Parrott (1979b) and it is interesting that there was a marked mouse strain effect on the pattern of increase, the pattern in slow-responder BALB/c being quite distinct from that in NIH.

Correlations between the course of infection and lymphoblast activity have also been recorded by Dobson & Soulsby (1974) working with *T. colubriformis* in guinea-pigs and by Johnstone, Leventhal & Soulsby (1981) with *Ascaris suum* in mice. The latter workers used a virus plaque assay to detect activated T lymphocytes and also monitored antigen- and mitogen-induced blastogenesis. They showed that the increased levels of activity were T-cell-dependent and that responses were accelerated in secondary infections.

The T-cell dependency of the responses described here confirms and extends previous observations on adoptive transfer of immunity in this system (Wakelin & Wilson, 1979) and the data suggest strongly that the cells capable of transferring immunity are a population of rapidly dividing T lymphoblast cells. The temporal restriction of the ability to transfer immunity with MLNC from infected animals is therefore explained by the short-lived nature of this T-cell response. After primary infections mice remain immune to challenge for prolonged periods and this must reflect the persistence of a memory cell population which can be rapidly restimulated on challenge. In this respect immunity to *T. spiralis* shows similarity to the

mechanisms proposed for immunity to the intracellular pathogen *Listeria monocytogenes*. In the latter immunity is mediated by an acquired population of short lived, sensitized T lymphocytes which activate macrophages. The capacity to transfer immunity is present only when the spleen contains an enlarged population of rapidly dividing T lymphoblasts (North, 1973). As with *T. spiralis*, secondary infection results in an accelerated clearance of the *Listeria* organism, and this is the consequence of a more rapid and more extensive production of lymphoblasts (North, 1975). It has been proposed that a major function of T cells in the expulsion of *T. spiralis* is the mediation of inflammatory changes in the intestine, in co-operation with myeloid cells of bone marrow origin (Wakelin & Wilson, 1979). Although the details of this mechanism are unknown, it has been shown that MLNC taken at times when T lymphoblast activity is high will transfer an enhanced mucosal mast cell response (Alizadeh & Wakelin, 1980). It therefore seems that it is the lymphoblast population or its immediate progeny which initiate the sequence of intestinal inflammatory changes that lead ultimately to worm expulsion. It will be important to determine more precisely the subset of T lymphocytes involves. Despite the extensive data now available concerning the roles of T-cell subsets in immunological phenomena only a few attempts have so far been made to apply this information to the field of immunoparasitology (Mitchell, 1980). If the interpretation of T-cell function proposed here is correct, the effectors of immunity to *T. spiralis* should have the phenotype of helper populations (i.e. be Ly $1^{+}2^{-}3^{-}$). It is known that cells of this phenotype are involved in delayed hypersensitivity responses and it may be significant that Ly 1^{+} cells are also known to regulate myeloid cell development *in vitro* (Nabel, Galli, Dvorak, Dvorak & Cantor, 1981). Determination of effector cell phenotype is now in progress.

ACKNOWLEDGMENTS

This work was supported by research training award J77/689b and project grant 977/65T from the Medical Research Council.

Summary Points

Chapter 5

(1) After a primary infection with *T. spiralis* NIH mice showed a short lived increase in cellularity of the MLN, which began between days 2 and 4, peaked at day 8 and had declined by day 12. The majority of cells contributing to this increase were Ig-ve cells.

(2) Coincident with the increase in cell number there was an increase in lymphoblast activity, largely associated with the T cell fraction.

(3) MLNC taken at intervals from mice during a primary infection successfully transferred immunity, when taken on days 4 and 8 but not on day 12 post infection. It was shown that cells effective in transferring immunity were present in the T-enriched fraction.

(4) Following a secondary infection given 21 days after a primary infection, the same sequence of changes was apparent in the MLN, but the time course was accelerated. Peak cellularity occurred on day 4 post challenge. Cells capable of transferring immunity were present in the MLN on days 2 and 4 post challenge.

Chapter 6. Analysis of the cells mediating immunity in mice to infection with *T. spiralis*. Positive and negative selection of MLNC.

The techniques used to enrich populations of MLNC for T cells or B cells, in the experiments described in previous chapters are open to criticism. Separation of cells on nylon wool columns is a relatively crude method, and has been shown to strip certain molecules from the cell membrane (Corrigan, O'Kennedy and Smyth, 1979). The complement mediated cytotoxicity technique also has disadvantages, for example, complement mediated cytotoxicity tests initially suggested that the Ly 1 antigen of the mouse T lymphocyte was expressed only on a subset of T cells (Cantor and Boyse, 1976) but was later shown to be expressed on all T cells in fluorescent binding studies (Scollay, Kochen, Butcher and Weissman, 1978).

Modern techniques of cell separation have become increasingly dependent upon monoclonal antibodies. The development of the cell fusion technique as a method for producing monoclonal antibodies has revolutionized studies of lymphocyte membrane antigens. If mouse myeloma cell clones are fused with spleen cells from allogeneic immunizations between inbred strains, mono specific antibodies may be raised against polymorphic determinants on cell surface antigens. Monoclonal antibodies are used in two types of assay for separating cell suspensions on the basis of their expression of certain cellular antigens:

(1) Negative selection, in which a cell population bearing a particular membrane antigen is effectively removed by an antibody mediated complement lysis mechanism,

or,

(2) <u>Positive selection</u>, in which a cell population bearing a particular membrane antigen is selected using the fluorescent properties of conjugates linked to a monoclonal antibody, with the cells remaining in a viable and functional condition.

It is generally agreed that positive selection is a more acceptable technique, in terms of cell treatment, and has been shown to be more accurate than negative selection. Positive selection is achieved by the use of a fluorescence activated cell sorter (FACS), using flow cytometry, a technique used extensively in modern medical and biological research (Mendelsöhn, 1981). The FACS can produce suspensions of cells which are greatly enriched in one particular cell type, and has provided much of the present day information about lymphocyte membrane antigens (Williams, 1980).

Thus, although the results of experiments from earlier chapters are clear-cut in showing that only T enriched MLNC populations transfer the ability to accelerate expulsion of <u>T. spiralis</u> from the gut, it seemed desirable to confirm these results using improved methods of cell selection and at the same time to give more information concerning the phenotype of the cells involved.

Materials and Methods

Mice

Male NIH and male B10 background H-2- congenic B10 G mice were obtained from Olac 1976 Ltd and Male CBA mice were kindly donated by Mr T D G Lee. All mice were approximately 8-weeks-old at the start of experiments.

Cell suspensions and transfer

MLNC suspensions were prepared according to the standard techniques described in the general materials and methods. After receiving the appropriate treatment, the required number of cells were injected into recipient mice in a volume not exceeding 0.5 ml.

Worm recovery and worm size

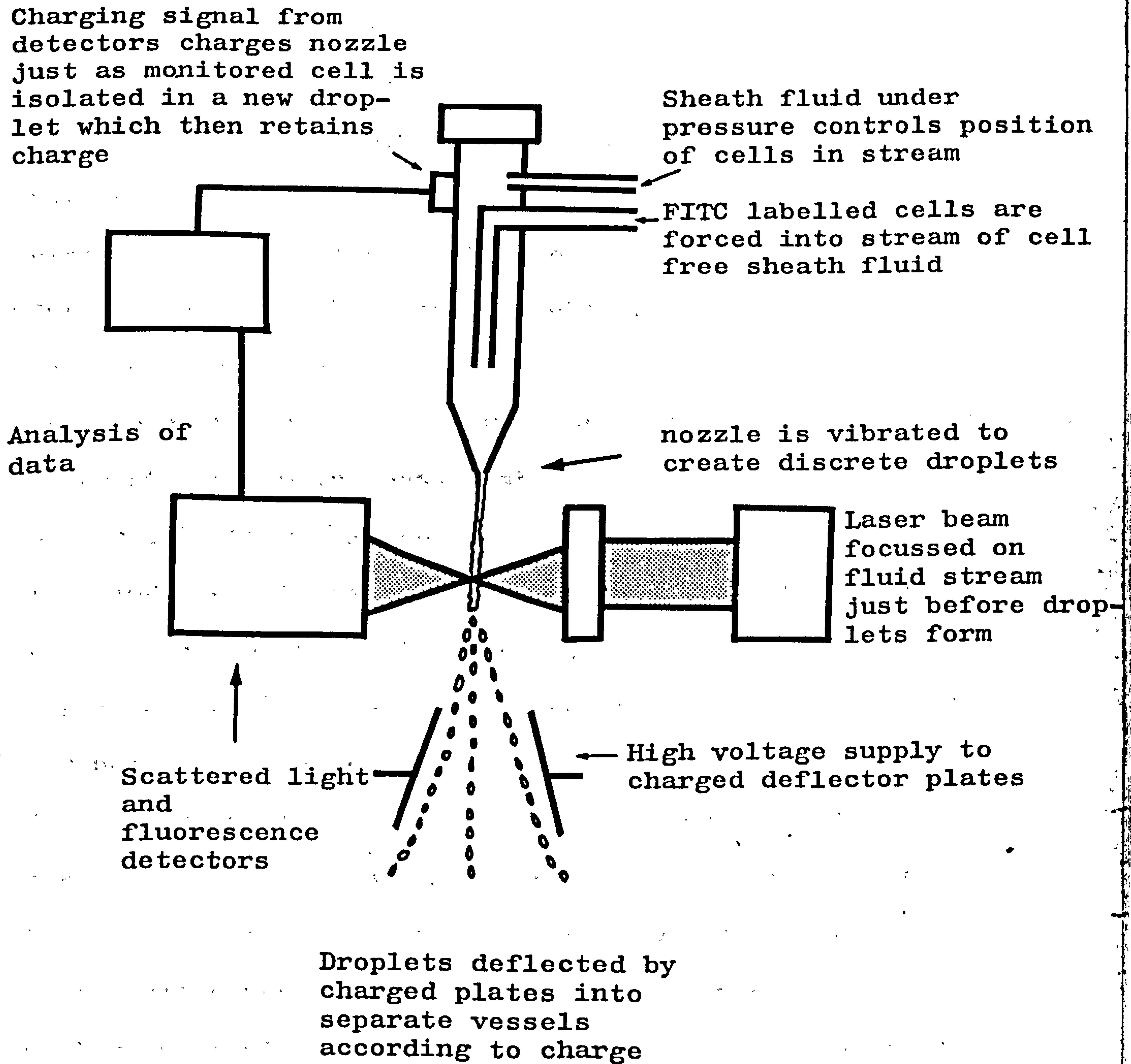
Adult worm recovery and worm length were measured as described in the general materials and methods.

Positive selection: The Fluorescent activated cell sorter

A FACS IV linked to a pre-programmed LS1-11 micro-computer (Becton Dickinson, FACS systems) was used by kind permission of Professor R W Baldwin, Cancer Research Campaign Laboratories, University of Nottingham.

The principle features of the FACS are shown in Fig 6.1. After labelling with a fluoroscein conjugated

Fig 6.1. Basic features of the fluorescence activated cell sorter FACS.



antibody, single cells pass through a laser beam and fluorescence and light scatter detectors register the light emitted from each cell. The optical signals are transformed into electrical pulses which are processed and stored for display and analysis. Upon sorting the stream of cells is subject to vertical ultrasonic vibration causing it to break into droplets a short but accurately fixed distance below the detection points. Thus, cells which have passed the detectors continue to pass down the stream, reach its tip and become enclosed in droplets. During the time taken for the cell to travel from the detectors to the stream tip, its fluorescence and scatter are processed and compared with pre-set parameters, determining which of two populations it should be assigned to. An electrical charge is applied to the stream at precisely the time the cell reaches the stream tip so that the droplet containing the cell will be charged either positively (labelled cell) or negatively (unlabelled cell). The charged and uncharged droplets from the stream continue to fall directly downward, until they pass through a constant electrical field created by a set of "deflection" plates. The charged droplets are deflected towards the left or right collection vessels depending upon charge. All uncharged droplets continue directly downwards and fall into the centre discard vessel.

All cell suspensions are kept on ice throughout the procedure, the sorted cells being collected into FCS coated sterile plastic tubes. Any red blood cells, cell debris or dead cells are excluded from analysis and collection

by appropriate setting of parameter thresholds. The results from FACS analysis are presented as histograms, measuring fluorescence or forward light scatter, on linear or log plots.

(i) Fluorescent Labelling of Cells

A suspension of MLNC from NIH mice were prepared in RPM1 1640 medium as normal. Positive selection for cells bearing the Thy 1.2 antigen was carried out after labelling with a fluoroscein conjugated anti-mouse Thy 1.2 monoclonal antibody (Clone TS; 4 mg protein/ml; Code 63-30) obtained from Miles Laboratories Ltd. Prior to use, the antibody solution (in PBS pH 7.2) was centrifuged at 3000g for 30 mins to remove any particulate material. To discover the optimum concentration of antibody required for staining MLNC, a titration of the monoclonal antibody was carried out using the FACS.

Antibody was reacted with MLNC at a cell concentration of 1 x 10^7 cells/ml (RPMI 1640 medium) on ice for 30 mins. After this time, the cells were analysed directly or washed x 1 in RPM1 1640 medium prior to analysis. The results demonstrate that the optimum concentration of monoclonal antibody to obtain two discrete populations, one staining, one not staining was 1/200. Washing the labelled cells, increased the efficiency of analysis (fig 6.2.).

Figure 6.2. Titration of FITC conjugated anti-Thy 1.2 antibody. Labelling efficiency of various concentrations of antibody.

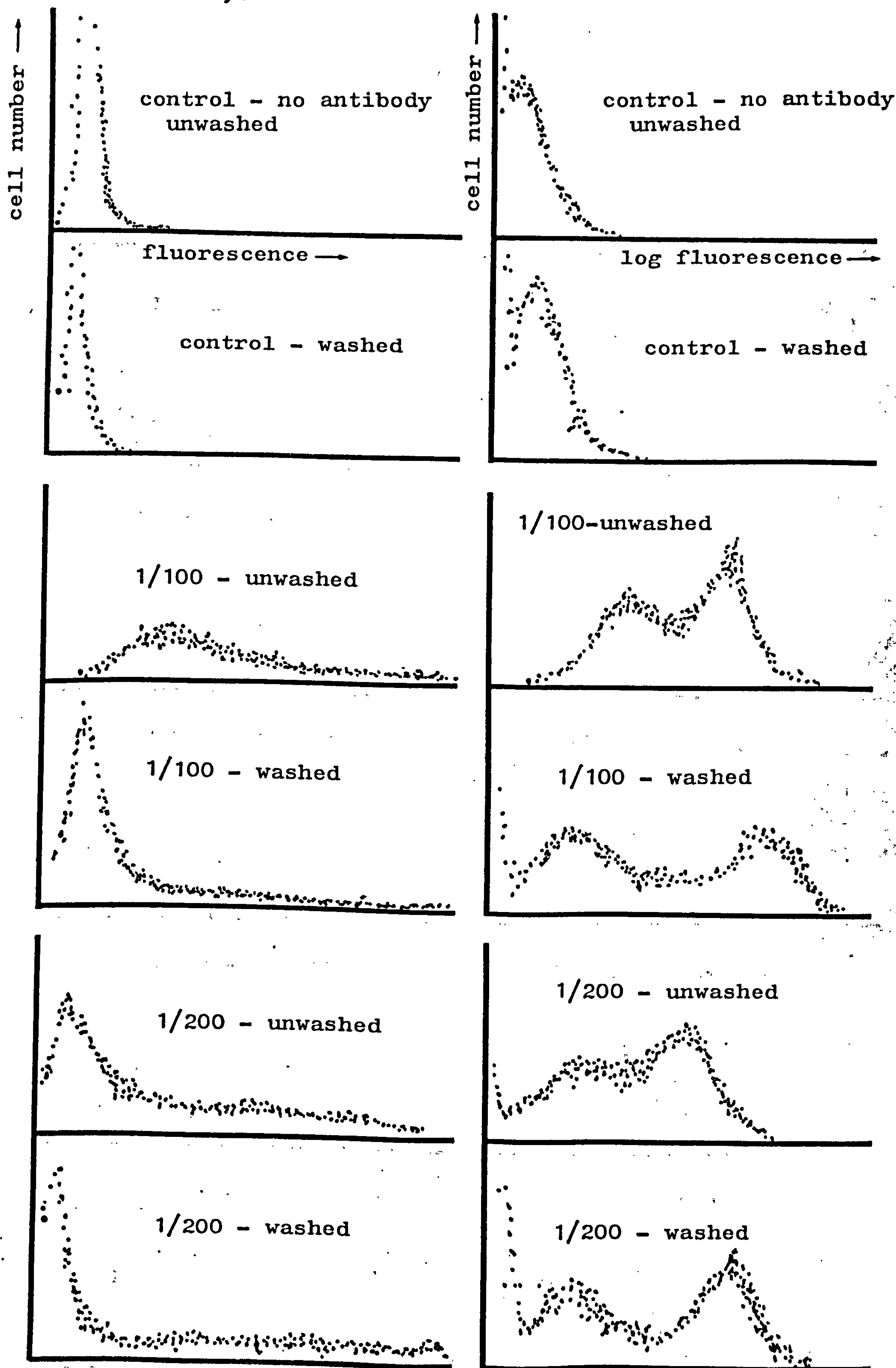
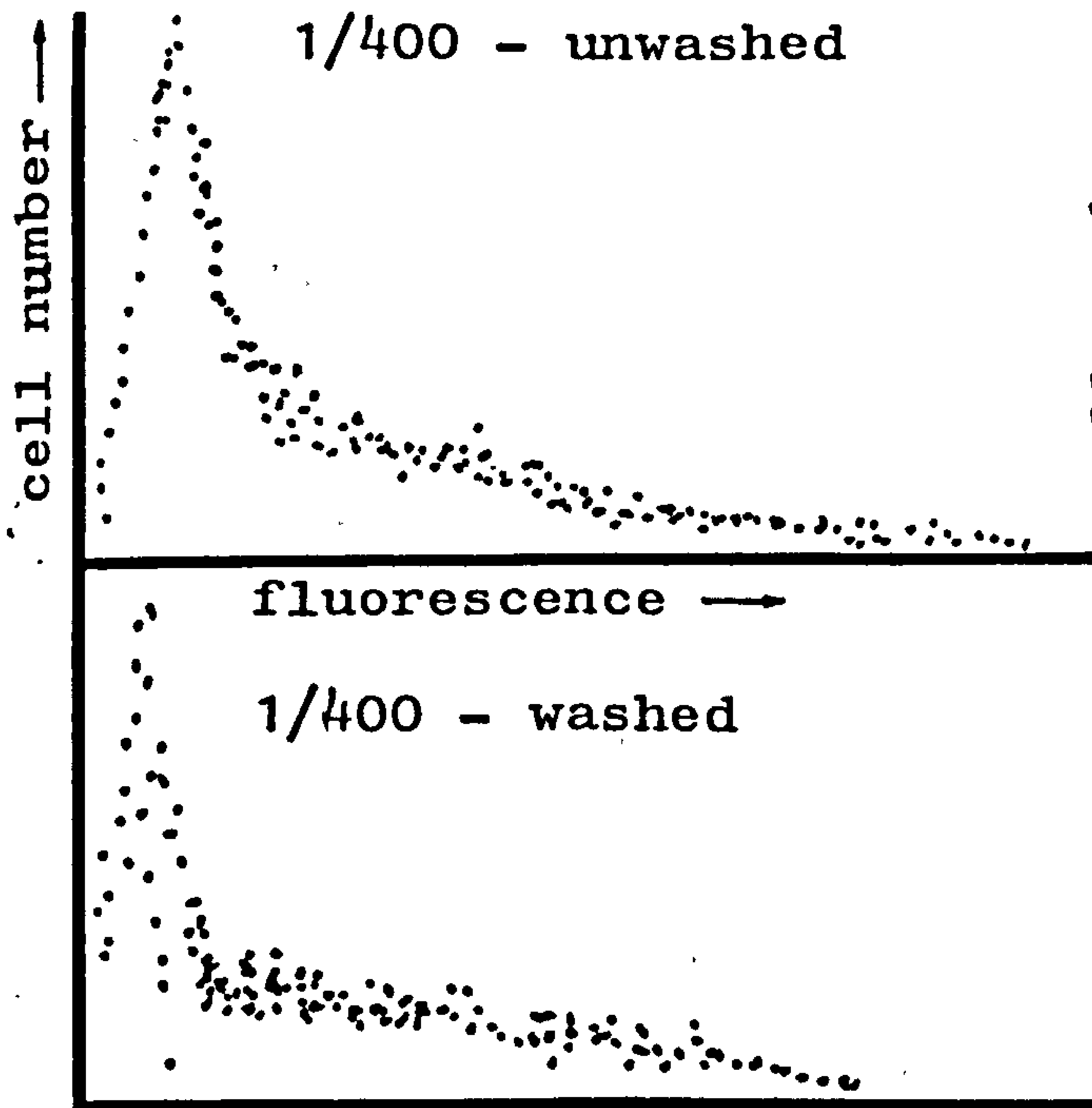
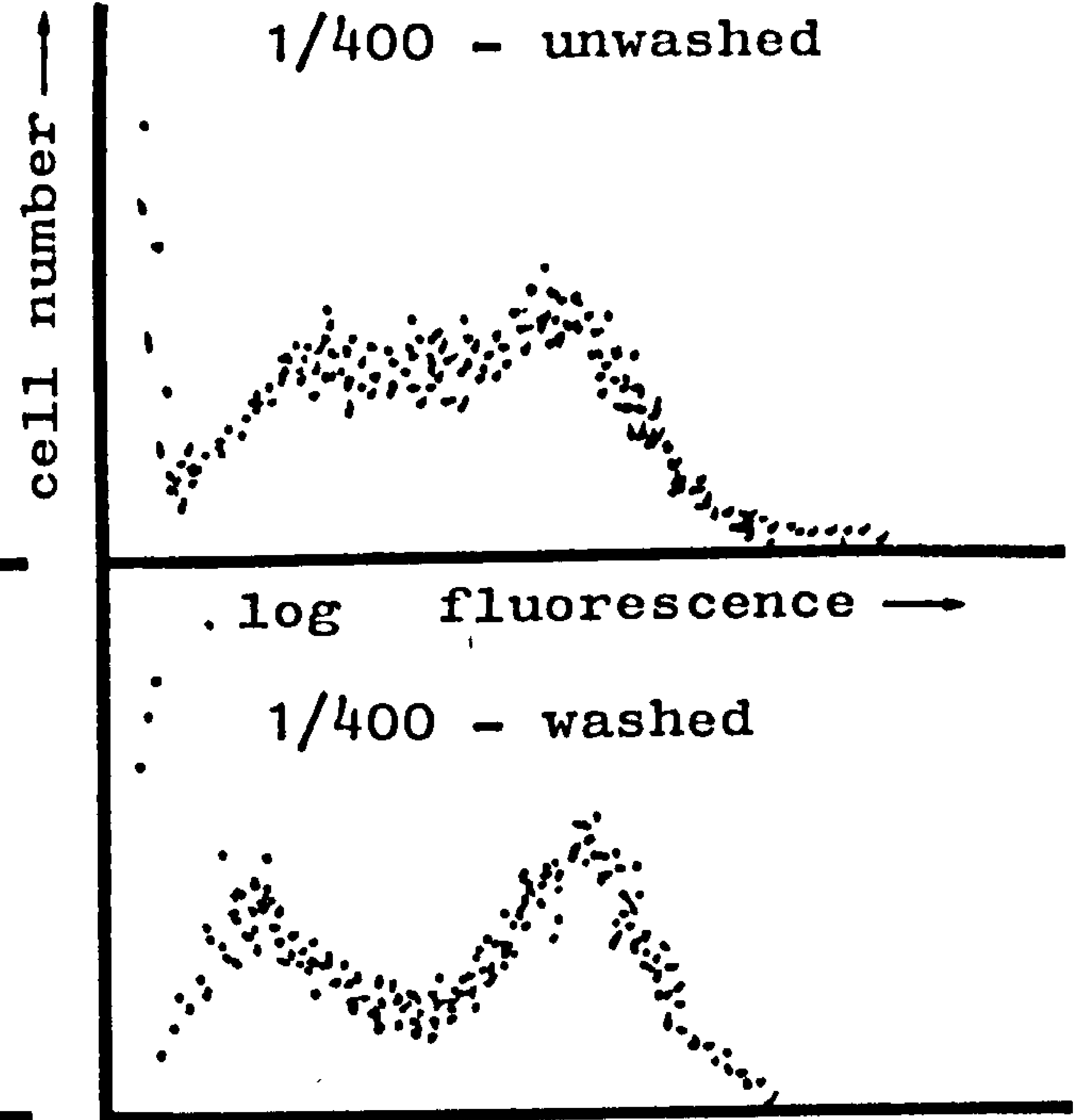
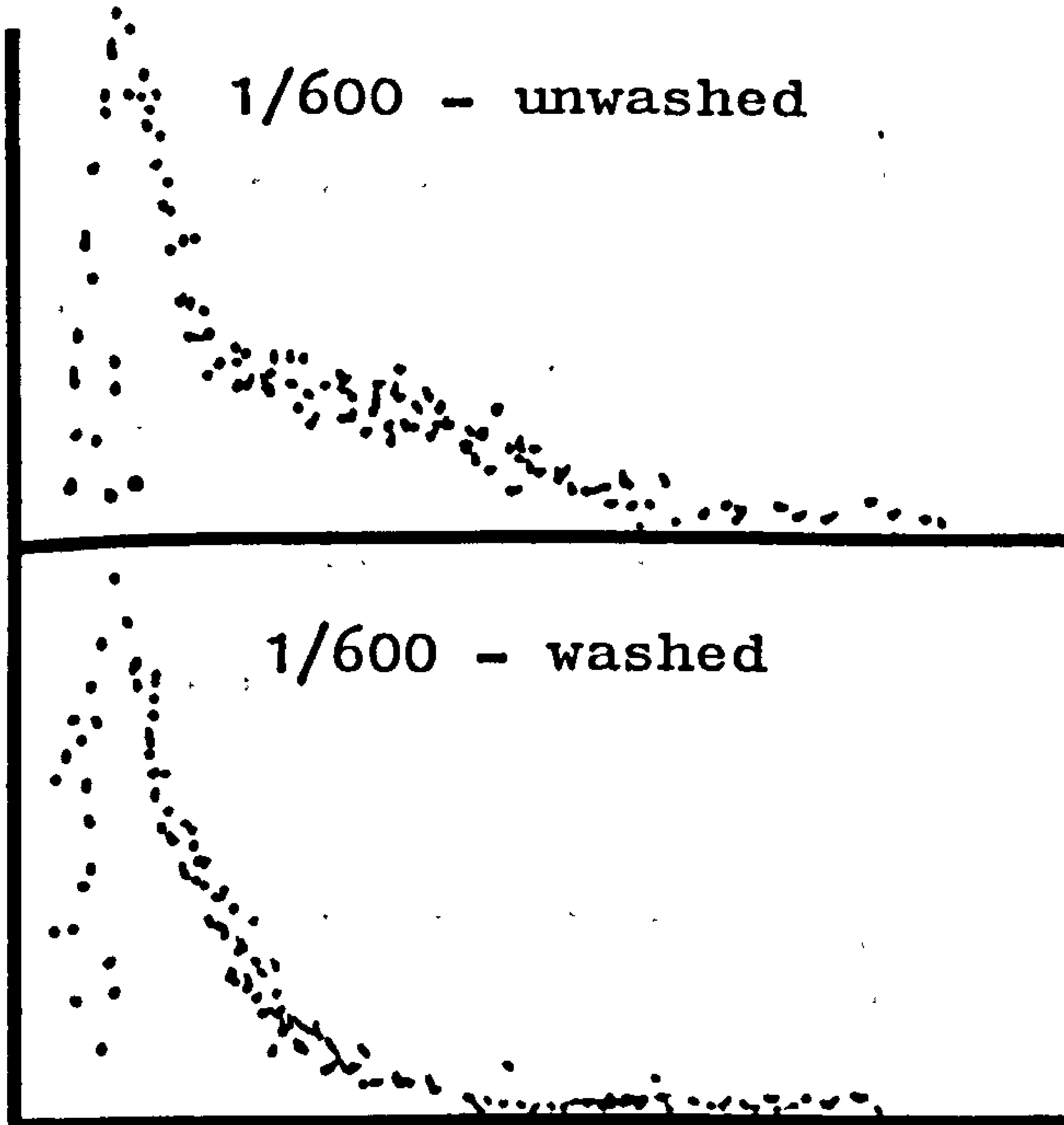
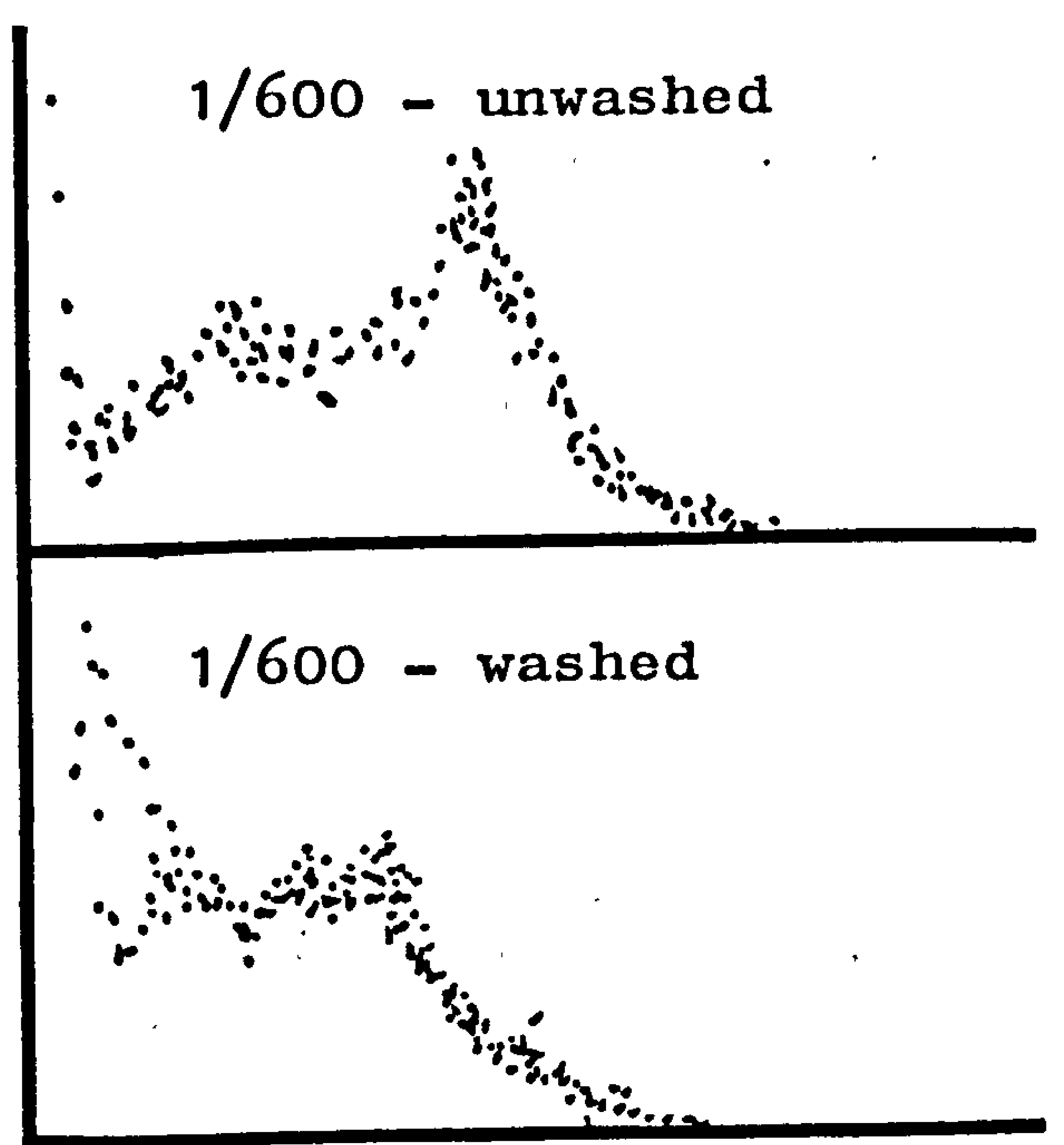


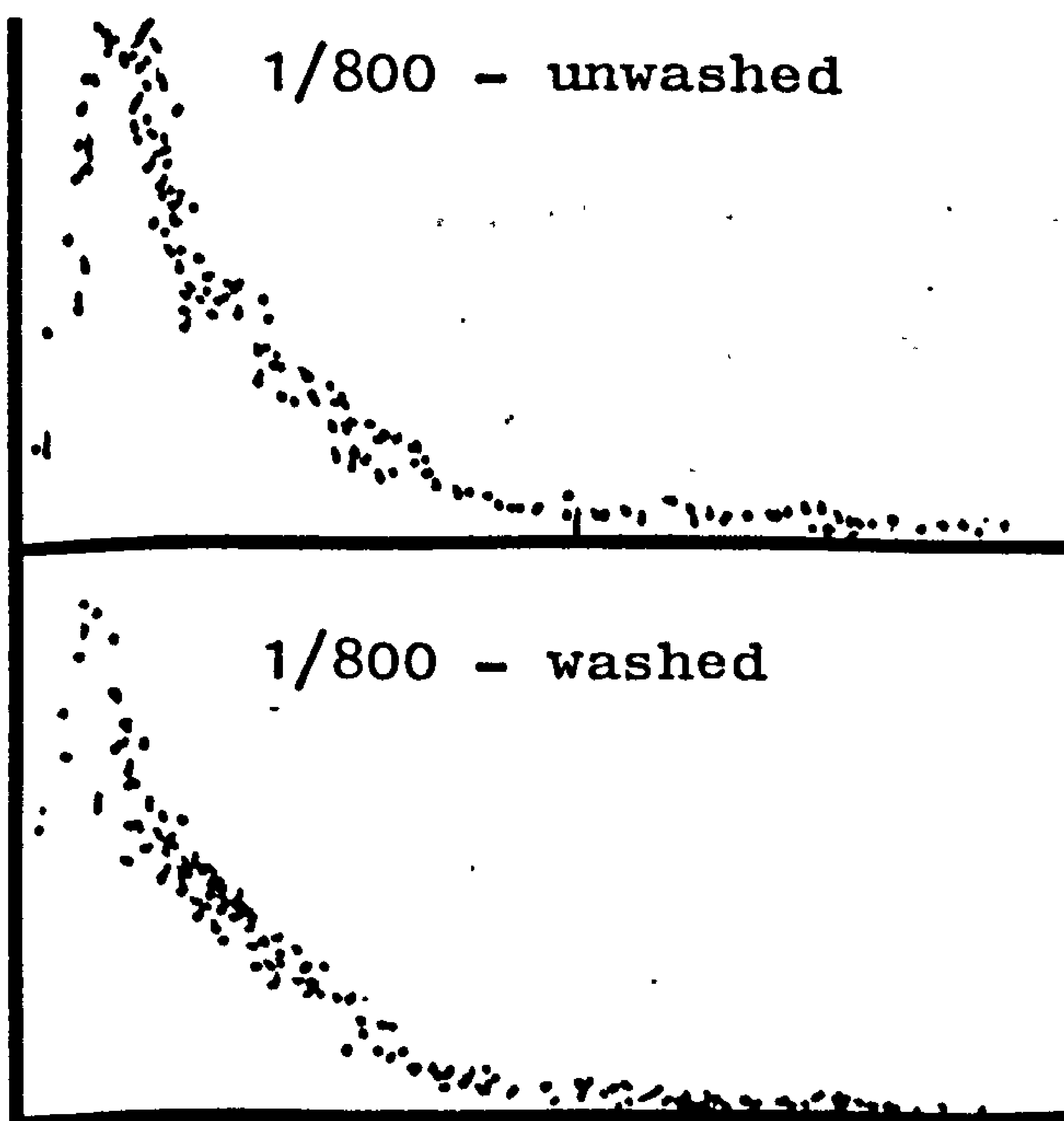
Figure 6.2. (continued)

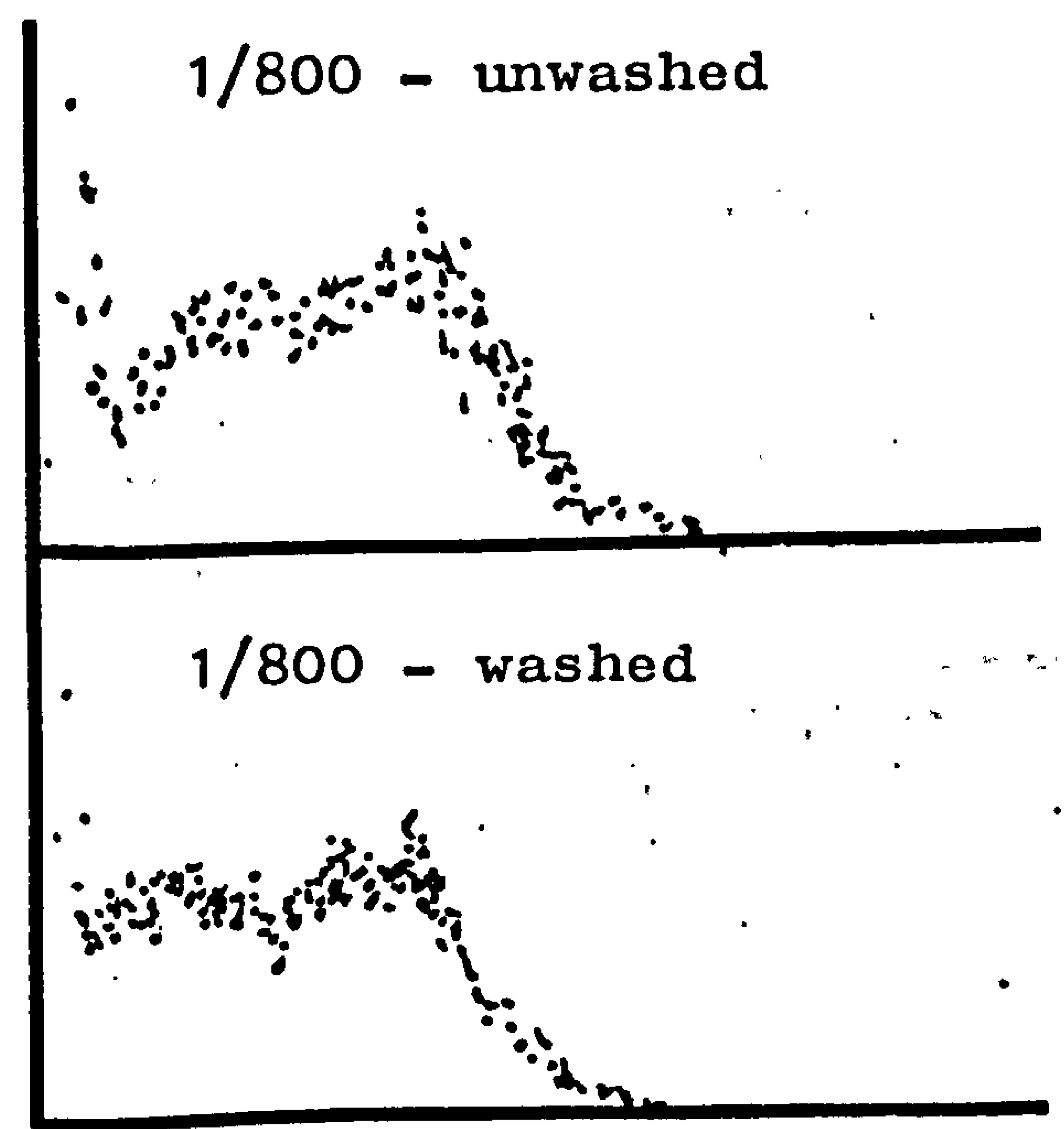












Complement Mediated Cytotoxicity Assay

A cytotoxic anti-Ly 2.2 monoclonal antibody, Clone HO.22 (Gottlieb, Marshak-Rothstein, Auditore-Hargreaves, Berkoben, August, Rosche and Benedetto, 1980) was kindly donated by Dr D B Thomas, Division of Immunology, NIMR, Mill Hill, London. A preliminary experiment was conducted to assess the cytotoxic titre of the antibody, previously ascribed at $>1:6 \times 10^4$ (Thomas and Calderon, 1982). Thymocyte suspensions were prepared from B10G (Ly 2.2), NIH (Ly phenotype unknown) and CBA (Ly 2.1.). 4×10^6 cells were suspended in 200μl of antibody at various concentrations in medium 199, and kept on ice for 30 mins. The cells were washed and resuspended in 200μl of absorbed guinea pig complement at a dilution of $\frac{1}{3}$ and incubated at 37°C for 40 mins. Samples were assayed in duplicate. Cell viability was assayed using trypan blue, and the results expressed as a cytotoxic index.

$$\text{Cytotoxic index} = \frac{(\%\ \text{dead test} - \%\ \text{dead control})}{100 - \%\ \text{dead control}} \times 100$$

The results demonstrate that the antibody had a cytotoxic titre of $1:1 \times 10^4$ when reacted with Ly 2.2. positive cells (B10G). However, the results also show that the antibody has no effect upon NIH cells, and therefore that NIH are Ly 2.1 positive, as are CBA mice, (Table 6.1.).

Table 6.1. Complement dependent cytotoxic activity of HO-2.2 antibody against thymocytes of inbred strains of mice.

Treatment	Cytotoxic Index					
	B10G		CBA		NIH	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
1/100 antibody only	18.6	15.3	19.6	9.6	6.5	7.9
C' only	23.5	12.6	14.5	11.9	5.9	6.3
1/100 antibody + C'	84.7	87.1	15.6	14.2	3.2	4.6
1/1,000 antibody + C'	76.5	89.1	20.1	11.7	10.2	5.9
1/10,000 antibody + C'	76.5	82.4	17.2	10.3	7.7	4.1
1/20,000 antibody + C'	18.6	14.3	n.d.	n.d.	9.1	13.0
1/30,000 antibody + C'	23.5	12.6	n.d.	n.d.	4.5	11.9

n.d. = not done

Results

Adoptive transfer of immunity against T. spiralis: Positive selection of Thy 1.2 positive cells using FACS

FITC conjugated monoclonal anti Thy 1.2 antibody was used at a concentration of 1/200 to label MLNC taken from NIH donors 8 days post infection. Cells were adjusted to a concentration of 1×10^7 cells/ml in RPMI 1640 medium; cell sorting was carried out at a flow rate of approximately 8×10^6 cells per hour and a total of 8×10^7 cells were sorted. Analysis of the unseparated population and sorted populations are shown in the histograms presented in fig 6.3. Thy 1.2 positive cells were 94.31% pure and Thy 1.2 negative cells were 98.07% pure. 52% of the cells applied to the FACS were deflected into the Thy 1.2 positive collection vessel and 44% of the cells were deflected into the Thy 1.2 negative collection vessel.

Six NIH cell recipients each received 2×10^7 unseparated MLNC, four NIH cell recipients each received 8×10^6 Thy 1.2 positive cells, and three NIH cell recipients each received 7.3×10^6 Thy 1.2 negative cells. Together with six untreated control NIH mice, all animals were infected with 300 T. spiralis muscle larvae one day after cell transfer (day 0). Seven days post infection, worm burdens in the small intestine, and worm lengths were measured.

The results (Table 6.2) show that unseparated immune MLNC do transfer immunity, as assessed by accelerated expulsion

Fig 6.3. Analysis of FACS cell sorting. Cells labelled with FITC conjugated anti-Thy 1.2

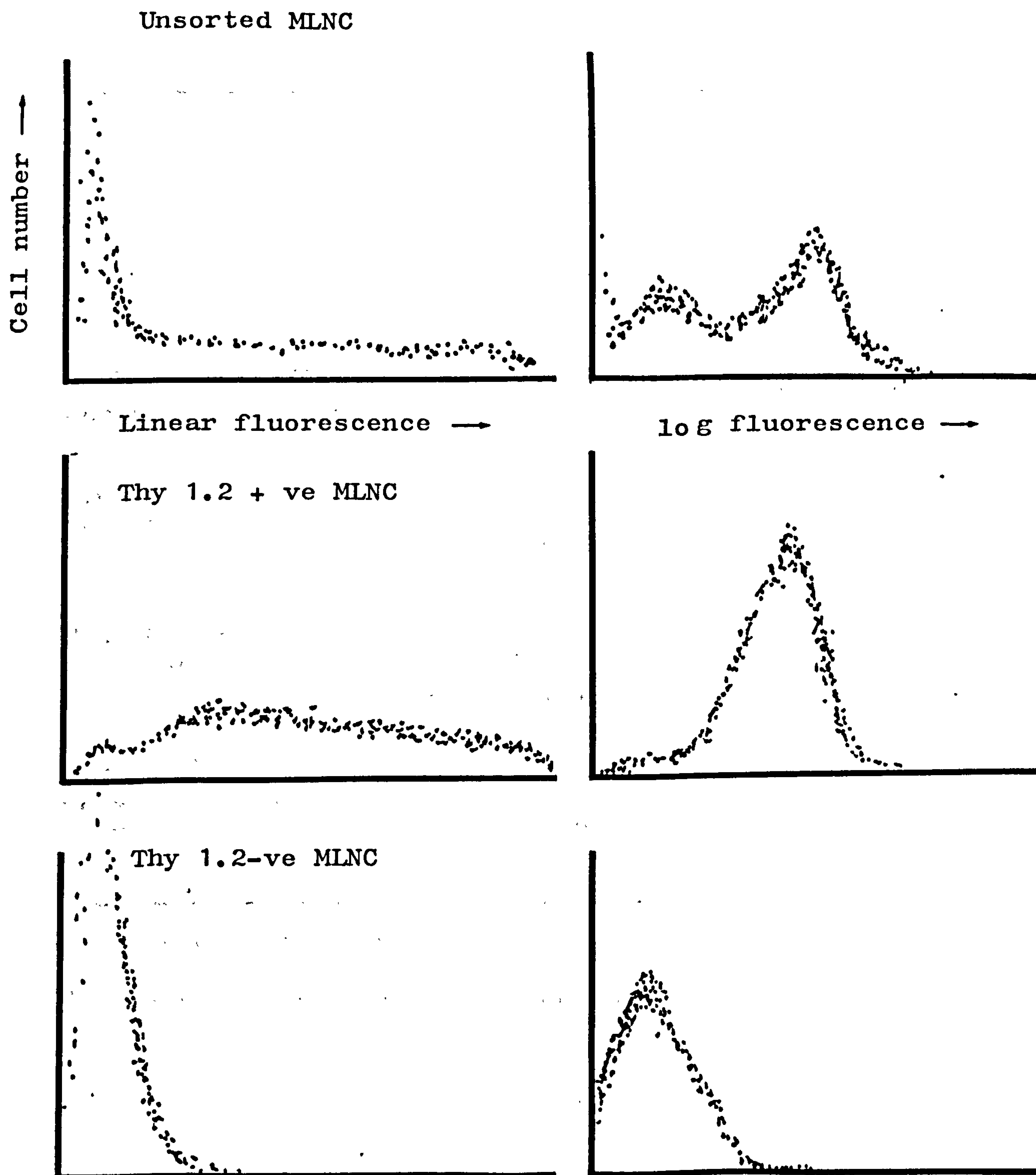


Table 6.2. Adoptive transfer of immunity against *T. spiralis* in mice with positively selected populations of MLNC.

Group	Day 7 post infection		
	Worm recovery from individual mice	Worm length m.m.	
		Mean	S.D.
Control, no cells	138, 174, 147, 206, 165, 144.	1.99	0.18
2.0×10^7 unseparated immune MLNC	35, 41, 49, 52, 22, 39. *	1.64*	0.18
8×10^6 Thy 1.2 positive immune MLNC	62, 101, 78, 107. [a]	1.54*	0.15
7.3×10^6 Thy 1.2 negative immune MLNC	152, 119, 132.[a]	1.76	0.14

* Mean significantly different from controls.

a statistics not conducted due to group size.

of the worms from the intestine, and clearly demonstrate that positively selected Thy 1.2 bearing cells also cause accelerated worm expulsion. Thy 1.2 negative cells also showed a small reduction in worm burden on day 7 post infection. Worm length was also significantly reduced in animals which had received unseparated cells or Thy 1.2 positive cells.

Adoptive transfer of immunity against T. spiralis: Negative selection of Ly subsets using a cytotoxic anti Ly 2+ antibody

T cells can be defined in terms of their expression of a variety of surface antigens or markers. Antigens present on the surface of immune T cells are coded for by genes from many chromosones, Thy 1 (Chromosome 9), Ly 1 (Chromosome 19) and Ly 2 (Chromosome 6). The expression of particular combinations of these antigens is characteristic of individual T cell subsets. Furthermore, both Thy 1 and Ly 1 and Ly 2 can be expressed in two different allelic forms dependent upon the strain of mouse.

Wakelin and Donachie (1980) have shown that the transfer of immune MLNC between the rapidly responding NIH mice (H-2q) and B10G mice (H-2q) resulted in accelerated worm expulsion, characteristic of a rapid responder. In the reciprocal transfer immune MLNC from BIOG mice transferred immunity to NIH mice as effectively and as rapidly as did NIH cells. The HO.22 clone produces a monoclonal antibody cytotoxic for cells which express Ly 2.2 antigens.

However, as described in the Materials and Methods NIH mice are Ly 2.1, and therefore, in order to discover the subpopulation of T cells which mediate immunity to T. spiralis, MLNC taken from BIOG mice (Ly 2.2) were treated with anti Ly 2.2 antibody and transferred into NIH (Ly 2.1) recipients.

BIOG cell donors were infected with 300 T. spiralis muscle larvae on day -8. On day 0 the MLNC were taken and treated with a 1/100 dilution of anti-Ly 2.2 monoclonal antibody, followed by a $\frac{1}{3}$ dilution of absorbed guinea pig complement. Treatment of BIOG MLNC with antibody and complement reduced the total cell number by 42.8%. NIH cell recipients each received, either 2.5×10^7 untreated MLNC, 2.5×10^7 MLNC treated with complement alone, 2.5×10^7 MLNC treated with antibody alone or 1.43×10^7 untreated MLNC. Together with controls, all animals were infected with 350 T. spiralis muscle larvae on the day of cell transfer (day 0). Seven days later worm numbers in the small intestine were measured. Recipients of 2.5×10^7 untreated immune MLNC had a mean worm burden of 135.2 on day 7 post infection; significantly lower than control animals (mean worm burden of 287.8), whereas recipients of 1.43×10^7 untreated immune MLNC showed a significant but less pronounced effect on worm expulsion (mean worm burden of 209.3). Treatment of immune MLNC with antibody alone or complement alone did not affect the level of immunity transferred. Also, treatment of immune MLNC with antibody and complement to deplete the suspension of Ly 2.2 bearing cells, did not affect the ability to adoptively transfer

immunity, recipients of 1.43×10^7 treated cells having a mean worm burden of 139.2 a comparable level to recipients of 2.5×10^7 untreated immune MLNC. These results suggest that the cells which mediate expulsion of T. spiralis from the small intestine are Ly $1^+ 2^-$, T lymphocytes. (Table 6.3).

Table 6.3. Adoptive transfer of immunity against *T. spiralis* in mice with negatively selected populations of MLNC. Negative selection using anti-Ly 2.2 antibody + C'.

	Worm recovery day 8 post infection		
	Mean		S.D.
Control - no cells	287.8		13.1
2.5 x 10^7 immune MLNC - no treatment	135.2	*a	11.2
2.5 x 10^7 immune+ MLNC - antibody only treatment	129.3	*a	20.2
2.5 x 10^7 immune+ MLNC - complement only treatment	132.0	*a	11.8
1.43 x 10^7 immune MLNC - antibody and complement treated	139.2	*a,b	11.8
1.43 x 10^7 immune MLNC - no treatment	209.3	*b	10.7

+n = 3
* = significantly different from control
a = no significant difference between groups
b = significant difference between groups

Summary Points.

Chapter 6

(1) Positive selection of Thy 1.2 + ve MLNC from infected mice, and their adoptive transfer into naive recipients confirmed that Thy 1.2 + ve cells (T cells) play a major role in the expulsion of the worm burden from the gastrointestinal tract. However, a role for Thy 1.2 - ve cells cannot be excluded.

(2) Negative selection of T cell subsets using a cytotoxic anti-Ly 2+ antibody coupled with adoptive transfer techniques demonstrated that Ly 1+ T cells were the major subset involved in the expulsion of the worm from the intestine.

Chapter 7: Short lived dividing cells mediate adoptive transfer of immunity to *T. spiralis* in mice. Characteristics of the cells involved.

Part of this chapter has been published in Immunology Vol 46, 451 - 457, 1982.

Immunology 1982 46 451

Short lived, dividing cells mediate adoptive transfer of immunity to *Trichinella spiralis* in mice

II. *IN VIVO* CHARACTERISTICS OF THE CELLS

D. WAKELIN, R. K. GRENCIS & ANNE M. DONACHIE *Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Bearsden Road, Bearsden, Glasgow*

Accepted for publication 17 December 1981

Summary. The *in vivo* characteristics of mesenteric lymph node cells (MLNC) capable of mediating the adoptive transfer of immunity to *Trichinella spiralis* have been examined. Mediator cells were diverted into the peritoneal cavity of infected donor mice following the induction of a peritoneal exudate and it was shown that these were nylon-wool, non-adherent (T) cells. After density gradient separation of [^{125}I]-UdR-labelled MLNC, the fractions that were most effective in transferring immunity were those containing a small proportion of cells but the largest proportion of incorporated activity. Treatment of the donors of MLNC with the mitotic inhibitor vinblastine effectively prevented both the transfer of immunity and increased incorporation of [^{125}I]-UdR characteristic of the mediator population.

In vitro irradiation of MLNC failed to affect their ability to transfer immunity.

Collectively these findings support the conclusion that mediator cells are T lymphoblasts, and suggest that mediation of immunity is effected directly by this population and not by their progeny.

INTRODUCTION

The accompanying paper (Grencis & Wakelin, 1982) has shown that the cells that mediate adoptive transfer of immunity to *Trichinella spiralis* are available in donor mice for only a limited period, corresponding with the duration of infection. In primary infections, cells are present in the mesenteric node by day 4 and their numbers decline after day 8. Their availability is related to the increase in total cell number that infection stimulates and to the increase in proportion of actively dividing, T lymphoblast cells. In secondary infections, cells appear earlier after infection, by day 2, and the increases in total cell number and lymphoblast activity are similarly accelerated. These observations point to the fact that the cells that transfer immunity are a short lived, dividing population stimulated by infection. Availability of the cells is restricted to the period of active intestinal response. In immune but unchallenged mice, mesenteric node cell populations do not contain a sufficient number of mediator cells to transfer immunity successfully, but cells are produced rapidly in response to challenge.

In the present paper the *in vivo* characteristics of mediator cells have been examined in terms of their ability to move into sites of inflammation, their status as dividing cells and their life span in recipients. The results confirm the view that mediator cells have the characteristics of T lymphoblasts.

Present address and correspondence: D. Wakelin, Department of Zoology, University of Nottingham, University Park, Nottingham NG7 2RD.

0019-2805/82/0600-0451$02.00
© 1982 Blackwell Scientific Publications

MATERIALS AND METHODS

Details of the mice and parasite used, the techniques

for infection and worm recovery, preparation, fractionation, isotope labelling and adoptive transfer of cells are described in the accompanying paper (Grencis & Wakelin, 1982). In the majority of experiments, mesenteric lymph node cells (MLNC) were taken from donors infected 8 days previously and recipients were given $2\text{–}4 \times 10^7$ cells. Donors and recipients were routinely infected with 300 larvae.

Peritoneal exudate

Mice were given 1 ml of a 10% solution of proteose peptone (Difco Ltd) in Hanks's balanced salt solution (HBSS) by intraperitoneal injection. Cells were collected 24 hr later by washing the peritoneal cavity with 5 ml of HBSS, centrifuging and resuspending in medium 199 (Gibco Europe Ltd). After counting, the cells were injected into recipient mice.

Vinblastine

The mitotic inhibitor vinblastine sulphate (Sigma) was dissolved in HBSS and injected intravenously at a dosage of 5 μg/g body weight.

Irradiation

Cells suspended in medium 199 were irradiated *in vitro* from a 500 Ci ^{60}Cobalt source (output 1050 rad/min).

Density gradient fractionation

Percoll (polyvinylpyrrolidone-coated colloidal silica, Pharmacia Ltd) was made isotonic for use with living cells by adding 5·5 ml of ten times concentrated HBSS to 50 ml of the commercial preparation. The isotonic solution was regarded as '100%' Percoll and dilutions were made from this using isotonic HBSS (Kurnick, Östberg, Stegagno, Kimura, Örn & Sjöberg, 1979). The refractive indices of each dilution were measured using a refractometer. MLNC suspensions ($5\text{–}10 \times 10^7$ cells in 1 ml of medium 199 or 2×10^7[^{125}I]-UdR-labelled cells in 1 ml of RPMI 1640) were layered onto discontinuous gradients in plastic, tissue culture grade tubes and centrifuged at 3000 r.p.m. for 15 min. Fractions were harvested by pipette, washed, counted and resuspended in medium 199 for adoptive transfer, or placed in vials for counting of radioactivity.

Statistics

The significance of differences in mean values between control and experimental mice was examined by Student's *t* test. *P* values $<0·05$ were considered significant.

RESULTS

Ability of effector cells to move into the inflamed peritoneal cavity

Lymphoblasts are known to migrate non-specifically into areas of inflammation and will accumulate in the peritoneal cavity once this is inflamed (McGregor & Logie, 1974; Love & Ogilvie, 1977). This property is not shared by small lymphocytes.

In an initial experiment peritoneal exudate cells (PEC) were recovered from the unstimulated peritoneal cavity of mice infected 8 days previously with *T. spiralis* and injected into recipient mice. An additional group was given MLNC from the donors and then both groups, plus controls, were challenged and killed 8 days later (Table 1). Whereas MLNC transferred immunity effectively, there was no effect on worm numbers in recipients of PEC.

In subsequent experiments PEC were taken from donors injected 24 hr earlier with proteose peptone. The results of one such experiment, comparing the activities of induced PEC and MLNC from mice infected for 8 days, are also shown in Table 1. In this case, cells from the stimulated peritoneal cavity transferred immunity as effectively as MLNC. A similar result was obtained using induced PEC and MLNC from mice infected for 4 days. An additional experiment confirmed that the effector PEC were T cells. Induced PEC and MLNC from day 8 donors were passed through nylon wool columns and the non-adherent (T-cell-enriched) fractions transferred into

Table 1. Adoptive transfer of immunity against *Trichinella spiralis* with mesenteric lymph node cells and with normal or induced peritoneal exudate cells taken from donor mice 8 days after infection

	No. of worms recovered 8 days after infection of recipients			
	Exp. 1		Exp. 2	
Cells transferred	Mean	SD	Mean	SD
None	202·4	9·3	210·2	27·4
MLNC	119·6*	44·1	124·5*	30·2
PEC	222·5‡	31·8	126·5*†	18·4

* Mean significantly lower than no cells control.

† PEC collected 24 hr after i.p. injection of 10% proteose peptone.

‡ PEC from unstimulated peritoneal cavity.

Table 2. Ability of density gradient (Percoll) separated fractions of mesenteric lymph node cells (MLNC) from infected mice to transfer immunity against *T. spiralis*

MLNC transferred	Recovery after separation of ^{125}IUdR-labelled cells		No. of cells transferred ($\times 10^6$)	No. of worms recovered 8 days after infection of recipients	
	% Of total cells	% Of total activity		Mean	SD
None	—	—	—	191·5	20·3
Unfractionated	—	—	20	87·7*	32·3
Density <48% Percoll	20·4	34·0	3	89·0*†	21·1
Density <52% Percoll	28·3	47·5	3	88·0*†	22·3
Density <58% Percoll	31·5	12·0	3	126·2*	33·6

* Mean significantly lower than no cells control.
† Mean significantly lower than <58% fraction.

recipients. Both transferred an effective immunity, the worm burdens being respectively 10% and 43% that of the controls.

Adoptive transfer of immunity with a lymphoblast-enriched population of MLNC

Several initial attempts were made to fractionate immune MLNC by density gradient centrifugation in order to obtain a blast-cell-enriched population. The best results were achieved when [^{125}I]-UdR-labelled MLNC from 8-day infected donors were spun through a discontinuous gradient formed by layering 22%, 48%, 52%, 58% and 72% dilutions of stock Percoll. Dead cells and debris remained above the 22% layer, live cells passed through into the denser layers. The majority of labelled cells had densities of less than 58% Percoll (1·072 g/ml). In two experiments the fractions of density <1·072 g/ml contained 47% and 49% of the cells recovered but 70% and 82% of the activity recovered. A comparison was then made of abilities of blast-enriched and blast-depleted fractions to transfer immunity, by transferring cells with densities <48% (1·059 g/ml), <52% (1·065 g/ml) and <58% (1·072 g/ml) Percoll. As blast enrichment and depletion were only relative, recipients were given a small number of cells (3×10^6) to accentuate any differences between the fractions. The number of unfractionated cells given to the control recipient group was equivalent to the total population from which the fractions were taken, i.e. 2×10^7 cells/mouse. The results are shown in Table 2. The two blast-enriched fractions transferred a degree of immunity comparable with that transferred by the unfractionated starting population and a significantly greater immunity than that transferred by the blast-depleted fraction. Similar results were obtained in a repeat infection.

Effect of vinblastine treatment on donors of MLNC

Vinblastine has an irreversible effect upon cells in, or entering into, mitosis but does not affect non-dividing cells. When used *in vivo* its effect is progressive, because of asynchrony in cell division, and thus for maximum inhibition donors are treated 15–20 hr before collection of MLNC (Bruchovsky, Owen, Becker & Till, 1965).

An initial experiment was carried out to confirm that short-term exposure to vinblastine itself had no effect on adoptive transfer in the *T. spiralis* system. One group of infected donors was injected 1 hr before collection of MLNC and their cells, together with cells from untreated infected donors were transferred into recipient mice. Both groups, together with controls were infected and killed after 8 days. Mean worm burdens were 156 for controls, 80 for recipients of MLNC from untreated donors and 51 for recipients of MLNC from donors treated with vinblastine. In subsequent experiments MLNC were collected 15–20 hr after treatment. Some cells were labelled with [^{125}I]-UdR to measure blast activity and the remainder were used for adoptive transfer. The results of one such experiment using cells from mice infected for 4 and 8 days are shown in Table 3. Cells from treated donors failed to transfer immunity and failed to show the characteristic increased incorporation of [^{125}I]-Udr

Table 3. Effect of donor pretreatment with vinblastine on the ability of mesenteric lymph node cells to transfer immunity against *T. spiralis*.

	No. of worms recovered 8 days after infection of recipients			
	MLNC taken day 4		MLNC taken day 8	
MLNC transferred	Mean	SD	Mean	SD
None	131·8	16·8	158·8	27·6
3×10^7 from untreated donors	66·0*	20·1	20·8*	14·1
3×10^7 from VBL-treated donors	117·2	10·7	156·0	22·1
Reduction (%) in [^{125}I]-UdR labelling index of MLNC†	90·1		76·7	

VBL given at 5 μg/g, 16 hr before cell collection.
* Mean significantly lower than no cells control.
† 100−[(c.p.m. 1×10^7 MLNC from VBL-treated mice)/(c.p.m. 1×10^7 MLNC from untreated mice) × 100].

indicative of blast cell activity. There was no difference in this respect between cells taken early and late during the course of a primary infection.

A similar experiment was then carried out using T-cell-enriched fractions of MLNC from untreated and vinblastine-treated, infected donors. The results shown in Table 4 confirm those obtained with unseparated populations, in that treatment inhibited blast cell incorporation of [^{125}I]-UdR and prevented an effective transfer of immunity.

Table 4. Effect of donor pretreatment with vinblastine on ability of nylon wool separated, T-enriched mesenteric lymph node cells (T-MLNC) to transfer immunity against *T. spiralis*

	No. of worms recovered 8 days after infection of recipients	
Cells transferred	Mean	SD
None	165·7	26·1
$3{\cdot}5 \times 10^7$ Unseparated MLNC from untreated donors	40·0*	16·3
$3{\cdot}0 \times 10^7$ T-MLNC from untreated donors	17·0*	9·0
$3{\cdot}0 \times 10^7$ T-MLNC from VBL-treated donors	122·8	27·7

VBL given at 5 μg/g, 16 hr before cell collection.
Reduction (%)† in [^{125}I]-UdR-labelling index of T-MLNC from VBL-treated mice = 78·5.
* Mean significantly lower than no cells control.
† 100−[(c.p.m. 1×10^7 T-MLNC from VBL-treated mice)/(c.p.m. 1×10^7 T-MLNC from untreated mice) × 100].

Effect of *in vitro* irradiation of MLNC on adoptive transfer

In a series of experiments MLNC and a T-enriched fraction of MLNC from infected donors were exposed to *in vitro* irradiation before transfer into recipients. The results of three such experiments, using 1000 rad are shown in Table 5. Irradiation had no significant effect upon transfer of immunity.

Life span of effector cells in recipients

In all previous adoptive transfer experiments the immune status of recipients of immune MLNC had been tested by challenging with *T. spiralis* on the day of transfer or shortly after. Several experiments were carried out to find out how long adoptive immunity would persist in recipients in the absence of challenge. In the first experiment cells were transferred into five groups of mice (day 0) from donors infected 8 days previously. One group was challenged immediately and the remainder were challenged at intervals up to 78 days after transfer. As a control for the ability of the batch of mice used to express immunity, an additional five groups were infected on day 0 and challenged at the same intervals. In each case mice were killed 8 days after challenge. The results are shown in Fig. 1. Cell recipients maintained a high degree of immunity throughout, with worm burdens of 37·5–48·3% of control values. Mice immunized by infection showed complete immunity.

As there was a possibility that the persistent immunity in recipients might have been stimulated by migrating newborn larvae transferred with the MLNC, the carcasses of three recipients killed on day 78 were digested in pepsin-HCl. Only one muscle larva was recovered from the total digest.

In a similar experiment three groups of recipient mice were given a T-cell-enriched fraction of immune MLNC. One group was challenged immediately, the other two were challenged 28 and 56 days later. The results are also shown in Fig. 1 and indicate that persistence of adoptively transferred immunity was a property associated with the T-cell fraction.

Table 5. Effect of 1000 rad *in vitro* irradiation on the ability of mesenteric lymph node cells or nylon wool separated, T-enriched MLNC to transfer immunity against *T. spiralis.*

	No. of worms recovered 8 days after infection of recipients					
	Exp. 1		Exp. 2		Exp. 3	
	Day 4 MLNC†		Day 8 MLNC†		Day 8 T-MLNC‡	
Group	Mean	SD	Mean	SD	Mean	SD
No cells	156·0	23·1	155·2	38·3	208·0	18·1
Normal cells	80·0*	19·6	66·0*	19·1	145·5*	39·1
Irradiated cells	108·7*	20·9	92·6*	29·9	126·6*	31·9

* Mean significantly lower than no cells control.
† $2 \cdot 5 \times 10^7$ Cells.
‡ 1×10^7 Cells.

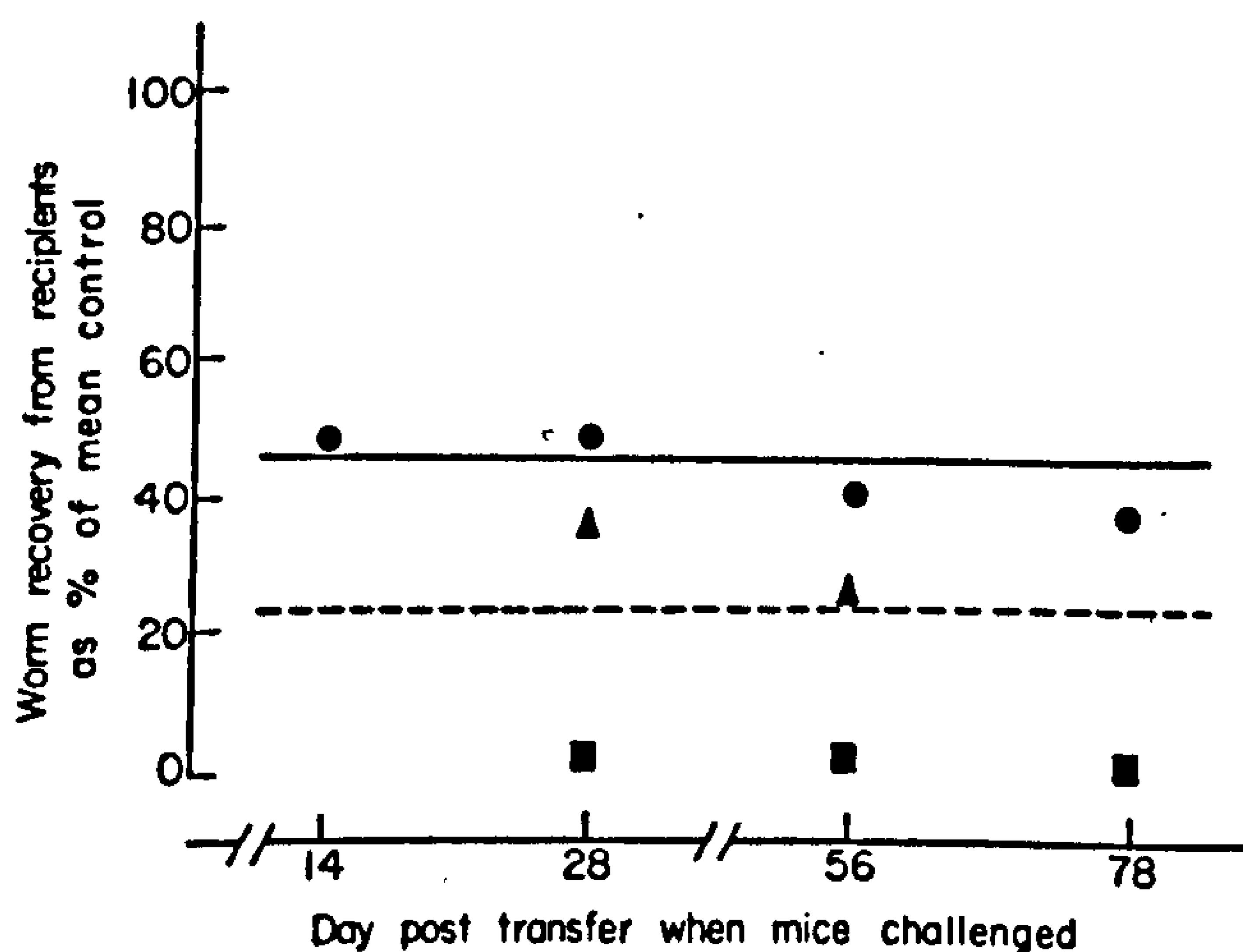


Figure 1. Persistence of immunity to *T. spiralis* in mice challenged at intervals after adoptive transfer with (●) mesenteric lymph node cells, (▲) nylon wool separated, T-cell-enriched MLNC, or (■) after immunization by prior infection. Cell transfers and immunizing infections on day 0. Level of immunity in mice challenged on day of transfer of MLNC (——); of T-MLNC (- - - -).

DISCUSSION

In all cases that have been adequately studied, it has been shown that immunity to parasitic helminths is thymus-dependent and transferable adoptively with T-cell populations (Mitchell, 1980). Whereas there are many descriptive accounts of T-cell involvement, there have been relatively few analyses of the kinetics of the response or of the nature and function of the cells concerned. It is clear that, in protective immunity to intestinal nematodes, T cells must be involved primarily in anti-worm antibody responses or in initiating and amplifying inflammatory responses. A direct cytotoxic role is unlikely. In the case of *T. spiralis*, immunity against the intestinal phase is expressed most strikingly in worm expulsion and there is strong circumstantial evidence that the direct causes of expulsion stem from the inflammatory changes which infection elicits and which are T-cell-mediated (Wakelin & Wilson, 1979a,b).

The close temporal relationship between the increase in number of T lymphoblasts in the MLN of mice infected with *T. spiralis* and ability to transfer worm expulsion adoptively with MLNC suspensions (Grencis & Wakelin, 1982) strongly suggests that these dividing cells are the major mediators of immunity. The results presented here confirm this view and show that the cells capable of transferring worm expulsion have the *in vivo* characteristics of lymphoblast cells. Thus transfer of immunity was associated with a population of large (low density), readily labelled cells which migrated into induced peritoneal exudates and whose capacity to function in adoptive transfer was suppressed after exposure to the mitotic inhibitor vinblastine, but was resistant to *in vitro* irradiation.

Many workers have shown that the normal migration patterns of lymphocytes are modified when inflammatory sites are present in the animal (e.g.

Koster & McGregor, 1971; Asherson, Allwood & Mayhew, 1973; McGregor & Logie, 1974; Rose, Parrott & Bruce, 1976a,b; Love & Ogilvie, 1977) and this phenomenon has been exploited in experimental studies on mediators of immunity. For example, the diversion of lymphoblasts to induced peritoneal exudates has been used to obtain cells capable of transferring protective immunity and delayed hypersensitivity reactions against *Listeria monocytogenes* (Koster, McGregor & Mackaness, 1971) and *Mycobacterium tuberculosis* (Lefford & McGregor, 1978). Love & Ogilvie (1977) found that in both uninfected rats and rats infected with the nematode *T. spiralis*, inflammation of the peritoneal cavity brought about a diversion of migration of intravenously injected thoracic duct lymphocytes, even though the majority of cells still homed to the small intestine. Rose, Parrott & Bruce (1978) were unable to demonstrate this phenomenon in NIH mice infected with *T. spiralis*, i.e. the system studied here. The reason for this discrepancy is not known.

The mitotic inhibitor vinblastine (VBL) has proved a useful tool with which to analyse the properties of cells mediating immune responses and the results obtained here convincingly demonstrate that mediator cells are dividing lymphocytes. In *T. spiralis* in mice, as in *L. monocytogenes* in mice and rats, the major cellular mediators of immunity are available for a comparatively short time during infection and their activity in adoptive transfer is VBL sensitive (Tables 2 and 3; McGregor & Logie, 1973; North 1973; 1975; North & Deissler, 1975). However this is not the case with other systems. During infection with *M. tuberculosis* in rats, for example, there is a shift from VBL-sensitive to VBL-resistant mediators of immunity (Lefford, McGregor & Mackaness, 1973) and VBL-resistant T cells have been shown to transfer immunity to *T. spiralis* in rats (Crum, Despommier & McGregor, 1977). It is difficult to make direct comparisons between the latter work and that described in this paper. Not only are different species of hosts involved, and there is evidence that rats and mice respond differently to *T. spiralis* (Alizadeh & Wakelin, 1981), but the cell populations studied by Crum *et al.* (1977) were obtained after multiple, drug-abbreviated infections, a protocol that does not generate mediator cell populations in mice, though it does result in substantial immunity to challenge (unpublished results). In rats both VBL-resistant T and VBL-sensitive B cells transfer immunity and it is interesting that although immunity to *Nippostrongylus brasiliensis* in the rat is transferred most efficiently with cells taken during a primary infection, mediator cells are still available after long term hyperimmunization (Nawa & Miller, 1978) and at this time B cells also transfer a slight but significant degree of immunity (Nawa, Parish & Miller, 1978).

The observation that *in vitro* irradiation of MLNC suspensions did not affect their *in vivo* capacity to transfer immunity implies that the mediators concerned need to function for only a short period after transfer in order to initiate the series of events leading to worm expulsion. Again, this implicates lymphoblasts as the mediator population, as small lymphocytes that require to proliferate and differentiate before becoming effector cells would be radiosensitive (Anderson & Warner, 1976).

Identification of the mediator cells as a population of short-lived, rapidly-dividing cells explains the apparently contradictory observations that immunity in the infected animal is long lasting, but the availability of cells capable of transferring immunity is limited to short periods during the active response to primary infection or challenge. An analagous situation has been observed in mice infected with *L. monocytogenes* (North, 1973, 1975; North & Deissler, 1975) and a similar explanation proposed. However, there is some evidence in this system that a low level of immunity can be transferred with cells taken after an acute infection has been cleared and these cells are non-replicating, i.e. are VBL-resistant. There was no evidence for this in the *T. spiralis*-mouse system, but it is possible that failure to transfer immunity from immune, unchallenged mice is a quantitative phenomenon or that it may reflect the relative insensitivity of the parameter of worm expulsion. For example the time course of expulsion in recipients challenged immediately after transfer, or challenged 2 weeks later is similar (unpublished results), even though the former would have available a 'ready-made' population of mediator cells. Clearly long term memory cells must be present in immune mice and the evidence suggests that the mediator population may differentiate into such cells after transfer, i.e. when challenge was delayed after adoptive transfer the recipients were still capable of an accelerated response (Fig. 1).

The fate of lymphoblasts after transfer to recipients is speculative, though there is evidence from labelling studies that they divide and give rise to smaller lymphocytes (McGregor & Logie, 1973). These cells may then enter the recirculating pool of lymphocytes and thus be unable to re-enter the intestinal mucosa

(Freitas, Rose & Parrott, 1977) until triggered into blast activity by infection. This population is therefore likely to be the memory population but, under the conditions employed in adoptive transfer, does not have appreciable mediator activity.

ACKNOWLEDGMENTS

This work was supported by Medical Research Council project grants 977/65/7 and 980/0058T (DW, AMD) and by MRC Research Training Award G77/6896 (RKG).

Additional experiments and discussion.

The ability to fractionate a complex population of cells into subpopulations differing in one or more physical characteristics and still be capable of normal function is a powerful test for the study of many problems in cellular immunology. Various techniques have been used for separation including sedimentation, density gradient centrifugation, counter-current distribution electrophoresis, and electronic separation by volume. In the previous chapter a negative selection technique utilizing the anti-mitotic vincalkaloid drug vinblastine was used to remove dividing (blast) cells from the MLN of mice during a primary infection with T. spiralis, and a positive selection technique, isopycnic centrifugation to fractionate MLNC into blast enriched and blast depleted cell populations. The results obtained suggested that rapidly dividing T blast cells mediate expulsion of T. spiralis from the small intestine. However, enrichment of MLNC populations for blast cells by centrifugation was relatively poor. Two techniques which have been shown to enrich complex cell populations in blast cells efficiently are velocity sedimentation at unit gravity (Miller and Phillips, 1969) and natural serum mediated cytotoxcicity (Kierszenbaum and Budzko, 1977). Fresh guinea pig serum has been shown to have a non specific, but selective complement mediated cytotoxic effect upon immune lymphocytes (Budzko, Kierszenbaum and Waksman, 1977), T lymphoblasts showing resistance to this effect (Kierszenbaum and Budzko, 1979). Preliminary experiments utilizing this technique with MLNC from T. spiralis infected mice provided conflicting

and inconsistent results (Grencis, unpublished observations) and therefore, was considered unsuitable for further study.

In velocity sedimentation, cells are allowed to settle through a low density medium ($\sim$1.01 g/cm^3). The sedimentation rate of a cell is proportional to the diameter of the cell, and because the diameter of immune lymphocytes usually varies between 5 - 15μm a maximum of a 9 fold range in sedimentation rates may be obtained on the basis of cell size differences. However, the densities of the majority of mouse lymphocytes vary between 1.04 and 1.09 g/cm^3, and as sedimentation rate is also proportional to the difference between the densities of cell and medium, less than a 3 fold range in sedimentation rates is obtained on the basis of differences in cell densities. Thus, sedimentation rate should provide a sensitive technique capable of separating large dividing lymphocytes.

The following experiments in addition to providing information from vinblastine studies, concerning the division status of MLNC which mediate expulsion of T. spiralis during a secondary infection, also utilize the technique of velocity sedimentation (using the recently developed Celsep apparatus) to analyse the size of the cells which mediate expulsion of T. spiralis from the gut during a primary infection.

Materials and Methods

Cell suspensions and cell transfer

MLNC suspensions were prepared, labelled, separated into T enriched populations and B enriched populations and transferred according to the techniques described in the general materials and methods. In vitro blast cell activity was measured using the macro culture and microculture methods, as described in the general materials and methods.

Vinblastine

Vinblastine has a short half life in vivo (Valeriote and Bruce, 1965) and acts selectively upon dividing cells by inhibiting spindle formation, resulting in metaphase arrest and cell death (Palmer, Livengood, Warren, Simpson and Johnson, 1960; Bruchovsky, et al 1965). Vinblastine sulphate (Sigma) was dissolved in HBSS and injected intravenously at a dosage of 5μg/g body weight.

Velocity sedimentation

Cells may be separated by sedimentation velocity in fluid at unit gravity according to Stokes Law:-

$$\text{Sedimentation velocity} = S = \frac{2\ a^2 g\ (Pp - Pm)}{9n}$$

Where a is the cell diameter

Pp is the density of the cell (particle)

Pm is the density of the fluid

n is the viscosity of the fluid

g is the acceleration due to gravity

For most cell suspensions the separation that can be achieved is primarily dependent upon cell size (a) ie, if the variations between the density of the cell and the density of the fluid are very small, and if a shallow gradient in the fluid is employed, then S is approximately equal to Ka^2 where K is a constant, thus separation is primarily a function of cell size. However, sedimentation and density separation are not completely independent procedures. Cell density is roughly inversely proportional to cell size since the nucleus of a cell is much more dense than the cytoplasm and larger cells tend to have a smaller nucleus/cytoplasm ratio. Therefore if variations in density between cells is great enough, density alone will be sufficient to effect a separation.

The objective of the method used is to form a thin layer of cells in suspension on top of a fluid column and to allow the cells to sediment through medium under the influence of gravity for an appropriate length of time, after which fractions containing cells which have moved different distances are collected.

The major limit to separation of cells by velocity sedimentation is the phenomenon of streaming. This occurs if the cell concentration in the cell band loaded onto the column exceeds a certain number, called the streaming limit. Shortly after loading, large numbers of filaments (streams) can be observed hanging down from the cell band. Resolution is therefore affected and the efficiency of the technique reduced.

A fluid column is unstable because of convection and mechanical jarring and therefore, to maintain stability a shallow density gradient is introduced to the column. The gradient is made as shallow as possible, with the density of the medium as low as possible to ensure separation on a basis of sedimentation, rather than density.

Sedimentation Chamber

The column used in the present experiments is a Celsep (Model 6000; Wescor Inc). Celsep consists of two basic units: (1) the sedimentation chamber, and (2) the motor base unit. The sedimentation chamber is basically a shallow cylinder 21.5cm in diameter and 2.75 cm in depth, containing a volume of approximately 1000 ml. The base unit enables accurate and efficient levelling and tilting of the sedimentation chamber.

Gradient

A two cylinder gradient maker was used to generate a shallow linear gradient in the Celsep. 4% and 8% solutions of Percoll (Pharmacia fine chemicals Ltd) in RPMI 1640 medium with 5% FCS were used to generate an effective linear gradient of between 4% and 6.65% percoll for separation.

Separation

The gradient was loaded, through the bottom loading port at a flow rate of approximately 20 ml/min with the chamber in the up (tilted) orientation (see Plate 1a).

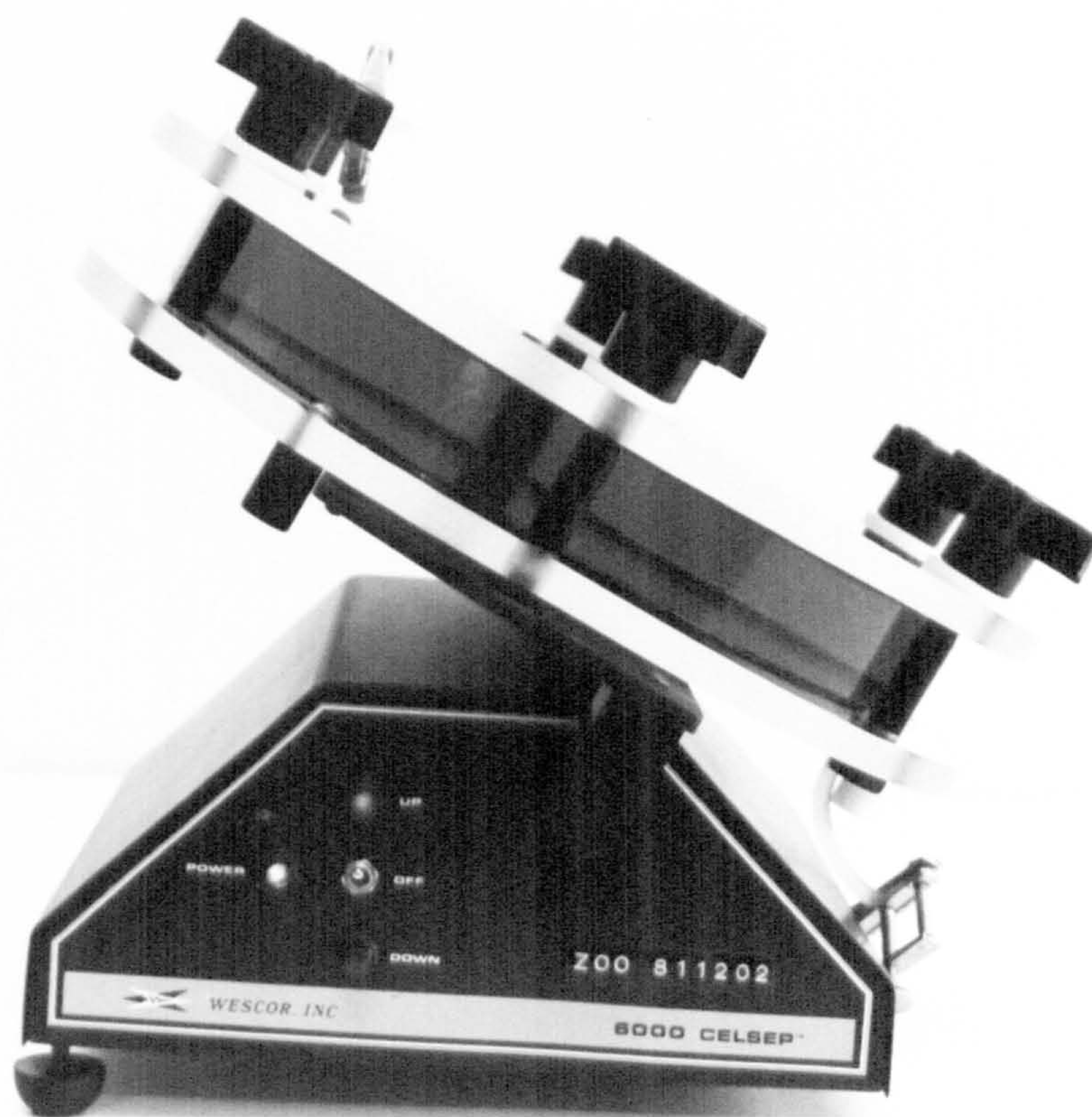


Plate 1a. Celsep chamber in tilted up position for loading and unloading of gradient.

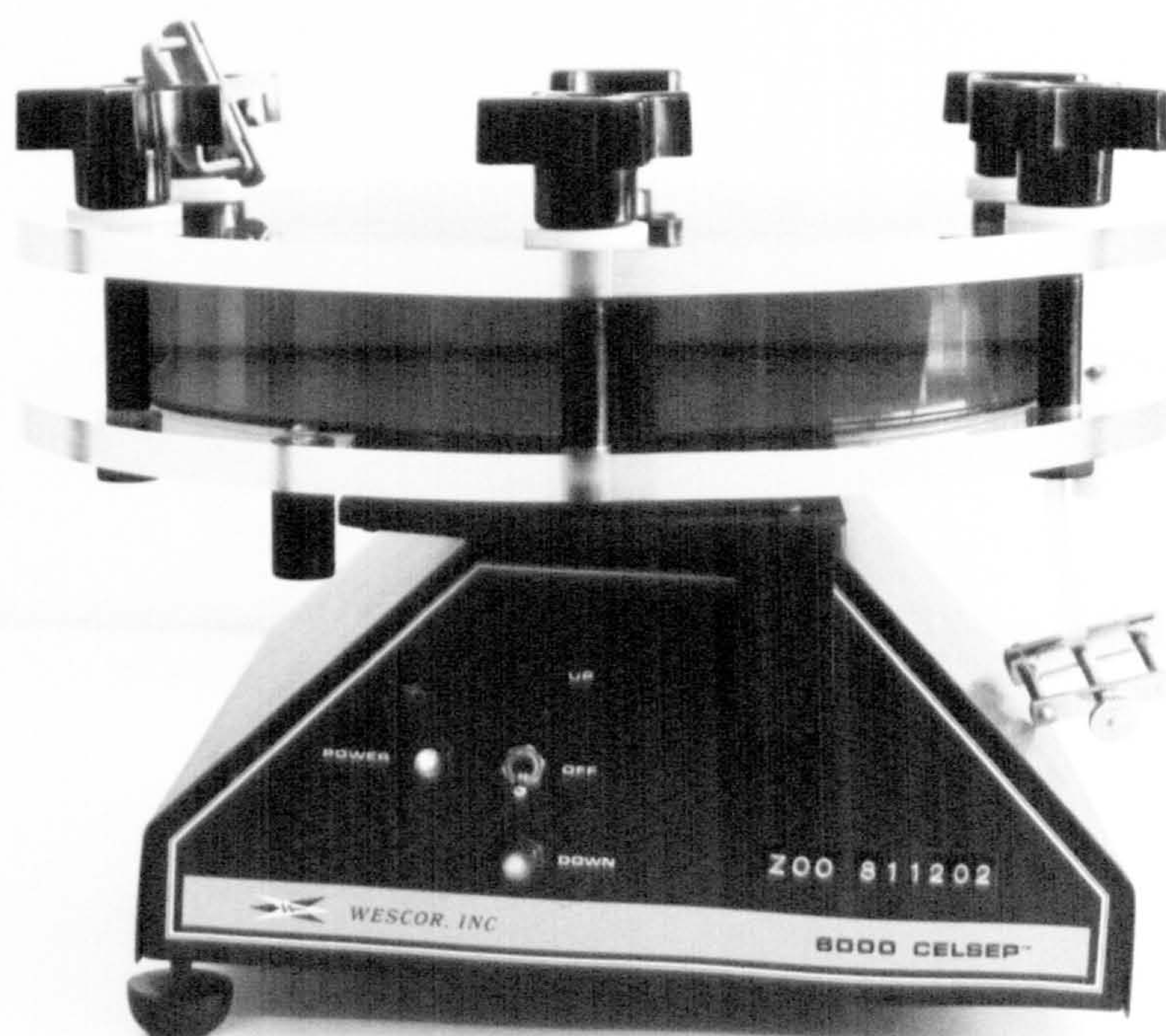


Plate 1b. Celsep chamber in horizontal down position for separation at unit gravity.

The cell suspension (streaming limit: 1.5×10^8 MLNC/50 ml) in RPMI 1640 medium with 5% FCS was then layered onto the gradient through the top loading port followed by 50 ml of PBS pH 7.2 overlay. Care was taken to avoid the inclusion of air bubbles in the chamber which disrupt the gradient. After closing off both loading ports, the chamber was orientated to the down (horizontal) position for separation (see Plate 1b).

The cells were allowed to separate for 2 hours after which time the chamber was re-orientated to the up (tilted) position for unloading. The gradient and cells was unloaded at a flow rate of approximately 30 ml/min through the bottom port, by displacement with PBS pH 7.2. The fractions were taken and centrifuged at 200 g for 5 minutes to pellet the cells, which were then washed with fresh medium before use. All operations were carried out at 4°C. Cell recoveries were usually between 50% and 80% of the cells applied to the chamber.

Results

The effect of donor pre-treatment with vinblastine on the capacity of MLNC taken after secondary infection, to transfer immunity against *T. spiralis* in NIH mice

It has previously been shown that treatment with vinblastine completely abrogated the capacity of MLNC taken from animals during a primary infection, to adoptively transfer immunity (Wakelin, Grencis and Donachie, 1982). Grencis and Wakelin (1982) have additionally shown that the population of MLNC responsible for mediating immunity during a secondary infection with *T. spiralis* are T cells, but the cell cycle status of these cells was not determined.

In order to discover whether the cells which mediate immunity during a secondary infection with *T. spiralis* in mice are rapidly dividing T cells, an experiment was designed in which MLNC were taken from animals which had received a secondary infection and intravenous vinblastine treatment. Donor NIH mice were infected with 300 *T. spiralis* on day -21 and challenged with a further 300 *T. spiralis* muscle larvae on day 0. 16 hours previous to collection of MLNC on day 4 post challenge, four groups of donors were injected intravenously with vinblastine. Cells from untreated, and vinblastine treated donors were taken, separated and transferred on day 0. Together with controls all recipients were infected with 320 *T. spiralis* muscle larvae on the day of cell transfer. *In vitro* blast cell activity of the various cell populations was assessed on day 0 before

transfer, and worm numbers in the small intestine were measured on day 8 post infection.

The results show that treatment of cell donors with vinblastine completely abrogated the capacity of T enriched fractions of MLNC to adoptively transfer immunity. Unseparated and untreated immune MLNC, and untreated T cell enriched fractions of immune MLNC did adoptively transfer immunity against T. spiralis as assessed by expulsion of the worms from the gut. Untreated B cell enriched fractions of immune MLNC did not transfer immunity, confirming earlier work. The efficacy of the vinblastine treatment upon dividing cells in the MLN was reflected by the in vitro blast cell activity of the various cell populations. The increase in labelling index normally shown by T cell enriched MLNC over control cells from uninfected mice was not evident in the T cell enriched population of MLNC taken from vinblastine treated mice (Table 7.6).

The results support the hypothesis that expulsion of T. spiralis from the gut during a secondary infection is mediated by rapidly dividing T lymphocytes, a situation similar to that operating in a primary infection.

Enrichment of MLNC populations for blast cells using velocity sedimentation

Preliminary experiments were designed to assess the feasibility and efficiency of velocity sedimentation using the Celsep apparatus to enrich or deplete populations of

Table 7.6. The effect of donor pre-treatment with vinblastine on the capacity of MLNC to transfer immunity against *T. spiralis*. Cells taken day 4 post secondary infection. Primary infection given day -21.

Group	Worm recovery days post infection		Mean labelling index
	Mean	S.D.	
Control - no cells	201.2	11.8	----
3.0×10^7 unseparated MLNC from untreated mice	55.0*	13.3	189*
2.5×10^7 immune⁺ T - MLNC from untreated mice	9.0*	6.1	237*
2.5×10^7 immune⁺ T - MLNC from Vb treated mice	209.0	8.4	117
2.0×10^7 immune B - MLNC from untreated mice	197.6	21.4	n.d.

* significantly different from control.

⁺n = 5

MLNC for blast cells.

In the first experiment 1.5×10^8 MLNC taken from NIH mice 8 days after infection with T. spiralis were layered onto the gradient in the Celsep chamber. Separation was carried out for two hours after which consecutive 25 ml fractions were collected from the gradient in the chamber and assessed for viable cell numbers and in vitro blast cell activity. 49% of the total number of cells applied to the chamber were recovered in a viable form. In order to ease interpretation for each recovered fraction, the results are expressed as % total recovered cells for cell numbers, and % total activity from all cells for in vitro blast cell activity. Of the 40 fractions recovered from the chamber only fractions 17-29 contained recoverable cells. The majority of the % total activity from all cells (blast cells) was contained in the first few fractions (Fractions 17-20/21) collected from the chamber, although % total recovered cells were highest in fractions 21-23/24. Fractions 25-28 contained few cells and little activity (Table 7.7).

To confirm the results from the first experiment, a second experiment was designed. 1.5×10^8 MLNC taken from animals infected 8 days previously with T. spiralis were loaded onto the Celsep chamber and allowed to separate for 2 hours. After this time the cells were collected for analysis. In this experiment the first 550 ml of the gradient were ignored and then three 100 ml fractions (A, B & C) were collected in succession. The remaining

Table 7.7. Velocity sedimentation of MLNC from mice. Cell recovery and *in vitro* blast cell activity of fractions recovered from Celsep chamber. Each fraction 25 ml volume.

Fraction Number	% total recovered cells	% total activity from all cells
1 - 16	nil	nil
17	3.2	11.2
18	5.5	16.3
19	7.2	17.7
20	7.8	14.7
21	12.6	18.0
22	13.3	9.7
23	15.0	7.4
24	8.0	4.5
25	8.1	2.6
26	6.5	1.5
27	7.8	1.0
28	4.9	0.7
29 - 40	nil	nil

350 ml gradient was discarded. As can be seen from Table 7.8 the results confirm those obtained in the first experiment. The first fraction taken off the chamber (fraction A) contained the smallest number of cells but exhibited the greatest in vitro blast cell activity. Fractions B and C between them contained approximately 90% of the cells but only 20% of the blast cell activity.

Adoptive transfer of immunity against *T. spiralis* with MLNC populations enriched in blast cells by velocity sedimentation

An experiment was designed in which blast cell enriched and blast cell depleted populations of MLNC were adoptively transferred into recipient mice. Cell donors were infected with 300 *T. spiralis* on day 0. On day 8 post infection MLNC were collected and 1.5×10^8 cells were applied onto the Celsep chamber and allowed to separate for 2 hours. Upon collection, the first 550 ml of gradient were discarded, and three 100 ml fractions (A, B and C) consecutively collected. The remaining gradient was also discarded. The cells from each fraction were washed, counted and after a sample had been removed for estimation of in vitro blast cell activity, prepared for cell transfer. 64% of the total number of cells applied to the column were recovered in viable form. Confirming earlier experiments, the first cells collected from the chamber contained the majority of the blast cell activity. One group of cell recipients each received 1.92×10^7 unseparated MLNC; and three other groups of recipients received separated MLNC from fractions A, B or C, in a number corresponding to

Table 7.8. Velocity sedimentation of MLNC from mice. Cell recovery and *in vitro* blast cell activity of fractions recovered from Celsep chamber.

Fraction	% total recovered cells	% total activity from all cells
First 550 ml	nil	nil
A (100 ml)	10.5	79.5
B (100 ml)	48.6	19.1
C (100 ml)	40.8	1.4
Remaining 350 ml	nil	nil

the proportion that would be present in 1.92×10^7 unseparated MLNC. All cell recipients together with controls were infected with 350 T. spiralis muscle larvae on day 0. Worm burdens in the small intestine were measured on day 7 post infection. The results clearly show a correlation between blast cell activity and adoptive transfer of immunity (Table 7.9).

These results confirm the results from earlier vinblastine and isopycnic density gradient centrifugation studies (Wakelin et al, 1982) and show that short lived dividing cells mediate expulsion of T. spiralis from the gastro-intestinal tract.

Table 7.9. Adoptive transfer of immunity against *T. spiralis* in NIH mice with MLNC populations enriched in blast cells by velocity sedimentation.

Fraction	% total cells recovered	% total activity from all cells	Group	Worm recovery Day 8	
				Mean	S.D.
			No cells	224.0	22.0
			1.92×10^7 unseparated MLNC	89.6*a	24.2
A	9.6	82.8	2.25×10^6 + MLNC	102.5*a	42.0
B	69.7	15.4	1.34×10^7 ++ MLNC	219.8	24.9
C	20.7	1.8	4.50×10^6 + MLNC	229.0	23.3

* significantly different from control
\+ n = 4
++ = 5
a = no significant difference between groups

Summary Points

Chapter 7

(1) Cells capable of mediating immunity to *T. spiralis* were shown to be diverted into the peritoneal cavity of infected mice following the induction of a peritoneal exudate. These cells were nylon wool non-adherent (T) cells.

(2) Separation of MLNC from infected mice using isopycnic centrifugation, and velocity sedimentation at unit gravity demonstrated that the most effective cell populations were those with a high proportion of large low density (blast) cells which incorporated high amounts of radiolabelled DNA precursors.

(3) Treatment of donors of MLNC after primary and secondary infections with the mitotic inhibitor vinblastine sulphate effectively prevented both the transfer of immunity and the increased incorporation of $[^{125}I]$ - UdR, characteristic of the mediator population.

(4) *In vitro* irradiation of the MLNC failed to affect their ability to transfer immunity.

Chapter 8: Memory to *T. spiralis* infections in mice; possible mechanisms involved.

From the early work of Glenny and Sudmersen (1921), which examined the immune response to diptheria toxoid in rabbits, the phenomenon of immunological memory has been known for many years. It is now generally accepted that successful priming induces the formation of a pool of specifically prepared memory cells ready to respond to further exposure to antigen by the rapid production of cells and antibody.

In addition to the well established B cell memory system (Cunningham, 1969; Pilarski, 1978; Dresser and Popham, 1979) a role for memory T cells has also been investigated. Miller and Sprent (1971) and Sprent and Miller (1976) showed that a proportion of T blast cells generated in a primary response survived and became small memory carrying cells in the recirculating lymphocyte pool. Work upon the facultative intracellular parasite Listeria monocytogenes has shown a role for specific long-lived, tissue-positioned T memory cells outwith the recirculating lymphocyte pool, generated from T blasts during a primary infection (Jungi, 1980a, b; Jungi and Jungi, 1981).

Resistance to re-infection with T. spiralis in mice has been shown to be a long lived phenomenon (Wakelin and Lloyd, 1976a). Work from the previous chapters has demonstrated that expulsion of the worm from the intestine of NIH mice is mediated by dividing T cells, which are recalled rapidly in response to a secondary infection, in a manner indicative of immunological memory. MLNC are capable of mediating

expulsion of the worm from the small intestine for up to at least 78 days after cell transfer (Wakelin et al, 1982), which suggests that the T blasts that mediate expulsion are capable of surviving for a considerable period of time. Other adoptive transfer experiments have shown that cells capable of transferring immunity do not remain in the MLN after expulsion of the worm from the gut in primary or secondary infections (Grencis and Wakelin, 1982). It is possible that a proportion of the T blasts become part of the recirculating lymphocyte pool, as small non-dividing cells, or take up a sedentary position in various tissues. Upon re-infection the cells could re-enter the MLN, proliferate and home back to the small intestine to mediate expulsion.

The recruitment of lymphocytes to the site of antigenic challenge has received considerable attention. Recent works (McConnell and Hopkins, 1981; Hopkins, McConnell and Pearson, 1981) have implicated a role for prostaglandin E_2 (PGE_2) in a cell shutdown phenomenon in the lymphnodes draining the site of antigenic stimulation, whereby recirculating cells are prohibited from leaving the node for a short period of time. The mechanisms involved in recall of memory to T. spiralis infections are unknown, although T blasts are known to home non-specifically to the gut (Rose, Parrott and Bruce 1976b) and inflammatory foci (Wakelin et al, 1982).

The work in this chapter examines some of the aspects of memory to T. spiralis infections, and the possible mechanisms involved in recall and lymphocyte recruitment upon challenge.

Materials and Methods

Cell Suspensions and Adoptive Transfer

Cell suspensions were prepared, enriched for T or B cells, labelled with fluorecein-conjugated antibodies, and transferred according to the methods described in the general materials and methods.

Assays for Delayed Type Hypersensitivity

(i) Antigen

Soluble crude worm antigen was prepared (total protein concentration 2.5 mg/ml) as described in the Materials and Methods of Section I. Prior to use antigen was sterilized by membrane filtration using a disposable 0.22μm Millex-or filter (Millpore).

(ii) Footpad Assay

Prior to injection, size measurements of the thickness of each hind footpad were made using a pocket thickness gauge (Mitutoyo Mfg. Co Ltd, Japan). After swabbing with 70% alcohol, each hindfootpad was injected intradermally with either 10μl of antigen (25μg total protein, right footpad) or 10μl of sterile PBS pH 7.2 (left footpad) using a 50μl Terumo microsyringe (Terumo corporation, Japan) mounted with a 30 -G needle. 24 hours post infection, the left and right hind footpads were measured. For each animal, the % increase in footpad thickness was calculated as follows:

$$\frac{\text{Thickness of footpad 24 hr post injection} - \text{Thickness of footpad prior to injection}}{\text{Thickness of footpad before injection}} \times 100$$

The mean percentage increase in footpad thickness ($\pm$ S.E) was calculated for each group of animals.

(iii) Radioisotopic ear assay

Delayed type hypersensitivity reactions were measured using the radio-isotopic ear assay as described by Vadas, Miller, Gamble and Whitelaw (1975). Briefly, on the day of the assay, 10μl of antigen (left pinna) or sterile PBS pH 7.2 (right pinna) were injected intradermally into the ear using a 50μl Terumo microsyringe mounted with a 30 -G needle. Ten hours later 2μCi of 5 - $[^{125}I]$ Iodo - 2' - deoxyuridine, $[^{125}I]$ -UDR, in a volume of 0.2 ml sterile 0.9 % saline was given intravenously to each animal. Thirty minutes prior to injection of the isotope, 0.1 ml of a 10^{-3} M solution of 5 - fluorodeoxyuridine (FUdR, Sigma) was injected intraperitoneally into each animal in order to increase labelling efficiency of the isotope $[^{125}I]$ - UdR by blocking incorporation of 2' - deoxyuridine -5' phosphate into deoxy-thymidine - monophosphate synthesis (Hughes, Commerford, Gitlin, Krenger, Schutze, Shah and Reilly, 1964). Twenty-four hours after injection of isotope mice were killed, both left and right ears cut off at the hairline, placed in plastic vials and radioactivity counted in a Packard Tribarb liquid scintillation spectrometer. The results were expressed as the ratio of radioactivity in the left ear (antigen) to that in the right ear (PBS - control).

Cell culture and assessment of cell trapping

Cell trapping was assessed using a modification of the methods used by Zatz and Lance (1971) and Frost and Lance (1974).

In order to label lymphocytes, MLNC taken from uninfected NIH donors were incubated with radioactive chromium (^{51}Cr, Sodium chromate, specific activity 1mCi/ml, 1x10^{8} cells/ml of culture medium (see solutions, general materials and methods) for 30 mins at 37°C in a gently shaking water-bath. After labelling, cells were washed three times in medium, counted and 3 x 10^7 viable cells injected intravenously via a lateral tail vein. An injected dose equivalent was retained to measure the injected radioactivity. 24 hours post infection animals were killed and various organs (MLN, spleen, small intestine, large intestine, and liver) were removed, placed individually in plastic tubes and the radioactivity for each organ counted for one minute in a Packard Tricarb liquid scintillation spectrometer. The amount of radioactivity in each organ was expressed as a % of the injected dose.

The presence of cell trapping was considered confirmed when the mean localization of ^{51}Cr labelled cells in the organ from infected animals was at least 20% greater than that found in the corresponding organ from the uninfected control animals (Frost and Lance, 1974).

Results

The recirculating lymphocyte pool: Involvement in immunity to *T. spiralis*

Previous experiments (Chapter 5) have shown that expulsion of *T. spiralis* from mice is mediated by rapidly dividing cells present in the MLN for only a limited period of time after infection (Grencis and Wakelin, 1982) although animals remain solidly immune for many months. A classical secondary response is elicited upon a secondary infection implicating the presence of a memory cell population. The location of such memory cells is unknown.

A preliminary experiment was designed to examine the role of cells in the recirculating lymphocyte pool in memory to *T. spiralis* infections. It is well known that at any one time the spleen contains a large proportion of recirculating lymphocytes (See Ford and Smith, 1979). Therefore, a suspension of spleen cells taken from infected animals at a time long after worm expulsion may contain recirculating T cells carrying memory for *T. spiralis*.

Spleen cells and MLNC were taken from NIH donor mice 31 days after a primary infection of 300 *T. spiralis* muscle larvae. Spleen cells were enriched for T or B cells before transfer. Cell recipients received either unseparated MLNC, unseparated spleen cells or spleen cells enriched for B or T lymphocytes, and together with controls, infected with 300 *T. spiralis* on the day of cell

transfer (day 0). Worm burdens in the small intestine and female worm fecundity were measured on day 8 post infection.

4×10^7 unseparated MLNC and 5×10^7 unseparated spleen cells from infected animals had no effect upon worm expulsion assessed on day 8 post infection. However, 4×10^7 and 9×10^7 spleen cells enriched for T cells from infected animals did accelerate worm expulsion in recipients, 9×10^7 spleen T cells being more effective than 4×10^7 spleen T cells. 7×10^7 spleen cells enriched for B cells did not accelerate worm expulsion. Fecundity was not depressed in recipients of any of the cell populations (Table 8.1).

The results present preliminary evidence for the existence of a population of T memory cells to T. spiralis residing in the spleen or as part of the recirculating lymphocyte pool.

Table 8.1. Adoptive transfer of immunity to *T. spiralis* in NIH mice. Involvement of spleen cells or recirculating cells. Cells taken from donors 31 days post infection.

	Day 8 post infection		
	Worm recovery		Worm fecundity
	Mean	S.D.	Larvae per female per hour
Control - no cells	161.2	7.4	4.3
4×10^7 unseparated MLNC	168.2	10.6	4.6
5×10^7 unseparated spleen cells	170.4	14.7	2.9
4×10^7 T cell enriched spleen cells	104.8*	12.5	3.8
9×10^7 T cell+ enriched spleen cells	72.8*	8.5	3.8
7×10^7 B cell enriched spleen cells	162.5	6.7	4.0

* significantly different from control - no cells

+n = 5

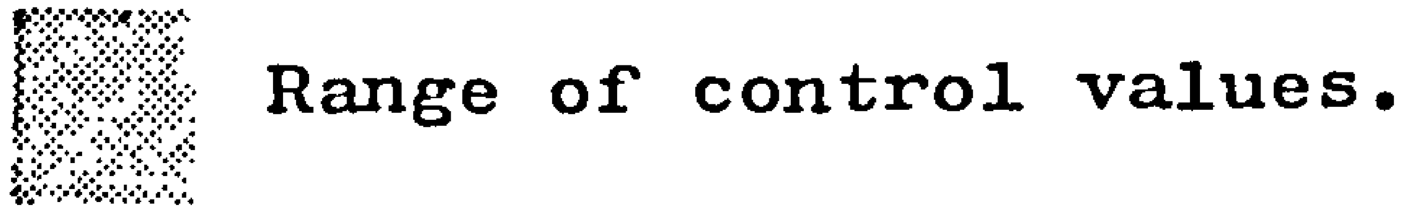
Sensitization of NIH mice to *T. spiralis* antigens by infection

An experiment was designed to examine the presence of recirculating T memory cells to *T. spiralis* antigens, identifying the cells by their ability to participate in delayed type hypersensitivity (DTH) reactions. In mice, it has been shown that DTH reactions are governed by recirculating T cells that bear the surface marker Ly-1, and that some of these cells are long lived (Huber, Devinsky, Gershon and Cantor, 1976; Vadas, Miller, McKenzie, Chism, Shen, Boyce, Gamble and Whitelaw, 1976).

Ten groups of mice were infected with 300 *T. spiralis* on day 0. Each type of DTH assay was performed on one group of infected mice and one group of control uninfected animals on days 2, 8, 16, 21 and 34 post infection. NIH mice became sensitized to *T. spiralis* antigens after infection with *T. spiralis* as assessed by both the footpad DTH assay and the radio-isotopic DTH ear assay. DTH reactions in infected mice were greater than controls by day 8 post infection and reached plateau levels by day 21, remaining at this level until the conclusion of the experiment (fig 8.1).

The results show that cells generated in response to infection with *T. spiralis* have the capacity to recognise *T. spiralis* antigen administered parenterally and participate in delayed type hypersensitivity reactions.

Fig 8.1. Delayed type hypersensitivity reactions to *T. spiralis* antigens in mice infected with *T. spiralis*


Range of control values.

Footpad assay

% increase in thickness of footpad ± S.E.

40
30
20
10

2 8 16 21 34

Radioisotopic ear assay

Ratio of left ear (Ag) to right ear (PBS) ± S.E

4.0
3.0
2.0
1.0

2 8 16 21 34

Days post infection

Cell trapping in NIH mice infected with *T. spiralis*

Experiments were designed to examine the possibility of a cell shutdown or cell trapping phenomenon occurring in the MLN of mice infected with *T. spiralis*. The cell trapping phenomenon has been described for a number of defined antigens, elicited in the lymph node tissue draining the site of antigenic stimulation (Frost and Lance, 1974; McConnell and Hopkins, 1981).

In the first experiment, recipient mice each received 3×10^7 ^{51}Cr labelled MLNC, either 2, 4, or 8 days after infection with 300 *T. spiralis* muscle larvae. An additional group of uninfected mice which received cells, served as a control group. 24 hours after injection of the cells the animals were killed and MLN, spleen, small intestine, large intestine and liver were removed for measurement of radioactivity. Only the MLN showed an increased accumulation of ^{51}Cr labelled MLNC, characteristic of cell trapping the levels of activity recovered showing an increase from day 2 to day 8 post infection. The other organs examined showed no difference in activity between uninfected and infected animals. (Table 8.2). Thus it is possible that a cell trapping effect does operate in the MLN of *T. spiralis* infected mice. However, previous work has shown a considerable increase in cell number of the MLN on days 4 and 8 post infection, partly due to rapidly dividing T cells (Grencis and Wakelin, 1982). It is possible that the increased accumulation of ^{51}Cr labelled cells in the MLN at these times as described above, was due to purely mechanical

Table 8.2. Cell trapping. Twenty-four hour localization of ^{51}Cr labelled MLNC in uninfected mice and mice infected with *T. spiralis*.

	Mean percentage radioactivity recovered from injected dose ± S.D.			
Organ	Uninfected control	Day 2 P.I.	Day 4 P.I.	Day 8 P.I.
MLN	8.48 ± 1.78	11.66* ± 1.45	11.89* ± 1.50	13.99* ± 1.39
Spleen	19.35 ± 1.02	18.87 ± 1.50	17.57 ± 2.60	17.86 ± 1.93
Small intestine	2.88 ± 0.19	2.18 ± 0.22	3.00 ± 0.15	2.89 ± 0.19
Large intestine	1.38 ± 0.46	1.28 ± 0.39	1.21 ± 0.15	1.23 ± 0.22
Liver	19.92 ± 1.94	20.85 ± 1.60	19.38 ± 1.55	20.19 ± 2.18

* Positive evidence of cell trapping. Mean percentage localization of ^{51}Cr labelled cells in organ at least 20% greater than mean of control animals.

retention of the circulating cells, a consequence of the large cell numbers and/or cell size in the MLN. To investigate this possibility an experiment was designed in which cell recipients received ^{51}Cr labelled cells 8 hr, 24 hr and 48 hrs after an infection of 300 T. spiralis. At these times, the cellularity of the MLN is comparable to that of uninfected mice. 24 hr after infection of the cells, the animals were killed and the MLN, spleen, small intestine and liver were removed and measured for radioactivity. Again, the only organ to show an increased accumulation of labelled cells, characteristic of a cell trapping response was the MLN (Table 8.3).

An additional experiment was conducted to examine whether the cell trapping response occurred in the MLN of mice during a secondary infection. Cell recipients were infected with 300 T. spiralis on day 0 and challenged with 300 T. spiralis on day 21. 8 hr, 24 hr, and 48 hr after injection of ^{51}Cr labelled MLNC, the animals were killed and the MLN, spleen, small intestine and liver collected for measurement of radioactivity. Only the MLN showed evidence of cell trapping as assessed by accumulation of ^{51}Cr labelled cells (Table 8.4). The levels of activity in the MLN increased between 8 hr and 48 hr post infection, and were greater than the levels observed following a primary infection (Table 8.3).

Rose, Parrott and Bruce (1976a) have shown that ^{51}Cr labelled lymphoblasts show an enhanced migration to the small intestine of T. spiralis infected mice, and it is

Table 8.3. Cell trapping in the MLN of _T. spiralis_ infected mice. Twenty four hour localization of ^{51}Cr labelled MLNC in uninfected mice and mice given a primary infection of 300 _T. spiralis_ muscle larvae.

Organ	Mean percentage radioactivity recovered from injected dose I.S.D.			
	Uninfected control	8 hr P.I.	24 hr P.I.	48 hr P.I.
MLN	5.68 ± 0.73	7.95* ± 0.83	7.90* ± 0.50	8.00* ± 0.53
Spleen	12.81 ± 1.67	13.34 ± 1.22	14.48 ± 1.02	13.94 ± 1.32
Small intestine	2.29 ± 0.34	2.69 ± 0.43	2.86 ± 0.37	2.65 ± 0.38
Liver	32.25 ± 6.31	32.99 ± 3.67	34.49 ± 1.76	33.75 ± 4.47

* Positive evidence of cell trapping (See Table 8.2.).

Table 8.4. Cell trapping in the MLN of *T. spiralis* infected mice. Twenty four hour localization of ^{51}Cr labelled MLNC in uninfected mice and mice given a secondary infection of 300 *T. spiralis* muscle larvae on day 0. Primary infection of 300 *T. spiralis* muscle larvae given on day -21.

	Mean percentage radioactivity recovered from injected dose ± S.D.			
	Day 21 post primary infection control	8 hr post secondary infection	24 hr post secondary infection	48 hr post secondary infection
MLN	4.84 ± 0.81	8.11* ± 0.86	8.34* ± 1.20	11.15* ± 0.56
Spleen	12.45 ± 1.79	11.76 ± 1.22	11.67 ± 1.32	11.89 ± 1.14
Small intestine	2.18 ± 0.25	2.48 ± 0.25	2.45 ± 0.42	2.49 ± 0.64
Liver	31.67 ± 5.65	32.56 ± 5.67	32.04 ± 4.44	31.20 ± 4.10

* Positive evidence of cell trapping (See Table 8.2).

known that lymphoblasts are labelled more heavily with ^{51}Cr than non-dividing cells (Eyre, Rosen and Perry, 1970). The labelled MLNC in these experiments did not show enhanced migration to the small intestine of T. spiralis infected mice, and therefore, the increased levels of activity observed in the MLN of infected mice were unlikely to be due to lymphoblasts. The evidence from these experiments does suggest that a cell trapping response does occur in mice early after infection with T. spiralis.

Summary Points

Chapter 8

(1) Adoptive transfer of immunity with large numbers of T cell enriched spleen cells taken from donor mice 31 days after a primary infection, presented evidence for the existence of a population of T memory cells to *T. spiralis* in the spleen or recirculating lymphocyte pool.

(2) Infection with *T. spiralis* was shown to sensitize NIH mice to *T. spiralis* antigen administered parenterally, as assessed by DTH reactions. DTH reactions were greater than control levels by day 8 post infection and reached plateau levels by day 21.

(3) Evidence was presented to show the occurrence of a cell trapping response in the MLN early after both primary and secondary infections in mice.

Chapter 9. Generation of cells in the MLN with the capacity to adoptively transfer immunity against *T. spiralis* in mice.

All five life cycle stages of Trichinella spiralis occur in one host and each generates a component of the total immune response to infection (See Wakelin and Denham, 1982). Chemically abbreviated infections have enabled workers to show that both the pre-adult and adult stages of T. spiralis are protectively immunogenic (Campbell, 1965; Denham, 1966; Despommier, McGregor, Crum and Carter, 1977b). James and Denham (1975) and James, Moloney and Denham (1977) have shown that immunity generated by newborn larvae was effective only against this stage and not against other life cycle stages. Recent immunochemical work has emphasized the strict stage specificity of anti-T. spiralis serum antibodies generated by infection in mice (See Maizels, Philipp and Ogilvie, 1982) and McKenzie, Preston and Ogilvie (1978) have shown in vitro that these antibodies mediate granulocyte adherence in a stage specific manner. However, there have been few studies which examine the role of different life cycle stages in the generation of specific protective immune responses known to operate against T. spiralis in vivo.

Previous work (Grencis and Wakelin, 1982) has shown a temporal correlation between expulsion of T. spiralis from the gut, high blast cell activity in the MLN and capacity of MLNC to adoptively transfer immunity. It is tempting to speculate that the high blast cell activity observed is related to the continual presentation of antigens by the worms in the intestine. The experiments in this chapter examine some of the factors involved in the generation of the MLN blasts responsible for mediating worm expulsion.

Materials and Methods

Cell suspensions, adoptive transfer and blast cell activity.

Cell suspensions, adoptive transfer of immunity, and assays for in vitro blast cell activity were carried out as described in the general materials and methods.

Transplantation of adults or muscle larvae

Five day old adult *T. spiralis* worms were recovered from the intestines of infected wistar rats using the modified Baermann technique; incubation was limited to approximately 45 minutes. Worms were selected randomly and no attempt was made to differentiate between male and female worms. *T. spiralis* muscle larvae were recovered by artificial digestion of infected mice as described in the general materials and methods. The adult worms required for each transplantation were washed with warm (37°C) Hanks' balanced salt solution (HBSS), placed into a small test tube and allowed to sediment in fresh HBSS at 37°C. The volume of HBSS containing worms was reduced to approximately 0.2 ml. Worms were normally transplanted not more than 2 hours after the donors had been killed.

Recipient mice were anaesthetized with sagatal (sodium pentobarbitone, 60 mg/ml, May and Baker Ltd) in a 1:10 solution of sterile 0.9% NaCl. This solution was injected intraperitoneally (i.p.) at a dose of 0.01 ml/g body weight. At this dosage, anaesthesia is induced within a few minutes and persists for 2 - 3 hours. Operations

were performed within 30 - 60 minutes of adminstration of anaesthetic. The mice were fixed to the operating surface using plastic tape and the skin over the upper abdomen shaved and sterilized with 70% alcohol. A small incision (approximately 1 cm long) was made through the skin near the midline. The body wall was pierced with a scalpel and the incision was extended using blunt forceps. The proximal duodenum was pulled out through the incision from under the liver using a small metal hook. A hole was made through the duodenal wall with a hypodermic needle and the adult worms or muscle larvae were injected from a drawn-out glass pipette. Parasites were expelled into the duodenum in HBSS (adults) or 0.2% agar (larvae) in a direction away from the stomach. After removal of the pipette the hole was sutured (0.7 Mer Silk Mersuture, Ethicon Ltd, Edinburgh). The peritoneal cavity and intestine were sprayed with an antibiotic mixture of bacitracin, neomycin and polymixin ('Rikospray', Riker Laboratories Ltd, Loughborough). The intestine was replaced in the body cavity and the body wall closed with sutures and sprayed again with 'Rikospray'. The skin was sutured, sprayed with 'Rikospray' and sealed with aerosol plastic skin (Nobecutane, Astra Chemicals Ltd, Watford). Operated animals and controls were provided with antibiotic (terramycin) in their drinking water (see general materials and methods).

Anthelmintic and Worm recovery

The use of the anthelmintic methyridine (Mintic) and the techniques employed in worm recovery were as described in the general materials and methods.

Results:

Adoptive transfer of immunity against *T. spiralis* with MLNC taken from animals after a chemically abbreviated infection.

Cells capable of adoptively transferring immunity against *T. spiralis* in NIH mice are available in the MLN for only a limited period of time after infection (Grencis and Wakelin, 1982). Cell transfer using MLNC is most effective when cells are taken 8 days post infection at the time of peak blast cell activity. Spontaneous cure begins shortly after day 8. An experiment was designed to examine if the efficacy of MLNC in adoptive transfer of immunity was dependent upon the continual presence of the worms in the small intestine.

NIH cell donors were infected with 300 *T. spiralis* on day -8. Some of the cell donors were given mintic on day -5 to terminate the infection. MLNC suspensions from treated and untreated donors were prepared on day 0 (8 days after infection) and *in vitro* blast activity of the different cell populations measured. Cells were transferred on day 0 and recipients together with controls were infected with 350 *T. spiralis* muscle larvae on the day of cell transfer. Eight days post infection worm numbers in the small intestine were measured.

The results show that MLNC populations from donors that had received complete (8 day) or abbreviated (3 day) infections were equally effective in adoptively transferring

immunity against T. spiralis. The ability to transfer immunity was associated with elevated levels of blast cell activity in each donor cell populations (Table 9.1).

The results suggest that continual presence of the worms in the gut is not essential for the generation and for maintenance of cells in the MLN capable of transferring immunity to T. spiralis in mice.

Adoptive transfer of immunity against T. spiralis with MLNC taken from animals exposed to transplanted adult worms

The previous experiment suggested that the early (pre-3 day old) phases of T. spiralis infections were adequate to stimulate cells in the MLN capable of adoptively transferring immunity. Therefore an experiment was designed to investigate the role of the post 3 day old phase T. spiralis infection upon generation of immunocompetent cells in the MLN using transplanted adult worms. The protocol for infection of cell donor groups is shown below:-

Group	Day -8	Day -5	Day 0
A	Infect 300 muscle larvae	---	Take MLNC for transfer and measurement of blast cell activity
B	Infect 300 muscle larvae	MINTIC	
C	Transplant 300 muscle larvae	---	
D	Transplant 300 muscle larvae	MINTIC	
E	Transplant 200 Adults	---	
F	Transplant 200 Adults	MINTIC	

Groups of cell recipients received MLNC on day 0 and,

Table 9.1. Adoptive transfer of immunity against *T. spiralis* with MLNC taken from mice after chemically abbreviated infection.

	Worm recovery Day 8		
	Mean	S.D.	Mean labelling index + of donor cells
Control - no cells	242.0	7.5	----
3.5×10^7 MLNC from normal (8 day) infection	87.8*	31.7	310*
3.5×10^7 MLNC from abbreviated (3 day) infection	129.8*	26.3	183*

* Mean significantly different from control.
\+ (c.p.m./For experimental MLNC)/(c.p.m./ for control MLNC) x 100.

together with controls all animals were challenged with 300 T. spiralis muscle larvae on the day of cell transfer. Eight days post infection worm burdens in the small intestine were measured.

MLNC from animals given 3 or 8 day muscle larvae infections via conventional oral infection or via laparotomy, transferred a strong degree of immunity to naive recipients as assessed by accelerated worm expulsion. However, MLNC taken from animals which had received an 8 day or 3 day adult only infection did not transfer immunity. The capacity of MLNC populations from animals given muscle larvae infections to adoptively transfer immunity was reflected by raised blast cell activity levels, whereas blast activity levels of MLNC from adult only infections were similar to control levels (Table 9.2).

A small experiment was performed in order to confirm these findings. NIH cell donors received 3 or 8 day adult only infections, or a convential 8 day oral infection of muscle larvae. MLNC were taken and transferred or measured for blast activity on day 0. Together with controls cell recipients were injected with 360 T. spiralis muscle larvae on the day of cell transfer. Worm burdens in the small intestine were measured on day 8 post challenge.

The results confirm those from the previous experiment by showing that MLNC taken from mice given 3 or 8 day adult infections are not capable of transferring immunity against T. spiralis. Blast cell activity of the MLNC

Table 9.2. Adoptive transfer of immunity against *T. spiralis* with MLNC taken from animals exposed to transplanted adult worms.

Group	Worm recoveries from cell donors on day of transfer		Worm recoveries of cell recipients Day 8		Mean labelling index+
	Mean	S.D.	Mean	S.D.	
Control - no cells	---	---	141.2	11.0	---
3×10^7 MLNC from group A donors	156.8	15.1	1.2*	1.8	285*
3×10^7 MLNC from group B donors	0.0	---	11.6*	9.1	186*
3.5×10^7 MLNC from group C donors	166.7	12.7	1.0*	1.7	315*
3.5×10^7 MLNC from group D donors	0.0	---	35.6*	20.8	225*
3×10^7 MLNC from group E donors	104.2	15.6	129.3	10.4	93
3×10^7 MLNC from group F donors	0.0	---	128.6	14.8	84
Sham operated controls++	---	---	---	---	108

* Mean significantly different from control
\+ labelling index, see Table 9.1.
++ n = 3.

populations from adult only infections remained similar to control levels, much lower than the levels shown by MLNC from animals given an oral 8 day muscle larvae infection (Table 9.3).

Level of muscle larvae infection required to generate cells in the MLN capable of adoptively transferring immunity against *T. spiralis*

An experiment was designed to discover if the size of the muscle larvae infection was important in the generation of cells in the MLN capable of mediating immunity to *T. spiralis*.

NIH cell donors were infected with either 300, 150 or 50 muscle larvae on day -8. On day 0 MLNC suspensions were prepared and transferred into recipients or measured for blast cell activity. Together with controls, cell recipients were infected with 350 *T. spiralis* muscle larvae on the day of cell transfer. Eight days post infection worm burdens in the small intestine were measured.

The results show that an infection with as few as 50 muscle larvae (mean of 27 established worms) was capable of generating cells in the MLN which adoptively transferred immunity. The capacity of MLNC to transfer immunity was reflected by increased blast cell activity of MLNC from donors given 50, 150 or 300 muscle larvae (Table 9.4).

Table 9.3. Adoptive transfer of immunity against *T. spiralis* with MLNC taken from animals exposed to transplanted adult worms.

	Worm recoveries from cell donors on day of transfer		Worm recoveries of cell recipients Day 8		Mean labelling index +
	Mean	S.D.	Mean	S.D.	
Control - no cells	---	---	232.0	12.0	---
2 x 10^7 MLNC from 3 day adult infection	0.0	---	229.5	14.4	119
2 x 10^7 MLNC from 8 day adult infection	116.0	22.7	231.0	8.7	123
2 x 10^7 MLNC from 8 day oral muscle larvae infection	186.6	11.5	106.4*	15.2	272*

* Mean significantly different from control
\+ labelling index, see Table 9.1.

Table 9.4. The effect of different levels of muscle larvae infection upon the capacity of MLNC to adoptively transfer immunity against *T. spiralis*. Recipients each received 2×10^7 MLNC from the appropriate donors.

Level of muscle larvae infection in donors No of Larvae	Mean % recovery of worms from donors day 8	Worm recovery from recipients day 8 Mean	S.D.	Labelling index +
Control - no cells	---	263.3	41.8	---
50	54.8	133.8*	33.7	199*
150	47.5	118.0*	17.5	227*
300	44.7	140.5*	26.5	405*

* Mean significantly different from control
\+ labelling index; see Table 9.1.

Immunization of NJH mice with adult T. spiralis

The previous experiments have shown that infections of adult worms (and their consequent new born larvae burden) are not capable of generating cells in the MLN which can adoptively transfer immunity as assessed by accelerated worm expulsion.

However, previous experiments have shown that infections of transplanted adult T. spiralis worms do confer resistence to normal challenge infections of muscle larvae, in mice (Kennedy, Wakelin and Wilson, 1979) and to a varying degree in rats (Bell, McGregor and Despommier, 1979; Bell and McGregor, 1979b). Therefore an experiment was designed to examine the degree of resistance stimulated by transplanted adult T. spiralis worms in NIH mice. NIH mice received either 250 transplanted adult worms or 300 T. spiralis muscle larvae on day -21. Three mice received sham operations as controls for transplantations. On day -14, three animals which had received adult worms were killed to check establishment of the transplanted T. spiralis. On day 0, together with controls, all animals were challenged with 300 T. spiralis muscle larvae and worm burdens in the small intestine measured on day 7.

Mice which had received transplanted adults (mean of 160.2 established worms) or an oral infection of muscle larvae were solidly immune to a challenge infection of muscle larvae, as assessed by worm numbers in the small intestine on day 7 (Table 9.5).

Table 9.5. Immunization of NIH mice with adult *T. spiralis* worms. Challenge infection of 300 *T. spiralis* muscle larvae given day 0.

Group	Day 7 post infection Worm recovery	
	Mean	S.D.
Control	153.8	21.9
250 adult T. spiralis worms day -21	6.6*	10.7
300 muscle larvae day -21	0.0*	0.0
Sham operation day -21 +	142.1	18.6

* Mean significantly different from control
\+ n = 3

The results confirm the earlier work by Kennedy *et al* (1979) and show that adult worms are capable of immunizing mice against re-infection with *T. spiralis*.

Summary Points

Chapter 9

(1) Adoptive transfer of immunity against T. spiralis with MLNC taken from mice exposed to a full or chemically abbreviated infection were equally successful and shown to be associated with elevated blast cell activity.

(2) Experiments in which adult worms were introduced directly into the gut suggested that the adult stage of infection contributed little to the generation of cells capable of transferring immunity.

(3) The muscle larvae stages of infection were shown to be potent stimulators of cells capable of adoptively transferring immunity, infections of as few as 50 larvae producing MLNC effective in transfer.

(4) Transplanted adult worms do protect mice against re-infection. It is possible that this is due to antigens released from the progeny (muscle larvae) of the transplanted adults and not to adult stage antigens.

Discussion

Analysis of the cellular basis of the immune response to helminth infection has received much attention for a number of systems (See Mansfield, 1982) although detailed studies examining the generation and characteristics of the cells involved are rare, save those concerning granulocyte adherence (See Ellner and Mahmoud, 1982). From adoptive transfer studies it has been shown that cells which are capable of mediating expulsion of N. brasiliensis from the rat are Ig negative (Nawa, et al, 1978). Ig negative cells have been shown to adoptively transfer immunity against T. muris in the mouse (See Lee, Wakelin and Grencis 1982), and T cell dependence of immunity has been inferred for a number of helminths including N. dubius, Aspiculuis tetraptera, Syphacia obvelata, S. ratti, S. mansoni, Taenia taeniaeformis and Mesocestoides corti (See Mitchell, 1980). Despommier et al (1977b) from work with T. spiralis in rats have suggested that IgA B blasts may play a major role in expulsion of the worm from the G.I. tract, although work by Wakelin and Wilson (1979a) on T. spiralis has shown that this is not the case in mice.

The experiments in the present study have utilized a number of approaches to examine the generation and characteristics of the cells which mediate expulsion of T. spiralis from the intestine of NIH mice. It has been consistently shown that MLN cells taken from NIH mice after a primary infection with T. spiralis are capable of adoptively transferring immunity as assessed by the

accelerated expulsion of the worms from the gut, and reduced female worm fecundity (Wakelin and Lloyd, 1976b; Wakelin and Wilson, 1977a; Wakelin and Wilson, 1979a; Wakelin and Donachie, 1980; Alizadeh and Wakelin, 1982). These observations were confirmed in the present study and extended to show that effective cells were present in the MLN for only a limited period of time after primary infection, days 4-8. After a secondary infection MLNC were also shown to be capable of adoptively transferring immunity, the effective cells being available in the MLN up to day 4 post challenge. In both cases the capacity to successfully transfer immunity with MLNC was associated with increased numbers of dividing T cells in the MLN. The presence of Thy 1 antigen on the surface of effective cells was confirmed by both negative selection using nylon wool and a cytotoxic anti-Thy 1.2 monoclonal antibody and positive selection techniques using the FACS. The results from the cell sorting experiments provided positive evidence for the involvement of Thy 1.2 + ve cells in mediating expulsion, but did not exclude some role for Thy 1.2 - ve cells. However, the technical and operational constrictions of using the FACS to provide adequate numbers of cells for <u>in vivo</u> work make it difficult to fully assess the significance of the latter finding.

Based on recent research there now exists a substantial amount of information concerning lymphocyte subsets of the mouse (Ahmed and Smith, 1982) and therefore parasite/mouse systems are ideally suited for investigations of the role of different lymphocyte subpopulations in immunity to parasites. In this respect the most detailed studies to date

have been conducted upon the protozoan parasite L. tropica. In this system Ly1+ T cell blasts isolated from primed lymph node cells could be maintained in culture for several days, and were shown to be capable of mediating a) helper activity for antibody responses in vitro in a hapten carrier system, b) the transfer of antigen - specific DTH responses to normal mice and c) the specific activation of parasitized macrophages resulting in the destruction of the parasites. It has also been possible to obtain parasite - specific Ly1+ T cell clones which exhibited similar functional properties, from these enriched blast cell populations (See Louis, et al, 1982). Studies on lymphocyte subset involvement in helminth systems are few and far between. Populations of Ly 1+T cells have been shown to have modulatory effects upon S. mansoni granulomata in mice (Doughty and Phillips, 1982), and Johnson, Nicholas, Metcalf, McKenzie and Mitchell, (1979) has reported that Ly 2- cells were better than Ly 1- cells at promoting the peritoneal eosinophilia observed in mice infected with M. corti. The experiments from the present study show that the cells which mediate expulsion of T. spiralis from the gut in mice are Ly2 negative, suggesting that Ly1 positive T cells are involved in mediating expulsion. However, the role which this subpopulation of T cells plays in the mechanism of expulsion is at present open to conjecture.

The lymphoblast status of the mediator cells of expulsion of T. spiralis from the gut, was confirmed using vinblastine sulphate to effectively remove dividing cells from the MLN at times after primary and secondary infections

when large numbers of blast cells were usually present. Adoptive transfer of Vb treated cells failed to confer protection against infection. Another approach confirming the blast nature of the cells, exploited the property of lymphoblasts to migrate non specifically into areas of inflammation (McGregor and Logie, 1974). Positive selection of blast cells using the techniques of isopycnic centrifugation and velocity sedimentation coupled with adoptive transfer, confirmed that in vivo, rapidly dividing cells in the MLN mediated expulsion of the worm from the gastrointestinal tract.

The function of the blast cell population in bringing about the changes that mediate expulsion of the worms from the intestine is unknown. It is likely that the lymphoblasts generated by infection play a variety of different roles, calling for the involvement of several functionally distinct cell clones. The present work has shown that irradiation of the mediator cells does not prevent their effectiveness as mediator cells and suggests that they do not need to divide after transfer to carry out their functions. Therefore it is reasonable to hypothesize that the lymphoblasts may be involved in the release of a variety of soluble mediators (See Rocklin, Bendtzen and Greineder, 1980) which are involved in a number of different processes. Those which are relevant to response effective against intestinal parasites include:-

(a) the recruitment of monocytes and polymorphs to the site of infection by chemotaxis,

(b) Localisation and retention of lymphocytes and macrophages at the inflammatory site due to lymphocyte inhibitory factor (LIF) and macrophage inhibitory factor (MIF),

(c) lymphocyte mitogenesis,

(d) Mast cell differentiation (Nabel, Galli, Dvorak, Dvorak and Cantor, 1981),

(e) mast cell degranulation (Askanase, Bursztajn, Gershon and Gershon, 1980),

(f) villous atrophy and crypt hyperplasia (Ferguson and Macdonald, 1977).

Furthermore, the vasoactive properties of some mediators may initiate other components of the inflammatory response, and bring into play other protein systems including the complement and Kinin systems (See Wesley-Leid and Williams, 1979).

The demonstration of adoptive transfer of the mucosal mast cell response with MLNC from <u>T. spiralis</u> infected animals (Alizadeh and Wakelin, 1982; Parmentier, Ruitenberg and Elgersma, 1982) provides evidence for MLNC involvement in the inflammatory reaction in the gut. Rose <u>et al</u> (1976a) have shown that lymphoblasts will home to the small intestine (ie the infection site) in <u>T. spiralis</u> infected animals and Manson-Smith, Bruce and Parrott, (1979a) have

demonstrated the T cell dependence of villous atrophy and crypt hyperplasia associated with T. spiralis infections. These additional observations present strong presumptive evidence for the involvement of soluble mediators in the inflammatory changes found in the gut of T. spiralis infected animals. Indeed, the expulsion of T. spiralis from the gut is probably a direct consequence of the environmental changes generated in the gut, which are detremental to worm survival (See Wakelin and Denham, 1982). Thus, the inflammatory responses resulting from lymphoblast derived mediators are likely to be the result of complex and multiple interactions. Investigation of the contributions made by particular components of these responses will depend upon the production of distinct functional cell clones and isolation of their products, and examination of their action upon worm survival, and their contribution to the overall inflammatory response.

Although the existence of immunological memory to parasitic infection is evident in a number of helminth systems (See Cohen and Sadun, 1976) and has been examined in a few systems (Adams and Rothwell, 1980; Hopkins, 1982), the study of the cellular basis of memory has received little attention. B cell memory to defined antigens has been under investigation for a number of years (See Dresser and Popham, 1979). T cell memory to defined antigens has also been studied (Sprent and Miller, 1976), although more recent work has examined the immune response and memory to the bacterium L. monocytogenes. Jungi (1980b) demonstrated the existence of memory T cells outside the recirculating

lymphocyte pool (RLP) and Jungi and Jungi (1981) provided evidence that these tissue positioned memory T cells are long lived progeny of the blast cells generated early in infection, or are short lived, but constantly replaced from other sources of memory cells.

The present study has briefly examined some of the aspects involved in memory to infection with T. spiralis. It is possible that T memory cells are derived from the population of T blast cells which is generated in response to infection. Due to technical difficulties a direct examination of the RLP of mice is difficult, but from an indirect study using the proportion of the RLP in the spleen evidence indicates the possible existence of a population of memory T cells within the RLP. This possibility is strengthened from the vaccination studies of the first section. The accelerated blast cell response observed in the MLN of challenged, vaccinated mice, is indicative of a secondary response, which implies the existence of a population of memory cells, and because of the site of immunization probably part of the RLP. However, it is possible that memory cells may be tissue positioned, as in L. monocytogenes infections. One useful approach to investigate the memory cell population to T. spiralis, and their anatomical location, would be to use an in vitro lymphocyte stimulation assay. Using T. spiralis antigen, it would be feasible to examine cells from different sources for specificity of response to T. spiralis antigen. Indeed, Krco, David and Wassom (1982) have shown the feasibility of such a technique, and demonstrated responsive Ly 1+T lymphocytes in the draining

lymph node of vaccinated mice.

The recruitment of lymphocytes to the site of infection has been investigated in T. spiralis infected NIH mice. Rose, Parrott and Bruce (1976a) have shown that T lymphoblasts show increased non specific localization to the gastro-intestinal tract during the early phases of infection, and this phenomenon is independant of altered vascular supply (Ottaway, Manson-Smith, Bruce and Parrott, 1980). However, these studies did not present any evidence for the involvement of the draining node (MLN) in lymphoblast homing. The present work has shown a definite involvement of the MLN in mediating expulsion of T. spiralis from the gut, and the experiments of lymphocyte trapping provide evidence of a mechanism whereby primed and unprimed lymphocytes may be recruited to the lymph node draining the site of infection. The components involved in the cell trapping response have been best studied using defined antigens in sheep (Hopkins, et al, 1981; McConnell and Hopkins, 1981) and mice (Zatz and Lance, 1971; Frost and Lance, 1974; Van Rooijen and Roeterink, 1980) which have demonstrated that both primed and unprimed lymphocytes may be involved, the response being regulated by complement, PGE_2 levels and possibly macrophages. Evidence from the present study does suggest that the cell trapping response in the MLN may involve an antigen specific component, as cell trapping after a secondary infection was greater than that after a primary infection.

The results from the present study show that continual

antigenic stimulation by the worm in the gut is unnecessary for the elevated blast cell activity observed in the MLN during the phase of active intestinal infection. The results suggest that the adult stage of T. spiralis contributes little if anything to the stimulation of cells capable of mediating worm expulsion. Therefore, it is reasonable to suggest that the immunogenic stage of infection is the muscle larvae or pre adult stage, at least in terms of expulsion immunity. This view is enforced by the experiments showing that very low numbers of muscle larvae are capable of stimulating cells which can mediate worm expulsion. Campbell, Hartman and Cuckler (1963) demonstrated that protective immunity against infection with T. spiralis can be stimulated by repeated intestinal infections of 1 day duration, and vaccination studies by Despommier et al (1977a) emphasized the strong immunogenic nature of muscle larvae antigens. Bell, McGregor and colleagues (Bell and McGregor, 1979, a, b; 1980; Bell, McGregor and Despommier, 1979) have examined the stage specificity of immunity to T. spiralis in rats and suggested that although the adult stage generated immune responses which were autospecific in their action, the early muscle larvae stages were the most immunogenic. It is probable that the protective muscle larvae antigens are derived from the secretory granules of the stichosome (Despommier and Muller (1970). Despommier (1974) has shown that nearly all supplies of muscle larval derived secretory granules are exhausted by 30-40 hr after oral infection, with over 50% of the granules secreted by the 8th hour. The stichosome undergoes extensive morphological changes during the worms' first 48 hrs in the intestine

resulting in the synthesis of new granules which are then secreted by the mature adult worm. This may account for the relatively poor immunogenicity of adult worms in stimulating cells capable of mediating worm expulsion. However, the present study does show that transplanted adults do confer protection against re-infection, expressed as an accelerated worm expulsion, confirming earlier work by Kennedy, Wakelin and Wilson (1979). Nevertheless, the transplanted adults were not sterile, and it is therefore possible that the resultant muscle larvae burdens were responsible for the protective immunity generated. Indeed, Pritchard (1977) has demonstrated the existence of cells, possible migratory macrophages, which contain T. spiralis antigen in the vicinity of muscle larvae cysts.

SECTION 3

Chapter 10: Immunity to *T. spiralis* in mice. Factors involved in direct anti-worm effects.

To be published in the *Proceedings of the International Commission for Trichinellosis*, 1983.

Introduction

Immunity to the intestinal nematode *Trichinella spiralis* results from the complex interaction between humoral, cellular and pathological changes generated in response to infection. Using the technique of adoptive cell transfer in mice, recent work has shown that expulsion of the adult worm population from the gut is mediated by short lived dividing T cells (Grencis and Wakelin, 1982; Wakelin, Grencis and Donachie, 1982). It has been suggested that T blasts mediate inflammatory changes in the intestine which lead to an environment detremental to worm survival (Wakelin and Wilson, 1979b).

The role of B cells, immunoglobulin (Ig) secreting cells and antibody in protective immunity to *T. spiralis* infection remains unclear. Recent studies using defined antigens have demonstrated how complex and varied are the patterns of development and distribution of Ig secreting cells in various lymphoid tissues during an immune response (See Benner, Hijmans and Haaijman, 1981). Therefore, it is not surprising that the role of Ig secreting cells and antibody in protective immunity to *T. spiralis* infections remains to be comprehensively described. The passive transfer of serum from *T. spiralis* infected animals has been examined by a number of workers and has yielded variable results (Larsh, Goulson and Weatherley, 1964a, b; Denham, 1969; Love, Ogilvie and McLaren, 1976; Moloney and Denham, 1979). Despommier, McGregor, Crum and Carter (1977b) have

suggested that in the rat, IgA producing lymphoblasts are involved in the expulsion of adult T. spiralis worms from the gut, although this has been shown not to be the case in the mouse (Wakelin and Wilson, 1979a).

Thus, although much of the work to date is fragmented and not directly comparable, most evidence does point towards a direct anti-worm effect of antibody which is reflected by a reduction in worm size, fecundity and often host muscle larvae burden.

This paper examines the direct anti-worm effects of antibody using the technique of passive transfer of serum and the anti-worm effects of different cell populations using the technique of adoptive cell transfer.

Materials and Methods

Mice

Inbred male NIH and outbred male CFLP mice were obtained from Olac 1976 Ltd, and were used at approximately 8 weeks of age, unless otherwise stated. Experimental and control groups normally consisted of six animals.

Preparation of cell suspensions and cell transfer

Suspensions of mesenteric lymph node cells (MLNC) were prepared from donors by standard methods (Wakelin and Wilson 1979a). Bone marrow (BM) cells were prepared according to the method ofWakelin and Wilson (1977b). Briefly, cells were prepared by flushing through the long bones of the hind limbs with cold (4°C) medium 199 (Gibco, Europe) supplemented with 5 i.u. heparin/ml (Gibco, Europe) and 5% heat inactivated foetal calf serum, (FCS, Gibco Europe). The cell suspension was left for 5 mins to allow large particles to settle, the supernatant collected and centrifuged at 200 g for 5 mins and resuspended in medium for counting and transfer.

Enrichment of MLNC populations for T cells or B cells was carried out as described by Grencis and Wakelin (1982). Cells were transferred intravenously via a lateral tail vein in a volume not exceeding 0.5 ml.

In vitro blast cell activity was assessed using the micro culture method as described by Lee, Wakelin and Grencis (1982).

Worm recovery

The incubation method used for recovery of adult worms from the small intestine has been described previously (Wakelin and Wilson, 1977a).

In vitro Assay of worm fecundity

To obtain a quantitative assessment of worm fecundity, the following method was employed. After 45 mins incubation of the opened intestines of infected animals 24 adult female worms were removed from each beaker, washed with warm (37°C) medium 199 and one worm placed in each individual well of a sterile flat bottomed microtitre plate (Flow Laboratories Ltd) containing warm medium 199 supplemented with 5% FCS, 10,000 i.u./ml penicillin/streptomycin (Gibco Europe), and 25μg/ml amphotericin B (Fungizone, Flow Laboratories Ltd). The microtitre plates were covered and incubated at 37°C in an atmosphere of 5% CO_2 in air and 95% relative humidity for 24 hrs. Immediately prior to and immediately after the culture period worm viability was assessed by worm mobility. Any worms not moving were excluded from the results. After culture the plates were cooled at -40°C for 3 mins and following cooling 30μl of 50% formalin/ethanol solution was added to each well. The plates

were kept at 4°C until ready for counting. The number of larvae shed per female over the 24 hr period was counted using a Leitz inverse compound microscope.

Worm Size

Adult female worm length was measured from camera lucida drawings using a bit pad digitizer linked to a D.E.C.P.P.P. 11/34 computer. Twenty five worms taken from the pooled burdens of each group were measured.

Muscle larvae burden

The skinned and evicerated carcasses of experimental and control animals were artificially digested, usually in groups in three, using standard techniques.

Production of antisera

Animals were bled by cardiac puncture. The blood was allowed to clot overnight at 4°C and subsequently centrifuged at 1500g for 15 mins. The serum was removed, aliquoted and frozen at -40°C prior to use. Presence of specific antibody was assessed using the standard double diffusion method of Ouchterlony using a water soluble crude muscle larvae preparation as antigen.

Irradiation of animals

Animals each received 650 rads on the day prior to infection, using a 60 Cobalt source, Department of Chemistry, University of Nottingham.

Statistics

Student's t test was used to assess the significance of differences between mean values in results. P values < 0.05 were considered significant.

Results

In vitro female fecundity following a primary infection with T. spiralis

A preliminary experiment was designed to examine female fecundity throughout the course of a primary infection with T. spiralis. Three groups of NIH mice were infected with 300 muscle larvae on day 0 and worm numbers and the in vitro female fecundity were assessed for each of six animals per group on days 6, 8 and 10 post infection.

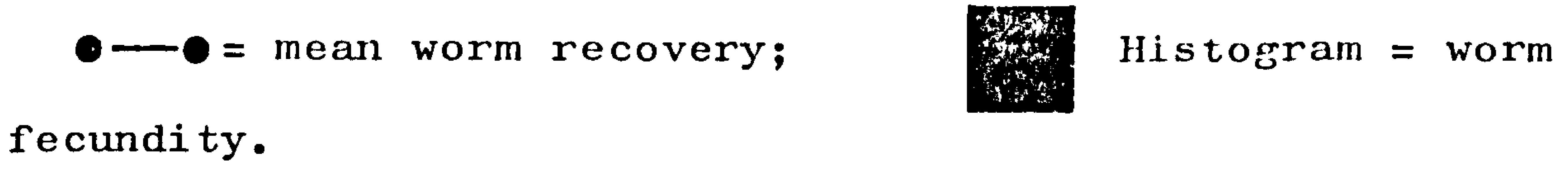
Worm expulsion did not begin until after day 8 post infection, although worm fecundity showed a reduction between day 6 and day 8, and larval release was virtually halted by day 10 post infection (Fig10.1).

Transfer of immunity with B cell enriched or T cell enriched populations of mesenteric lymph node cells

Previous work has shown that sub-lethal irradiation of the host provides a useful approach for the analysis of the components involved in the immune response to infection with T. spiralis, presumably by effectively removing most of the immuno-competent cells from the lymphoid system, (Wakelin and Wilson, 1980).

MLNC were taken from donor NIH mice infected with T. spiralis 8 days previously. Cell populations were

Figure 10.1. The course of a primary infection with T. spiralis in NIH mice. Mice given 300 T. spiralis muscle larvae on day 0.



●—● = mean worm recovery; Histogram = worm fecundity.

Mean worm number ± S.D.

180
160
120
80
40
0

Mean number of larvae shed/♀/24 hr ± S.E.

70
60
50
40
30
20
10
0

6 7 8 9 10

Table 10.1. Transfer of immunity against *T. spiralis* with MLNC populations enriched in T cells or B cells in mice irradiated with 650 rad 1 day prior to infection.

	Day 8 post infection				Day 12 post infection			
	Worm recovery		Fecundity Larvae/♀/24 hr		Worm recovery		Fecundity Larvae/♀/24 hr	
	Mean	S.D.	Mean	S.E.	Mean	S.D.	Mean	S.E.
Control No cells	125.2	12.8	70.7	2.8	0.0+	0.0	n.d.	n.d.
Irradiated Control No cells	126.0	16.5	126.6	6.3	47.6	17.4	10.3	8.1
Irradiated 4×10^7 T cells	136.2	9.7	29.6*	11.1	45.0	25.0	5.6	3.3
Irradiated 3.5×10^7 B cells	120.2	7.1	35.6*	8.6	63.8	13.8	4.8	3.5

+ n = 3 animals
n.d. = not done
* = significantly lower than control value.

enriched for T cells or B cells using nylon wool. Contaminating T cells were removed from the B cell enriched fraction by treatment with a cytotoxic monoclonal anti-Thy 1.2 antibody (Clone F7D5, Olac 1976 Ltd) and guinea pig complement. T cell enriched populations were approximately 13% Ig+ve and B cell enriched populations were approximately 90% pure as assessed by surface Ig immonofluorescence. A cell donor/recipient ratio of 3:1 was used.

Cell recipients were sublethally irradiated on day -1 and received the appropriate cell inoculum on day 0. Together with irradiated and unirradiated controls all animals were infected with 300 T. spiralis muscle larvae on the day of cell transfer. Worm numbers in the intestine and worm fecundities were assayed on day 8 and 12 post infection.

There was no significant difference in worm numbers between the groups on day 8 or day 12 post infection, although by day 12 expulsion had begun in all groups. However, on day 8 there was a marked reduction in worm fecundity in those groups that had received MLNC populations enriched in T or B cells. As would be expected from earlier work (Wakelin and Wilson, 1980) irradiated animals have a considerably increased fecundity when compared to unirradiated controls. By day 12 post infection, worm fecundity in cell groups was considerably depressed on comparison with day 8 values.

However, there was no significant difference between groups (Table10.1).

The results demonstrate that both T cell enriched and B cell enriched populations of MLNC do adoptively transfer direct anti-worm effects as assessed by a reduction in worm fecundity.

Transfer of immunity with immune serum

The previous experiment and other work (Wakelin and Wilson, 1979a) have shown that B cell enriched populations of MLNC are capable of mediating direct anti-worm effects and it has been suggested that serum antibody taken from infected donors may have a similar effect upon passive transfer (Wakelin, unpublished observations).

An experiment was designed to examine the effect of immune serum upon worm survival, fecundity, size and host muscle larvae burden using the technique of passive transfer. Immune serum was taken from CFLP mice which had been infected with 300 T. spiralis muscle larvae 30-35 days previously. This serum gave strong precipitation lines on reaction with worm antigen in a standard double diffusion Ouchterlony test. Control serum was taken from CFLP mice which had not received an infection, and this gave a negative result when examined for antibody.

NIH recipients each received either a total of 3 ml of serum given in three doses on days -1, +1, and +3 or, a total of 2 ml of serum given in two doses on days -1 and +1. Together with controls all animals were infected with 320 T. spiralis muscle larvae on day 0. Worm survival, fecundity, and adult female length were measured on day 5 and 8 post infection and host muscle larvae burden was measured after day 35 post infection, experimental and control groups consisted of 9 animals per group.

There was no difference between experimental and control groups in terms of worm establishment as assessed by worm numbers on day 5 post infection. In addition, there were no differences in worm fecundity or worm length between experimental and control groups at this time (Table 102). By day 8 post infection, although the results suggest that worm expulsion had begun, there were no significant differences between control and experimental groups. However, worm fecundity and worm length was markedly depressed in the groups which had received immune serum, 3 ml of immune serum being more effective than 2 ml (Table 103). Host muscle larvae burdens were also considerably reduced in animals which had received immune serum. Mice that had received 2 ml of immune serum had a mean of 13,346 muscle larvae, those which had received 3 ml of immune serum a mean of 8,026 muscle larvae; both values show a considerable reduction over treatment controls (mean of 21,660 muscle larvae) and mice which had received 3 ml of control serum (mean of 20,800 muscle larvae).

Table 10.2. Transfer of immunity against T. spiralis with serum. Parameters assessed on day 5 post infection.

n		Day 5 post infection					
		Worm number		Fecundity larvae/♀/24 hr		Size length mm	
		mean	S.D.	mean	S.E.	mean	S.D.
9	Control no serum	189.8	19.6	71.1	7.1	2.17	0.16
5	3 ml Control serum+	189.0	18.4	63.6	3.1	2.11	0.18
9	2 ml Immune serum	188.8	19.3	62.7	4.3	2.00	0.13
9	3 ml Immune serum	188.2	11.0	60.3	6.1	1.98	0.17

+ = estimation of female fecundity based on 4 females less than control no serum

Table 10.3. Transfer of immunity against _T. spiralis_ with serum. Parameters assessed on day 8 post infection.

	Day 8 post infection					
	Worm number		Fecundity larvae/♀/24 hr		Size length mm	
	Mean	S.D.	Mean	S.E.	Mean	S.D.
Control	119.0	58.5	50.7	3.2	1.96	0.15
3 ml Control serum	90.2	45.8	46.9	1.8	1.95	0.20
2 ml Immune serum	95.3	46.5	27.6*	5.5	1.62*	0.11
3 ml Immune serum	110.0	63.0	13.9*	7.2	1.50*	0.13

* significantly different from control

These results clearly demonstrate that immune serum has a direct anti-worm effect as reflected by a stunting in worm length, and reduction in worm fecundity and hence host muscle larvae burden.

Transfer of immunity with bone marrow cells

It is clear from the previous experiments that antibody and B cell or Ig secreting cell enriched fractions of MLNC populations have a direct anti-worm effect. The time of appearance of maximum levels of specific anti-worm antibodies in the serum of T. spiralis infected mice varies between strains but is usually after the worm population has been expelled from the intestine (Jungery and Ogilvie, 1982). The anatomical location of the Ig secreting cells at this time is unknown. It has been recently shown that in adult mice the majority of all Ig secreting cells of the individual are localised in the bone marrow (See Benner, Hijmans and Haaijman, 1981). Utilizing this finding an experiment was designed to examine the role, if any, of bone marrow cells taken from mice previously infected with T. spiralis to produce direct anti-worm effects, using the technique of adoptive cell transfer.

Twenty week old NIH bone marrow cell donors were infected with 300 T. spiralis on day 0 and cells taken for transfer on day 8 (prior to worm expulsion) or day 26 (after worm expulsion) post infection. Control bone marrow donors received no infection. Cell recipients each

received 2.5 x 10^7 bone marrow cells on day 0 and together with untreated controls infected with 360 *T. spiralis* on the day of cell transfer. To assess the numbers of dividing cells in the donor bone marrow populations *in vitro* blast cell activity was measured on the day of cell transfer. Experimental and control animals were kept in groups of 6, two animals being retained for estimation of muscle larvae burden after day 35 post infection. Seven days post infection worm survival, worm fecundity and worm length were measured.

There were no differences between experimental and control groups in terms of worm numbers. However, worm fecundity and worm length were increased in animals which had received bone marrow from infected or uninfected donors. This "suppression" of direct anti-worm effects showed no correl ation with blast cell activity in the respective bone marrow populations, but was reflected by increased muscle larvae burdens of cell recipients when compared to controls (Table 10.4).

Table 10.4. Transfer of immunity against T. spiralis with Bone marrow cells. Cell recipients each received 2.5×10^7 cells on the day of infection (Day 0).

	In vitro blast cell activity[+] c.p.m. 5×10^5 cells		Day 7 post infection: Worm number		Fecundity larvae/♀/24 hr		Size length mm		Muscle larvae burden number/animal
	Mean	S.D.	Mean	S.D.	Mean	S.E.	Mean	S.D.	Mean
Control no cells	---	---	264.6	18.8	36.1	11.6	1.72	0.12	17,375
Control BM cells	44768	5071	267.7	16.5	65.4*	8.7	2.43*	0.27	31,250
Day 8 BM cells	54619*	2808	238.7	22.7	63.7*	9.2	2.49*	0.18	38,750
Day 26 BM cells	32871*	3773	266.3	10.7	64.3*	5.2	2.48*	0.16	36,250

+ = Each cell sample assayed in triplicate

* = significantly different from control

Discussion

A central role for antibody in protective immunity against *T. spiralis* infections has yet to be confirmed. Many studies have examined serum for specific Ig to *T. spiralis* and the numbers of Ig secreting lymphocytes in various lymphoid organs of animals infected with *T. spiralis*. Crandall and Crandall (1972) found an increase in the levels of serum IgG and IgM after infection of mice with *T. spiralis*. Infection with this nematode also induces an increase in IgE antibody levels shortly after infection (Gabriel and Justus, 1979) and Dessain, Parker, James and David (1981) have shown that suppression of the total IgE response in rats infected with *T. spiralis* leads to elevated muscle larvae burdens. Recently advanced immunochemical studies have demonstrated the stage specificity of serum antibodies produced in response to infection with *T. spiralis* (Philipp, Parkhouse and Ogilvie, 1981) although correl ation with protective immunity has not been studied (Jungery and Ogilvie, 1982).

Experiments examining the passive transfer of immunity with immune serum have often yielded inconsistencies and have therefore often escaped comprehensive explanation. Love, *et al* (1976) and Wakelin and Lloyd (1976b) did successfully transfer immunity with serum and concluded that antibodies probably damage the adult worm reducing fecundity, but are not solely responsible for expulsion of the worm. One report has described the accelerated

expulsion of worms from the intestines of outbred Swiss mice previously passively sensitized with immune serum and suggested that IgG and IgE antibodies played a major role in an immediate hypersensitivity type of response (Gabriel and Justus, 1979). However, this type of response has not been demonstrated in the inbred NIH strain of mice (Alizadeh, personal communication). To date, the only Ig that has been shown to have a direct anti-worm effect is IgA. An IgA enriched extract from the intestinal secretions of infected animals depressed the _in vitro_ release of newborn larvae by female worms (Jacqueline, Vernes, Bout and Biguet, 1978).

The experiments described in this paper support the hypothesis that antibody does have a direct anti-worm effect as shown by a reduction in worm fecundity and worm size. The reduction in host muscle larvae burden in passively sensitized mice, although due in part to direct anti-worm effects could also reflect an antibody mediated killing mechanism active against newborn larvae as described _in vitro_ by Kazura and Grove (1978) and discussed in relation to anti-newborn larvae immunity _in vivo_ by Moloney and Denham (1979).

The results from this paper also suggest that during the intestinal phase of infection cells responsible for mediating direct anti-worm effects are present in the mesenteric lymph node. Adoptively transferred B cell enriched fractions of MLNC have been shown not to accelerate

expulsion of the worm from the gut (Grencis and Wakelin, 1982) but clearly mediated a direct anti-worm effect presumably due to the production of anti T. spiralis antibody (Table 10.1). The T cell enriched fraction of MLNC also mediated direct anti-worm effects possible through T-B co-operation resulting in production of antibody (See Wakelin and Wilson, 1979a), or as a consequence of the pronounced T cell mediated inflammatory response in the intestine which would lead to an environment detremental to worm survival (See Wakelin and Denham, 1982).

The demonstration that BM cells do not produce direct anti-worm effects upon adoptive transfer, suggest that the anti-T. spiralis antibody producing cells responsible for producing high specific Ig levels readily observed after T. spiralis infections in NIH mice (Jungery and Ogilvie, 1982) must reside elsewhere in the host lymphoid system, or be part of the recirculating lymphocyte pool. It is possible that these cells reside in the intestinal tissue, and more research into local antibody production, ie, along the gastrointestinal tract is needed (See Befus and Bienenstock, 1982). The "suppressive" phenomenon shown by recipients of BM cells upon direct anti-worm effects, clearly needs further examination, but such effects are not unknown. Duwe and Singhal (1979) have shown that BM cells from normal mice suppressed the in vitro IgM antibody response of spleen cells to defined antigens, and the cell responsible for this direct anti-B cell effect has been characterized as

a non macrophage, medium to large lymphocyte cell (Drury and Singhal, 1974).

It is well established that mice produce specific anti-*T. spiralis* antibodies upon infection, although the time of production of phase specific antibodies varies between strains (Jungery and Ogilvie, 1982). The work presented here presents definite evidence of a direct anti-worm effect of antibody causing a reduction in size and fecundity, a situation known to occur in other gastro-intestinal nematode/host systems eg, *Nippostrongylus brasiliensis* (Ogilvie and Love, 1974), *Nematospiroides dubius* (Behnke and Parish, 1979) and *Strongyloides ratti* (Moqbel, 1977).

Summary Points

Chapter 10

(1) Adoptive transfer of both B and T cell enriched populations of MLNC, taken from infected mice, and transferred into sublethally irradiated recipients caused a reduction in worm fecundity but did not accelerate expulsion of the worms from the small intestine.

(2) Passive transfer of primary infection immune serum, was successful in reducing worm fecundity, worm size and host muscle larvae burden, although worm numbers in the small intestine were not affected.

(3) An examination of bone marrow as a source of specific anti-*T. spiralis* antibody producing cells, capable of adoptively transferring direct anti-worm effects, proved negative.

SECTION 4

Chapter 11: Specific cross-immunity between *Trichinella spiralis* and *Trichuris muris*: Immunization with heterologous infections and antigens and transfer of immunity with heterologous immune mesenteric lymph node cells.

Published in Parasitology (1982) Vol 84, 381-389.

Parasitology (1982), **84**, 381–389
With 3 figures in the text

Specific cross-immunity between *Trichinella spiralis* and *Trichuris muris*: immunization with heterologous infections and antigens and transfer of immunity with heterologous immune mesenteric lymph node cells

T. D. G. LEE, R. K. GRENCIS *and* D. WAKELIN
Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Bearsden Road, Bearsden, Glasgow G61 1QH

(*Accepted* 2 *September* 1981)

SUMMARY

Infections with either 300 infective *Trichinella spiralis* larvae or 400 embryonated eggs of *Trichuris muris* were effective in eliciting accelerated expulsion of heterologous challenge infections given 20 days after the primary infection. Accelerated expulsion could also be achieved by the administration of soluble crude worm antigen given 12 days prior to heterologous challenge or by adoptive transfer of mesenteric lymph node cells taken from mice infected with the heterologous parasite. Each species is capable of eliciting an accelerated secondary expulsion response in hosts that have been actively or adoptively immunized against the other species and these results are taken to indicate that there is a specific cross-immunity between *T. spiralis* and *T. muris* due to shared antigens. It is postulated that these shared antigens are derived from stichocyte granules.

INTRODUCTION

Interactions between intestinal helminth parasites have often been studied using concurrent infections, that is, with two different parasite species inhabiting the intestinal tract simultaneously. The effects that can be observed on either or both species under these conditions are frequently absent when the infections are administered sequentially. This fact has prompted some authors to speculate that, in general, the consequences of worm–worm interactions result from the non-specific action of host inflammatory responses and do not depend upon immunological cross-reactivity.

This speculation probably holds true for many concurrent infections whether the parasites are given as primary infections or as challenge infections following an initial infection with one species. Dineen, Gregg, Windon, Donald & Kelly (1977) have shown that whereas sheep immunized by infection with *Trichostrongylus colubriformis* are refractory to homologous challenge they are susceptible to infection with *Nematodirus spathiger*, unless the latter is given concurrently with a challenge infection of *T. colubriformis*. Thus, although the expulsive response to a challenge infection is initiated in a specific manner, the effects of the expression of this response may operate non-specifically. Specificity in initiation must be

attributable to the antigens presented to the immunized host and if two closely related species present similar antigens they will both be able to elicit a secondary response after priming by only one of the species.

This relationship has been found to exist between the two trichostrongyle nematodes *T. colubriformis* and *T. vitrinus* (Dineen *et al.* 1977) and, more recently, between *Strongyloides ratti* and *Trichinella spiralis* (Moqbel & Wakelin, 1979) and it has also been suggested to exist between *Nippostrongylus brasiliensis* and *Nematospiroides dubius* (Jenkins, 1975) although this was expressed in terms of longevity of infection rather than accelerated expulsion. Unfortunately, follow up experiments to confirm antigenic similarities have not been done on any of these groups.

The demonstration of shared immunogens would be of great interest since it would make possible comparisons of host responses to similar antigens presented by different parasites.

It is reasonable to assume that the investigation of possible consequences of antigenic similarity between species of parasites is best facilitated by the investigation of a model system in which the parasites are phylogenetically related and physiologically similar but in which they inhabit different sites in the intestinal tract. Such a system is provided by infection of the mouse with *T. spiralis* and *T. muris*. These species are members of the Trichuroidea but they occupy distinct habitats in the host, *T. spiralis* being parasitic in the small intestine, *T. muris* in the large intestine. Both species evoke strong immunity in NIH mice, as assessed by accelerated worm loss on challenge infection (Wakelin, 1975; Wakelin & Lloyd, 1976) and both possess the characteristic trichuroid stichosome which is thought to be the major source of functional antigens (*T. spiralis* – Despommier & Muller (1970), *T. muris* – Jenkins & Wakelin (1977)). They have also been shown previously to be susceptible to non-specific concurrent effects by Bruce & Wakelin (1977) and although these authors saw no evidence of specific cross-immunity, recent evidence in this laboratory has prompted the re-examination of this aspect.

The experiments described in this paper examine the degree of immunity evoked by heterologous infection, by heterologous immunization using a soluble crude worm antigen, and by adoptive transfer using heterologous mesenteric lymph node cells.

MATERIALS AND METHODS

Mice

Male NIH mice (3 star – Hacking and Churchill Ltd) were used at 5–8 weeks of age in all experiments. Experimental and control groups usually consisted of 6 mice. All mice were maintained under conventional laboratory conditions.

Parasites

The strain of *T. spiralis* and the methods used for infecting mice and recovering worms were as described previously (Wakelin & Wilson, 1977). The methods used for infections with *T. muris* and for recovery of worms from infected mice were as described by Wakelin (1967).

Antigen preparation

Whole adult male *T. muris* were removed from the large intestine and washed at least 5 times in phosphate-buffered saline, (pH 7·2), (PBS) before use. *T. spiralis* muscle larvae were obtained from rats that had been infected at least 5 weeks previously and were washed at least 10 times in PBS before use.

The worms were disrupted in a glass tissue homogenizer. The homogenate was allowed to extract overnight at 4 °C and then centrifuged at 1500 *g* for 30 min to remove coarse particulate matter. The supernatant fluid was then ultracentrifuged at 100000 *g* for 1 h. The resulting supernatant fluid was assayed for protein using the method of Schacterle & Pollack (1973) and adjusted to a final total protein concentration of 2·5 mg/ml.

Preparation of cell suspensions

Mesenteric lymph nodes were stripped of fat and stored in Hanks' balanced salt solution on ice until processed. Suspensions were made by dicing the nodes with fine scissors and pressing the pieces through a nylon mesh sieve into a Petri dish containing Medium 199 (Gibco Europe Ltd) supplemented with 5% (v/v) foetal calf serum (Gibco Europe Ltd) and 10 i.u./ml heparin (B.D.H. Chemicals Ltd). Cell clumps and debris were allowed to settle and the supernatant fluid was centrifuged at 200 *g* for 5 min. The cells were resuspended in a known volume of fresh medium and cell viability was assessed by the Trypan blue exclusion test. Viability was usually greater than 90%. The cell concentration was adjusted to give the appropriate number in a volume not greater than 0·5 ml. Cells were injected intravenously into recipient mice via a lateral tail vein.

Mesenteric lymph node cells were taken from donors 8 days after a primary infection of 300 *T. spiralis* muscle larvae or 20 days after a primary infection of 450 *T. muris* eggs. In all cell transfer experiments cell recipients were challenged on the same day with either *T. spiralis* or *T. muris*.

Statistics

Student's *t*-tests were used to assess the significance of differences between mean values in results. *P* values of less than 0·05 were considered significant.

RESULTS

Infections

The timing of the expulsive response of NIH mice to primary infections of *T. spiralis* and *T. muris* has been well characterized (see Wakelin, 1978*b*), complete expulsion of the parasite burdens being achieved by day 12 and day 14 post-infection respectively.

In the first experiment 2 groups of mice were given primary infections of 300 *T. spiralis* larvae and 2 groups were given primary infections of 400 embryonated *T. muris* eggs. Twenty days later, challenge infections of 300 *T. spiralis* larvae were given to 1 of the groups previously infected with *T. spiralis*, to 1 of the groups

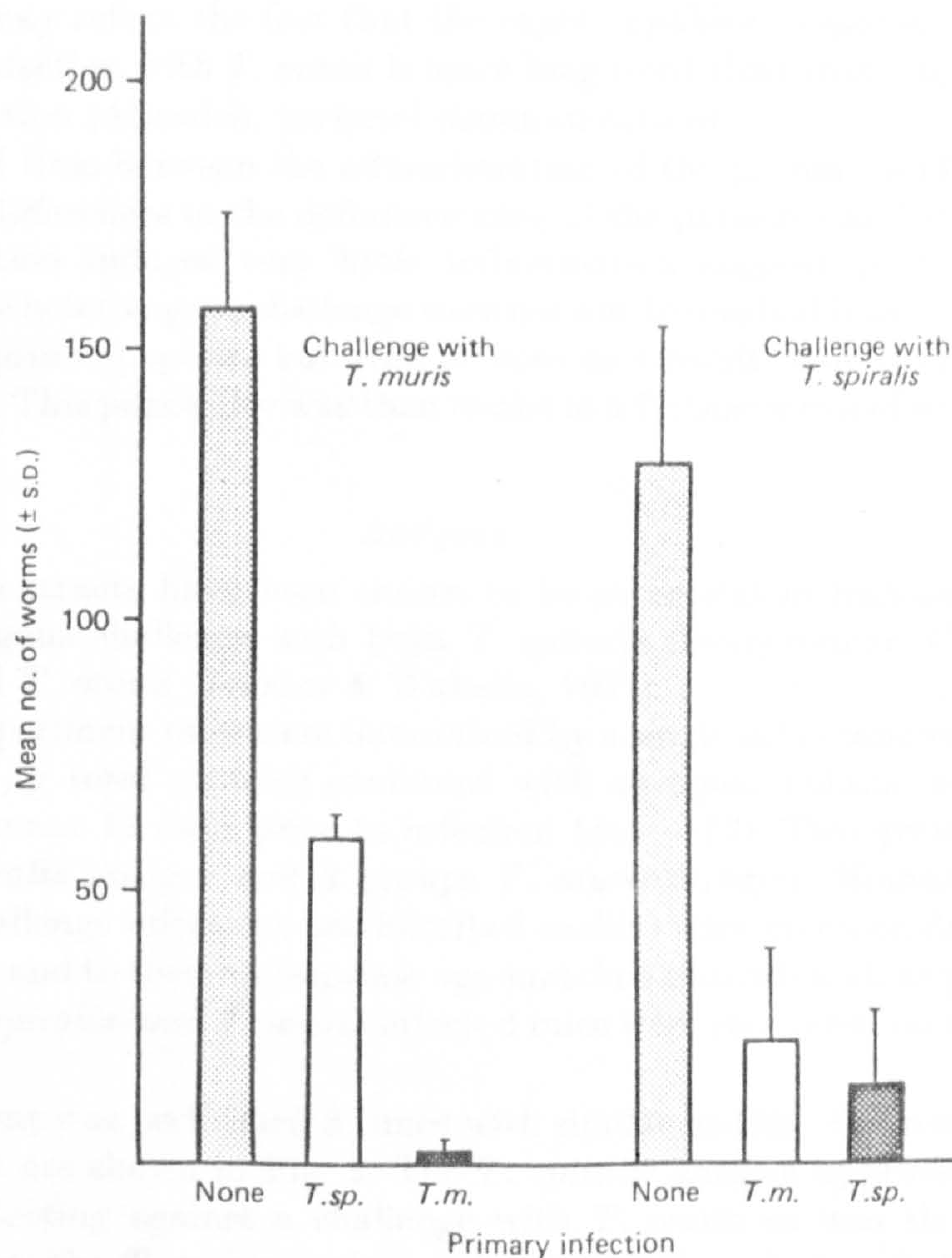


Fig. 1. The effect of prior infections with *Trichuris muris* (*T.m.*) or *Trichinella spiralis* (*T.sp.*) on homologous or heterologous challenge. Primary infections with either 300 *T. spiralis* larvae or 400 *T. muris* eggs were given 20 days before the administration of the challenge infections. Worm numbers were assessed on day 8 (*T. spiralis*) or day 10 (*T. muris*) post-challenge.

previously infected with *T. muris* and to an age-matched naive (control) group. Infections of *T. muris* eggs were similarly given to one of the groups previously infected with *T. spiralis* and to an age-matched naive (control) group. Thus, the previously infected groups of mice received either an homologous or heterologous challenge infection.

Worms were recovered from *T. spiralis*-infected mice on day 8 post-infection and from *T. muris*-infected mice on day 10 post-infection.

This experiment was performed 3 times with similar results. The results of one of these experiments are shown in Fig. 1 and show clearly that a primary infection with either parasite not only affects the course of an homologous challenge infection but also of an heterologous challenge. In all cases there was a highly significant reduction ($P < 0{\cdot}001$) in worm recoveries as compared with the control values. Although there was no significant difference between the protection to challenge with *T. spiralis* engendered by either homologous or heterologous primary infections, a challenge with *T. muris* was affected much more significantly

($P < 0{\cdot}001$) in mice primed with the homologous as opposed to the heterologous parasite. This may reflect the fact that the rapid expulsion response engendered by a primary infection with *T. muris* is more long-lived than that engendered by *T. spiralis* infection (Alizadeh, personal communication).

The length of time between the administration of the primary and secondary infections, the differences in the definitive sites of the parasites and the fact that *T. muris* infection induces very little inflammation suggest that the effects exerted upon the heterologous challenge were not due to residual intestinal changes elicited by the priming species but arise *de novo* as a result of an immunological cross-reactivity. This possibility was then tested in a further series of experiments.

Antigens

Crude worm extracts have been shown to be successful in immunizing mice against homologous challenge with both *T. spiralis* (Despommier, Campbell & Blair, 1977a)and *T. muris* (Jenkins & Wakelin, 1977).

In the first experiment mice were immunized by a single subcutaneous injection of antigen (250 μg total protein) combined with an equal volume of Freund's incomplete adjuvant 12 days prior to infection (day -12). Two groups of mice received *T. spiralis* antigen and 2 groups *T. muris* antigen. Homologous and heterologous challenge infections (as described earlier) were given on day 0 to the immunized mice and to their appropriate age-matched controls and, as previously, worms from *T. spiralis*- and *T. muris*-infected mice were recovered on days 8 and 10 respectively.

This experiment was performed 3 times with similar results. The results of one such experiment are shown in Fig. 2. The *T. spiralis* antigen appeared to be as effective in protecting against a challenge with *T. muris* as was the *T. muris* antigen. Likewise, the *T. muris* antigen protected well against a challenge with *T. spiralis* ($P < 0{\cdot}001$) and, although in the experiment shown the homologous antigen provided a significantly higher degree of protection than the heterologous antigen ($P < 0{\cdot}001$), this was not a consistent finding in the repeat experiments.

These results imply that there are some common antigens present in the antigen preparations since both protected mice well against heterologous challenge.

Cell transfers

Adoptive transfer of immunity, as measured by accelerated expulsion of the worm population, can be achieved by the transfer of immune mesenteric lymph node cells (IMLNC) from infected animals, provided these cells are taken within a limited time period after infection. This procedure has been well demonstrated in both of the species employed in these experiments (see Wakelin, 1978*a*).

Two groups of mice were given 4×10^7 IMLNC from *T. spiralis*-infected donors and 2 groups were given 4×10^7 IMLNC from *T. muris*-infected donors. These mice, along with their appropriate age-matched controls (which received no cells), were given homologous or heterologous challenge infections on the day of cell transfer. Control mice were not given cells from control donors as it had been shown repeatedly that such transfers have no effect upon the course of infection with

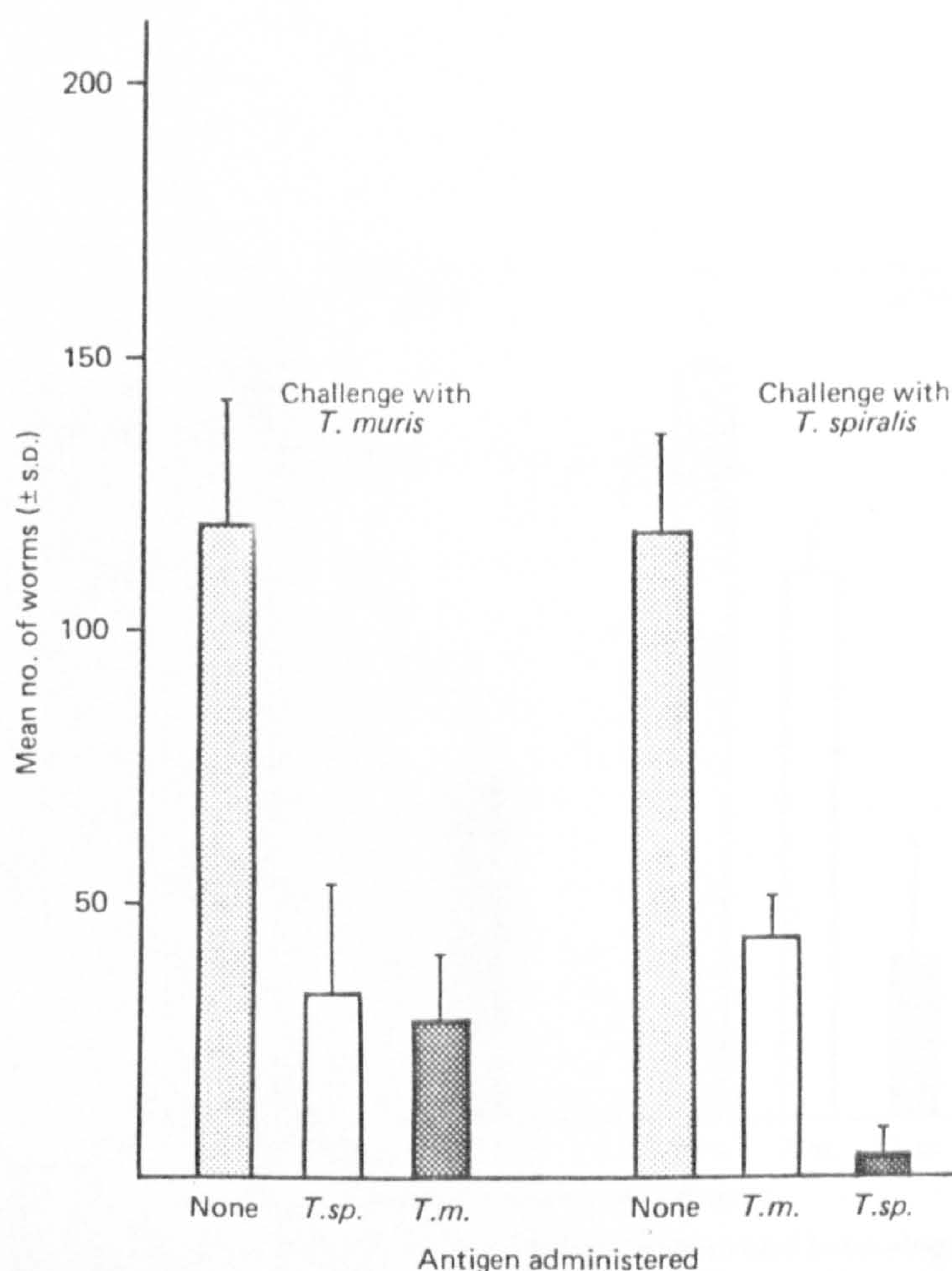


Fig. 2. The effect of prior immunization with crude worm antigens on homologous or heterologous challenge. Antigens were given subcutaneously on day −12 (day 0 being the day of challenge). Worm numbers were assessed on day 8 (*Trichinella spiralis*) or day 10 (*Trichuris muris*) post-challenge.

either parasite (unpublished observations). Mice were killed for worm recovery on days 8 (*T. spiralis*) and 10 (*T. muris*) post-infection.

This experiment was performed twice with nearly identical results. The results of one of these experiments are shown in Fig. 3. Although heterologous IMLNC were not as effective in inducing accelerated expulsion as homologous cells, the differences in worm recoveries between control and recipient groups were, in both cases, significant ($P < 0{\cdot}05$) and reproducible.

DISCUSSION

The experiments outlined above show that immunization of mice against *T. spiralis* or *T. muris* using infections or antigens or the transfer of IMLNC will affect the course of both homologous and heterologous challenge infections. These results suggest strongly that there are antigenic similarities between the two species

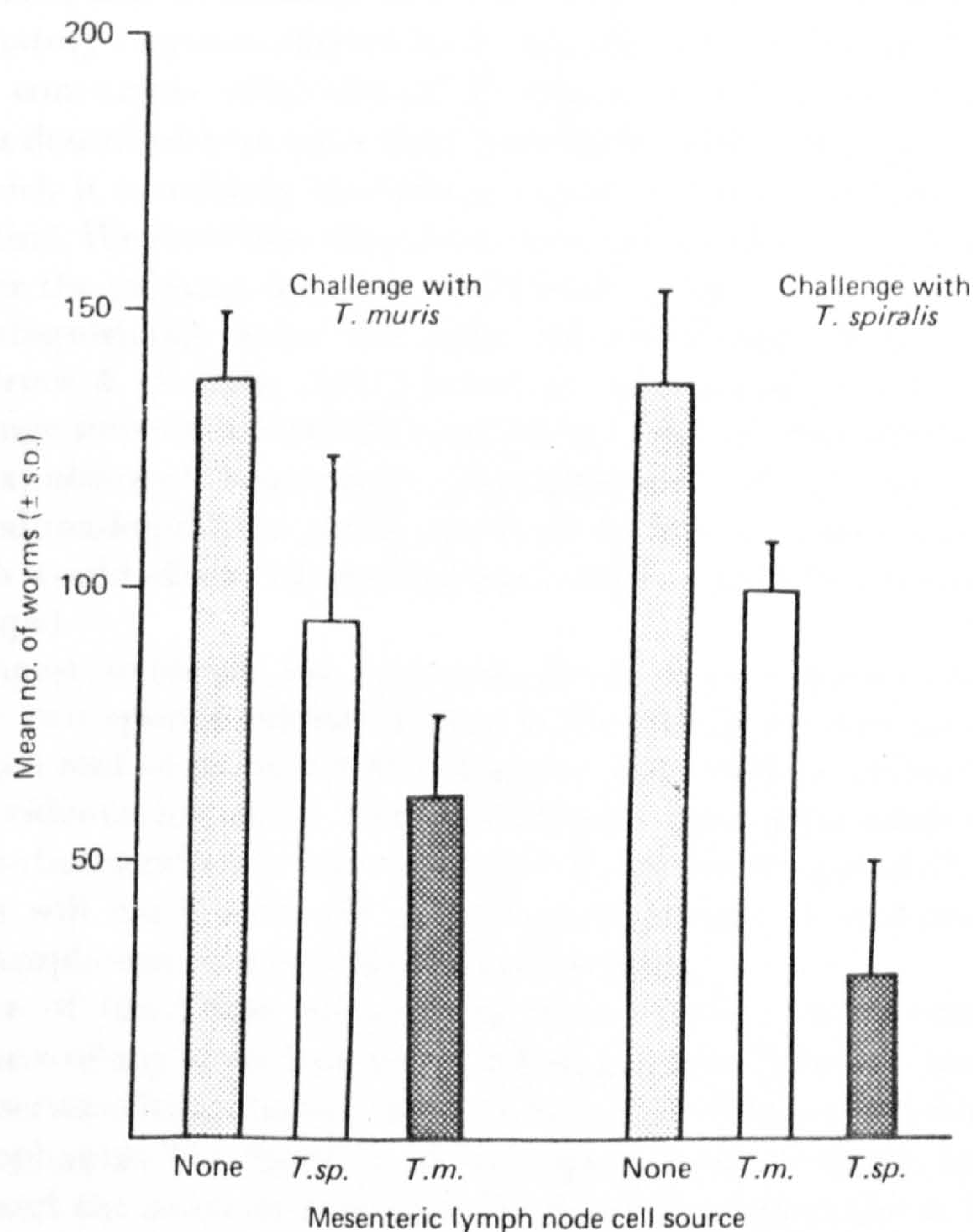


Fig. 3. The passive transfer of immunity with homologous and heterologous mesenteric lymph node cells. Cells were taken from either *Trichinella spiralis*- or *Trichuris muris*-infected mice (on days 8 or 20 respectively) and injected intravenously into naive recipients. These recipients then received either an homologous or heterologous challenge on the day of the cell transfer. Worm numbers were assessed on day 8 (*T. spiralis*) or day 10 (*T. muris*) post-infection.

and that the accelerated expulsion of heterologous challenge infections is the result of specific responses to shared antigens.

In those experiments where priming was achieved by active infection, it is necessary to consider the possibility that the cross-immunity observed was the result of the inflammatory changes in the intestine caused by the immunizing species as there is growing evidence that, although the initiation of a response to parasite infection is antigenically specific, the expression of the response is mediated by non-specific factors. It is highly unlikely that prior immunization with a *T. muris* infection could affect a challenge infection with *T. spiralis* in this way, since *T. muris* is known to cause very little inflammation in the gut and any changes would presumably be localized primarily in the large intestine. However, this possibility is relevant to those experiments where *T. spiralis* was used as the immunizing species. Other authors have implicated inflammatory changes in the gut, induced by *T. spiralis*, as being responsible for accelerated expulsion of challenge infections with other nematodes (Kazacos, 1975; Au & Ko, 1979;

Kennedy, 1980) and, in addition, Bruce & Wakelin (1977) have demonstrated that the inflammatory response elicited by *T. spiralis* exerts a profound effect upon the survival of concurrent infections of *T. muris*. It is for these reasons that the experiments described here use a time interval between priming and challenge (21 days) at which it is unlikely that there would be residual inflammatory changes in the intestine. We have also examined the response to challenge infections given 42 days after the primary infection and found a degree of cross-immunity at this time, indistinguishable from the data presented here (unpublished results). Although Bruce & Wakelin (1977) found no evidence of specific cross-immunity when challenge infections were delayed, in the light of what is now known of the kinetics of expulsion of these species, the experimental design they employed must be considered inadequate to justify their conclusions (i.e. mice were not killed at a time which would effectively demonstrate differences between experimental and control groups).

The strongest evidence for immunologically based specific cross-immunity between the two species examined here is the ability to immunize with heterologous antigen and to passively transfer immunity with heterologous IMLNC.

Further evidence has come from preliminary serological analysis, which has shown that antisera raised in rabbits against *T. spiralis* or against *T. muris* antigen preparations will react with the heterologous antigen in immunodiffusion and immunoelectrophoresis (unpublished observations).

The source of functional antigens in these species, and therefore the most probable source of any shared antigens, is thought to be the stichosome. This organ consists of a series of large discoid cells (stichocytes) which surround and are ducted into the oesophagus. The function of the organ is still uncertain but considering its location and the mode of existence of these nematodes (Lee & Wright, 1978; Wright, 1979) it is most likely that it has some role in extracorporeal digestion.

The ultrastructure of the stichosome has been studied extensively in both species (Bruce, 1970, 1974; Wright, 1972; Despommier, 1974; Despommier & Muller, 1976) and a striking feature of the constituent cells is the presence of a large number of cytoplasmic granules. Despommier & Muller (1976) have attributed at least 4 unique antigens to each of 2 types of granules found within the stichocytes of *Trichinella* and have previously shown (Despommier & Muller, 1970) that it is these granules which contain the functional antigens. It is conceivable that if the stichocytes of *Trichuris* were fractionated in a similar manner, shared antigens could be identified.

If it proves to be the case that the species share major protective antigens it will be necessary to determine why such antigens elicit such different effects when presented by *T. spiralis* rather than by *T. muris* (in the former the response to infection is one of gross inflammation with rapid cellular infiltrate of the mucosa and characterized by high levels of reaginic antibodies, whereas in the latter there is little inflammation, cellular infiltration is delayed and there appears to be no reaginic antibody response), on what level the differences are expressed and what further factors, such as the site of presentation of antigen or the contributory effects of migrating larvae, are involved.

Work is currently in progress, using the more sensitive analytical techniques of radio-immunoassay and polyacrylamide gel electrophoresis in attempts to isolate and characterize shared antigens.

Summary Points

Chapter 11

(1) Infections with either 300 T. spiralis muscle larvae or 400 embryonated eggs of T. muris were effective in generating the accelerated expulsion of heterologous challenge infections.

(2) Accelerated expulsion of either parasite could be achieved by prior administration of soluble crude worm antigen prior to challenge.

(3) Transfer of MLNC from either T. spiralis or T. muris infected mice, caused the accelerated expulsion of both homologous and heterologous challenge.

Summary Discussion

Although much is known concerning the immune response to parasites, a detailed knowledge of the mechanisms involved is lacking in many systems. The spontaneous cure phenomenon exhibited by many laboratory models of gastro-intestinal parasitism is poorly understood, and a comprehensive view of the immune mechanisms operating remains unclear. In the present study, the generation of the immune responses leading to expulsion of *T. spiralis* from the gastrointestinal tract of mice, and the mechanisms involved, were examined by two approaches, namely 1) by the controlled stimulation of immunity by vaccination with worm antigen, and 2) by the stimulation of immunity by infection.

The first section of the thesis was concerned with establishing the course of primary and secondary infections of *T. spiralis* in mice, and examining the capacity of a soluble muscle larvae antigen to protect against infection. Vaccination with worm antigen was successful in protecting against an oral infection of *T. spiralis*. Vaccinated animals expelled their challenge worm burden earlier than controls, in a temporally similar manner to that of a secondary infection. These results confirm work done by other workers, notably Despommier and colleagues, who have shown a definite protective role for stichosome granule antigens (See Despommier *et al*, 1977a).

The comparison of immune responses occurring after infection with those occurring after vaccination suggests that *T. spiralis* antigen given parenterally may be handled in a manner similar to that following presentation of antigen via the intestine during infection. It is tempting to speculate that vaccination with *T. spiralis* antigen generates a population of memory cells which can be rapidly mobilized after a challenge infection, and indeed vaccinated challenged mice show a rapid increase in MLNC blast activity similar to that observed after a secondary infection.

Section two, formed the major part of the present study, and examined in detail the cellular immune response to infection with *T. spiralis* which resulted in expulsion of the worm burden from the intestine. In NIH mice, successful adoptive transfer of immunity with MLNC confirmed earlier work (Wakelin and Lloyd, 1976b; Wakelin and Wilson, 1977a; 1979a) and both negative and positive selection of Thy 1.2. positive cells strengthened the evidence for T cell involvement in the expulsion process. Isopycnic centrifugation, velocity sedimentation and vinblastine sulphate treatment techniques confirmed the blast-like nature of those cells, and the use of monoclonal antibodies against Ly antigens presented evidence which suggested that Ly1+ cells are the major T cell subset involved. This latter finding provided an interesting corollary with recent evidence from Krco, David and Wassom (1982) who have shown that Ly1+ T cells are responsible for the *in vitro* proliferation of lymph node cells taken from the draining node of mice vaccinated with *T. spiralis* antigen. However, their data also imply

that Ly2+ amplifier cells may be involved, and that there is a requirement for macrophage involvement, possibly for effective antigen presentation.

The expression of a classical secondary response to T. spiralis infection implies the presence of a population of memory cells. Evidence of memory cell involvement to T. spiralis was inferred from experiments in which T. spiralis antigens were shown to elicit DTH type reactions, long after expulsion of a primary infection from the intestine. The nature and anatomical location of the memory cells (presumably T cells) remains to be discovered although adoptive transfer of spleen cells taken long after expulsion of the intestinal worm burden, suggests that they may be part of the recirculating lymphocyte pool. The mechanism of recall of memory cells to the site of infection may occur via two mechanisms,

1) selective recruitment of antigen specific cells from the RLP on passage through the MLN, or
2) the homing of antigen stimulated blasts to the site of infection (small intestine) after stimulation outwith the MLN.

Positive evidence for cell trapping in the MLN of infected mice was presented, and it is possible that the initial shutdown is followed by an increased lymphocyte traffic through the MLN, as is known to occur for other antigen/node systems (Cahill et al 1976). Both mechanisms would effectively increase antigen/cell interactions.

Evidence was also presented which suggested that the muscle larvae/pre-adult stages of the parasite are the major source of protective antigens, again confirming the work of Despommier _et al_ (1977a). If the adult stages contribute little to the generation of cells in the MLN capable of mediating expulsion, then the results suggest that continual antigenic stimulation by the worm in the gut is unnecessary for the generation of the expulsion response. Indeed, it is now well known that continual antigen presentation is unnecessary for the clonal expansion of antigen reactive lymphocytes (See Möller, 1982b).

The role of specific antibody in mediating immunity to _T. spiralis_ is a much discussed subject, and to date, there is no widely accepted conclusion as to its protective role, if any. The evidence presented in this thesis does support the previously proposed hypothesis of a direct anti-worm effect of specific antibody, which manifests its effect in a reduction of worm fecundity and worm length. The results also present evidence for the existence of antibody secreting cells or plasma cell progenitor cells in the MLN during the phase of intestinal infection. It is likely that T cells may play a helper function in antibody production as proposed by Wakelin and Wilson (1979a). Serum anti-_T. spiralis_ antibody levels generally attain peak levels after the worms have been expelled from the intestine (Jungery and Ogilvie, 1982) although the anatomical location of the antibody secreting cells is unknown. In other systems, the bone marrow has been shown to be the major site of antibody production

(Benner, Hijmans and Haaijman, 1981). However, this was shown not to be the case in the *T. spiralis*/mouse system. It is possible that the plasma cells are present in the intestinal tissue,including peyers patches.

The final part of the thesis presented an investigation of the cross protection between *T. spiralis* and the related colon dwelling nematode *Trichuris muris*. Cross reactivity was shown to operate and to be of an immunological nature, as shown by adoptive transfer of immunity with heterologous immune MLNC. Indeed, Lee (1982) using sensitive serological techniques has shown minor shared antigens between *T. spiralis* and *T. muris* antigens preparations. Adoptive transfer of immunity with MLNC in the *T. muris* system has shown that immunity is T cell mediated, and that the effective cells are associated with a high *in vitro* blast cell activity (Lee, Wakelin and Grencis, 1983).

The *T. spiralis*/mouse system is arguably, the most intensively studied intestinal nematode model. The following discussion will attempt to assimilate information from other sources (See Wakelin and Denham, 1982) with information from the present study, in order to present an overview of the possible immune mechanisms operating against *T. spiralis* infections in mice.

It is reasonable to suggest that the major source of protective antigens is contained in the stichosomal granules of the muscle larval stage. Upon infection, this antigen is released into the intestinal tissue, and is presumably presented to lymphocytes in the draining node

(MLN) by accessory cells possibly dendritic cells (See Steinman and Nussenzweig, 1980). Antigen/cell interaction (and cell recall in secondary infections) is promoted by cell trapping and upon stimulation Thy1+ve Ly1+ lymphocytes will divide into several functionally distinct T cell clones. It is known that T blasts home to the small intestine of T. spiralis infected mice (Rose et al, 1976a) and therefore, it is reasonable to presume that a proportion of the T blasts generated in the MLN will home to the site of infection. The T blasts may effect their function by the release of a variety of soluble mediators, and through a variety of amplification mechanisms generate the acute inflammation observed in the gut during infection. The resulting intestinal environment would be unsuitable for worm survival, and expulsion of the worm burden would occur.

It is reasonable to suggest that the role of specific antibody is minor, at least in terms of the expulsive mechanism. However, antibody may contribute by damaging the worm, as evidenced by reduced fecundity and worm stunting. The control of antibody production may be dependent upon T cell help, and presumably operate at a local level.

The strong immunity generated by both infection and vaccination with muscle larvae antigen suggests that memory T cells (and possible B memory cells) from the RLP or tissues are rapidly brought into action upon challenge infection, and an accelerated series of the events observed after primary infection will occur, resulting in the accelerated loss of worms from the small intestine. The phenomenon

of rapid expulsion observed in mice for a short period of time after primary infection is probably controlled by related but distinct mechanisms, and has been thoroughly reviewed by Alizadeh (1981).

The immune response to infection with T. spiralis is extremely complex, involving as it does both cellular and humoral events, and is completely interrelated with a number of non-immunological mechanisms (Fig 1). Analysis and identification of the components of these responses cannot be attempted in vitro and will require the use of new and sophisticated techniques recently introduced to the field of immunology. The development of functional T cell clones derived from antigen stimulated T lymphoblasts will facilitate examination of the mediators involved in the generation and modulation of the immune and inflammatory responses and will permit isolation and study of particular components and their effects. Detailed studies of local immunity at the intestinal level are still relatively few and far between (See Befus and Bienenstock, 1982) and investigation of selected, intestinal cell populations in this way cannot fail to provide much important information which should have wide relevance to the field of protective immunity against intestinal pathogens.

Figure 1. Immunity to *T. spiralis*: An Overview

Adams, D B and Rothwell, T L W (1977).
Trichostrongylus colubriformis: host factors influencing the transfer of immunity in guinea pigs using mesenteric lymph node cells. Experimental Parasitology 42, 121-128.

Adams, D B and Rothwell, T L W (1980).
The role of lymphocytes in immunological memory for resistance to infection by Trichostrongylus colubriformis in guinea pigs. Cellular Immunology. 55, 1-11.

Ahmed, A and Smith, A H (1982).
Surface markers, antigens and receptors on murine T and B cells: Part 1. CRC Critical Reviews in Immunology. 3, 331-369.

Alizadeh, H (1981).
Immunological and inflammatory responses in the intestines of mice infected with the parasitic nematode Trichinella spiralis. PhD Thesis, University of Glasgow.

Alizadeh, H and Wakelin D (1981a).
Mechanism of rapid expulsion of Trichinella spiralis from mice. In: Trichinellosis (Ed. by C W Kim, E J Ruitenberg and J S Teppema) p 81 Reedbooks Ltd, Chertsey.

Alizadeh, H and Wakelin, D (1981b).
Comparison of rapid expulsion of Trichinella spiralis in mice and rats. International Journal of Parasitology, 12, 65-73.

Alizadeh, H and Wakelin, D, (1982).
Genetic factors controlling the intestinal mast cell response in mice infected with Trichinella spiralis. Clinical and Experimental Immunology, 49, 331-337.

Anderson, R E and Warner, N L (1976).
Ionizing radiation and the immune response. Advances in Immunology, 24, 216-335.

Askanase, P W (1979).
Immunopathology of parasitic diseases: involvement of basophils and mast cells. Springer Seminars in Immunopathology 2, 417-442.

Askanase, P W, Bursztajn, S, Gershon, M D, and Gershon, R K (1980)
T cell dependent mast cell degranulation and release of serotonin in murine delayed-type hypersensitivity. Journal of Experimental Medicine, 152, 1358-1374.

Au, A C S and Ko, R C (1979).
Cross-resistance between Trichinella spiralis and Angiostrongylus cantonensis in laboratory rats. Zeitschrift für Parasitenkunde 59: 161-168.

Bachman, G W and Molina, I (1933).
Resistance to infestation with _Trichinella spiralis_ in hogs.
American Journal of Hygiene 18, 266-294.

Bartlett, A and Ball, P A J (1974).
The immune response of the mouse to larvae and adults of _Nematospiroides dubius_.
International Journal of Parasitology 4, 463-470.

Bawden, M P, Palmer, T T , Leef, M F and Beaudin, R L (1979).
Malaria vaccination with irradiated sporozoites; serological evaluation of the antigen and antibody responses. In: _Bulletin of the World Health Organisation_ Vol 57. Supplement No 1 pp 205-210.

Befus, A D and Bienenstock, J (1982).
Factors involved in symbiosis and host resistance at the mucosa-parasite interface, _Progress in Allergy_, 31, 76-177.

Behnke, J M, and Parish, H A, (1979).
Expulsion of _Nematospiroides dubius_ from the intestine of mice treated with immune serum, _Parasite Immunology_, 1, 13-26.

Behnke, J M and Parish, H A (1981).
Transfer of immunity to _Nematospiroides dubius_. Co-operation between lymphoid cells and antibodies in mediating worm expulsion. _Parasite Immunology_ 3, 249-259.

Bell, R G , and McGregor, D D (1979a)
Trichinella spiralis: expression of rapid expulsion in rats exposed to an abbreviated enteral infection, Experimental Parasitology 48: 42-50.

Bell, R G and McGregor, D D (1979b).
Trichinella spiralis: Role of different life cycle phases in induction, maintenance and expression of rapid expulsion in rats, Experimental Parasitology 48: 51-60.

Bell, R G and McGregor, D D (1980)
Variation in anti-Trichinella responsiveness in inbred mouse strains. In Genetic control of natural resistance to infection and malignancy, Ed by Skamene, E, Kingshavn, P A L and Landy, M, pp 67-73, Academic Press Inc, New York.

Bell, R G , McGregor, D D , and Despommier, D D (1979).
Trichinella spiralis: mediation of the intestinal component of protective immunity in the rat by multiple, phase-specific antiparasite responses, Experimental Parasitology, 47: 140-157.

Benner, R, Hijmans, W, and Haaijman, J J,(1981).
The bone marrow: the major source of serum immunoglobulins, but still a neglected site of antibody formation, Clinical and Experimental Immunology 46, 1-8.

Bienenstock, J and Befus, A D (1980).
Mucosal Immunology. Immunology 41: 249-270.

Bridgen, P J, Cross, G A M and Bridgen, J (1976).
N-terminal amino acid sequence of variant specific surface antigens from Trypanosoma brucei, Nature (Lond) 288, 624-626.

Bruce, R G (1970).
Structure of the oesophagus of the infective juvenile and adult Trichinella spiralis. Journal of Parasitology 56, 540-9.

Bruce, R G (1974).
The formation and utilization of stichosome secretory globules in Trichinella spiralis. In Trichinellosis (ed. C. Kim), pp 49-57 . New York: In text Educational Publishers.

Bruce, R G and Wakelin, D (1977).
Immunological interactions between Trichinella spiralis and Trichuris muris in the intestine of the mouse. Parasitology 74, 163-73.

Bruchovsky, N, Owen, A A, Becker, A J and Till, J E (1965).
Effects of vinblastine on the proliferative capacity of L cells and their progress through the division cycle. Cancer Research 25, 1232-1237.

Budzko, D B, Kierszenbaum, F and Waksman, B H (1977).
Cytotoxic effects of normal sera on lymphoid cells.
III. Selective killing of mouse T cells by normal
guinea pig serum.
Journal of Immunology 119, 381-6.

Cahill, R N P, Frost H and Trnka, Z (1976).

The effects of antigen on the migration of recirculating lymphocytes through single lymph nodes.

Journal of Experimental Medicine, 143, 870-888.

Campbell, C H (1955).

The antigenic role of the excretions and secretions of *Trichinella spiralis* in the production of immunity in mice.

Journal of Parasitology 41, 483-491.

Campbell, W C (1965).

Immunizing effect of enteral and enteral-parenteral infections of *Trichinella spiralis* in mice.

Journal of Parasitology 51, 185-194.

Campbell, W C, Hartman, R K and Cuckler, A C (1963).

Induction of immunity to trichinosis in mice by means of chemically abbreviated infections.

Experimental Parasitology 14, 29-36.

Cantor, H and Boyse, E A (1976).

Regulation of cellular and humoral immune responses by T- cell subclasses.

Cold Spring Harbor Symposium of Quantitative Biology 41, 23-32.

Capron, A , Dessaint, J P, Capron, M, Joseph, M and Pestel, J (1980).

Role of anaphylactic antibodies in immunity to schistosomes.

American Journal of Tropical Medicine and Hygiene 29, 849-857.

Castro, G A (1976).

Spatial and temporal integration of host responses to intestinal stages of *Trichinella spiralis*: retro- and prospective views, in: *Biochemistry of Parasites and Host-Parasite Relationships*, (H van den Bossche, ed), Elsevier/North Holland Biomedical Press, Amsterdam, pp 343-358.

Chensue, S W, Wellhausen, S R and Boros, D L (1981).

Modulation of granulomatous hypersensitivity II Participation of Ly 1+ and Ly 2+T lymphocytes in the suppression of granuloma formation and lymphokine production in *Schistosoma mansoni* infected mice.

Journal of Immunology 127, 363-367.

Clegg, J A and Smith, M A (1978).

Prospects for the development of dead vaccines against helminths.

Advances in Parasitology 16, 165-218.

Cohen, A and Schlesinger, M (1970).

Absorption of guinea pig serum with agar. A method for elimination of its cytotoxicity for murine thymus cells.

Transplantation 10, 130-132.

Cohen, S and Sadun, E H (1976).

Eds *Immunology of Parasitic infections*. Blackwell Scientific Publications.

Coker, C M (1955).

Cellular factors in acquired immunity to Trichinella spiralis, as indicated by cortisone treatment of mice. Journal of Infectious diseases 98, 187-197.

Corrigan, A, O'Kennedy, R and Smyth, H (1979).

Lymphocyte membrane alterations caused by nylon wool column separations.
Journal of Immunological Methods 31, 177-182.

Crandall, R B and Crandall, C A, (1972),

Trichinella spiralis: Immunologic response to infection in mice, Experimental Parasitology 31, 378-398.

Crum, E D, Despommier, D D and McGregor, D D (1977).

Immunity to Trichinella spiralis. I Transfer of resistance by two classes of lymphocytes. Immunology 33, 787-795.

Cunningham, A J (1969).

Studies on the cellular basis of IgM immunological memory.
Immunology 16, 621-632.

Cypess, R H (1970).

Artificial production of acquired immunity in mice by footpad injections of crude larval extract of Nematospiroides dubius.
Journal of Parasitology 56, 320.

Day, K P, Howard, R J, Prowse, S J, Chapman, C B and Mitchell, G F (1979).

Studies on chronic versus transient intestinal nematode infections in mice. I. A comparison of responses to excretory/secretory (ES) products of Nippostrongylus brasiliensis and Nematospiroides dubiusworms. Parasite Immunology, 1, 217-239.

Denham, D A (1966).

Immunity to Trichinella spiralis. I. The immunity produced by mice to the first four days of the intestinal phase of the infection, Parasitology 56: 323-327.

Denham, D A (1968).

Bordetella pertussis vaccine as an adjuvant for helminth antigens.

Journal of Parasitology, 54, 68.

Denham, D A (1969).

Immunity of Trichinella spiralis. IV. Passive immunity in mice, Folia parasit-ologica 16: 183-187.

Despommier, D D (1974).

The stichocyte of Trichinella spiralis during morphogenesis in the small intestine of the rat. In: Trichinellosis (ed C. Kim), Intext Educational Publishers, New York. pp 239-254.

Despommier, D D (1977).
Immunity to *Trichinella spiralis*.
American Journal of Tropical Medicine and Hygiene 26, 68-75.

Despommier, D D (1981).
Partial purification and characterization of protection inducing antigens from the muscle larva of *Trichinella spiralis* by molecular sizing chromatography and preparative flatbed isoelectric focusing.
Parasite Immunology 3, 261-272.

Despommier, D D and Laccetti, A (1981).
Trichinella spiralis. Proteins and Antigens isolated from a large particle fraction derived from the muscle larva.
Experimental Parasitology 51, 279-295.

Despommier, D D and Muller, M (1970).
Functional antigens of *Trichinella spiralis*. *Journal of Parasitology* 56, (Section II, Part I): 76.

Despommier, D D and Muller, M (1976).
The stichosome and its secretion granules in the mature muscle larvae of *Trichinella spiralis*.
Journal of Parasitology 62: 775-85.

Despommier, D D, Müller, M, Jenks, B and Fruitstone, M (1974).
Immunodiagnosis of human trichinosis using coutercurrent electrophoresis and agar gel diffusion techniques.
American Journal of Tropical Medicine and Hygiene. 23, 41-44.

Despommier, D D, Campbell, W C and Blair, L S (1977a).
The in vivo and in vitro analysis of immunity to Trichinella spiralis in mice and rats. Parasitology 74: 109.19.

Despommier, D D, McGregor, D D, Crum, E D, and Carter, P B, (1977b).
Immunity to Trichinella spiralis. II. Expression of immunity against adult worms, Immunology 33: 797-805.

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 eosinophil response to Trichinella spiralis infection, Journal of Experimental Medicine, 153, 423 - 436.

Dineen, J K and Wagland, B M (1966).
The cellular transfer of immunity to Trichostrongylus colubriformis in an isogenic strain of guinea pig. II. The relative susceptibility of the larval and adult stages of the parasite to immunological attack. Immunology 11, 47 - 57.

Dineen, J K, Kelly, J D and Love, R J (1973).
The competence of lymphocytes obtained from immune and non-immune donors to cause expulsion of Nippostrongylus brasiliensis in the rat (DA strain). International Archives of Allergy and Applied Immunology 45, 504-512.

Dineen, J K, Gregg, P, Windon, R G, Donald, A D and Kelly, J D (1977).
The role of immunologically specific and non-specific components of resistance in cross-protection to intestinal nematodes.
International Journal for Parasitology 7, 211-15.

Dobson, C and Soulsby, E J L (1974).
Kinetics in guinea pigs infected with Trichostrongylus colubriformis: tritiated thymidine uptake in gut and allied lymphoid tissue, humoral Ig and hemaglutinating antibody responses, delayed hypersensitivity reactions, and in vitro lymphocyte transformation during primary infections.
Experimental Parasitology 35, 16-34.

Doughty, B L, and Phillips, S M (1982).
In vitro delayed hypersensitivity granulomas. I. Formation and modulation by lymphoid supopulations.
Journal of Immunology 37-42.

Dresser, D N and Popham, A M (1979).
The influence of T cells on the initiation and expression of immunological memory.
Immunology 38, 265-274.

Drury, P J, and Singhal, S K, (1974).
Isolation of θ-isoantigen, antibody forming cells from normal bone marrow according to their density and surface adherent properties. International Archives of Allergy and Applied Immunology 46, 707-724.

Duwe, A K and Singhal, S K (1979).

The Immunoregulatory role of bone marrow. I. Suppression of the induction of antibody responses to T-dependent and T-independent antigens by cells in the bone marrow. Cellular Immunology 43, 362-371.

Ellner, J J and Mahmoud, A F (1982).

Phagocytes and Worms: David and Goliath revisited. Reviews of Infectious Diseases 4, 698-714

Eugui, E M and Allison, A C (1979).

Malaria infections in different strains of mice and their correlation with natural killer activity.

In: Bulletin of the World Health Organisation

Vol 56 Supplement No 1. pp 231-238.

Ewart, A and Olson, L J (1961).

The use of a mouse oral L.D. 50 to evaluate the immunogenicity of Trichinella metabolic antigens.

Texas Report of Biology and Medicine 19, 580-584.

Eyre, H J, Rosen, P J and Perry, S. (1970).

Relative labelling of leukocytes, erythrocytes and platelets in human blood by 51 chromium.

Blood 36, 250-253.

Ferguson, A and MacDonald, T T (1977).

Effects of local delayed hypersensitivity on the small intestine; in CIBA Foundation Symposium 46 pp 305-327 (Association of Scientific Publishers, Elsevier Scientific Publishing Co Exceppta-Med, North-Holland Publishing Co Amsterdam).

Ford, N L and Atkins, R C (1972).
Specific unresponsiveness of recirculating lymphocytes after exposure to histocompatability antigens in F_2 hybrid rats.
Nature, New Biology 234, 178.

Ford, N L and Smith, M E (1979).
Lymphocyte recirculation between the spleen and the blood.
In: Role of the spleen in the immunology of parasite diseases, Tropical diseases research, Series 1. pp 29-41.

Freitas, A A de, Rose, M L and Parrott, D M V (1977).
Murine mesenteric and peripheral lymph nodes: a common pool of small T cells. Nature (London) 270, 731.

Gabriel, B W, and Justus, D E (1979),
Quantitation of immediate and delayed hypersensitivity responses in Trichinella-infected mice, International Archives of Allergy and Applied Immunology, 60: 275-285.

Glenny, A T and Sudmersen, H J (1921).
Notes on the production of immunity to diptheria toxin.
Journal of Hygiene, Cambridge. 20 176-

Gottlieb, P D, Marstiak-Rothstein, A, Auditore-Hargreaves, K, Berkoben, D B, August, D A, Rosche, R M and Benedetto, J D. (1980).
Construction and properties of new Lyt-Congenic strains and Anti-Lyt-2.2 and Anti-Lyt-3.1 monoclonal antibodies.
Immunogenetics 10, 545-555.

Grencis, R K and Wakelin, D (1982).

Short lived, dividing cells mediate adoptive transfer of immunity to Trichinella spiralis in mice.

I. Availability of cells in primary and secondary infections in relation to cellular changes in the mesenteric lymph node.

Immunology 46: 443-450.

Hall, J G and Morris, B (1965).
The immediate effect of antigens on the cell output of a lymph node.
British Journal of Experimental Pathology 46, 450-454.

Handman, E, Cereding, R and Mitchell, G F (1979).
Murine cutaneous leishmaniasis: disease patterns in intact and nude mice of various genotypes and examination of some differences between normal and infected macrophages.
Australian Journal of Experimental Biology and Medical Science, 57, 9-29.

Handwerger, B S and Schwartz, R H (1974).
Separation of murine lymphoid cells using nylon wool columns. Recovery of the B cell enriched population.
Transplantation 18, 544-547.

Hay, J B, Lachmann, P J and Trnka, Z (1973).
The appearance of migration inhibition factor and a mitogen in lymph draining tuberculin reactions.
European Journal of Immunology, 3, 127-131.

Hopkins, C A (1982).
Immunological memory in mice to adult Hymenolepis diminuta (Cestoda).
Journal of Parasitology, 68, 32-38.

Hopkins, C A and Stallard, H E (1974).
Immunity to intestinal tapeworms: the rejection of Hymenolepis citelli by mice.
Parasitology 69, 63-76.

Hopkins, J, McConnell, I and Pearson, J D (1981).
Lymphocyte traffic through antigen-stimulated lymph nodes. II. Role of prostaglandin E_2 as a mediator of cell shutdown.
Immunology 42, 225-231.

Howard, J G, Hale, C and Liew, F Y (1981).
Immunological regulation of experimental cutaneous leishmaniasis.
Journal of Experimental Medicine 153, 557-568.

Huber, B, Devinsky, O, Gershon, R K, and Cantor, H (1976).
Cell mediated immunity: Delayed type hypersensitivity and cytotoxic responses are mediated by different T- cell subclasses.
Journal of Experimental Medicine 143, 1534-1539.

Hughes, W L, Commerford, S L, Gitlin, D, Kreuger, R C, Schultze, B, Shah, V and Reilly, P (1964).
Deoxyribonucleic acid metabolism in vivo. I.
Cell proliferation and death as measured by incorporation and elimination of iododeoxyuridine.
Federation Proceedings 23, 640-648.

Jacqueline, E, Vernes, A, Bout, D, and Biguet, J (1978).
Trichinella spiralis: Facteurs immuntaires inhibiteurs de la production de larves. II Premier analyse in vitro des facteurs humoraux et secretoires actifs chez les souris, le rat, et la miniporc infestes ou immunises,
Experimental Parasitology 45: 42-54.

James, E R, and Denham, D A (1975).
Immunity to Trichinella spiralis VI. The specificity of the immune response stimulated by the intestinal stage, Journal of Helminthology 49, 43-47.

James, E R, Moloney, A and Denham, D A (1977).
Immunity to Trichinella spiralis VII. Resistance stimulated by the parenteral stages of the infection, Journal of Parasitology, 63, 720-723.

Jenkins, D C (1975).
The influence of Nematospiroides dubius on subsequent Nippostrongylus brasiliensis infections in mice. Parasitology 71, 349-55.

Jenkins, S N (1977).
Studies on the immune response of the mouse to the nematode Trichuris muris. Ph.D. Thesis, University of Glasgow.

Jenkins, S N and Wakelin, D (1977).
The source and nature of some functional antigens of Trichuris muris. Parasitology 74, 153-61.

Johnson, G R, Nicholas, W L, Metcalf, D, McKenzie, I F, and Mitchell, G F (1979).
Peritoneal cell population of mice infected with Mesocestoides corti as a source of eosinophils. International Archives of Allergy and Applied Immunology 59, 315-322.

Johnstone, C, Leventhal, R and Soulsby, E J L (1981).
Ascaris suum: T-cell responses of C57 BL/6J mice in vitro and in vivo.
Experimental Parasitology: 51, 243-256.

Julius, M H, Simpson, E and Herzenberg, I A (1973).
A rapid method for the isolation of functional thymus derived murine lymphocytes.
European Journal of Immunology 3, 645-649.

Jungery, M and Ogilvie, B M (1982).
Antibody response to stage-specific Trichinella spiralis surface antigens in strong and weak responder mouse strains.
Journal of Immunology 129, 839-843.

Jungi, T W (1980a).
Non recirculating T lymphocytes in cellular resistance to infection.
Cellular Immunology 55, 499-505.

Jungi, T W (1980b).
Immunological memory to Listeria monocytogenes in rodents: Evidence for protective T cells outside the recirculating lymphocyte pool.
Journal of the Reticuloendothelial Society 28, 405-417.

Jungi, T N and Jungi, R (1981).
Immunological memory to Listeria monocytogenes in rodents. IV. Studies on origin and origin and fate of tissue-positioned T memory cells.
Immunology 44, 789-798.

Kagan, I G (1960).
Trichinosis: a review of biologic, serologic and immunologic aspects.
Journal of Infectious diseases 107, 65-93.

Kazacos, K R (1975).
Increased resistance in the rat to Nippostrongylus brasiliensis following immunization against Trichinella spiralis. Veterinary Parasitology 1, 165-74.

Kazura, J N, and Grove, D I, (1978).
Stage specific antibody dependent eosinophil-mediated destruction of Trichinella spiralis, Nature (Lond) 274, 588-589.

Kelly, J D and Dineen, J K (1972).
The cellular transfer of immunity to Nippostrongylus brasiliensis in inbred rats (Lewis strain).
Immunology 22, 199-212.

Kennedy, M W (1980a).
Effects of the host immune response on the longevity, fecundity and position in the intestine of Trichinella spiralis in mice. Parasitology 80, 49-60.

Kennedy, M W (1980b).
Immunologically mediated non-specific interactions between the intestinal phases of _Trichinella spiralis_ and _Nippostrongylus bràsiliensis_ in the mouse.
Parasitology 80, 61-72.

Kennedy, M W, Wakelin, D and Wilson, M M (1979).
Transplantation of adult _Trichinella spiralis_ between hosts: worm survival and immunological characteristics of the host-parasite relationship,
Parasitology, 78, 121-130.

Keusch, G T (1982).
Ed. The biology of parasitic infection: _Reviews of Infectious diseases_. 4, 735-911.

Kierszenbaum, F and Budzko, D B (1977).
Cytotoxic effects of normal sera on lymphoid cells.
I. Antibody independent killing of heterologous thymocytes by guinea pig, rabbit and human sera. Role of the alternative pathway of complement activation.
Cellular Immunology 29, 137-46.

Kierszenbaum, F and Budzko, D B (1979).
Changes occurring on the surface of mouse T cells during concanavalin A induced lymphoblastic transformation.
Immunology 38, 827-834.

Kim, C W, Ruitenberg, E J and Teppema, J S (1980).
Eds _Trichinellosis_, Reedbooks Ltd, Chertsey.

Koster, F T and McGregor, D D (1971).
The mediator of cellular immunity. III Lymphocyte traffic from the blood into the inflamed peritoneal cavity. Journal of Experimental Medicine. 133, 864-76.

Koster, F T, McGregor, D D and Mackaness, G B (1971).
The mediator of cellular immunity. II Migration of immunologically committed lymphocytes into inflammatory exudates.
Journal of Experimental Medicine 133, 400-409.

Krco, C J, David, C S and Wassom, D L (1982).
Characterization of an *in vitro* proliferation response to solubilized *Trichinella spiralis* antigens: Role of Ia antigens and $Ly\text{-}1^{+}$ T cells.
Cellular Immunology 68, 359-367.

Kurnick J T, Ostberg, L, Stegagno, M, Kimura, A K, Örn, A and Sjöberg, O (1979).
A rapid method for the separation of functional lymphoid cell populations of human and animal origin on PVP-Silica (Percoll) density gradients.
Scandanavian Journal of Immunology, 10, 563-

Kwa, B H and Liew, F Y (1977).
Immunity in taeniasis-cysticercosis. I. Vaccination against *Taenia taeniaeformis* in rats using purified antigen.
Journal of Experimental medicine, 146, 118-131

Landsteiner, K and Chase, M W. (1942).
Experiments on transfer of cutaneous sensitivity to simple chemical compounds.
Proceedings of the Society for Experimental Biology (New York) 49, 688-690.

Lang, B Z and Hall, R F (1977).
Host-parasite relationships of *Fasciola hepatica* in the white mouse. VIII. Successful vaccination with culture incubate antigens and antigens from sonic disruption of immature worms.
Journal of Parasitology 63, 1046-1049.

Larsh, J R (1963).
Experimental Trichiniasis, Advances in Parasitology 1: 213-286.

Larsh, J E (1970).
Immunity, in: Trichinosis in Man and Animals (S.E. Gould, ed), Charles C Thomas, Springfield, Illinois, pp 129-146.

Larsh, J E, Goulson, H T and Weatherley, N F (1964a).
Studies on delayed (cellular) hypersensitivity in mice infected with *Trichinella spiralis* I. Transfer of lymph node cells.
Journal of the Elisha Mitchell Scientific Society. 80, 133-135.

Larsh, J E, Goulson, H T, and Weatherley, N F (1964b). Studies on delayed (cellular) hypersensitivity in mice infected with _Trichinella spiralis_ II. Transfer of peritoneal exudate cells, _Journal of Parasitology_ 50, 496-498.

Lee, G B and Ogilvie, B M (1981). The mucus layer in intestinal nematode infections. In: _The Mucosal Immune System in Health and Disease_, Ed Ogra, P and Bienenstock, J Ross Laboratories, Columbus, Ohio.

Lee, T D G (1982). Immunological consequences of infection of the mouse colon with _Trichuris muris_. Ph.D. Thesis, University of Glasgow.

Lee, T D G and Wright, K A (1978). The morphology of the attachment and probable feeding site of the nematode _Trichuris muris_. _Canadian Journal of Zoology_ 56, 1889-1905.

Lee, T D G, Wakelin, D and Grencis, R K (1982). Cellular mechanisms of immunity to the nematode _Trichuris muris_. _International Journal of Parasitology_ In Press.

Lefford, M J and McGregor, D D (1978). The lymphocyte mediators of delayed hypersensitivity: the early phase cells. _Immunology_ 34, 581-590.

Lefford, M J, McGregor, D D and Mackaness, G B (1973).
Properties of lymphocytes which confer immunity to tuberculosis in rats. Immunology 25, 703-715.

Leskowitz, S and Waksman, B H (1960).
Studies in immunization. The effect of route of infection of bovine serum albumin in Freunds adjuvant on production of circulating antibody and delayed hypersensitivity.
Immunology 4, 58-71.

Lloyd, S (1981).
Progress in immunization against parasitic helminths.
Parasitology 83, 225-242.

Louis, J A, Zubler, S G, Coutinho, G, Lima, G, Behin, R, Mauel, J and Engers, H D (1982).
The *in vitro* generation and functional analysis of murine T cell populations and clones specific for a protozoan parasite, *Leishmania tropica*.
Immunological Reviews 61, 215-243.

Love, R J and Ogilvie, B M (1977).
Nippostrongylus brasiliensis and *Trichinella spiralis*: Localization of lymphoblasts in the small intestine of parasitized rats. Experimental Parasitology 41, 124-132.

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.

Lowry, O H, Rosebrough, N J, Farr, A L and Randall, R J. (1951).

Protein measurement with the folin-phenol reagent.

Journal of Biological Chemistry 193, 265-

Mackenzie, C D, Preston, P M and Ogilvie, B M (1978).

Immunological properties of the surface of nematodes.

Nature (London) 276, 826.

Maizels, R M, Philipp, M and Ogilvie, B M (1982).

Molecules on the surface of parasitic nematodes as probes of the immune response to infection.

Immunological Reviews Vol 61, 109-136.

Mansfield, J M (1981).

Ed. Parasitic Diseases. Volume 1. The Immunology.

Marcel Dekker, Inc. New York and Basel.

Manson-Smith, D F, Bruce, R G and Parrott, D M V (1979a).

Villous atrophy and expulsion of intestinal

Trichinella spiralis are mediated by T- cells, Cellular Immunology 47, 285-292.

Manson-Smith, D F, Bruce, R G, Rose, M L and Parrott, D M V (1979b).

Migration of lymphoblasts to the small intestine.

II. Strain differences and relationship to distribution and duration of Trichinella spiralis infection.

Clinical and Experimental Immunology 38, 475-482.

Markell, E K (1958).
The effect of cortisone treatment upon the longevity and productivity of Trichinella spiralis in the rat.
Journal of Infectious Diseases 102, 158-161.

McConnell, I and Hopkins, J (1981).
Lymphocyte traffic through antigen-stimulated lymph nodes. I. Complement activation within lymph nodes initiates cell shutdown.
Immunology 42, 217-223.

McConnell, I, Lachmann, P J and Hobart, M J (1974).
The restoration of specific immunological virginity.
Nature (London) 250, 113.

McCoy, O R (1935).
Artificial immunization of rats against Trichinella spiralis.
American Journal of Tropical Medicine and Hygiene 21, 200-213.

McDonald, V and Phillips, R S (1978).
Plasmodium chabaudi in mice. Adoptive transfer of immunity with enriched populations of spleen T and B lymphocytes.
Immunology 34, 821-830.

McGregor, D D and Logie, P S (1973).
The mediator of cellular immunity. VI Effect of the antimitotic drug vinblastine on the mediator of cellular resistance to infection Journal of Experimental Medicine 137, 660-674.

McGregor, D D and Logie, P S (1974).
The mediator of cellular immunity. VII Localisation of sensitized lymphocytes in inflammatory exudates.
Journal of Experimental Medicine, 139, 1415-1430.

Mendelsöhn, M L (1981).
The attributes and applications of flow cytometry.
In. Acta pathologica et microbiologica Scandanavia. Section A, supplement No 274, (Eds Laerum, O D, Lindmo, T and Thorud, E). Universit et for laget 1980. pp 15-27.

Miller, J F A P and Sprent, J (1971).
Cell to cell interaction in the immune response, VI. Contribution of thymus-derived cells and antibody forming cell precursors to immunological memory.
Journal of Experimental Medicine 134, 66-82.

Miller, R G and Phillips, R A (1969).
Separation of cells by velocity sedimentation.
Journal of Cellular Physiology 73, 191-202.

Mitchell, G F (1979).
Responses to infection with metazoan and protozoan parasites in mice.
Advances in Immunology, 28, 451-511.

Mitchell, G F (1980).
T cell dependent effects in parasitic infection and disease. In: Progress In Immunology, Vol 4 (Ed Fougereau, M and Dausset, J) Academic Press, London.

*Mitchell, G F and Anders, R F (1982).
Parasite antigens and their immunogenicity in infected hosts. In: The Antigens, Vol VI, Academic Press Inc pp 69 - 149.

Mitchell, G F (1981a).
Exploiting mouse strain susceptibility differences in immuno parasitology.
Immunology Today 2, 61-63.

Mitchell, G F (1981b).
Hybridoma antibodies in immunodiagnosis of parasitic infection.
Immunology Today 2, 140-142.

*

Mitchell, G F, Curtis, J M, Scollay, R G and Handman, E (1981).
Resistance and abrogation of resistance to cutaneous leishmaniasis in reconstituted BALB/c nude mice.
Australian Journal of Experimental Biology and Medical Science 59, 539-554.

Mitchell, G F, Garcia, E G, Cruise, K M, Tui, W U and Hocking, R E (1982).
Lung granulomatous hypersensitivity to eggs of Schistosoma japonicum in mice analysed by a radioisotopic assay and effects of hybridoma (idiotype) sensitization.
Australian Journal of Experimental Biology and Medical Science 60, 401-416.

Möller, G (1982a).
Ed Immunological Reviews. Vol 61.
Immunoparasitology. Munksgaard. Copenhagen K, Denmark.

Möller, G (1982b).
Ed Immunological Reviews, Vol 63.
Interleukins and lymphocyte activation. Munksgaard, Copenhagen K, Denmark.

Moloney, A and Denham, D A (1979).
Effects of immune serum and cells on newborn larvae of *Trichinella spiralis*, *Parasite Immunology*, 1, 3-12.

Moqbel, R (1977).
"Studies on the host-parasite relationship of *Strongyloides ratti* in Rats." Ph.D Thesis, University of London, England.

Moqbel, R and Wakelin, D (1979).
Trichinella spiralis and *Strongyloides ratti*: Immune interaction in adult rats. *Experimental Parasitology* 47, 65-72.

Murray, M, Robinson, P B, Grierson, C and Crawford, R A (1979).
Immunization against *Nippostrongylus brasiliensis* in the rat. A study of the use of antigen extracted from adult parasites and the parameters which influence the level of protection.
Acta Tropica 36, 297-322.

Nabel, G, Galli, S J, Dvorak, A M, Dvorak, H F and Cantor, H (1981).
Inducer T lymphocytes synthesize a factor that stimulates proliferation of cloned mast cells *Nature* (Lond) 291, 332-334.

Nakashima, I, Ohta, F, Kobayashi, T, Kato, O and Kato, N (1974).
Effect of antigen doses and time intervals between antigen injections and secondary, tertiary and quaternary antibody response.
Immunology, 26, 443-454.

Nawa, Y and Miller, H R P (1978).
Protection against Nippostrongylus brasiliensis by adoptive immunization with immune thoracic duct lymphocytes.
Cellular Immunology. 42, 225-239.

Nawa, Y, Parish, C R and Miller, H R P (1978).
The protective capacities of fractionated immune thoracic duct lymphocytes against Nippostrongylus brasiliensis. Cellular Immunology 37, 41-50.

North, R J (1973).
Cellular mediators of anti-Listeria immunity as an enlarged population of short-lived, replicating T cells.
Kinetics of their production.
Journal of Experimental Midicine 138, 342-355.

North, R J (1975).
Nature of memory in T-cell mediated antibacterial immunity.
Anamnestic production of mediator T cells.
Infection and Immunity 12, 754-760.

North, R J and Deissler, J F (1975).
Nature of "memory" in T-cell-mediated antibacterial immunity: cellular parameters that distinguish between the active immune response and a state of "memory".
Infection and Immunity 12, 761-767.

Ogilvie, B M and Jones, V E (1971).
Nippostrongylus brasiliensis: A review of immunity and the host/parasite relationship in the rat.
Experimental Parasitology 29, 138-177.

Ogilvie, B M and Love, R J (1974).
Co-operation between antibodies and cells in immunity to a nematode parasite.
Transplantation Reviews 19, 147-168.

Ogilvie, B M, Love, R J, Jarra, W and Brown, K N (1977).
Nippostrongylus brasiliensis infection in rats. The cellular requirement for worm expulsion.
Immunology 32, 521-528.

Ottaway, C A, Manson-Smith, D F, Bruce, R G and Parrott, D M V, (1980).
Regional blood flow and the localization of lymphoblasts in the small intestine of the mouse II. The effect of a primary enteric infection with Trichinella spiralis,
Immunology 41, 963-971.

Ottesen, E A, Smith, T K and Kirkpatrick C H, (1975).
Immune response to *Trichinella spiralis* in the rat.
I. Development of cellular and humoral responses.
International Archives of Allergy and Applied Immunology
49, 396-410.

Parmentier, H K, Ruitenberg, E J and Elgersma, A (1982).
Thymus dependence of the adoptive transfer of intestinal
mastocytopoiesis in *Trichinella spiralis* infected mice.
International Archives of Allergy and Applied Immunology
68, 260-267.

Palmer, C G, Livengood, D, Warren, A K, Simpson, P J
and Johnson, I S (1960).
The action of Vincaleukoblastine on mitosis *in vitro*.
Experimental Cell Research 20, 198-201.

Pery, P and Luffau, G (1979).
Antigens of helminths (Chapter 2). In: *The Antigens*
Vol V. Academic Press.

Philipp, M, Parkhouse, R M E, and Ogilvie, B M (1980).
Changing proteins on the surface of a parasitic nematode.
Nature (London) 257, 538-540.

Pierce, N F and Koster, F T (1980).
Priming and suppression of the intestinal immune
response to cholera toxoid/toxin by parenteral toxoid
in rats.
Journal of Immunology 124, 307-311.

Pilarski, L M (1978).

An analysis of B cell memory. I. Interaction of helper and suppressor effects in the *in vitro* expression of IgM memory.

Cellular Immunology 40, 376-388.

Playfair, J H L (1979).

Lethal *Plasmodium yoelii* malaria: the role of macrophages in normal and immunized mice. In: *Bulletin of the World Health Organisation* Vol 57, Supplement No 1, pp 245-246.

Playfair, J H L (1982).

Workshop report: Suppressor cells in infectious disease.

Parasite Immunology 4, 299-304.

Poulain, J, Pery, P and Luffau, G (1976).

Protection of rats against *Nippostrongylus brasiliensis* with worm antigens by oral administration. *Annales of Immunology* 127C, 209-213.

Pritchard, D I (1977).

Immunology of trichinosis in the guinea pig. The role of immediate hypersensitivity. Ph.D Thesis, University of Birmingham.

Pritchard, D I and Eady, R P (1980).

Eosinophilia in athymic nude (rnu/rnu) rats-thymus independent eosinophilia. *Immunology* 43, 409-416.

Pritchard, D I, Eady, R P, Jackson, D M, Orr, T S C, Richards, I M, Trigg, S, and Wells, E (1982).
Laboratory infection of primates with _Ascaris suum_ to provide a model of allergic bronchoconstriction. _Clinical and Experimental Immunology_, In Press.

Rappaport, J and Wells, H S (1951).
Studies in Trichinosis I. Immunity to re-infection in mice following a single light infection. _Journal of Infectious Diseases_ 88, 248-253.

Rocklin, R E, Bendtzen, K and Greineder, D (1980).
Mediators of Immunity: Lymphokines and Monokines. _Advances in Immunology_ 29, 55-136.

Rose, M L, Parrott, D M V and Bruce, R G (1976a).
Migration of lymphoblasts to the small intestine. I Effect of _Trichinella spiralis_ infection on the migration of mesenteric lymphoblasts and mesenteric T lymphoblasts in syngeneic mice. _Immunology_ 31, 723-730.

Rose, M L, Parrott, D M V and Bruce, R G (1976b).
Migration of lymphoblasts to the small intestine. II Divergent migration of mesenteric and peripheral immunoblasts to sites of inflammation in the mouse. _Cellular Immunology_ 27, 36-46.

Rose, M L, Parrott, D M V and Bruce, R G (1978).
The accumulation of immunoblasts in extravascular tissues including mammory gland, peritoneal cavity, gut and skin.
Immunology, 35, 415-423.

Rosenstreich, D L, Weinblatt, A C, and O'Brien, A D (1982).
Genetic control of resistance to infection in mice.
CRC Critical Reviews in Immunology 3, 263-330.

Rothwell, T L W (1978).
Vaccination against the nematode Trichostrongylus colubriformis III. Some observations on factors influencing immunity to infection in vaccinated guinea pigs.
International Journal of Parasitology 8, 33-37.

Rothwell, T L W and Dineen, J K (1973).
The response of regional lymph nodes of the guinea-pig to primary and challenge infection with the nematode Trichostrongylus colubriformis.
International Journal of Parasitology 3, 201-208.

Rothwell, T L W, and Love R J (1974).
Vaccination against the nematode Trichostrongylus colubriformis. I. Vaccination of guinea pigs with worm homogenates and soluble products released during in vitro maintenance.
International Journal of Parasitology 4, 239-299.

Schacterle, G R and Pollack, R L (1973).
A simplified method for the qualitative assay of small amounts of protein in biological material.
Analytical Biochemistry 51, 654-5.

Schwartz, R H (1982).
The cloning of T lymphocytes.
Immunology Today 3, 43-46.

Scollay, R, Kochen, M, Butcher, E and Neissman, I (1978).
Lyt markers on thymus cell migrants.
Nature (London) 276, 79-80.

Scott, M T (1981).
Delayed hypersensitivity to Trypanosoma cruzi in mice: specific suppressor cells in chronic infection.
Immunology 44, 409-418.

Sheldon, A J (1937).
Studies on active acquired resistance, natural and artificial in the rat to infection with Strongyloides ratti.
American Journal of Hygiene, 25, 53-56.

Sher, A, Hieny, S, James, S L and Asofsky, R (1982).
Mechanisms of protective immunity against Schistosoma mansoni infection in mice vaccinated with irradiated cercariae. II. Analysis of immunity in hosts deficient in T lymphocytes, Blymphocytes, or complement.
Journal of Immunology, 128, 1880-1884.

Silverman, P H (1970).

Vaccination: Progress and Problems. In "Immunity to parasitic animals". Vol 2. (G J Jackson, R Herman, and I Singer eds. pp 1165-1185.). Appleton-Century and Crofts, New York.

Silverman, P H, Poynter, D and Podger, K R (1962).

Studies on larval antigens derived by cultivation of some parasitic nematodes in simple media: protection tests in laboratory animals. Journal of Parasitology 48, 562-571.

Soulsby, E J L (1972).

Immunity to animal parasites. Academic Press. New York and London.

Sparrow, S (1980).

Ed Immunodeficient animals for cancer research. MRC laboratory animals centre symposium No 2. Macmillan Press Ltd.

Spencer, J C, Waldman, R M and Johnson, J E (1974).

Local and systemic cell-mediated immunity after immunization of guinea pigs with live or killed M. tuberculosis by various routes. Journal of Immunology 112, 1322-1328.

Sprent, J and Miller, J F A P (1973).

Effect of recent antigen priming on adoptive immune responses. Journal of Experimental Medicine, 138, 143-162.

Sprent, J and Miller, J F A P (1976).
Fate of H2-activated lymphocytes in syngeneic hosts III. Differentiation into long-lived recirculating memory cells.
Cellular Immunology 21, 314-326.

Steinman, R M and Nussenzweig, M C (1980).
Dendritic cells: Features and functions, pp 127-148.
Ed Möller, G. Immunological Reviews Vol 53.
Munksgaard, Copenhagen K. Denmark.

Stoll, N R (1961).
The worms: Can we vaccinate against them?
American Journal of Tropical Medicine and Hygiene, 10, 293-303.

Swarbrick, E T, Stokes, C F and Soothill, J F (1979).
Absorption of antigens after oral immunization and the simultaneous induction of specific systemic tolerance.
Gut 20, 121-125.

Tanner, C E, Lim H-C and Faubert, G (1978).
Trichinella spiralis: changes caused in the mouse's thymic, splenic and lymph node populations.
Experimental Parasitology 45, 116-127.

Taylor, D W and Siddiqui, W A (1979).
A study of the cellular and humoral immune responses in owl monkeys (Aotus trivirgatus) following vaccination against Plasmodium falcipanum. In: Bulletin of the world Health Organisation Vol 57. Supplement No 1 pp 247-254.

Thomas, D B and Calderon, R A (1982).
T helper cells change their Lyt-1.2 phenotype during an immune response.
European Journal of Immunology 12, 16-23.

Tilney, N L (1971).
Patterns of lymphatic drainage in the adult laboratory rat.
Journal of Anatomy, 109, 369-383.

Tomasi, T B, Jr (1980).
Oral tolerance.
Transplantation 29, 353-356.

Trizio, D and Cudkowicz, G (1974).
Separation of T and B lymphocytes by nylon wool columns: Evaluation of efficacy by functional assays *in vivo*.
Journal of Immunology 113, 1093-1097.

Urquhart, G M (1980).
Application of immunity in the control of parasitic disease.
Veterinary Parasitology 6, 217-239.

Vadas, M A, Miller, J F A P, Gamble, J and Whitelaw, A (1975).
A radioisotopic method to measure delayed type hypersensitivity in the mouse. I. Studies in sensitized and normal mice.
International Archives of Allergy and Applied Immunology 49, 670-692.

Vadas, M A, Miller, J F A P, McKenzie, I F C, Chism, S E, Shen, F W, Boyce, E A, Gamble, J R and Whitelaw, A M (1976).
Ly and Ia antigen phenotypes of T cells involved in delayed-type hypersensitivity and in suppression.
Journal of Experimental Medicine, 144, 10-19.

Valeriote, F A and Bruce, W R (1965).
An in vitro assay for growth inhibiting activity of vinblastine.
Journal of the National Cancer Institute 35, 851-856.

Van Rooijen, N and Roeterink, C H (1980).
Phagocytosis and lymphocyte migration: evidence that lymphocyte trapping in the spleen following carbon injection is not due to direct lymphocyte - macrophage adherence.
Immunology 39, 571-576.

Vernes, A (1976).
Immunization of the mouse and minipig against Trichinella spiralis. In Biochemistry of parasites and host parasite relationships. Ed. Van den Bossche. Elsevier/ North Holland Biomedical Press, Amsterdam, pp 319-324.

Wagland, B M and Dineen J K (1965).
The cellular transfer of immunity to Trichostrongylus colubriformis in an isogenic strain of guinea-pig.
Australian Journal of Experimental Biology and Medical Science, 43, 429-438.

Wakelin, D (1967).

Acquired immunity to *Trichuris muris* in the albino laboratory mouse. *Parasitology* 57, 515-24.

Wakelin, D (1975).

Immune expulsion of *Trichuris muris* from mice during a primary infection: analysis of the components involved.
Parasitology 70, 397-405.

Wakelin, D (1978a).

Immunity to intestinal parasites.
Nature 273, 617-20.

Wakelin, D (1978b).

Genetic control of susceptibility and resistance to parasitic infection. In *Advances in Parasitology* Vol 16 (ed W H R Lumsden, R Muller and J R Baker). New York and London: Academic Press.

Wakelin, D (1980).

Genetic control of immunity to parasites. Infection with *Trichinella spiralis* is inbred and congenic mice showing rapid and slow responses to infection.
Parasite Immunology 2, 85-98.

Wakelin, D and Denham, D A (1982).

Immunity. In *Trichinosis*, Ed W C Campbell, Plenum Press, New York. In Press.

Wakelin, D and Donachie, A M (1983).
Genetic control of Immunity to *Trichinella spiralis*. Influence of H-2 linked genes on the immunity to the intestinal phase of infection.
Immunology, In Press.

Wakelin, D and Lloyd, M (1976a).
Immunity to primary and challenge infections of *Trichinella spiralis* in mice: a re-examination of the conventional parameters.
Parasitology 72, 173-82.

Wakelin, D and Lloyd, M (1976b).
Accelerated expulsion of adult *Trichinella spiralis* in mice given lymphoid cells and serum from infected donors.
Parasitology, 72, 307-315.

Wakelin, D and Selby, G R (1973).
Functional antigens of *Trichuris muris*. The stimulation of immunity by vaccination of mice with somatic antigen preparations.
International Journal for Parasitology 3, 711-715.

Wakelin, D and Wilson, M M (1977a).
Transfer of immunity to *Trichinella spiralis* in the mouse with mesenteric lymph node cells in donors and expression of immunity in recipients.
Parasitology 74, 215-224.

Wakelin, D and Wilson, M M (1977b).
Evidence for the involvement of a bone marrow-derived cell population in the immune expulsion of _Trichinella spiralis_, _Parasitology_ 74, 225-234.

Wakelin, D and Wilson, M M (1979a).
T and B cells in the transfer of immunity against _Trichinella spiralis_ in mice.
Immunology 37, 103-109.

Wakelin, D and Wilson, M M (1979b).
Trichinella spiralis: immunity and inflammation in the expulsion of transplanted adult worms from mice.
Experimental Parasitology 48, 305-312.

Wakelin, D and Wilson, M M (1980).
Immunity to _Trichinella spiralis_ in irradiated mice,
International Journal of Parasitology 10, 37-41.

Wakelin, D, Grencis, R K and Donachie, A (1982).
Short lived dividing cells mediate adoptive transfer of immunity to _Trichinella spiralis_ in mice.
II. _In vivo_ characteristics of the cells.
Immunology, 46, 451-457.

Wassom, D L, David, C S and Gleich, G J (1979).
Genes within the Major Histo compatability Complex influence susceptibility to _Trichinella spiralis_ in the mouse.
Immunogenetics 9, 491-496.

Wassom, D L, David, C S and Gleich, G J (1980).
MHC-linked genetic control of the immune response to parasites: Trichinella spiralis in the mouse. In Genetics of Natural Resistance to Infection and Malignancy (Ed by Skamene, E, Kongshavn, P A L, and Landy, M) pp 75-82. Academic Press Inc, New York.

WesleyLeid, R, and Williams, J F (1979).
Helminth parasites and the host inflammatory system. Chemical Zoology Vol XI, pp 229-271, Academic Press.

W.H.O. (1976).
Immunological adjuvants. Technical Report Series, No 595.

W.H.O. (1981).
Intestinal protozoan and helminthic infections. Technical Report Series No 666.

Williams, A O (1980).
Cell-surface antigens of lymphocytes: Markers and Molecules. In: Biochemical Society Symposium No 45; The lymphocyte cell surface. (Eds Garland, P B and Crumpton, M J) pp 27-44.

Willmott, S (1980).
The eosinophil in Tropical disease. Transactions of the Royal Society of Tropical Medicine and Hygiene, Vol 74. Supplement

Wright, K A (1979).

Trichinella spiralis: An intracellular parasite in the intestinal phase.

Journal of Parasitology 65, 441-5.

Yoshida, N, Nussenzweig, R S, Potocnjak, P, Nussenzweig, V and Aikawa, M (1980).

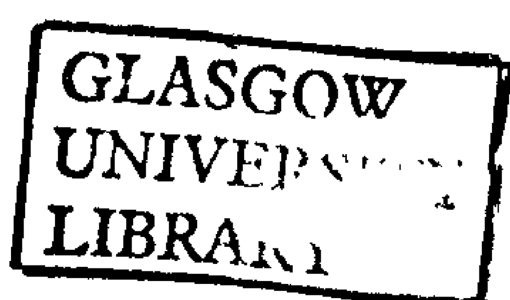
Hybridoma produces protective antibodies directed against the sporozoite stage of malaria parasite.

Science 207, 71-73.

Zatz, M M and Lance, E M (1971).

The distribution of ^{51}Cr- labelled lymphocytes into antigen-stimulated mice.

Journal of Experimental Medicine 134, 224-241.


GLASGOW UNIVERSITY LIBRARY