The genetic control of the antibody repertoire during infection with the parasitic nematode, *Ascaris*

by

Lesley A. Tomlinson B.Sc. (Hons)

Zoology Department

University of Glasgow

A thesis submitted for the degree of Doctor of Philosophy in the University of Glasgow.

May 1989

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ACKNOWLEDGEMENTS

I wish to express my sincere thanks to:

Dr. D.A.P. Bundy for providing the human sera and *Ascaris lumbricoides* parasites.

Dr. M. Haswell-Elkins and Dr. D. Elkins for *A. lumbricoides* parasites.

Prof. D. Wakelin and colleagues for providing certain mouse sera.

Dr. J. Christie for use of the affinity purified 14KDa.

Prof. D.W.T. Crompton and Dr. E. Hall, for helpful discussion and constructive comments.

Mr P. Rickus for photographic work.

Mrs F. McMonagle and Mrs E. Denton for help with the graphics.

Mrs. A. MacIntosh, Mrs. F. McMonagle and Mr. D. McLoughlin for expert technical assistance.

The team of very patient proof readers.

The supervisor for this project Dr. M.W. Kennedy for his help over the last three years.

This work was funded by a grant to M.W.K. from the Medical Research Council.

To Davie, Sheila, Lynne, Fiona, Colin and Lizzie for always knowing when to sympathise, criticise, or just ignore, a very special Thank-you!

Abbreviations

ABF, *Ascaris* body fluid

Ag, antigen

AP, alkaline phosphatase

BSA, bovine serum albumin

°C, degree Celsius

CFA, complete Freund's adjuvant

CO_2, carbon dioxide

CMI, cell mediated immunity

cm, centimeter

cpm, counts per minute

DSM, dried skimmed milk

ELISA, enzyme-linked immunosorbent assay

epg, egg per gramm

E/S, excretory/secretory

FITC, fluorescein isothiocyanate

h, hour

HLA, human histocompatability leukocyte antigens

HRP, horse radish peroxidase

IFA, incomplete Freund's adjuvant

Ig, immunoglobulin

i.p., intraperitoneal

Ir, immune response

Is, immune suppression

IU, international unit

i.v., intravenous

kDa, kilo dalton

L2, second stage larvae

L3/4, third-fourth stage larvae

M, molar

mA, milli-amp

MHC, major histocompatability complex

min, minute

mm, milli meter

M_r, relative molecular mass

NaOCl, sodium hypochlorite

ND, not determined

nm, nano meter

OD, optical density

O/N, overnight

PAGE, polyacrylamide gel electrophoresis

PBS, phosphate-buffered saline

PCA, passive cutaneous anaphylaxis

RT, room temperature

s, second

SAPU, Scottish antibody production unit

SDS, sodium dodecyl sulphate

Th cell, T helper cell

Tris, tris(hydroxymethyl)aminomethane

Ts cell, T suppressor cell

V/V, volume per volume

WB, worm burden

W/V, weight per volume

WLEP, Wellcome Laboratories for Experimental Parasitology

SUMMARY

The heterogeneity of host resistance or susceptibility to parasitic infection and the consequent overdispersion of the parasite population are well documented phenomena. This thesis presents an investigation of the genetic control of the antibody repertoire in infection with *Ascaris*, in a rodent model. Inbred and H-2 congenic strains of mice and rats were infected with *A. suum*, or a purified *Ascaris* component, and the antibody response was examined. The specificity of the response was studied using immunoprecipitation of radio-labelled antigens and SDS-PAGE. Infection sera were immunoprecipitated with excretory/secretory (ES) antigens, somatic antigen (ABF) or a purified 14kDa molecule, one of the main conponents of both ES and ABF. The level of antibody response was assessed using an ELISA system. The binding capacity of serum, from *Ascaris* infected animals, to the surface of living larvae was also examined, using immunofluorescence. All strains of mice and rats examined showed restricted recognition of the potentially antigenic components, in the context of infection. This restricted recognition could however, be overcome if a purified antigen was presented with adjuvant. The antibody repertoire produced as a result of *Ascaris* infection is controlled by MHC-linked genes, with non-MHC linked genes affecting the kinetics and level of antibody production.

Helminth infections characteristically, induce high titres of IgE and special attention was, therefore,

focused on the production and control of IgE, in this model. Parasite-specific IgE was examined using passive cutaneous anaphylaxis (PCA) and IgE-specific Western blotting. This analysis revealed that the repertoire of parasite-specific IgE, produced as a result of *Ascaris* infection, is identical to that of IgG antibody, exhibiting the same restricted recognition of parasite antigen and both MHC-linked and non-MHC linked genetic control.

Finally, the antibody repertoire produced by human subjects, living in an endemic area, and naturally infected with *A. lumbricoides* was examined. Serum from these individuals demonstrated restricted recognition of *Ascaris* components. There was variation in the level and specificity of the human antibody response, but no apparent correlation could be demonstrated between antibody production and infection status or age. The differences in antibody levels and specificity could also not be explained in terms of age or exposure to infection, suggesting that the differences observed may reveal that there is also genetic control of the human antibody response to infection with *Ascaris*. This system, therefore, provides a means of studying the genetic control of antibody production in response to infection, which may identify protective antigens or resistant host haplotypes.

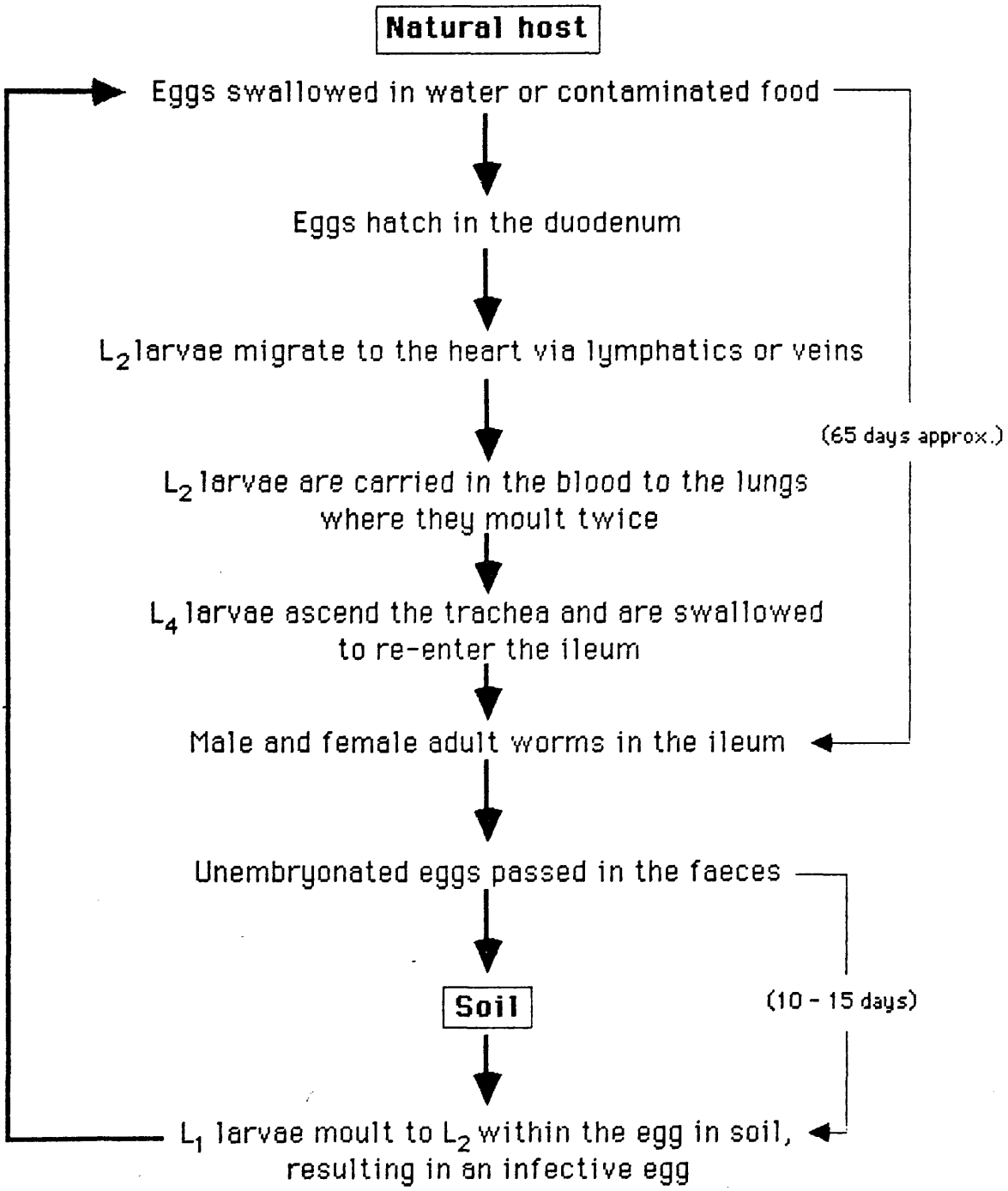
GENERAL INTRODUCTION

1.1; *Ascaris lifecycle*

Ascariasis in man is caused by the roundworm parasite, *Ascaris lumbricoides*. It is estimated that, worldwide, approximately 1.3 billion people are infected with *A. lumbricoides* (WHO Technical Report, 1981). Ascariasis is a persistent infection, and, in endemic areas, where prevalence may approach 100% in early childhood, it is possible that an infant becoming infected may harbour the parasite for the rest of its life. Infection occurs by ingestion of embryonated eggs, containing the L2 stage of the parasite which hatch in the gut (Figure 1). The L2 larvae migrate via the liver to the lungs where they moult to the L3 and then L4 stages of the parasite. The L4 larvae re-enter the gut where they mature into adults. From ingestion to maturity usually takes between 50 and 80 days and adult worms can survive for up to 2 years in the gut. Once mature, the adult worms mate and a female worm will produce somewhere in the region of 240,000 eggs per day during her reproductive lifespan (Cabrera, 1984; Pawlowski and Arfaa, 1984; Stephenson, 1987). These unembryonated eggs pass out with the faeces and, under conditions of poor sanitation, contaminate the environment.

The factors which regulate the population of *A. lumbricoides* are complex. Ecological factors such as landscape, weather and type of soil effect the viability and infectivity of the eggs. The survival time for these eggs is dependent largely on ecological factors, for

Figure 1:
The life cycle of *Ascaris*.



example, *A. lumbricoides* eggs can survive for more than 6 years in a temperate climate but for only a few hours under tropical conditions (WHO Technical Report, 1981). Unfavourable climatic conditions such as the cold winter in Europe, and the brief deviations from the normally warm, arid conditions in Saudia Arabia, are responsible for seasonal breaks in transmission. However, in many regions of the world with a warm, moist climate the transmission of *Ascaris* occurs throughout the year. Transmission to humans is largely dependent on socioeconomic factors, the most relevant of which appear to be density of human population, their involvement with agriculture and the general standard of sanitation (Crompton, Nesheim, and Pawłowski, 1985).

I1i; The Pathology of infection with *Ascaris*

Human hosts are often tolerant of intestinal infection with adult worms, resulting in asymptomatic infection. Complications due to migration of adult worms and intestinal obstruction are relatively rare, but can be the cause of mortalities in ascariasis (Lloyd and Soulsby, 1985). However, mass larval migration, due to intermittent exposure can cause pneumonitis which may be an even greater public health problem than the intestinal infection it precedes (WHO, Technical Report, 1981). This sort of lung involvement is relatively rare in populations continuously exposed to *Ascaris*. This lack of lung involvement has been attributed to a relatively low level of infection, as the intensity of the host's

pulmonary response may be proportional to the number of larvae destroyed during their migration (Phills *et al*, 1972). However, pulmonary changes were observed in experimental infections in man with as few as 6 to 45 *A. suum* eggs (Phills *et al*, 1972). An alternative suggestion is that some form of tolerance develops in populations continuously exposed to infection, and that this tolerance prevents lung disease (Spillman, 1975; Arfaa and Ghadirian, 1977).

Intensive infections in young, undernourished children, whose immune systems are constantly challenged by other infections are usually symptomatic. It is well known that nematode infections of the gut are less well tolerated in animals on diets deficient in protein or iron, and that in such animals antihelmintics may be less efficient (Suskind, 1977; Beisel, 1982). All these effects have been associated with the reduction in the immune response due to malnutrition. Studies have shown that even light infections with *Ascaris* may adversely affect the nutritional status of children (Gupta *et al* 1977, Stephenson *et al* 1980), and that the presence of ascariasis in undernourished children is of genuine public health concern.

The pathology and symptomology differ widely between the tissue phase of infection, caused by migrating larvae, and the intestinal phase, caused by pre-adult and adult worms. The difference is mainly in the type and

intensity of the reaction. The inflammatory and immunological reactions prevail in the tissue phase, whereas the host's intestinal functions seem to be the most affected in the intestinal phase (Pawlowski, 1982). The transit time of food may be increased in these infections, and there are disturbances in myoelectric activity and pancreatic hormone and gastrin secretion (Lloyd and Soulsby, 1985). Villous atrophy is often seen in intestinal nematode infections and may, at least in part, be a consequence of the immune response (Ferguson and Jarrett, 1975). Intestinal nematode infection results in increased permeability of the intestine which has two separate consequences: there is an enhanced loss of serum proteins from the circulation into the intestine, and enhanced intestinal permeability results in increased absorption of lactulose or potential antigenic molecules such as albumen (Bloch *et al*, 1979; Cobden *et al*, 1979).

Although there are IgE antibodies to *Ascaris* antigens in infected individuals, there are few reports of severe anaphylactic reactions in these people (WHO technical report, 1981). Moreover, there are fewer cases of Loeffler's syndrome (which has the characteristics of immediate type hypersensitivity response), in continuously exposed, than intermittently infected individuals (Spillman, 1975). In contrast to this lack of anaphylactic symptoms in infected individuals, naive individuals exposed to the parasite can develop acute

allergic reactions against *Ascaris* antigens (Coles, 1985).

Iiii; Prevalence of infection

The prevalence of infection in endemic areas may be very high. For example, in a sample from Jazin, Iran, 86% of the population examined were infected with *A. lumbricoides* (Croll *et al*, 1982), but the distribution of worms within the host population was highly aggregated, such that most people harbour a few or no worms, while a small percentage of the population harbour most of the total parasite population (Elkins *et al*, 1986; Bundy *et al*, 1986). Many factors may contribute to this overdispersion, including ecology, the genetic make-up of the host and behaviour, which may be particularly relevant in the infection of children.

This overdispersion is an important consideration in the design of chemotherapy programmes. The magnitude of natural transmission of ascariasis in endemic areas can be measured by examining the re-infection rate after successful mass chemotherapy. This rate is a function of infection pressure and the host's responses, which regulate the number of adult worms that develop. In some areas the re-infection rate is as high as 30% of the population per month, which means that, allowing for the 2 month lag after chemotherapy, the previous level of infection will be re-established within 6 months (Hlaing *et al*, 1987) This rapid re-infection makes mass

chemotherapy an expensive procedure, but, if the minority of the population that harbour heavy worm burdens could be readily identified, then treatment could be administered to this group alone. This targeted chemotherapy means that the most heavily infected individuals can be treated in a much more efficient and cost effective method. Alternatively, the rate of re-infection can be reduced by improving sanitation and general levels of hygiene. If the rate of exposure could be significantly reduced before mass chemotherapy, then the re-acquisition of infection is less likely.

Iiv: The immune response against *Ascaris* in the natural host.

Helminth infection results in a variety of host responses involving both immunological and non-immunological mechanisms. There is some confusion about the extent of pathogenicity of infection with *A. lumbricoides*, and the role of the host immune response in the relationship has been controversial (Lloyd and Soulsby, 1985). The evidence most often cited to support, or refute, the importance of the immune response in man is from epidemiological studies.

Data showing the age distribution of infection, or the acquisition of infection, or re-infection, can be used to look for a possible role for the immune response in resistance (Elkins *et al*, 1986). However, it is important to remember that the immune response may operate against worm growth, or fecundity, as well as being important in initiating worm expulsion.

The role of antibody in the response against infection with *A. lumbricoides* has been studied in several situations. Jones (1977) studied three populations infected with *Ascaris* in Papua New Guinea. In the population with the highest infection level in childhood (approximately 95%) only about 21% of adults were infected, and there was a negative correlation between the antibody levels measured by haemagglutination and eosinophil levels; antibody levels in the adults were much higher than in adults from populations with a lower childhood intensity of infection. He suggested

that continuous reinfection occurred in adults with low egg counts and high antibody titres, but the worms did not establish because these people had developed immunity.

Johannson *et al* (1968) were the first to report the increase in IgE levels characteristic of *A. lumbricoides* infection in man. They observed infected Ethiopian children had 15-20 fold increases in IgE, 2 fold IgG, 5 fold IgD and that IgA and IgM levels were not increased significantly. It has been suggested that migrating larvae stimulate classes of antibody other than IgE (O'Donnell and Mitchell 1980). In contrast Phills *et al* (1972), observed four patients accidentally infected with *A. suum* and found that only two had increased IgM and IgE responses, and in these the clinical symptoms were most marked.

Most of the IgE found in infections with *Ascaris* and certain other helminths is not directed against parasite antigens, as was demonstrated by Turner *et al* (1979). These authors investigated the specificity of serum IgE in individuals with high levels of total serum IgE from two populations; non-parasitised Australian subjects of European descent naturally sensitised to environmental allergens, and patients from Papua New Guinea infected with *Ascaris* and/or hookworm. A significant proportion of the IgE in the non-parasitised individuals was specific for common inhalant antigens (Gleich and Jacob, 1975). In parasitised individuals, although specific IgE antibodies to helminth allergens were demonstrated, their absolute level was low and it was not

possible to demonstrate any parasite antigen specificity for the bulk of the IgE. This study clearly shows that the regulation of IgE production in parasitised individuals differs from that in atopic patients.

It has been demonstrated that giving repeated doses of *A. suum* eggs to pigs can render them immune as demonstrated by fewer larvae in the lungs after a challenge infection (Roberts 1934, Naumycheva and Malakhova 1958, Taffs 1964, Kelley and Nayak 1964; Bindseil, 1971). Benkova (1982), showed protection in terms of the number of migratory larvae, could also be achieved using antigen prepared from larvae by ultrasound treatment, and soluble antigen from adults. This protection can be transferred with hyperimmune serum and colostrum (Kelley and Nayak, 1964;1965). The serological response in pigs is bi-phasic with the first response stimulated by the tissue stages and the second by the gut stage. There is also evidence that *A. suum* in pigs and *T. canis* in dogs may be expelled in a self-cure reaction (Taffs 1964, Fernando 1968).

Iv: The immune response against nematodes in animal models.

Most of the information available on the immune response to helminths comes from work involving rodents. In general helminth infections cause an increase in IgE, IgM and IgG antibodies, a T lymphocyte response, and eosinophilia. Nematodes of the small intestine also produce an increase in IgA levels, basophilia, proliferation of the mast cells associated with the intestinal mucosa and an increase in the

number of goblet cells on the intestinal villi (Ogilvie and De Savigny, 1982). The production of anti-parasite antibodies including IgE and the increase in accessory cells is T cell dependent, and the presence of T cells seems essential if resistance is to develop (Ogilvie and De Savigny, 1982).

Helminth infections are often associated with marked intestinal inflammation, reflected both in cellular changes in the mucosa itself and in altered physiochemical conditions in the intestinal lumen. It is thought that in primary infection the immediate cause of worm expulsion lies with inflammatory changes in the intestine (Wakelin, 1978).

The importance of accessory cells in worm expulsion was first demonstrated by Wakelin and Donachie (1980) using mice infected with *Trichinella spiralis*. Their work showed that sensitised lymphocytes taken from strong or weak responder strains of mice could function equally well in mice with a strong responder genotype, but were unable to accelerate worm expulsion in mice with a weak responder genotype. Experiments using irradiation and reconstitution have shown that expulsion of *N. brasiliensis* and *T. spiralis* occurred only in animals reconstituted both with immune lymphocytes and bone marrow cells.

Several non-lymphoid populations are present in inflamed mucosa, the two most important being the mast cell-basophil series, and eosinophils. *In vitro* studies have demonstrated

that eosinophils, in collaboration with antibody against the surface of the nematode, can kill larval stages of the parasite (Ogilvie *et al*, 1980). There is also some evidence that eosinophils may inhibit the establishment of larval nematodes *in vivo*. The antibodies required for the killer cell role of eosinophils may be either IgG or IgE. These cells are known to release a variety of factors such as enzymes and prostaglandins which have been considered as possible effectors of worm expulsion, either directly by causing worm damage, or indirectly by altering the intestinal environment (Wakelin, 1978). Levels of these enzymes do increase in intestinal helminth infections and peroxidases from lamina propria cells are known to kill *T. spiralis in vitro* (Castro *et al*, 1975), but there remains no direct evidence for eosinophil involvement in expulsion. Indeed in at least one case, *T.spiralis* infection in mice, expulsion is unaffected by eosinophil depletion (Grove *et al*, 1977). Macrophages may have a similar function to eosinophils but utilise either IgE or IgM antibodies (Ogilvie *et al*, 1980).

There has been much interest in whether expulsion from the gut involves mast cells or basophils. By introducing biologically active factors or their precursors directly into the gut, it has been shown that amines derived from basophils induce the expulsion of *Trichostrongylus colubriformis* from guinea pigs, and prostaglandin E induces expulsion of *N. brasiliensis* in rats (Rothwell *et al*, 1974; Richards *et al*, 1977).

The intestinal mucosal mast cells that differentiate and proliferate in response to parasitic infections are histochemically, biochemically and functionally distinct from mast cells widely distributed in other tissues (Jarrett and Haig, 1984; Befus, 1986). Mast cells are important in both the immediate and late phase of allergic reactions, but also in the generation of delayed-type hypersensitivity (Askenase *et al*, 1983). Histamine has been shown to be an important factor in immunoregulation and prostaglandins and serotonin from mast cells have immunoregulatory functions (Beer and Rocklin, 1984). In rodents infected with *N. brasiliensis*, resistance was expressed in animals in which the mast cell population had been ablated by irradiation or did not occur because of a genetic defect in the host. The present indication is, therefore, that a primary infection is terminated by a mechanism not involving mast cells, but immunity to re-infection may involve a local anaphylactic response. Evidence has been obtained that parasites invading the intestine of a resistant rodent may be trapped in the mucus blanket and rapidly expelled before they are able to establish in the gut (Lee and Ogilvie, 1981; Miller *et al*, 1981). It is possible that this mucus discharge is triggered by an anaphylactic reaction. If inflammatory changes do cause worm expulsion, in an essentially non-specific fashion, the changes induced by immune responses against one species of intestinal parasite should effect other species present concurrently, and several studies have shown this to be the case (Wakelin, 1978)

Immunity against helminths has been transferred with immune serum, but it has proved difficult to correlate protection with circulating antibody levels (Wakelin, 1978). Protective roles have been described for IgG and IgM antibodies (Wakelin, 1985) but the most important antibodies are thought to be those at the mucosal surface. In intestinal parasitic infection increased levels of IgA have been detected in the serum, milk, intestinal secretions and bile (Befus and Bienenstock, 1984) but specific anti-worm IgA has been identified rarely. However, IgA is known to be involved in the immune response against *Taenia taeniaeformis* (Wakelin, 1978), *N. brasiliensis* (Sinski and Holmes, 1977; Jones *et al*, 1970), *H. contortus* (Duncan *et al*, 1978) and *Trichinella spiralis* (Despommier *et al*, 1977).

Anti-worm antibodies may not cause expulsion directly, but perhaps by metabolic interference, may render worms susceptible to attack by subsequent components of the response. Immune suppression of serum recipients prevents worm expulsion even though the worms show reduced growth and decreased reproductive potential. In two experimental systems, *N. brasiliensis* and *T. spiralis* infections in mice, it has been shown that such worms can be expelled only if the irradiated host is reconstituted with the appropriate cell populations (Wakelin, 1978). This has been interpreted to mean that two steps are involved in worm expulsion.

The most striking antibody response against intestinal nematodes is IgE production, both specific anti-worm IgE and

non-specific potentiation. Whether IgE contributes to worm expulsion is a matter of dispute, but it cannot be obligatory as spontaneous cure can occur in its absence and its presence does not guarantee expulsion (Jarrett *et al*, 1969).

Heavy *Ascaris* infection in the mouse induces an acute self-limiting disease, and during the second week mice often exhibit signs of infection such as inactivity, ruffled coat and weight loss correlated with the pneumonitis and bronchial pneumonia induced by larval migration and tissue damage (Bindseil 1970, Sprent and Chen 1949). During the second week of infection there is a 10-20 fold increase in serum IgM levels, paralleled by an IgM antibody response to helminth antigens with phosphorylcholine determinants. During the third week, IgG levels are increased and homocytotropic antibodies (measured by passive cutaneous anaphylaxis) to helminth antigens were detected; reaginic antibodies are also detected at low levels during the second week of infection. During the course of this infection there is a definite but selective immunosuppression (Crandall and Crandall 1971; Crandall 1976). Primary resistance to *Ascaris* is reputed to be T lymphocyte dependent (Bindseil 1971). The relative importance of cell mediated immunity and antibody in natural and acquired resistance is uncertain. In 1977, Brown *et al*, looked at the antibody production in two substrains of inbred mice infected with *A. suum*. These authors demonstrated a possible link between reduced levels of IgG1 and IgE antibody levels and acquired resistance to *Ascaris* infection.

Immunity has not been successfully transferred from infected mice to recipients with cells (Crandall 1965, Mitchell *et al* 1976). Guinea pigs, however, have been passively protected either with serum or cells from hepatic, mesenteric or mediastinial lymph nodes, but not with spleen cells from infected animals which appear to contain a population of suppressor cells that enhanced the infection in recipients (Khoury *et al* 1977).

The adult worms of most nematode parasites and the larvae of *T. canis* often seem to resist immunity and persist in the host for long periods. The available evidence suggests that these worms are immunogenic but survive despite the induction of an immune response. Studies in mice have shown that nematodes such as *Nematospiroides dubius* and *Ascaris* induce a degree of immunosuppression in the host, but direct evidence that immunosuppression contributes to the longevity of these infections is lacking (Ogilvie and De Savigny, 1982). In the case of strongyloidiasis in man, the reverse is true; immunosuppression of the host may result in an overwhelming and often lethal infection (Ogilvie and De Savigny, 1982). T suppressor cells or suppressor macrophages are also induced during the course of many types of infection. For example, during infection of guinea pigs with *Ascaris* (Khoury *et al*, 1977). T suppressor cells have been shown to cause the overwhelming, uncontrolled infections that invariably result in BALB/c mice infected with *Leishmania tropica* (Howard *et al*, 1980). Mechanisms other than direct suppression may affect immune activity in parasitic

infection. For example, in experimental trichinellosis, antigenic competition has been suggested to explain the depressed antibody response to heterologous antigens (Lubiniecki and Cypess, 1975), and agents toxic to lymphocytes have been found in the helminth and in the host sera during infection (Faubert and Tanner, 1975).

There are several situations in which suppressor cells or immuno-depression may be induced. Several nematodes infect young animals either *in utero* or via the colostrum and when infected early in life their immune response to the parasite is likely to be impaired permenantly. Normal spontaneous cure might fail to take place in young animals, and there is evidence from experimental studies with *N. brasiliensis* that this failure involves a deficiency in the lymphoid mediated component. A similar lymphoid deficiency has been suggested for the failure of immunity against *N. brasiliensis* and *T. muris* in lactating rodents. The depression of immunity in sheep and cattle during lactation leads to the contamination of pasture with infective stages at a time when parasite-susceptible young hosts are available. The failure of immunity in young hosts leads to heavy infection and may render control by vaccination impractical.

There seems to be no clearcut protective immunity in several host parasite relationships. However, the immune response is involved in limiting the intensity of infection and re-infection. A variety of explanations could be proposed for the lack of sterile immunity; the parasite as a result of

evolutionary adaptation may show reduced immunogenicity, it may actively suppress immunity against itself, or a percentage of the host population may be incapable of mounting a completely protective response. This later possibility would explain the overdispersion of the parasite within the host population, whereby children and heavily infected individuals could be regarded as low immunological responders.

Genetic control of the immune response

Ivi; Innate resistance

The effects of host genetics on the outcome of infection have been documented for a number of years (Ackert, 1942). The host response against invasion by parasites is a complex process involving several pathways, therefore, genetic control may be present at more than one stage. For example, host genetics may be important in resistance to a primary challenge, susceptibility to the pathological effects of infection, and the resistance to subsequent challenge infections. Genetic control can be exerted by single genes or by a number of genes working in unison (polygenic control).

Initial invasion by a parasite is influenced by innate immunity, i.e., the host's in-built ability to protect itself from invasion by pathogens. Single genes have in some cases been shown to affect the susceptibility of the host to primary helminth infection, for example *Hymenolepi.. citelli* in the deer mouse (Arnason et al, 1986; Wassom et al, 1974). It seems more usual, however, for susceptibility to primary infection, e.g. the number of adult worms that become established, to be under polygenic control. This is the case for *Schistosoma mansoni* in mice of different genetic constitutions (Fanning and Kazura, 1984) in which some strains of mice have up to twice as many adult worms as other strains, if a low infective dose of cercariae is used.

One of the most studied and best understood systems for looking at variation in host response is the infection of mice with *T. spiralis*. Mice provide a good model system for studying genetic control because of the wealth of information available on the genetics of these animals, and the ease with which breeding experiments can be performed. With the *T. spiralis* model, variation exists in all the parameters studied so far, but these variations are quantitative, in that all strains of mice, with the exception of some mutant strains, expel the intestinal stage of infection by means of an immune response (Wakelin, 1985). This genetic control over the outcome of infection with *T. spiralis* is exerted by a number of genes affecting different stages in the response (Wassom et al, 1984).

Genetically determined differences in the kinetics of the antibody response, particularly complement fixing isotypes, have been proposed as the basis for mouse-strain dependent differences in resistance to the larval stages of *T. taeniaeformis* (Mitchell *et al*, 1980). The early larval stages are susceptible to complement mediated attack, but later become resistant by virtue of anti-complement activity. Resistant strains of mice, for example, C57BL/6, develop antibody early enough after primary infection to prevent larval maturation; mice susceptible to primary infection for example C3H, do develop protective antibody but much later, therefore, they can resist a subsequent challenge.

Nematospiroides dubius infections in laboratory mice are characterised by chronicity. In many strains of mice, primary infections, which can exceed 8 months, are little affected by host immunity, although some strains do expel adult worms after a period of time. Under normal conditions, therefore, it can be assumed that genetic variability within host populations does not seriously interfere with the survival of the parasite and its ability to reproduce. In the mouse system there is an association with pronounced immunodepression against both homologous and heterologous antigens (Behnke *et al*, 1983). Recent findings suggest that there is strain variability in the effects exerted by *N. dubius* upon the immunological competence of the host, some strains being immunodependent to a greater degree, which may reflect mouse strain variation in the ease with which suppressor cells are induced (Pritchard *et al*, 1984).

Ivii: Acquired Resistance

In *T. spiralis* infection in mice a single gene, Ihe-1, controls the ability to express the rapid expulsion response after challenge (Bell et al, 1984). Differences in antibody recognition patterns have also been established in mice infected with *T. spiralis*; mice that show a greater overall level of resistance recognise surface antigens of the parasite more rapidly than poor responders (Jungery and Ogilvie, 1982). Because of the complex interrelationship between immune and inflammatory responses in the intestine, it is difficult to distinguish primary and secondary

phenomena, but it is important to note that several other parameters of intestinal inflammation, such as changes in permeability, villous architecture, and lymphoblast homing, have been identified as strain-dependent variables in the infected mouse (Manson-Smith *et al*, 1979). Variations within parameters of resistance in the intestine may operate independently, reflecting the ability of different components. Thus mice which can expel adult *T. spiralis* only slowly may nevertheless control worm fecundity more efficiently than some rapid responder strains (Wassom *et al*, 1984a).

Dipetalonema viteae is a parasite of the jird. Mice cannot be infected by exposure to infective larvae, but it is possible to implant adult worms subcutaneously and these worms will survive, reproduce and establish a patent infection. Under these conditions, strain-dependent variation in microfilaraemia is readily apparent, being reflected in the duration and level of microfilaremia (Haque et al, 1980; Storey *et al*, 1985). The differences between inbred strains of mice are independent of the survival time of adult worms, as implanted females die at approximately the same time in all strains of mice, and of non-immunological, structural, and physiological characteristics of the host, as response phenotype is reversed in radiation chimaeras given bone marrow cells from donors of the opposite phenotype. The immunological basis for resistance is not fully analysed but seems to involve IgM antibodies with specificity for the

microfilarial cuticle. The inability to produce such antibodies in BALB/C mice or in CBA/N mice probably results in prolonged microfilaraemia.

Serological responses, against *D. viteae* in the mouse, are strain dependent, but within each strain of mouse there is independent variation in the development of the antibody response. This independent variation of antibody responses to each biochemically defined antigen on the surface of microfilariae of *D. viteae* is very similar to the variation observed in the murine response to defined antigens of *T. spiralis* (Almond and Parkhouse, 1986). Such complex variation could, therefore, be a feature of murine humoral immune responses to nematodes, and this may extend to the level of the epitope as witnessed by comparing the IgM and IgA responses. Resistance was associated with IgM antibodies but there is no association between isotype and antigen, which may explain this. The final possibility is that some additional component of the immune response is operating in conjunction with IgM to eliminate microfilariae in resistant mice, for example, a particular cell or cell subset is activated (Almond et al, 1987).

In *D. viteae* infection in mice, the ability to respond rapidly, in terms of microfilariae clearance is inherited as a dominant trait (Wakelin, 1985). This responsiveness in F_1 hybrids has also been reported for several other nematode infections, including *T. spiralis, N. dubius, T. muris* and *T. taeniaeformis*.

The first gene identified for its effects on controlling the early response of mice to visceral infection, following i.v. inoculation of *Leishmania donovani* amastigotes, was the Lsh gene located between Idh-1 and In on chromosome 1. The resistant allele, transposed onto a C57BL10 background, suggested that this gene controls some form of T cell-independent macrophage activation process, which appears to be triggered by Lps-like molecules in the parasite surface and does not involve production of reactive oxygen intermediates, as the effector mechanisms for resistance. The outcome of infection with *L. donovani* is also influenced by genes at two other loci, genes linked to the MHC and the Sc1 gene, located on chromosome 8 (Blackwell 1983).

I viii: Genetic control of the pathology of infection

Genetically determined differences in pathology are visible in several parasitic infections, for example, in schistosomiasis, immunopathologic responses appear to be under distinct control. Several studies have shown that inbred mice vary considerably in the degree of pathology associated with egg deposition. For example, with *S. japonicum*, hepatic fibrosis was 3-4 times more severe in ICR mice than in C57BL/6 mice (Cheever *et al*, 1983). A similar variation occurred in granuloma size, but there was little correlation between the two parameters. Marked individual variation in pathology is observed in human populations, infected with schistosomes or filarial nematodes (Cohen and Warren, 1982) and both genetic and

immunological correlates have been described (Ellner *et al*, 1981; Dean *et al*, 1981). It has been shown with *S. mansoni* that strain variability in the level of resistance, induced by a 12 week infection, ranging from 14% to 95%, correlated closely with portal blood pressure elevation and the number of egg granulomata in the lungs (Dean *et al*, 1981). The rank order of strains used in this work was closely similar to that obtained when immunity was elicited by infection of BCG (Civil and Mahmoud, 1978). This suggests that a genetically determinded ability to respond to inflammatory agents might be important in determining levels of resistance and may imply that in chronically infected mice killing of challenge worms in inflammatory foci as well as diversion of migrating stages are both involved.

Iix: The implications of genetic control on vaccination against parasite infections

Genetic differences in response against infection must be considered when designing vaccination programmes and producing vaccines. If the population varies in its ability to respond in a protective way against the parasitic antigens, then control by vaccination may not be possible. Variation in the response of different strains of mice, to vaccination with irradiated larvae of *S. mansoni* has been studied, and distinct MHC-linked control of response has been demonstrated (Sher *et al*, 1984). Failure to achieve successful vaccination may imply some defect in antigen recognition or in a non-immunological,

perhaps inflammatory component required for effective resistance. A converse situation holds for mice infected with *T. taeniaeformis*, in which failure to show resistance to infection arises from a delay in antibody production, and can easily be corrected by vaccination (Mitchell *et al*, 1980).

The most common form of genetic control on the outcome of parasitic infection is a combination of the effects of a number of genes. One of the most frequently involved loci is the major histocompatability complex (MHC). This group of genes is known to affect the immune response and MHC-linked control is frequently apparent in helminth infection.

Ix: The major histocompatibility complex

The major histocompatibility complex (MHC) is a group of closely related genes clustered on one chromosome in a species. These genes were first identified by transplantation studies and are the body's means of identifying self and non-self. The proteins encoded by MHC genes act as regulatory factors for the activation of T cells and the expression of these molecules may play a role in the intrathymic development of the T cell repertoire. MHC genes can be divided into distinct categories, the most important of which in terms of T cell activation are Class I and Class II genes. These genes differ in their tissue distribution and function (Klein, 1982; Williamson and Turner, 1987) and there is a great deal of polymorphism within Class I and Class II alleles.

The proteins encoded by Class I genes are found in all somatic tissues to various degrees, with the most concentrated expression being found in cells of the immune system. Class I molecules are composed of one heavy glycoprotein chain, anchored in the cell membrane, and one light protein subunit. The predominant function of Class I molecules is the regulation of cytotoxic T cell (CD8+ in humans, Lyt2+ in mouse) proliferation. Class II molecules are expressed on B and T lymphocytes, macrophages, some epidermal and epithelial cells and other cell groups depending on their stage of activation and the effects of lymphokines. These Class II molecules

are glycoproteins consisting of an alpha and a beta chain and their function is the control of regulatory T cells (CD4+ in humans, L3T4+ in mouse). Class II molecules may also be involved in the regulation of cytotoxic T cells in some instances. Class II molecules are expressed on antigen presenting cells, and T cells recognise a combination of antigenic peptide and Ia (Rosenthal and Shevach, 1973; Schwartz, 1985: Brown *et al*, 1988; Davis and Bjorkman, 1988).

In mice the MHC, or H-2, is located on chromosome 17. Class I genes are coded for by two loci, K and D, which map to opposite ends of the H-2. The Class II loci occupy a region between the Class I loci, (see Figure 2). Polypeptides coded for by the Class II A-alpha and A-beta loci combine to form functional I-A molecules, and likewise with E-alpha and E-beta. The region containing the A and E loci is known as the I region and the genes located in this region were originally characterised as immune response (Ir) genes, because of the effects they have on immune responsiveness. All strains of inbred mice express I-A molecules but some strains do not express functional I-E molecules on the cell surface (Mathis et al, 1983). Specific I-A and I-E molecules have been identified as the crucial restriction elements in the response to certain pathogens (Wassom et al, 1987). The molecular composition of the human MHC, HLA, located on chromosome 6,and the rat MHC, RT1, located on chromosome 14, are comparable to that in the mouse (see Figure 2).

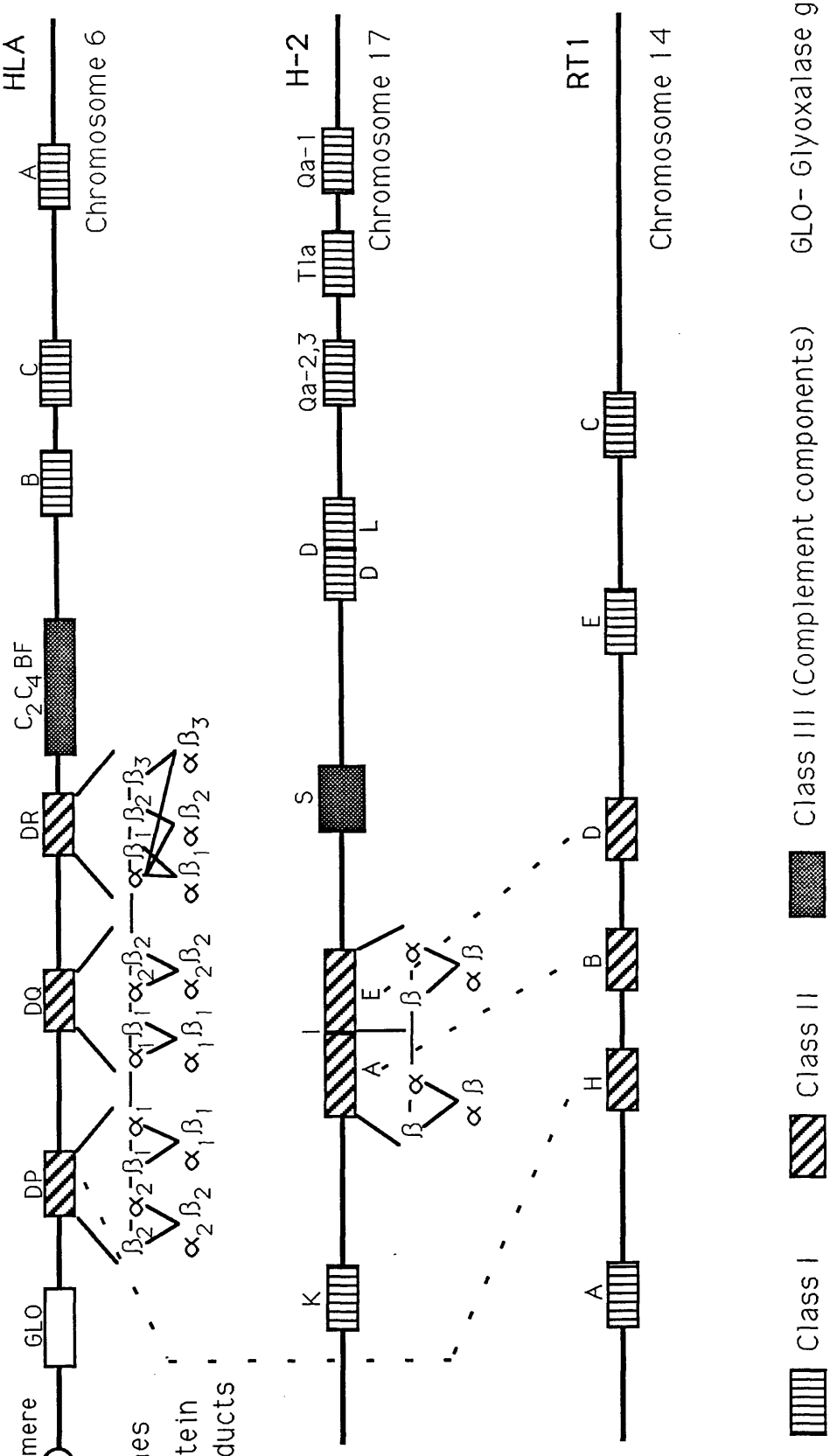

HLA
Chromosome 6
Centromere
GLO
DP
DQ
DR
B
C
A
C_2 C_4 BF
Genes
Protein products
H-2
Chromosome 17
K
I
S
D
L
Qa-2,3
Tla
Qa-1
RT1
Chromosome 14
Class I
Class II
Class III (Complement components)
GLO- Glyoxalase gene

Figure 2

Genetic control of immune responsiveness by MHC was first shown by McDevitt and Chintz (1969) and over the last two decades a large volume of literature on MHC control of immune responsiveness has accumulated. H-2 linked genes have been found to affect the response of mice to a variety of infectious organisms (Wakelin and Blackwell, 1988), perhaps the best understood genetic control of a host parasite relationship is that of mice infected with *T. spiralis*. In this host-parasite relationship H-2-linked genes affect the host response in terms of resistance to primary and secondary infections, worm expulsion and worm fecundity (Wassom et al, 1979; Wakelin and Donachie, 1983; Wakelin, 1985). These H-2 effects can also be monitored *in vitro* by the level of proliferative responses in lymphocyte cultures (Wakelin, 1985).

In *T. spiralis* infection in mice, H-2 control has been associated with 2 alleles; Ts-1, located in the A-beta locus and Ts-2, located between the S and D regions (Wassom et al, 1984b). Ts-1 is thought to act as an Ir gene, affecting the reponse of lymphocytes to parasite antigens. Ts-2 is thought to affect T lymphocyte interactions in the control of an inflammatory response in the gut. Genetic defects expressed at the level of secondary T cell populations have been demonstrated in other systems (Strassman *et al*, 1980). Under such circumstances non-responders produce activated Th cells but lack the subsequent T cell population, therefore, animals sharing the same H-2 haplotype would not

necessarily have the same phenotype for an H-2 linked response.

In *T. spiralis* infection resistant strains of mice, (those expressing H-2 b, s, f and q), only express I-A gene products and, consequently, antigen presentation and T cell interactions are mediated by I-A products (Wassom et al, 1987). These authors have proposed that antigen presentation in the context of I-E molecules may preferentially induce Ts cells. The I-E region has also been implicated as controlling the IgE response against *S. japonicum* in mice (Kaji et al, 1983).

Despite the clear H-2 effects on immune response, infection with *T. spiralis* is also regulated by non-H-2 linked genes (Wakelin and Donachie, 1981). Several effector mechanisms are active in the response against this infection and some of these mechanisms are known to function independently (Wassom et al, 1984a), it is therefore possible that each mechanism is controlled by different gene groups. Genetic control of immune responsiveness can be mediated by bone marrow derived cells and extrinsic factors (Wakelin and Donachie, 1981).

MHC linked influences have been demonstrated in a number of other host-parasite relationships, for example, mice infected with *S. mansoni* exhibit H-2 linked effects on the immune response (Claas and Delder, 1979). These H-2 associated differences can also occur in mice vaccinated

with irradiated cercariae of *S. mansoni* (Sher et al, 1984), where H-2^{b} and H-2^{d} haplotypes are associated with higher levels of immunity than H-2^{k} or H-2^{q}. Ir genes have also been associated with the degree of pathology seen in schistosome infections in mice (Cheevar et al, 1984), and the T cell response against *S. japonicum* (Kaji et al, 1983). In mice infected with *S. mansoni* both H-2 linked and non-linked genes have been shown to influence the humoral response against discrete parasite antigens (Kee et al, 1986).

Studies on *Leismania* infection of mice have revealed a complex pattern of control involving several genes at different loci (Blackwell, 1985). There are H-2 linked effects on the response against this parasite but non-H-2 linked genes play an equally important role, and the presence of a particular H-2 haplotype is not sufficient to predict the outcome of infection. The importance of H-2 linked genes on antibody specificity has also been demonstrated in mice infected with *M. tuberculosis* (Ivanyi and Sharp, 1986).

In rats, the MHC (RT1) has been shown to influence the immune response against a range of antigens (Gunther et al, 1973; Inomata et al, 1983). In rats infected with *Streptococcus mutans* three distinct groups are observed, high, intermediate and low responders (Niyama et al, 1987). These differences are thought to be controlled by

genes located at the RT1.D locus which influence antigen presentation and consequently T cell proliferation.

Several studies have examined the possible association between HLA and resistance to infection. The level of T cell proliferation against antigens from *S. japonicum* has been linked to particular HLA alleles (Sher and Scott, 1982; Sasazuki *et al*, 1980) and this type of association has also been found in T cell responses against antigens of *Streptococci* (Sasazuki et al, 1985) . Family studies of patients with leprosy demonstrated a link between HLA and both the tuberculoid and the lepromatous forms of the disease (DeVries *et al*, 1976; Fine *et al*, 1979; Van Eden *et al*, 1980, 1985).

The heterogeneity in the level of infection within a host population and the variation in the efficacy of the immune response mounted against the parasite are well documented phenomena. The work presented here has examined the antibody repertoire produced during infection with *Ascaris*. Parasite-specific IgG and IgE antibodies may play an important role in immunity and therefore, the specificity of these antibodies and the genetic control of this antibody response is examined. IgE antibodies may also be involved in the clinical symptoms associated with infection, and particular attention is focused on the genes controlling this response in a rodent model. Finally, the antigen recognition profiles of individuals living in *Ascaris* endemic areas were also examined, to

compare the recognition profiles produced in natural infection with those found in the rodent model, and to examine the possible relationship between antibody titre and age, or antibody titre and level of infection.

Materials and Methods

PARASITES

Fresh adult *Ascaris suum* worms were obtained from the intestines of infected pigs at a local abbatoir. Eggs were removed from the uteri of females and suspended in a solution of 5% sodium hypochlorite (NaOCl, 10-14% w/v chlorine, BDH, 30169), to dissolve excess tissue and sterilise the egg suspension. Eggs were then washed, a minimum of 8 times, in deionised water and embryonated in a solution of 2% formalin (37-41% Formaldehyde, BDH) and 125 units/ml of Nyastatin (Nystan, Peadiatric Oral Suspension, SquiBB) at 25°C for 40 days and stored at 4°C until used. Embryonated eggs were treated with 35% NaOCl solution, until microscopic examination showed removal of most of the shell. Following thorough washing by centrifugation in deionised water, the infective second-stage larvae (L2), were hatched by disruption of the remaining shell layers in a glass tissue homogeniser. The larvae were separated from shell fragments by allowing them to migrate overnight (O/N) through a cotton wool plug, into defined serum-free medium, at 37°C, as described by Kennedy and Qureshi (1986). This *Ascaris* culture medium (see appendix) was adapted from Stromberg *et al* (1977) and Urban and Douves (1984).

The lung-stage larvae were recovered from the lungs of infected rabbits (New Zealand White, Interfauna UK. Ltd.) seven days after an oral infection with 50,000 eggs. The larvae were separated from chopped lung tissue by migration through a 250 micrometer sieve (Endecotts)

washed twice in phosphate buffered saline (PBS), then twice in *Ascaris* medium. Larvae were then separated from any remaining debris by migration through a cotton wool plug into *Ascaris* medium, at 37°C. The larvae recovered are undergoing moulting at this point, and are a mixture of third- and fourth-stage larvae (L3/4).

The perienteric fluid of fresh adult worms (ABF) was collected, on ice, by cutting the end of the worm and draining out the fluid. The ABF was then dialysed against PBS and stored at -70°C, or stored without dialysing.

Ascaris lumbricoides worms were collected by Dr. M. R. Haswell-Elkins and Dr. D. B. Elkins, Imperial College, London, by mass chemotherapy using pyrantel pamoate (Mexin Pharmaceuticals, Bombay) at 10mg/kg body weight in Tamil Nadu, India. Adult female *Ascaris* worms were recovered from fæces passed within the first 24 hours (h) after treatment. The worms were rinsed with water, placed in 4% formalin, on ice and dispatched, by airfreight to Glasgow within 24h. Transit time approximately 7 days.

Adult worms were also provided, from St. Lucia, by Dr. D. A. P. Bundy, Imperial College, London, using a similar protocol to that described above.

EXPERIMENTAL ANIMALS

Mice and rats were initially infected orally at 7-12 weeks of age with 500-2,000 and 1,000-6,000 eggs respectively in an agar/water suspension. Subsequent infections were at approximately 28 day intervals.

Wellcome Labs. for Experimental Parasitology (WLEP) rats have been inbred at WLEP from an outbred Wistar stock for 12 generations.

F_1 HYBRID ANIMALS

F_1 animals were bred at WLEP from stock obtained from OLAC 1976 Ltd. and from WLEP stock.

RABBITS

Rabbits (approximately 3kg, New Zealand White, Interfauna UK Ltd.) were orally infected with 50,000 eggs, and the lungs removed 7 days later.

ANTISERA

Blood samples were collected by cardiac puncture either from a group of at least 3 animals, or from individual animals, before infection, 28 days after a primary infection and 14 days after subsequent infections. The blood was left to clot, then held either O/N at 4°C, or for 30 min at 37°C. The supernatant was removed and spun at 3,000 rpm for 10 min. The serum was then aliquoted and stored at -20°C. Subsequent freeze-thawing was kept to a minimum.

Human sera were transported from the Caribbean on ice to London where it was stored at -20°C. It was subsequently transported to Glasgow on ice and on arrival was mixed with an equal volume of glycerol (May and Baker) and stored at -20°C.

INTRAVENOUS IMMUNISATION

Naive animals were inoculated intravenously (iv) with approximatley 2,000 L2 for mice, or 6,000 L2 for rats in the tail vein at 28 day intervals. Serum was taken 28 days after a primary and 14 days after subsequent infections.

INTRAPERITONEAL IMMUNISATION

Naive animals were inoculated intraperitoneally (ip) with approximately 2,000 L2 for mice, or 6,000 L2 for rats at 28 day intervals. Serum was taken 28 days after a primary infection and 14 days after subsequent infections.

INTRANASAL IMMUNISATION

Mice were exposed at 14 day intervals to aerolised parasite material, approximately 500 microgrammes protein/mouse in PBS, using a Nebuliser (Aerosol Products, Colchester, Ref. No. C-F-18). They were subsequently bled 14 days after a tertiary and subsequent exposures.

INBRED MICE

Name	Abbreviation	Source	Genetic Background	H-2 Haplotype
CBA/Ca	CBA	OLAC 1976 Ltd.	CBA	k
BALB/c	BALB/c	"	BALB	d
DBA/2	DBA/2	"	DBA	d
C57BL/10ScSn	C57BL	"	C57	b
NIH	NIH	"	NIH	q
SJL	SJL	"	SJL	s
C3H/He	C3H	"	C3H	k
BALB/c	BALB/c	WLEP	BALB	d
NIH	NIH	"	NIH	q
CBA/Ca	CBA	NIMR London	CBA	k
CBA/N	CBA/N	"	CBA	k

CONGENIC MICE

Name	Abbreviation	Source	Genetic Background	H-2 Haplotype	H-2 Donor
BALB.B	BALB.B	OLAC 1976 Ltd.	BALB	b	C57BL/10Sn
BALB.K	BALB.K	"	BALB	k	C3H
B10.S	B10.S	"	C57BL	s	A.SW
B10.D2/n	B10.D2	"	C57BL	d	DBA/2
B10.G	B10.G	"	C57BL	q	Gray-lethal linkage stock
B10.BR	B10.BR	"	C57BL	k	C57BL/cd

Name	Abbreviation	Source	Genetic Background	RT1 Haplotype	RT1 Donor
INBRED RATS					
AGUS	AGUS	OLAC 1976 Ltd.	AGUS	l	
AO	AO	"	AO	u	
PVG	PVG	"	PVG	c	
WLEP	WLEP	WLEP	Wistar	u	
CONGENIC RATS					
PVG-RT1^{U}	RT1^{U}	OLAC 1976 Ltd.	PVG	u	AO
PVG-RT1^{l}	RT1^{l}	"	PVG	l	AGUS

Parents Female	Male	F_1	F_1 MHC Haplotype
F_1 HYBRID MICE			
BALB/c	SJL	BALB/c X SJL	d/s
B10.D2	B10.S	B10.D2 X B10.S	d/s
C57BL	B10.S	C57BL X B10.S	b/s
BALB/c	CBA	BALB/c X CBA	d/k
SJL	CBA	SJL X CBA	s/k
C57BL	SJL	C57BL X SJL	b/s
F_1 HYBRID RATS			
WLEP	PVG	WLEP X PVG	u/c
AGUS	PVG	AGUS X PVG	1/c

IMMUNISATION WITH ADJUVANT

Mice or rats were inoculated in two sites on the back with 5 microgrammes of purified 14kDa protein, measured using a Pierce Protein Assay (Pierce, USA), in Freund's complete adjuvant (Gibco, 660-5721). After 28 days the animals were boosted with 5 microgrammes 14kDa in Freund's Incomplete adjuvant (Gibco, 660-5720). After a further 28 days animals were boosted iv with 20 microgrammes of 14kDa protein in PBS and bled 14 days later. A subsequent group of animals were iv boosted 28 days after the primary iv boost and bled 14 days later.

EXCRETORY/SECRETORY MATERIAL

The excretory/secretory products (ES) from L2 were produced by artificially hatching eggs as described, culturing the larvae *in vitro* in *Ascaris* medium, at 37°C and 5% CO_2, and collecting the culture medium at approximately weekly intervals, for 2-3 weeks.

The L3/4 ES was produced from *in vitro* cultures of the L3/4 larvae, collected from the lungs of rabbits 7 days after an oral infection and cultured as for L2, collecting the culture medium at approximately weekly intervals. The L3/4 cultures were maintained until the viability of the larvae fell below 95%.

ANTISERA

Sheep anti-mouse gamma globulin (Scottish Antibody Production Unit (SAPU), Carluke, Scotland, S021-220).

Rabbit anti-*A. suum* infection serum was produced at Wellcome Labs. for Experimental Parasitology (WLEP) by infecting Sandy Lop rabbits with 3,000 viable eggs at 28 day intervals and collecting serum at the peak of the antibody response (see Kennedy *et al*, 1987).

Antiserum to the 14kDa molecule was prepared by immunising Sandy Lop rabbits with approximately 100 micrograms of 14kDa excised from ABF gels (Kennedy *et al*, 1987), in Freund's complete adjuvant (Gibco Labs., 660-5721). Animals were boosted 40 days later with 100 micrograms 14kDa in Freund's incomplete adjuvant (Gibco Labs., 660-5720) and bled on days 12, 14, 17 and 19.

Mouse anti-rat immunoglobulin (Ig) epsilon-chain specific monoclonal antibody, clone designation MARE 1 (Serotec, Oxford, MCA 193).

Goat anti-mouse IgG-Alkaline phosphatase (AP) conjugate (Sigma, A-4656).

Goat anti-mouse IgA, IgM, IgG-AP (Sigma, A-0162).

Rabbit anti-human IgG-AP (Dakopatts, Denmark, D336).

Rabbit anti-human IgA, IgM, IgG-AP (Dakopatts, D342).

Sheep anti-rat gamma globulin (SAPU, S023-220).

Donkey anti-sheep gamma globulin-horse radish peroxidase (HRP) (SAPU, S084-201).

Goat anti-rat IgG-FITC (Sigma, F-6258).

Rabbit anti-mouse-FITC (Sigma, F-7506).

Donkey anti-rabbit-FITC (SAPU).

RADIO-IODINATION

Iodine labelling of soluble ES material (see Kennedy and Qureshi, 1986) was carried out by the IODO-GEN method (Markwell and Fox, 1978). IODO-GEN tubes were prepared by allowing 200 microlitres of a 1mg 1,3,4,6-tetrachloro-3a, 6a-diphenyl glycoluril (Pierce Chemical Co.)/ml chloroform (May and Baker) solution to evaporate O/N, at room temperature (RT), to coat the sides of a 500 microlitre polyethene microfuge tube (Starstedt, 72.690). These coated tubes were stored desiccated at RT. For the labelling 200 microlitres of PBS-dialysed culture medium were incubated in the IODO-GEN tube with 300 microcuries for L3/4, and 100 microcuries for L2, $Na^{125}I$ (West of Scotland Radionucleotide Dispensary, or Amersham International PLC, IMS 30) for 10 minutes (min) on ice with repeated agitation. Excess iodine was consumed by the addition of 10% by volume of saturated tyrosine solution, and labelled macromolecules were separated from ^{125}I-Tyrosine by gel filtration on Sephadex PD 10 columns (Pharmacia, 17-0851-01).

Radio-iodination of antibodies was carried out in a similar method as above with approximately 100 microgrammes of protein added to 300 microcuries of I^{125}.

RADIO-IMMUNOPRECIPITATION

Radio-labelled antigens were immunoprecipitated in a protein-A based assay with serum from various host species and strains, using a 10% suspension of heat-

killed, formalin-fixed *Staphylococcus aureus* bacteria (Pansorbin Standardised: Calbiochem 507861, binding capacity 2.3mg human IgG/ml) as a solid-phase absorbent (Kessler, 1975). To 50 microlitres PBS/0.5% Triton X-100 (Sigma, T-6878) were added 2.5 microlitres of serum and 50-200 x 10^3 counts per minute (cpm) of antigen. I^{125} counts were obtained using a 1280 Ultrogamma Counter (LKB, Wallac). Following O/N incubation at 4°C, 50 microlitres *S. aureus* suspension were added and the mixture incubated for 1h at RT, with occasional shaking, then 1h at 4°C. The bacteria were then washed 3 times at 4°C, with PBS-Triton, the radioactivity in the pellet was measured and the pellet prepared for SDS-PAGE. This method selects for IgG antibody (Reis *et al*, 1984) and, when a broader specificity was required, 10 microlitres of a 1:9 dilution of Sheep anti-mouse gamma globulin (SAPU) was added with the *S. aureus*.

POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

This was carried out in a Pharmacia GE-2/4LS slab gel apparatus according to the manufacturer's instructions. Gradient gels consisted of 120mm separating and 10mm stack gel, and were 0.7mm thick (see appendix). Samples were prepared by mixing to 20 microlitres with deionised water and then 20 microlitres of sample buffer (see appendix) were added, containing 5% mercaptoethanol (Sigma, G-7126) or 1mg/ml iodoacetamide (BDH, 444181) for reducing or non-reducing conditions, respectively, followed by immersion in a boiling waterbath for 10 min.

The samples were centrifuged to remove particles using a microfuge (MSE, Scientific Instruments, 41137-2182) and the supernatant loaded onto gels. Following electrophoresis, gels were fixed for 30 min in a 25% methanol, 10% acetic acid and 1% glycerol (May and Baker) solution and then dried, at 80°C using a slab gel dryer (BIO-RAD, 1125B).

AUTORADIOGRAPHY

Dried gels were exposed to flashed Fuji RX x ray film (Technical Phot Systems, Cumbernauld) using Du Pont Cronex Lightining-Plus intensifying screens (H. A. West, Edinburgh), and the autoradiographs exposed at -70°C, in Harmer x-ray film cassettes (H. A. West, Edinburgh). Molecular weights were estimated by mobility relative (M_r) to standard marker proteins (Pharmacia, 17-0446-01 and Boehringer Mannheim, 750 115).

ELISA

General Protocol

Dynatech 96 well Microtitre plates (M129B) were coated with antigen, in carbonate/bicarbonate buffer, pH 9.6 (see appendix), at 50 microlitres/well. Plates were then covered and incubated for 2h at 37°C, washed 3 times with 0.05% (v/v) Tween 20 (Sigma, P 1379) in PBS (PBS-Tween), dried and used immediately. Appropriate antibody dilutions were then added in PBS-Tween containing 0.25% (w/v) bovine serum albumin (BSA) (Sigma, A-9647), at 50 microlitres/well. Plates were covered and incubated at 37°C for 1h. The plates were then washed 3 times, dried and the enzyme substrate and chromagen (see appendix) were added at 50 microlitres/well, and incubated at 37°C. The reaction was stopped 15-60min later with 3M sodium hydroxide, for alkaline phosphatase reactions or 2.5M hydrochloric acid, for horse radish peroxidase reactions, once the required colour had developed, as shown by positive controls. The optical density (OD) was read at a wavelength of 405nm for phosphatase, or 492nm for peroxidase, using a Titertek Multiskan MC plate reader (Flow Laboratories Ltd., Uxbridge). All plates included known positive and negative control wells.

The optimum antigen and antibody concentrations for each ELISA system were arrived at using the standard checker-board procedure and were as follows:

CONJUGATE	ANTIGEN	ANTIGEN CONCENT. MICROGRAM/ ML	ANTIBODY DILUTION	CONJUGATE DILUTION
Mouse IgG	ABF	1.0	1:400	1:500
	14k	1.0	1:400	1:500
	L3/4	1.0	1:100	1:500
	L2	1.0	1:100	1:500
Mouse IgA, IgG, IgM	ABF	1.0	1:400	1:500
Human IgG	ABF	0.2	1:400	1:500
	14k	1.0	1:400	1:500
	L3/4	1.0	1:400	1:500
	L2	1.0	1:400	1:500
Human IgA, IgG, IgM	ABF	0.2	1:400	1:500

PASSIVE CUTANEOUS ANAPHYLAXIS

Naive adult WLEP rats were injected intradermally, on the back, with 100 microlitre samples of test serum, or dilutions thereof. The animals were challenged 2-48h later, with an intravenous (iv) injection of antigen with 5mg Evans Blue (BDH, 241 152L) in 0.5ml PBS. Approximately 30 min later, the number of blue reaction spots, on the animal's back were recorded. All tests were carried out in duplicate, in two separate animals, and known positive and negative controls were used in each animal. The specificity of this test for IgE antibodies of both rat and mouse has been demonstrated previously (Prouvost-Danon, 1972; Ogilvie, 1964).

PERIODATE TREATMENT OF ANTIGENS

To 3mg of ABF, or 500 microgrammes of 14kDa was added 12ml of 10mM sodium acetate (Sigma, S5636) buffer (pH 5.7), see appendix, containing 0.1M sodium periodate (BDH, 10259). The mixture was incubated at RT, in the dark, O/N and then dialysed against PBS (pH 7.2). The dialysed solution was concentrated to approximately 1ml by ultrafugation using an amicon protein concentrator with a molecular weight cut off at 10kDa (Centricon 10, Amicon, USA, 4206). The protein concentration was measured using a Pierce protein assay (Pierce, USA).

AUTOCLAVE TREATMENT OF ANTIGENS

To 300mg of ABF, or 500 microgrammes of 14kDa was added 6ml of PBS (pH 7.2) and the mixture was autoclaved at

120°C for 20 min. After cooling the solution was concentrated as above and the protein concentration measured.

REDUCTION AND ALKYLATION OF ANTIGENS

To 1ml of Tris-HCl buffer (pH 8.2) containing 30mg/ml ABF, was added dithiotreitol (Sigma, D-0632) to a final concentration of 0.02M. The solution was incubated for 1h at RT. After incubation, iodoacetamide was added, to a final concentration of 0.12M and the pH was kept at 8 for 1h by the dropwise addition of sodium hydroxide. This method results in the reduction and subsequent alkylation of the solution (Hudson and Hay, 1980). The solution was then dialysed and concentrated as above.

WESTERN BLOTTING

SDS-PAGE gels were loaded with 750 microgrammes of unboiled ABF and run as previously described. The protein was then transferred from the gel onto nitrocellulose membranes (Hybond-C extra, 0.45 micron, Amersham International) using a TRANS-BLOT cell (Bio-Rad Labs., 170-3910) according to manufacturer's instructions. The transfer buffer used was Tris/Glycine. 0.1% SDS, pH 7.0 (see appendix). Electroblots were run at a constant 25mA, O/N, at 4°C. After transfer the membrane was air dried and the transferred protein was visualised using either amido black stain (see appendix) (BDH, 44291 2X) or an enzyme-linked detection system (see below).

ENZYME-LINKED ANTIBODY DETECTION SYSTEM FOR WESTERN BLOTS

The nitrocellulose membrane was air dried and cut into strips approximately 1cm in width. these strips were blocked using 10% dried skimmed milk, in wash buffer (Tris buffered saline plus 0.05% Tween, see appendix) 4ml per strip, for 1h, on a rocking table. All subsequent steps involved 4mls per strip on a rocking table.

The primary layer was added at a 1:50 dilution in wash buffer and incubated for 90 min at RT. After this the blot was washed 3 times, for 5min/wash, in wash buffer.

The secondary layer, sheep anti-rat gamma globulin (SAPU, S023-220), was added at a 1:500 dilution in wash buffer and incubated for 60min, at RT. After this incubation the blot was washed as before.

The tertiary layer, donkey anti-sheep gamma globulin-horse radish peroxidase-conjugate (SAPU, S084-201), was added at a 1:250 dilution in wash buffer and incubated for 60 min, at RT. The blot was subsequently washed as before.

4-chlor-1-napthol (Sigma, C8890) was used as the chromagen to visualise the antibody conjugate. The chromagen solution consisted of 48mg of chloronapthol dissolved in 16ml of methanol and added to 80ml of Tris buffered saline, pH 7.2, just before use 32 microlitres of hydrogen peroxide (30% w/v, BDH, 45202) were added, as enzyme substrate. The reaction was stopped after 20-60 min by removing the chloronapthol solution and washing the nitrocellulose with deionised water. The stained blot was then air dried.

PROTEIN DETECTION ON WESTERN BLOTS USING I^{125}-LABELLED ANTIBODIES

Western blots were produced as described previously, but the protein concentration was increased to 4.2mg/gel.

The nitrocellulose was cut into strips and blocked using skimmed milk, as described previously.

The primary layer was added at a 1:10 dilution, in wash buffer and incubated and washed as described previously.

The secondary layer, I^{125} labelled anti-rat gamma globulin, was added at approximately 100,000cpm/track, or I^{125} labelled mouse anti-rat epsilon (Serotec, MCA 193) was added at approximately 250,000cpm/track. The blot was incubated for 60 min at RT and washed a minimum of 5 times in wash buffer, 5 min per wash. The blot was then air dried and exposed with pre-flashed film at -70°C, as described in gel autoradiography.

ELECTRO ELUTION OF 14kDa PROTEIN FROM PREPARATIVE SDS-PAGE GELS

ABF was separated on 1.5mm thick 20% homogeneous gels, with the samples unboiled and laced with radiolabelled ABF. The unfixed gels were dried at 60°C and the 14kDa band excised using an autoradiograph as template. Protein was recovered from the matrix of the gel by electro-elution in a BIO-RAD electroelutor (Richmond, CA, 165-2976), using a 25mM Tris (Boehringer Manheim, 708 976), 192mM Glycine (Sigma, G-7126), 0.1% SDS (BDH, 44244) buffer. SDS was removed by dialysis against methanol, using 2,000 molecular weight cut-off membrane

(Sigma, D 2272), at 4°C for 48h, followed by dialysis against PBS and concentration by ultrafiltration, using an amicon protein concentrator with a molecular weight cut off at 10kDa (Amicon, Centricon 10, 4206).

AFFINITY PURIFIED 14kDa PROTEIN

The affinity purified 14kDa molecule was kindly provided by Dr. J.F.Christie, WLEP. Briefly, the electro-eluted 14kDa was used to immunise rabbits to provide antibody for immunoaffinity chromatography. Protein-A purified rabbit anti-14kDa antibody was bound to Affigel 10 (BIO-RAD, 153-6046) and eluted with a 0.2M, pH 2.3, glycine/HCl buffer.

INDIRECT IMMUNOFLUORESCENCE ON LIVING PARASITE LARVAE

For each serum sample to be tested approximately 0.5ml of larval culture was aliquoted and washed 3 times with PBS-0.1% azide (Sigma, S-2002). After the last wash the supernatant was removed leaving a final volume of 100 microlitres. The serum being tested was then added to result in a final volume of 200 microlitres and an optimum dilution of the sera. The tubes were then incubated on ice for 30 min with occasional resuspension. The larvae were then washed 3 times with PBS-azide leaving a final volume of 100 microlitres and a FITC-conjugated antibody added at the appropiate dilution. The tubes were again incubated for 30 min on ice. After the final incubation the larvae were washed again and as much of the supernatant as possible was removed. The

larval surface was measured by photon counting using a Leitz MPV Compact 2 microscope photometer linked to an Epsom PX 4 computer. The photometer field diaphragm was set to measure a rectangular area, in the range 230 to 555 micrometers square using a Leitz NPL Fluotar x 40 objective. The measured area was kept constant in any one experiment and the selected area did not include edge fluorescence. The fluorescence of one of the brightest specimens (rabbit) was used to standardise the photometer at the arbitary value 650.

The appropiate dilutions for infection sera and conjugates were titrated using a checker board and are shown below.

SPECIES	ANTIGEN	ANTISERA DILTUION	CONJUGATE DILUTION
Mouse	L2	1:10	1:30
	L3/4	1:10	1:30
Rat	L2	1:10	1:30
	L3/4	1:10	1:30
Rabbit	L2	1:30	1:30
	L3/4	1:30	1:30

DATA HANDLING

The data was analysed using an Apple Macintosh and Cricket Graph, Version 1.2 (Cricket Software, Malvern, P.A.).

Chapter 1

Ascaris in a laboratory model

Introduction

Laboratory rodents are commonly used to model parasitic infections, and it has been possible to study parasites of humans and domestic animals in this way. These studies range from immunology (Ogilvie and De Savigny, 1982) to epidemiology (Scott, 1988). Several studies on the immunology of ascariasis have used the mouse as experimental host, and as a model system it is relatively well defined (Crandall and Crandall, 1971; Crandall, 1976; Brown *et al*, 1977).

When mice are orally infected with embryonated eggs, the eggs hatch in the small intestine and L2 larvae migrate, via the liver, to the lungs where they moult to the L3 and L4 stages. After approximately seven days L3 and L4 larvae can be found in the lungs of the infected mouse, as in their natural host (Mitchell *et al*, 1976; Sprent, 1952). The life cycle, however, proceeds no further in rodents (Ransom and Foster, 1919). The murine host is, therefore, exposed to antigens of the infective and tissue-penetrating, migratory stages of the parasite, as is the natural host. When infected with *A. suum*, mice show a marked increase in eosinophils, IgM, IgG1 and IgA antibodies (Crandall and Crandall, 1971), and develop acquired resistance to infection (Bindseil, 1969, 1970; Crandall and Arean, 1965; Lejinka, 1965; Sprent and Chen, 1949; Brown *et al*, 1977).

Mitchell *et al* (1976) compared several strains of mice infected with *A. suum* and found that the C57Bl strain

was relatively more susceptible to a primary infection in terms of the number of worms recovered from the liver and lungs. In subsequent infections, however, all strains studied showed similar levels of resistance. In the same study, hypothymic nu/nu mice did not appear to be any more susceptible to a primary infection, implying that T cells are not involved in resistance to a primary exposure. In subsequent infections, however, nude mice did not appear to have developed any resistance to challenge, implying that T cells were necessary for acquired resistance. The liver has been implicated in resistance to infection with *Ascaris* and *Toxocara* (Kerr, 1938; Sprent and Chen, 1949; Fernando, 1968) and this was supported by Mitchell's study, which suggested that resistance was expressed at the liver, or during migration to the lungs.

There are several possible ways to examine the immune response against an intestinal parasite. One of the most accessible means of monitoring the immune system is to study serological responses. Antibodies may play a direct role in resistance and immunity to parasitic infection (Miller, 1984). Serological studies can also provide information for serodiagnosis and may be a useful means of detecting susceptible individuals in the population. The antibody production stimulated by helminth infection is T cell dependent, hence, antibody production can be used as an indication of T cell activity.

There is growing evidence that the excretory/secretory components of parasites may be important antigens in terms of the immunobiology of the infection. The activity of these antigens in protection has been demonstrated in several studies, involving a number of different parasites (Rickard and Adolph, 1977; Kwa and Liew, 1977; Silverman *et al*, 1962; Rothwell and Love, 1974; Murrell and Clay, 1972). In 1979 Stromberg demonstrated that the L3/4 ES from *A. suum* could protect guinea pigs from a challenge infection. These results support the conclusion that antigens released by the living worm are major determinants of the nature of the immune response. These antigens are presumably secreted by the parasite within the host and hence, along with the parasite surface, provide the most accessible site for immune attack (Ogilvie and de Savigny, 1982). Certainly, laboratory animals infected with *Ascaris*, produce substantial amounts of antibody against these ES components (Kennedy *et al*, 1987).

In this chapter the specificity of the antibody repertoire, in the context of infection with *Ascaris* in the laboratory model is examined. The antigens used are the perienteric fluid of adult worms (ABF), the *in vitro* released antigens of both the L2 and L3/4 larvae (ES) and a purified preparation of a 14kDa molecule. The latter is a major component of both ES and ABF and was later found to be allergenic (see Chapter 3). It has been shown previously that the L3/4 ES is

contaminated with rabbit serum albumin (Kennedy and Qureshi, 1986). These preparations consist of components common to all life-cycle stages, for example the 14kDa molecule and stage-specific components (Kennedy and Qureshi, 1986). Use of the ES antigens has been prompted by the knowledge that they are important in the immunobiology of infection (Stromberg, 1979a; 1979b; Ogilvie and De Savigny, 1982). The antibody repertoire produced in response to infection by various species and strains of host was examined, as were the effects of varying the immunising dose and the route of administration.

Chapter 1.

Results.

***Ascaris* antigens;**

In the analysis of the antibody repertoire to *Ascaris* in the model system, four antigens have been used. The SDS-PAGE profiles of radio-iodinated preparations of which are shown in Figure 3. Of the two ES materials available we have concentrated on the L3/4 ES because this preparation is available in greater concentrations and is more stable than the L2 ES.

The antigenicity of ES in the context of infection;

Serum from rabbits infected with *A. suum* was immunoprecipitated with radio-labelled L3/4 ES to verify the antigenicity of these products (Figure 4). All the labelled components appear to be antigenic to the infected rabbit. The immunoprecipitated antigens were run on SDS-PAGE under reducing or non-reducing conditions. The relative mobilities of three of the antigenic components, 41kDa, 225kDa and 410kDa, were found to alter under reducing conditions. The 225kDa and 410kDa molecules appear to be composed of di-sulphide linked dimers or hetero-dimers; consequently, immunoprecipitation of the native protein, if analysed under reducing conditions, would suggest, incorrectly, that more than one gene product had been precipitated. For this reason all subsequent analyses were performed under non-reducing conditions.

Figure 3; The SDS-PAGE profiles of IODO-GEN I^{125} labelled *Ascaris* antigens, (a) *Ascaris* body fluid (ABF), (b) L3/4 excretory/secretory products (ES), (c) L2 ES, (d) 14kDa, run on 5-25% gradient gels, under non-reducing conditions. Molecular weights were estimated by mobility relative to standard marker proteins (M).

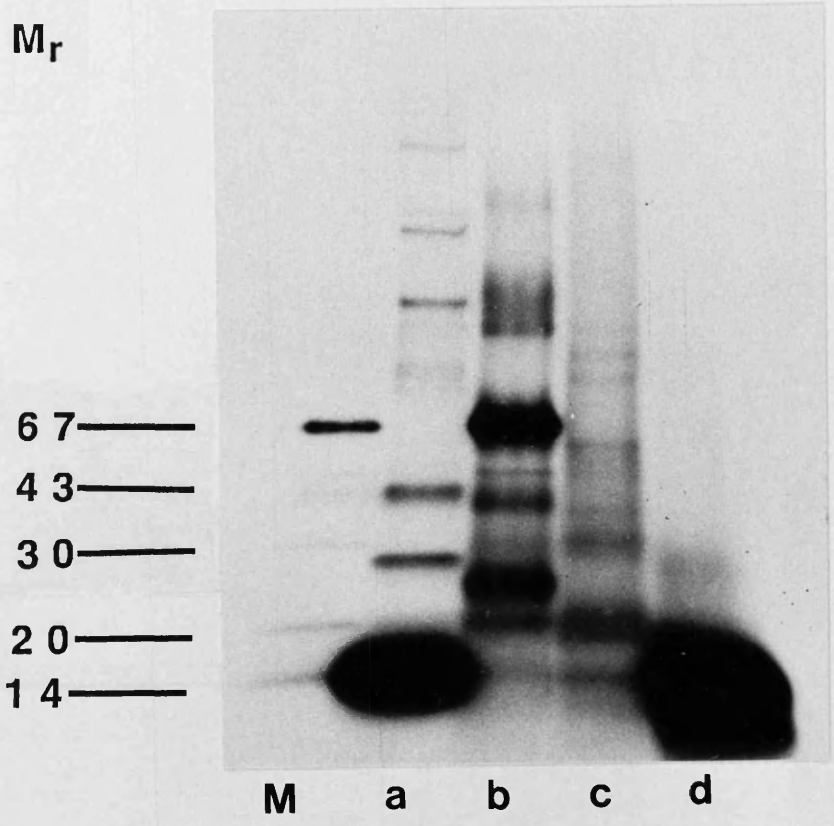

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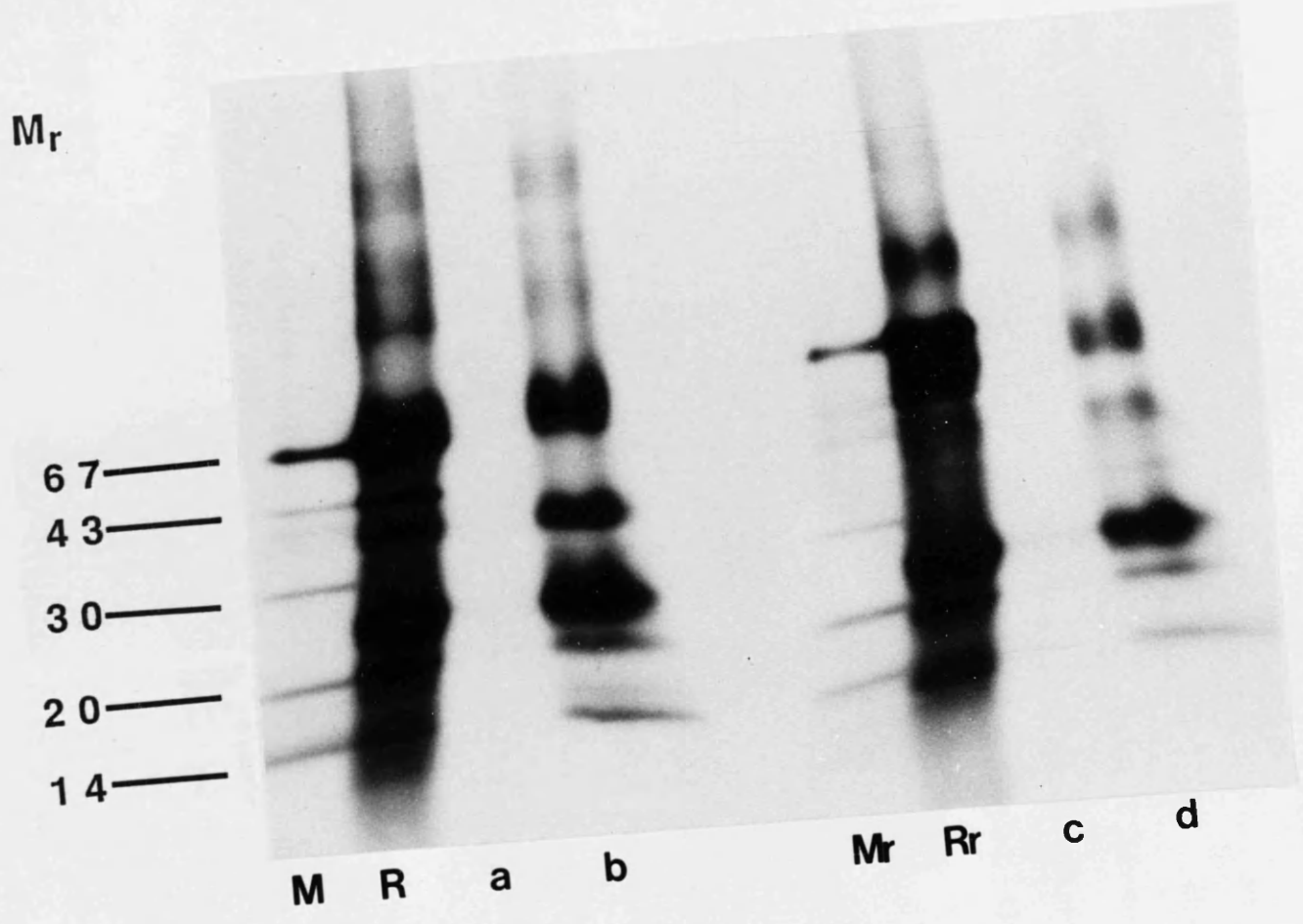
Figure 4; The antigen recognition profiles produced by immuno-precipitating I^{125} labelled L3/4 ES (R), with Normal rabbit serum (a), Rabbit anti-*A. suum* (b), Normal rabbit serum (c), Rabbit anti-*A. suum* (d), tracks a and b are run under non-reducing conditions and tracks c and d are run under reducing conditions.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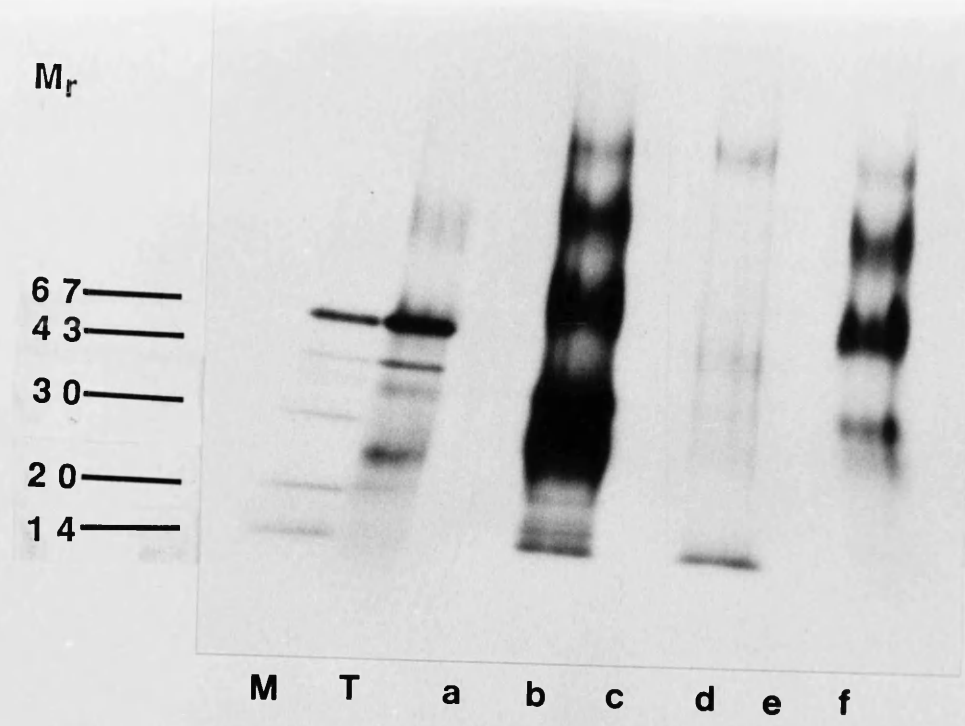
The recognition profiles of three species of laboratory animal;

The antigen recognition profiles of rabbits, rats and mice infected with A. suum are shown in Figure 5. The rabbits (Outbred Sandy lops) recognise all the discernible components of ES and ABF, while rats (Inbred, WLEP) and mice (Inbred, BALB/c) only respond to a subset of these. The molecules recognised vary between the rodent species, the most notable difference being the recognition of the "14kDa" in rats but not mice..

Genetic restriction of antigen recognition;

Given that different species produced different recognition patterns, the responses of a panel of inbred mice and rats were examined. The radio-labelled components precipitated by experimentally infected animals, when screened against L2 ES, L3/4 ES or ABF, are shown in Figure 6-10. An example of the percentage of antigen immunoprecipitated in this analysis is given in Tables 1 and 2. The components precipitated show that all strains of animals examined demonstrated restricted recogniton of *Ascaris* antigens in the context of infection, that is, no strain recognised all the potentially antigenic components. The profiles produced by different inbred strains varied from each other, but strains with the same MHC haplotype produced similar profiles, for example Wistar and AO which are both $RT1^{u}$. Of the 6 inbred strains of mice examined only one, SJL, recognised the 14kDa molecule, one of the major

Figure 5; The antigen recogntion profiles of 3 species of laboratory animal infected with *A. suum*. I^{125} labelled L3/4 ES (**Panel A**) or ABF (**Panel B**) were immuno-precipitated with Normal rabbit serum (a), Rabbit anti-*A. suum* (b), Normal rat serum (WLEP) (c), WLEP anti-*A. suum* (d), Normal mouse serum (BALB/c) (e), BALB/c anti-*A. suum* (f), and run on gradient gels under non-reducing conditions.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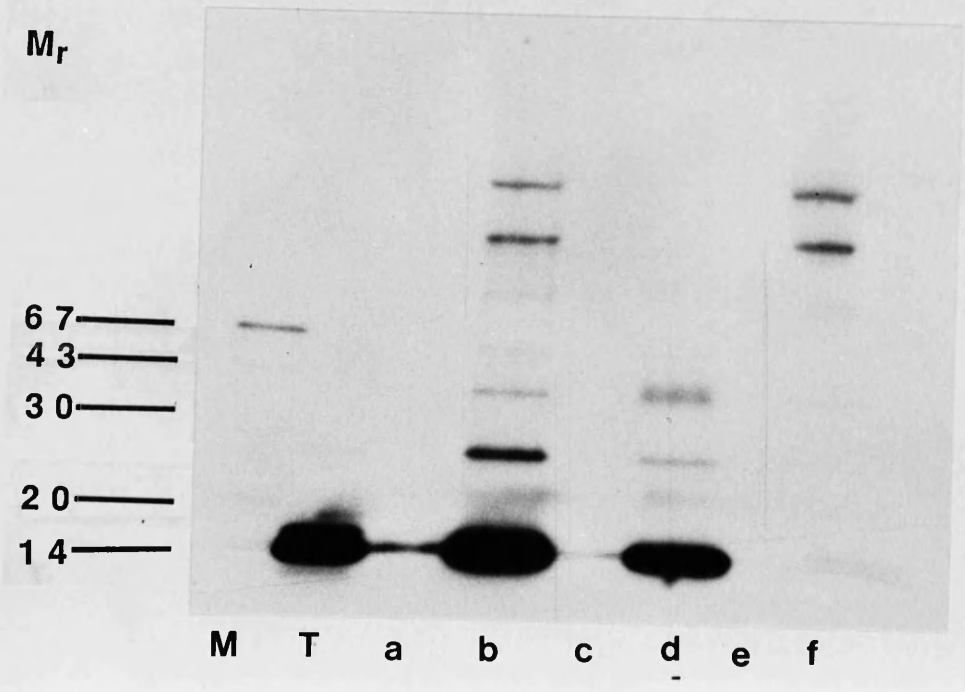
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Figure 6; The antigen recognition profiles of five inbred strains of mice infected with *A. suum*. I^{125} labelled L3/4 ES was immunoprecipitated with normal mouse serum (a), or sera taken 14 days after a tertiary infection BALB/c (b), SJL (c), NIH (d), C57BL (e), CBA (f). Precipitated antigen was run on gradient gels under non-reducing conditions.

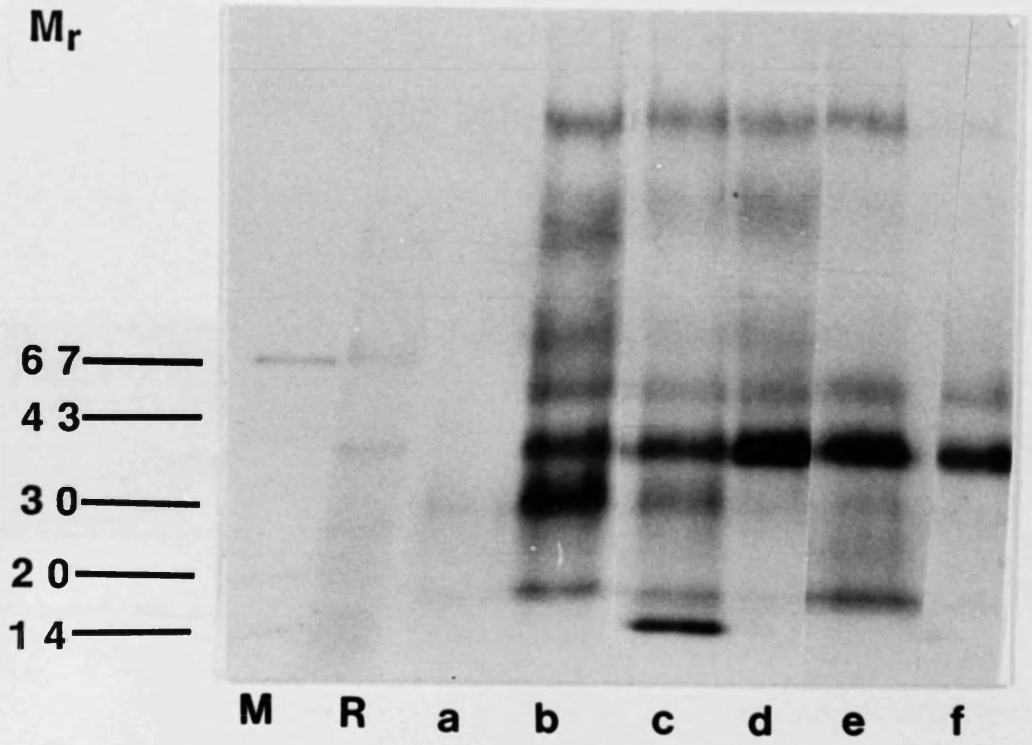
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TABLE 1

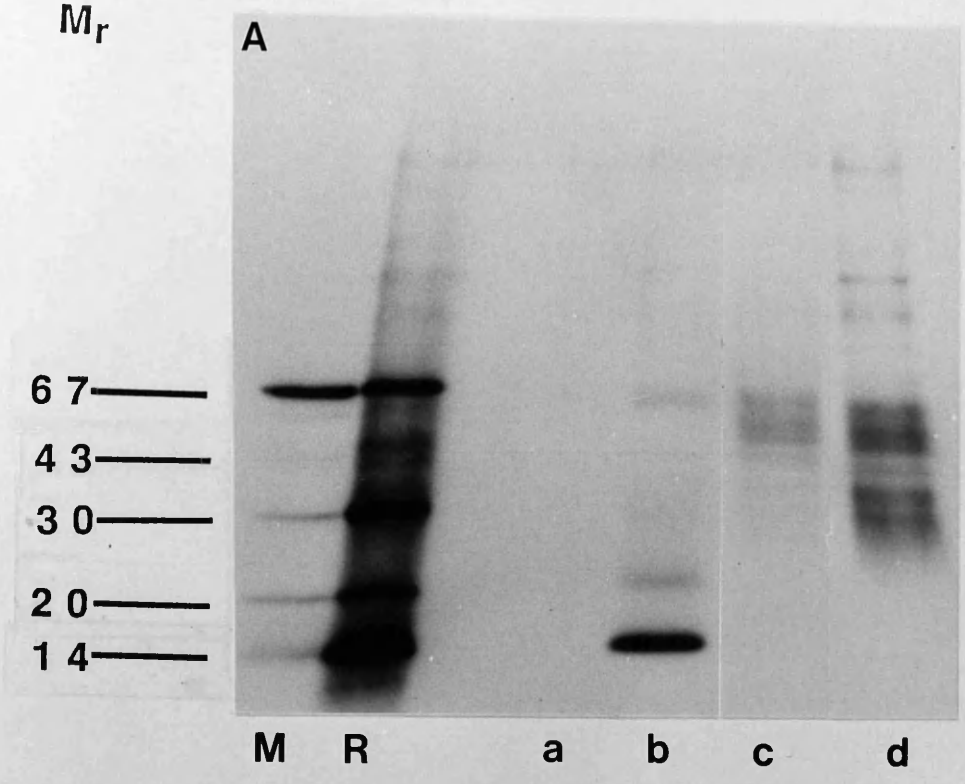
Immunoprecipitation of infection serum with I^{125} labelled L3/4 ES.

Strain	% precipitated
NMS	3.07
BALB/c	12.80
BALB/c anti-14k	4.66
DBA2	11.86
SJL	7.37
C3H	10.18
C57BL10	7.65
NIH	9.24
CBA	10.79

Figure 7; The antigen recognition profiles of sera from inbred strains of mice infected with *H. suum*. I^{125} labelled L2 ES (R) was immunoprecipitated with infection serum and the precipitated antigen was run on SDS-PAGE gels.

Panel A shows the profiles produced by normal mouse serum (a), or sera taken 14 days after a tertiary infection, SJL (b), CBA (c), DBA2 (d).

Panel B shows the antigen recognition profiles produced when infection sera is immunoprecipitated with I^{125} labelled L2 ES in a Protein A based assay, with, or without 10 microliters of a 1:9 dilution of anti-mouse gamma globulin. SJL serum from day 14 after a tertiary infection (a), normal mouse sera plus anti-gamma globulin (b), tertiary SJL plus anti-gamma globulin (c), normal mouse serum (d), tertiary NIH serum (e), normal mouse serum plus anti-gamma globulin (f), tertiary NIH plus anti-gamma globulin (g).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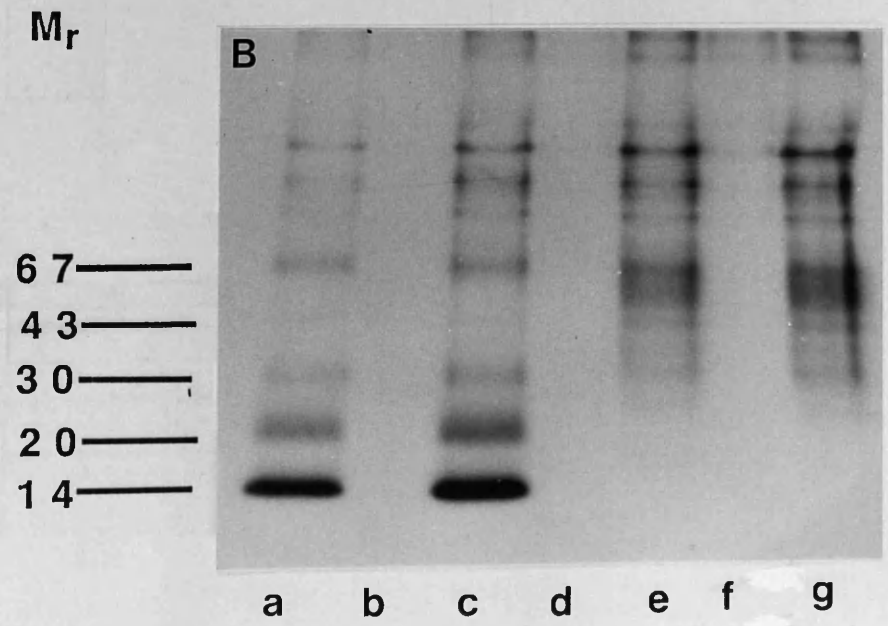
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Figure 8; The antigen recognition profiles of six inbred strains of mice infected with *H. suum*. I^{125} labelled ABF (R) was immunoprecipitated with normal mouse sera (g), or sera taken 14 days after a tertiary infection BALB/c (a), BALB/c anti-14kDa (b), DBA2 (c), SJL (d), CBA (e), C57BL (f), NIH (h), or BALB/c anti-ABF (i) (anti-ABF serum was provided by Miss E. Smith). The precipitated antigen was run on gradient SDS-PAGE gels under non-reducing conditions.

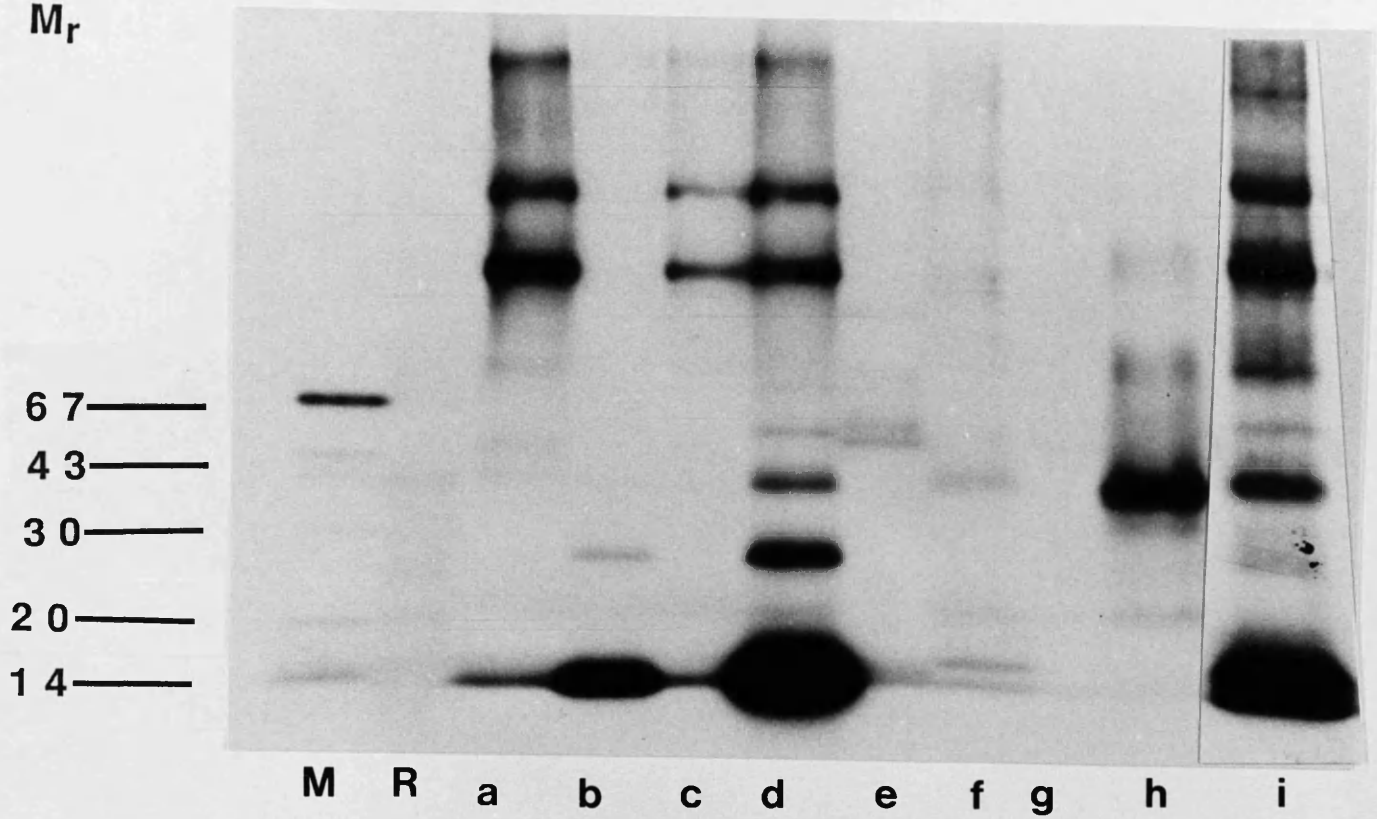
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Figure 9; The antigen recognition profiles of inbred and congenic strains of rats infected with *A. suum*. I^{125} labelled L3/4 ES (R) was immunoprecipitated with normal rat serum (b), or sera taken 14 days after a sixth infection, WLEP (a), AO (c), AGUS (d), PVG (e) PVG-$RT1^u$ (f), PVG-$RT1^l$ (g) precipitated antigen was run on gradient SDS-PAGE gels under non-reducing conditions.

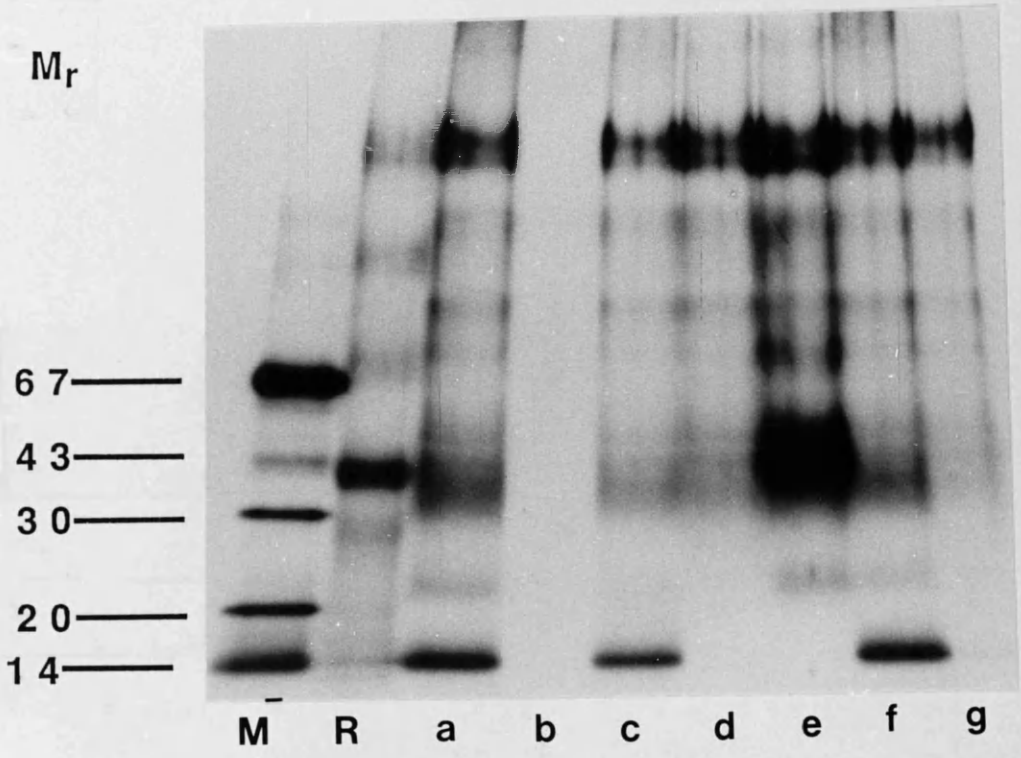
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Figure 10; The antigen recognition profiles of inbred and congenic strains of rats. I^{125} labelled ABF was immunoprecipitated with normal rat serum (a), or sera from 14 days after a quaternary infection WLEP (b), AGUS (c), AO (d), PVG (e), PVG-$RT1^u$ (f), PVG-$RT1^l$ (g) The precipitated antigen was run on gradient SDS-PAGE gels under non-reducing conditions.

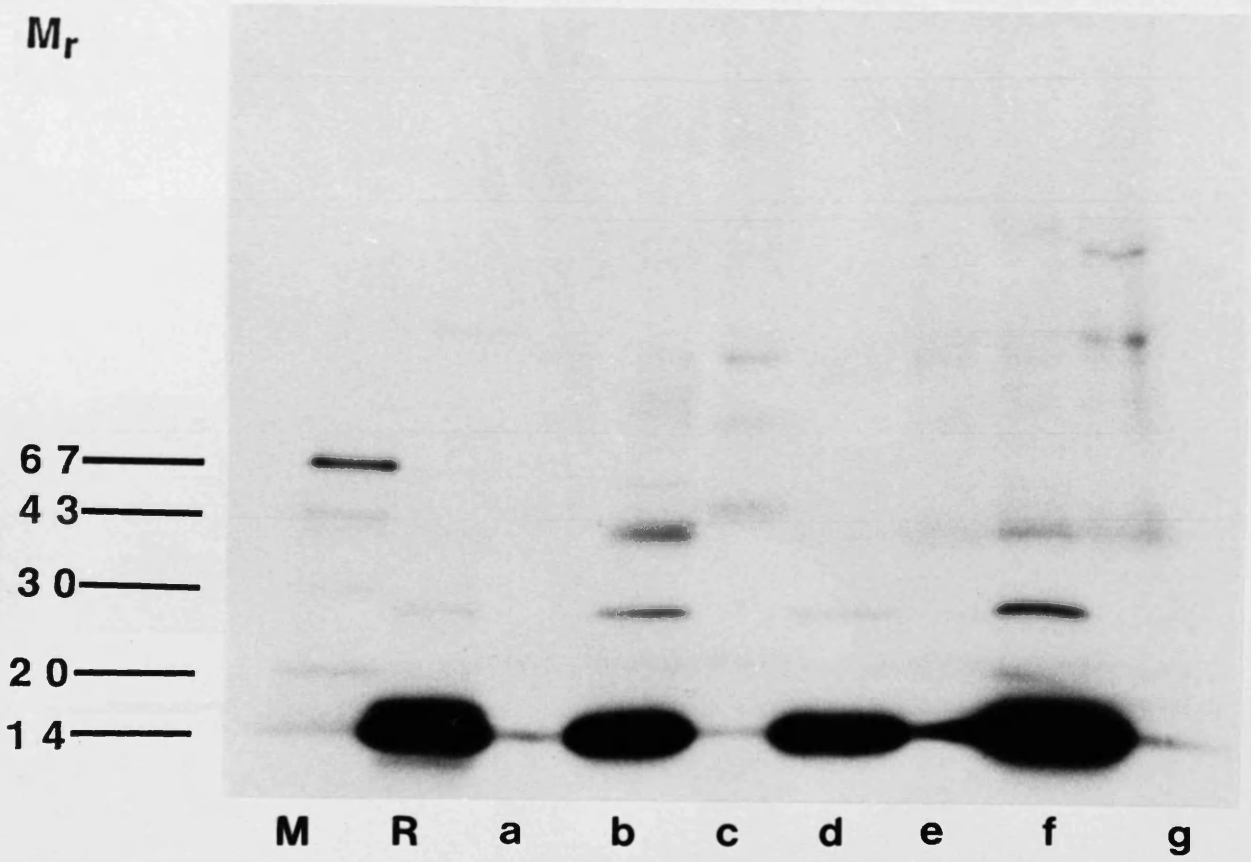
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TABLE 2

Immunoprecipitation of infection serum with I^{125} labelled L3/4 ES or ABF

Strain	% precipitated
A. L3/4 ES	
NRS	3.96
WLEP	7.64
AGUS	5.87
AO	6.84
PVG	11.83
PVG-$RT1^u$	7.14
PVG-$RT1^l$	7.70
B. ABF	
NRS	1.17
WLEP	4.73
AGUS	2.35
AO	4.59
PVG	2.62
PVG-$RT1^u$	15.71
PVG-$RT1^l$	3.27

components of both ES and ABF. This molecule was only recognised by certain rat strains and these strains shared the same MHC haplotype, RT1^{u}.

There are several explanations for these differences in recognition profiles, for example, they could be related to the age or sex of the host, the kinetics of the response, the dose of the antigen or the antibody isotype precipitated in the assay.

Individual differences;

The differences observed between different strains of animals may merely represent differences in the antigen recognition profiles of individual animals. To exclude this possibility the profiles of several individual animals were examined (see Figure 11). There were no significant differences in the components precipitated by the 7 BALB/c animals examined. Consequently, in subsequent analyses pools of equal volumes of sera from a minimum of 3 animals were used.

The kinetics of antigenic recognition;

The profiles produced by different strains after a tertiary infection may differ due to a difference in the kinetics of the response. This hypothesis was tested by examining the recogniton profiles after multiple infections. Figure 12 shows the recognition profiles of BALB/c mice that have received 6 infections. Once the mature response had developed, after a tertiary

Figure 11; The antigen recognition profiles of seven BALB/c mice, 14 days after a tertiary infection with *A. suum*. I^{125} labelled L3/4ES (T) was immunoprecipitated with normal mouse serum (a), or infection serum from one of 7 individual BALB/c mice (b)-(h). Precipitated antigen was then run on gradient SDS-PAGE gels under non-reducing conditions.

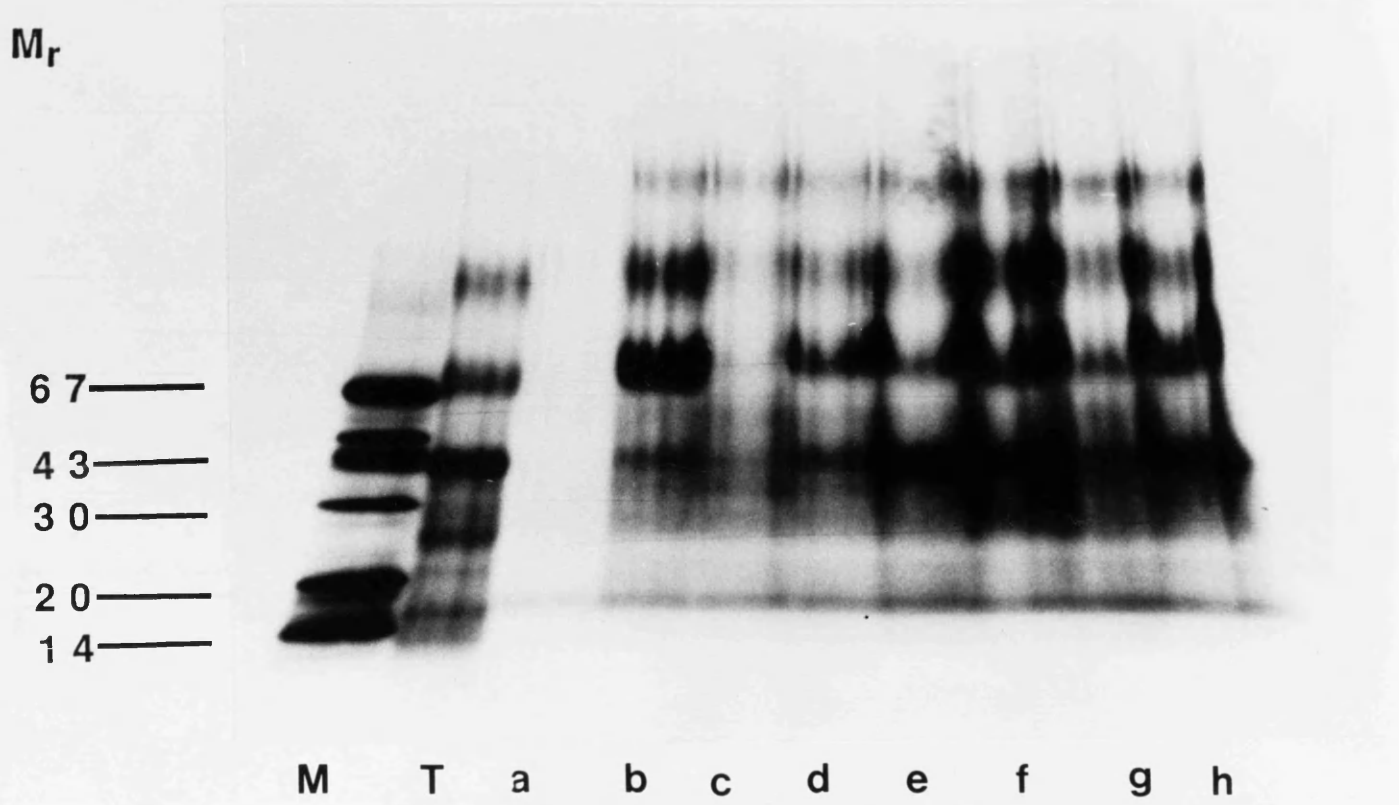
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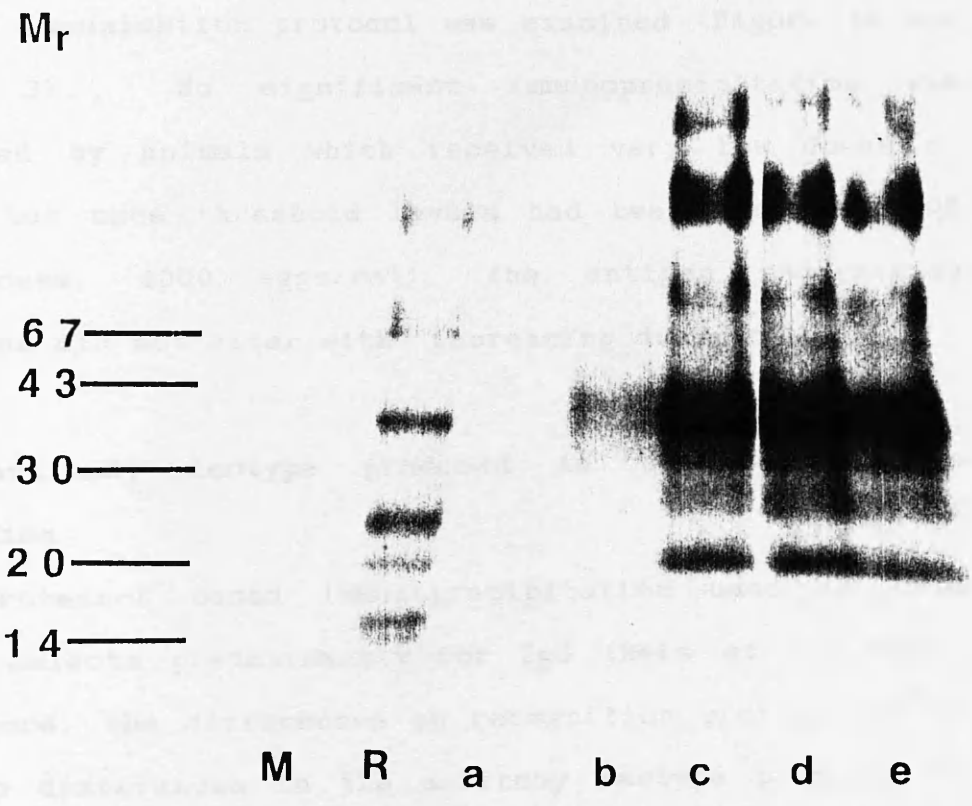
Figure 12; The antigen recognition profiles of BALB/c mice at various stages of infection with *H. suum*. I^{125} labelled L3/4 ES (R) was immunoprecipitated with normal mouse serum (a), serum from 28 days after a primary infection (b), or serum from 14 days after a tertiary infection (c), 14 days after 5 infections (d), 14 days after 7 infections (e).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infection, the profile remained the same even after subsequent infections. This phenomenom was also demonstrated in rats; Figure 13, shows the kinetic profiles of PVG and Wistar rats.

Dose response curve

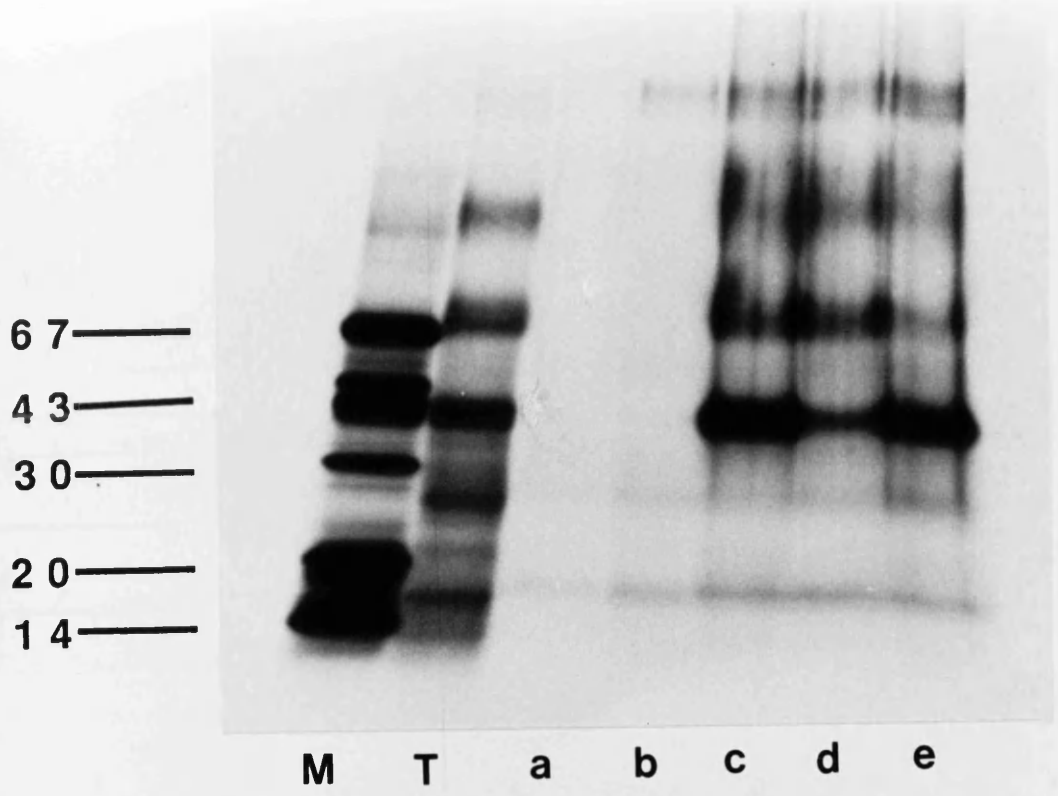
The effect of altering the dose of embryonated eggs used in the immunisation protocol was examined (Figure 14 and Table 3). No significant immunoprecipitation was produced by animals which received very low doses of eggs, but once threshold levels had been reached (2000 eggs/mouse, 4000 eggs/rat), the antigen recognition profiles did not alter with increasing doses of eggs.

The antibody isotype produced in the response to infection

The protein-A based immunoprecipitation used in this assay selects predominantly for IgG (Reis *et al*, 1984). Therefore, the differences in recognition profile may be due to differences in the antibody isotype produced in response to infection and not antigen recognition. To remove this bias a broad spectrum anti-immunoglobulin (anti-Ig) reagent was added to the immunoprecipitation (Figure 7B). This demonstrates that the antigen recognition profile produced is identical with protein-A or a broad based anti-Ig reagent.

Figure 13; The antigen recognition profiles of PVG (**Panel A**) and WLEP (**Panel B**) rats at various stages of infection with *A. suum*. I^{125} labelled L3/4 ES was immunoprecipitated with (**A**) normal PVG serum (a), serum from 28 days after a primary infection (b), 14 days after three infections (c), 14 days after four infections (d), 14 days after six infections (e).

(**B**) Normal WLEP serum (a), serum from 28 days after a primary infection(b), 14 days after three infections (c), 14 days after four infections (d), 14 days after seven infections (e), 14 days after eight infections (f).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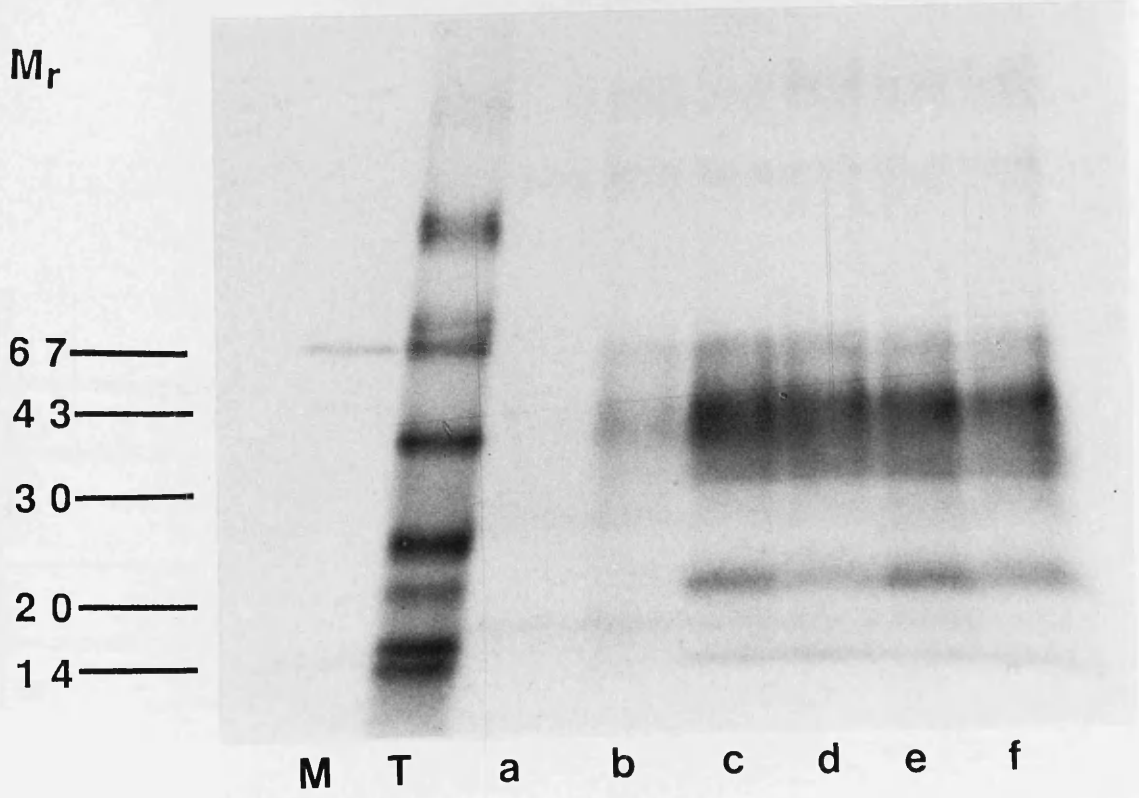
Mr
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4 3
3 0
2 0
1 4
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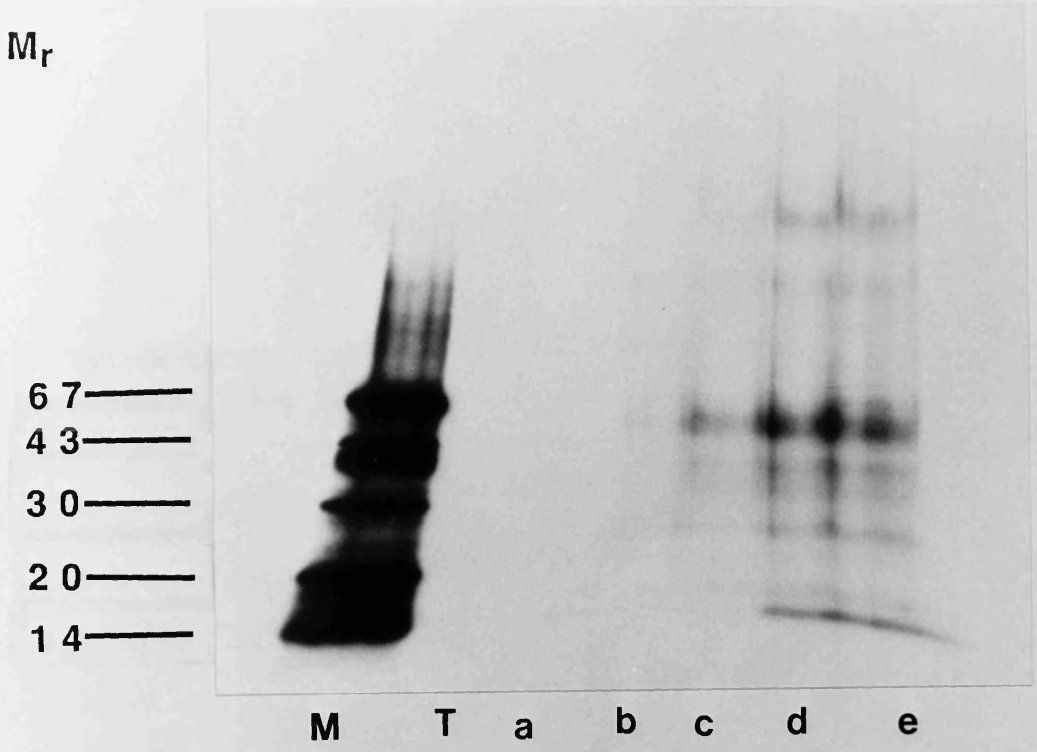
Figure 14; Depicts the antigen recognition profiles produced when immune sera from (**PANEL A**) WLEP rats and (**PANEL B**) BALB/c mice, infected with various doses of eggs, were immunoprecipitated with *A. suum* L3/4 ES (R).

(A) Normal rat sera (a), tertiary infection sera from animals which had been infected with 1,000 eggs/rat (b), 2,000 eggs/rat (c), 4,000 eggs/rat (d) or 6,000 eggs/rat (e).

(B) Normal mouse serum (a), tertiary infection serum from individual animals infected with 2,000 eggs/mouse (b, c, d), 4,000 eggs/mouse (e, f, g), or 8,000 eggs/mouse (h, i, j).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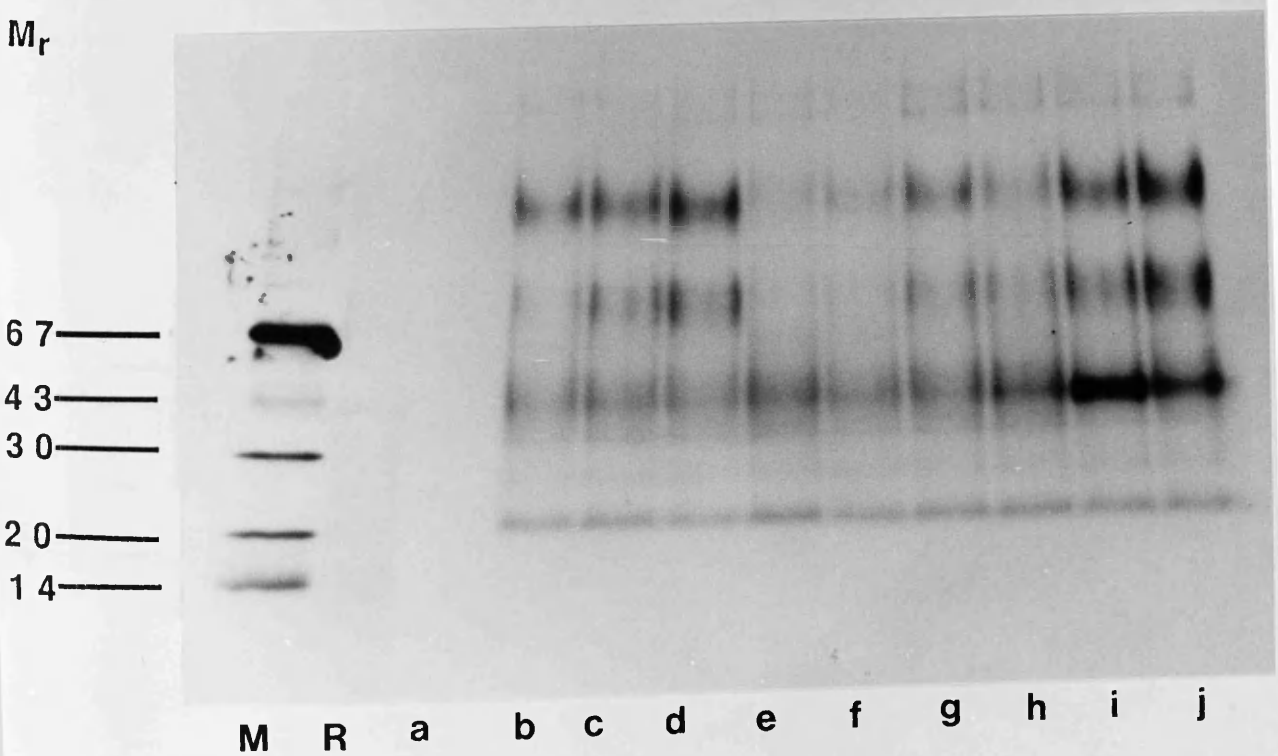
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Recognition profiles produced by immunisation with live larvae or purified "14kDa" and adjuvant

To examine the apparent inability of some strains of rodents to respond to the 14kDa molecule the method of immunisation was altered. BALB/c and SJL mice and WLEP rats were immunised using L2 larvae administered intraperitoneally or intravenously (Figure 15). This shows that the recognition profiles were unaltered by either immunisation technique. Alternatively BALB/c mice were immunised with the purified 14kDa molecule in Freund's adjuvant (Figure 16, Table 4). These non-responders were capable of producing a response against the 14kDa molecule when administered with adjuvant.

TABLE 3

Immunoprecipitation of infection serum with I^{125} labelled L3/4 ES.

Serum	Infective dose eggs/mouse	% Precipitated
NMS	0	5.73
BALB/c	500	8.41
	1000	9.66
	2000	14.43
	4000	10.45
	8000	15.16

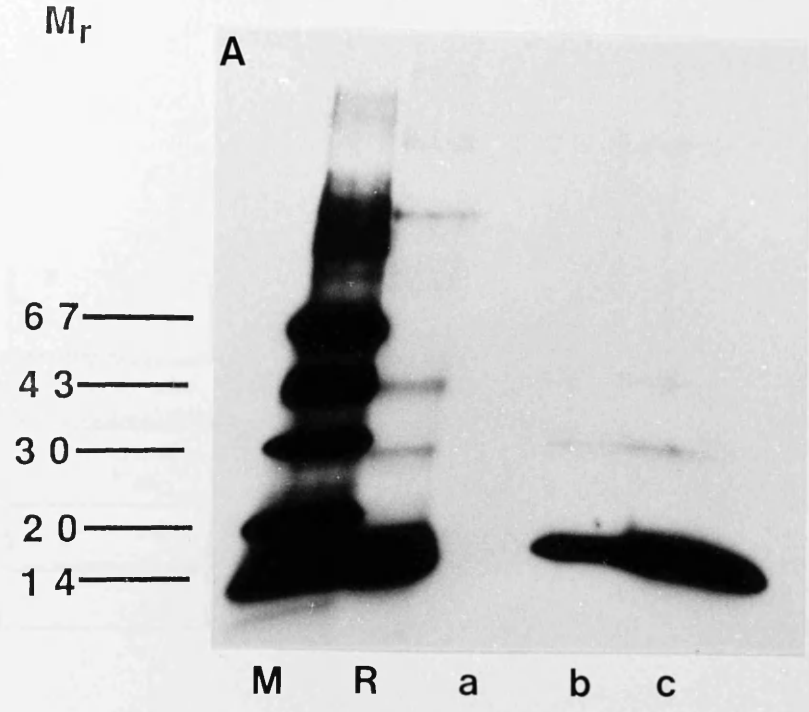
Recognition profiles produced by immunisation with live larvae or purified "14kDa" and adjuvant

To examine the apparent inability of some strains of rodents to respond to the 14kDa molecule the method of immunisation was altered. BALB/c and SJL mice and WLEP rats were immunised using L2 larvae administered intraperitoneally or intravenously (Figure 15). This shows that the recognition profiles were unaltered by either immunisation technique. Alternatively BALB/c mice were immunised with the purified 14kDa molecule in Freund's adjuvant (Figure 16, Table 4). These non-responders were capable of producing a response against the 14kDa molecule when administered with adjuvant.

Figure 15; The recognition profiles produced by immunoprecipitating serum from infected SJL mice (**Panel A**) or infected WLEP rats (**Panel B**) with I^{125} labelled ABF.

(A) Normal mouse sera (a), tertiary sera from mice that had been infected orally with 2,000 *Ascaris* eggs (b), tertiary sera from mice immunised iv with 2,000 L2 larvae.

(B) Normal rat sera (a), tertiary sera from rats infected orally with 6,000 *Ascaris* eggs (b), tertiary sera from rats immunised iv with 6,000 L2 larvae (c), tertiary sera from rats immunised ip with 6,000 L2 larvae (d).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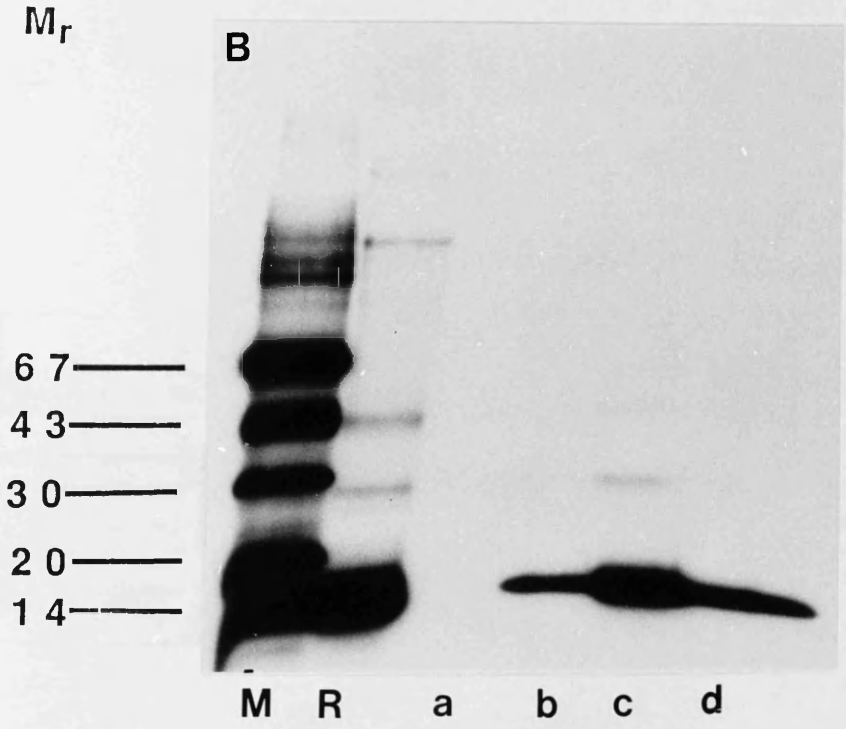
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Figure 16; The antigen recognition profiles of inbred strains of mice immunoprecipitated with I^{125} labelled 14kDa. The purified 14kDa molecule was immunoprecipitated with normal mouse serum (a), and sera from 14 days after a tertiary infection BALB/c (b), BALB/c anti-14kDa (c), CBA (d), SJL (e), C57BL (f), NIH (g). Precipitated antigen was run on gradient SDS-PAGE gels under non-reducing conditions.

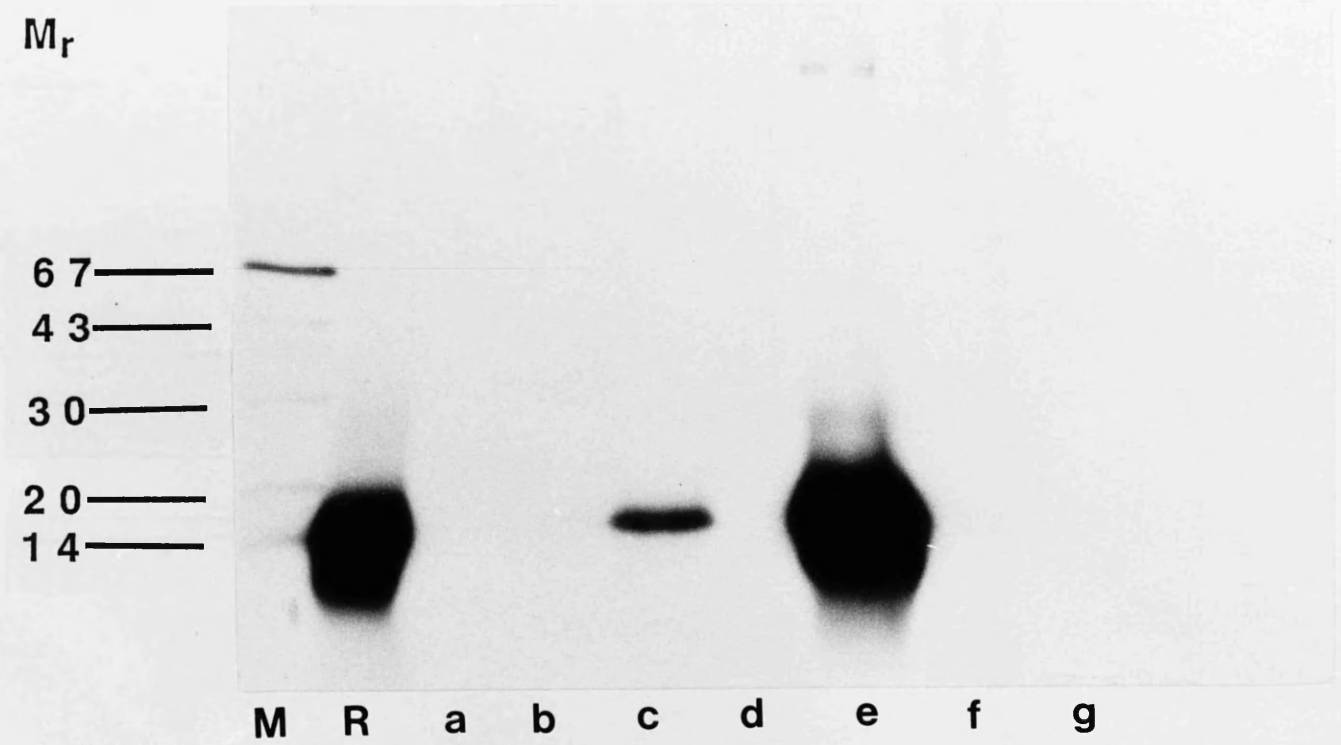
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TABLE 4

Immunoprecipitation of infection serum with I^{125} labelled 14kDa.

Sera	% Precipitated
NMS	2.22
BALB/c	4.46
BALB/c anti-14K	4.19
CBA	1.88
SJL	27.09
C57BL10	3.19
NIH	2.19

Chapter 1

Discussion

The effects the host species or strain can have on the response to, and outcome of infection have been documented over a number of years (Mitchell *et al*, 1982). Indeed, the effect of host strain on the response against *Ascaris* has been studied previously (Mitchell *et al*, 1976). These authors demonstrated that different strains of mice varied in their ability to resist a primary infection with *Ascaris*, in terms of the number of larvae reaching the lungs. The results presented in this chapter focus on the specificity of the antibody response to infection.

Ascaris is a complex multi-cellular organism with discrete stages to its life-cycle, consequently, it probably presents the host with an array of potentially antigenic components. It has been demonstrated that the ES components consist of both cross-reactive antigens, in that the rodents which are only exposed to the larval stages of the parasite precipitate adult antigens, and stage-specific antigens (Kennedy and Qureshi, 1986). Of the three species of animals studied, only the rabbit responded to all the potentially antigenic components during the course of infection. Rats and mice demonstrate restricted recognition and the target molecules varied with strain (Figure 5, 8, 10).

These differences may be explained in a number of ways. The use of inbred strains of animals which are presumably genetically identical should result in uniform responses

to a given challenge. It has been shown, however, that certain inbred strains do not respond uniformly. When 129/J mice are infected with *S. japonicum*, approximately 50% respond to a 26kDa antigen and this appears to render them resistant to challenge infections (Mitchell *et al*, 1985). These individual differences within a strain have also been demonstrated in inbred strains of mice infected with *S. mansoni* (Kee *et al*, 1986). When the response profiles of individuals within a strain infected with *Ascaris* were examined, no such differences were apparent. The profiles produced were therefore strain dependent and did not alter with age (within mature animals), sex or between individuals.

Studies have shown that, in terms of antibody production, different strains of animals respond at varying rates to infection (Jungery and Ogilvie, 1982). This may explain the differences between recognition profiles at a given time during infection. However, when the response profiles produced after each infection of 6 or more subsequent infections were studied the immune response, in terms of antigen recognition, appeared to be complete after the tertiary infection. Further infection did not alter this profile, and animals still produced restricted profiles even after multiple infections. This suggests that the differences seen are not due to differences in the kinetics of the response.

Another important criterion in determining the immune response produced is the dose of parasite used (Kayes *et al*, 1985; Wassom *et al*, 1984; Vaz *et al*, 1971). In the present study the effects of giving mice 4 times the optimum dose failed to produce recognition of all the antigenic components; the antigen recognition profiles of BALB/c mice were constant whether they were immunised with 2000 or 8000 *Ascaris* eggs. Therefore, the dose of infective eggs does not alter the antigen recognition profile within these limits.

The protein-A based immunoprecipitation method used in this study is predominantly specific for IgG antibodies (Reis *et al*, 1984). The importance of the antibody isotype produced, in the context of resistance, was demonstrated by Almond *et al* (1987). It is possible that the differences in recognition seen with *Ascaris*, reflect the fact that the profiles are produced predominantly by IgG. When a broad spectrum anti-Ig reagent was used the antigen recognition profile produced demonstrated that the differences seen were not due to isotype differences in the responses of the various strains of mice.

One explanation for the apparent inability of some strains of mice to respond to certain *Ascaris* components, is that these animals lack the necessary T cell receptors for recognition of these antigenic epitopes (Vidovic and Matzinger, 1988). This hypothesis was tested by altering the means of immunisation. Non-responders presented with

the purified 14kDa molecule in adjuvant were able to produce antibody against this component. This suggests that these animals do not respond in the context of infection because this molecule is not presented in the correct way, but this failure to respond is not due to a total inability of their immune systems to see this molecule. This finding, of recognition in the context of adjuvant, which does not occur in the course of infection, emphasises the advantages of using a model system which does not require adjuvants. The model infection will presumably present antigen to the host in a way comparable to that which occurs in the natural infection and enable the study of recognition in the context of infection. The reasons for the differences in recognition between infection and adjuvant presented components is not clear, they may be due to the route of administration as this is known to alter the responses produced (Ambler *et al*, 1973).

The results presented in this chapter suggest that there is a strain dependent difference between animals which is not due to the kinetics of response or differences in the immunoglobulin isotypes produced. These genetic differences occur only in the context of infection. The fact that animals with the same MHC haplotype produce identical recognition profiles when infected with *Ascaris*, warrants a further analysis of the genetics controlling the antibody response to this parasite.

Chapter 2

The genetic control of the antibody repertoire in experimental infection with *Ascaris*.

Introduction

Genetic variations in the responses of animals to infection have been documented for many years (Ackert, 1942). These genetic differences have been demonstrated in many species and against a whole range of infecting organisms (Wakelin and Blackwell, 1988). In parasitic helminth infections, genetic variation can be demonstrated in all the parameters used to measure primary and subsequent challenge infections. For example, parasite growth, fecundity, duration of initial infection and speed of elimination from the host (Wakelin, 1985). The study of these genetic differences has been pursued in several systems, the most popular host being the mouse, because of the wealth of information available on its genetics. The differences observed in the outcome of infection must lie either with the animal's innate immunity, or the ability to mount an effective response against the infection. Most of the effector mechanisms useful in the fight against infection are immune processes (Wassom *et al*, 1974; Wakelin, 1985). Consequently, the search for the genes controlling these genetic differences has concentrated on the genetic constitution of the host, which effects its innate immunity, and the MHC which is involved in the recogniton of antigen and hence can have a regulatory role in the immune response.

Response to a particular parasite antigen and resistance to infection need not correlate, but there is at least one example involving a helminth parasite where there is a correlation between these two factors. The most direct

correlation between effective antigen recognition, immune response and resistance to infection comes from infection of 129/J mice with *S. japonicum* (Mitchell *et al*, 1985). A proportion of mice do not develop adult worms after infection with cercariae. Sera from these mice recognise a 26kDa adult worm antigen which is not recognised efficiently by susceptible individuals. There are other examples of the importance of antibody production; the specificity, isotype and kinetics of the response are known to affect the outcome of infection, and these are all variables which may be altered by changing the host (Jungery and Ogilvie, 1982; Mitchell *et al*, 1980; Piessens *et al*, 1980; Thompson *et al*, 1979; Storey *et al*, 1985).

The immune response against, and the outcome of infection with a given parasite can vary depending on the strain of mouse infected (Almond *et al*, 1987; Wassom *et al*, 1983; Blackwell, 1983; Else and Wakelin, 1989; Kennedy *et al*, 1986). These differences are associated with the genetic constitution of the host. Several of the genes responsible for these differences have now been recognised. For example, the ability of mice to express the rapid expulsion response when challenged with *T. spiralis* is controlled by an allele termed Ihe-1 (Bell *et al*, 1984). One gene, Lsh, is responsible for the innate resistance to *Salmonella*, *Leishmania donovani*, *Mycobacterium tuberculosis* and *M. leprae* (Brown *et al*, 1982; Skamene *et al*, 1984; Bradley *et al*, 1979).

As well as the individual autosomal genes thought to be involved in the response to infection, a group of MHC-linked genes have been implicated in these strain differences. The most common means of genetic control appears to be a partnership between background and MHC genes (Wassom *et al*, 1984; Wakelin, 1985; Wassom *et al*, 1983; Hormaeche *et al*, 1985; Ivanyi and Sharp, 1986; Else and Wakelin, 1989; Deedler *et al*, 1978; Pond, Wassom and Hayes, 1988; Gibbens, Harrison and Parkhouse, 1986).

The involvement of these MHC-linked genes in the response to infection is not surprising, as the proteins they encode are associated with antigenic recognition and cell to cell communication, and hence, play a role in regulation of the immune response (McDevitt and Chintz, 1969; Ball and Stastny, 1984; Gunther *et al*, 1973; Inomata *et al*, 1983; Dorf, 1981). Evidence for the involvement of MHC alleles in the outcome of infection is now available in several model systems involving parasitic infections in the mouse (Wakelin, 1985; Else and Wakelin, 1989; Ivanyi and Sharp, 1986; Claas and Delder, 1979; Sher *et al*, 1984; Vadas, 1980; Kee *et al*, 1986).

The H-2 complex plays a role in the infection of mice with *T. spiralis*. Two alleles have now been identified, Ts-1, which is thought to act as an Ir gene, controlling lymphocyte responsiveness to parasite antigen. This Ir gene maps in the I region of the H-2 complex and is associated with the A_{β} locus. The second of these two

alleles, Ts-2, lies between the S and D loci and is thought to be involved in the control of a cell population necessary to amplify the host's response (Wassom *et al*, 1983).

In this chapter, the genetic control of antigen recognition in the context of infection with *Ascaris* is examined in both qualitative and quantitative terms. Preliminary experiments implicated MHC-linked genes in the response to discrete parasite components, and consequently particular attention was focused on this gene complex. The antibodies produced by a range of inbred and congenic rodents, as measured by SDS-PAGE, ELISA and surface immunofluorescence are presented. The antibody responses of a number of F_1 hybrids are also examined to study how the antibody repetoire is inherited.

Chapter 2

Results

Antigen recognition profiles of congenic mice.

The antigen recognition profiles of B10 and BALB MHC (H-2) congenic mice infected with *A. suum* were examined by SDS-PAGE. The profiles produced varied within these congenics against ABF and L3/4 ES (Figure 17, B10 vs ABF; Figure 18, B10 vs L3/4 ES; Figure 19, BALB vs L3/4 ES). Animals with the same background genes produced different recognition patterns but when animals with the same H-2 haplotype were compared the profiles produced were identical (Figure 20, $H\text{-}2^{q}$ animals; Figure 21 $H\text{-}2^{k}$ animals). There are slight differences in the primary profiles of the three $H\text{-}2^{q}$ strains but the tertiary profiles are the same. Only strains expressing the $H\text{-}2^{m}$ haplotype (SJL and B10.S) recognise the 14kDa molecule, and the 16kDa molecule of ABF is only recognised by animals with the $H\text{-}2^{b}$ haplotype (Figure 22).

Antigen recognition profiles of congenic rats.

An examination of the recognition profiles of MHC (RT1) congenic rats revealed that animals with the same genetic background did not produce the same recognition profiles when infected with *A. suum* (see Figure 9 and 10, Chapter 1; Figure 23, Table 5).

Antigen recognition profiles of F_1 hybrids.

The antigen recognition profiles of several F_1 hybrids were examined. The (BALB/c **x** SJL)F_1 hybrid showed that recognition of different molecules was inherited in different ways (see Figure 24). The 14kDa molecule was

Figure 17; The antigen recognition profiles produced by immunoprecipitating I^{125} labelled ABF (R) with normal mouse serum (a), or tertiary infection sera from C57BL10 (b), B10.D2 (c), B10.G (d), B10.S (e) and B10.BR (f) mice infected with *A. suum*. The immunoprecipitated antigen was run on SDS-PAGE under non-reducing conditions, molecular weights were estimated by comparision with radio-iodinated marker proteins (M).

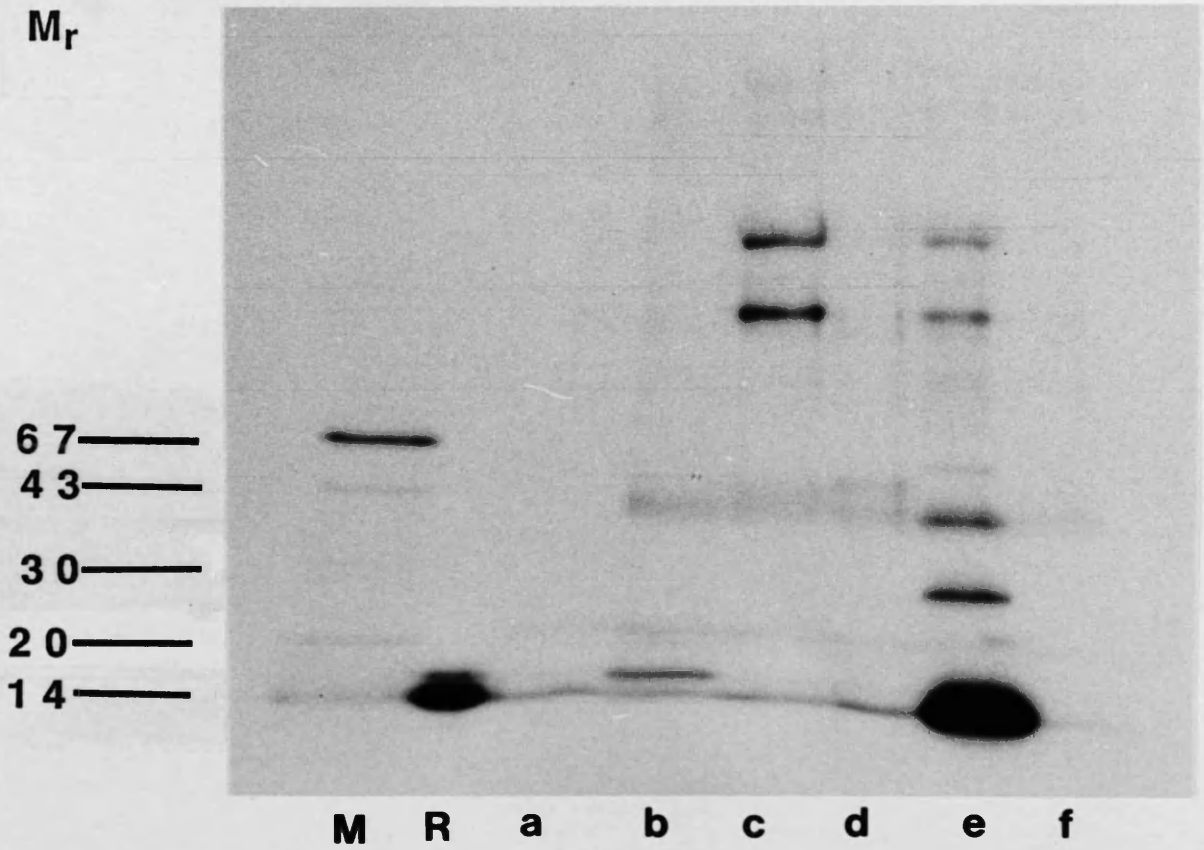
Mr
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Figure 18; The recognition profiles of mice of differing H-2 haplotype, but identical genetic background (B10). Radio-iodinated L3/4 ES (R) was immunoprecipitated with normal serum from each strain (a) or serum from mice infected three times with *A. suum* (c).

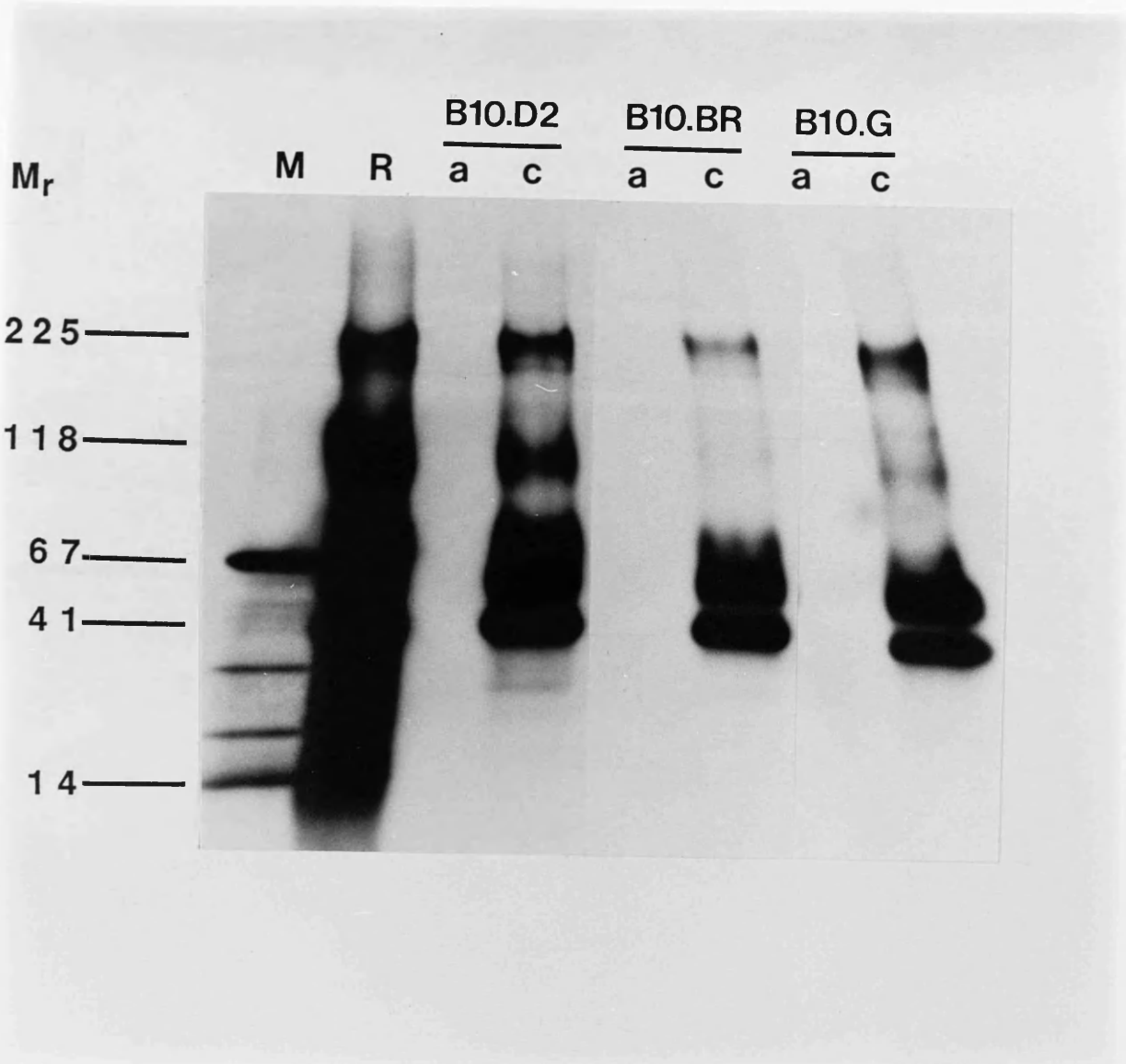

B10.D2
B10.BR
B10.G
Mr
M
R
a
c
a
c
a
c
225
118
67
41
14

Figure 19; The recognition profiles of mice of differing H-2 haplotype, but identical genetic background (BALB). Radio-iodinated L3/4 ES (R) was immunoprecipiated with normal serum from each strain (a) or serum from mice infected three times with *A. suum* (c).

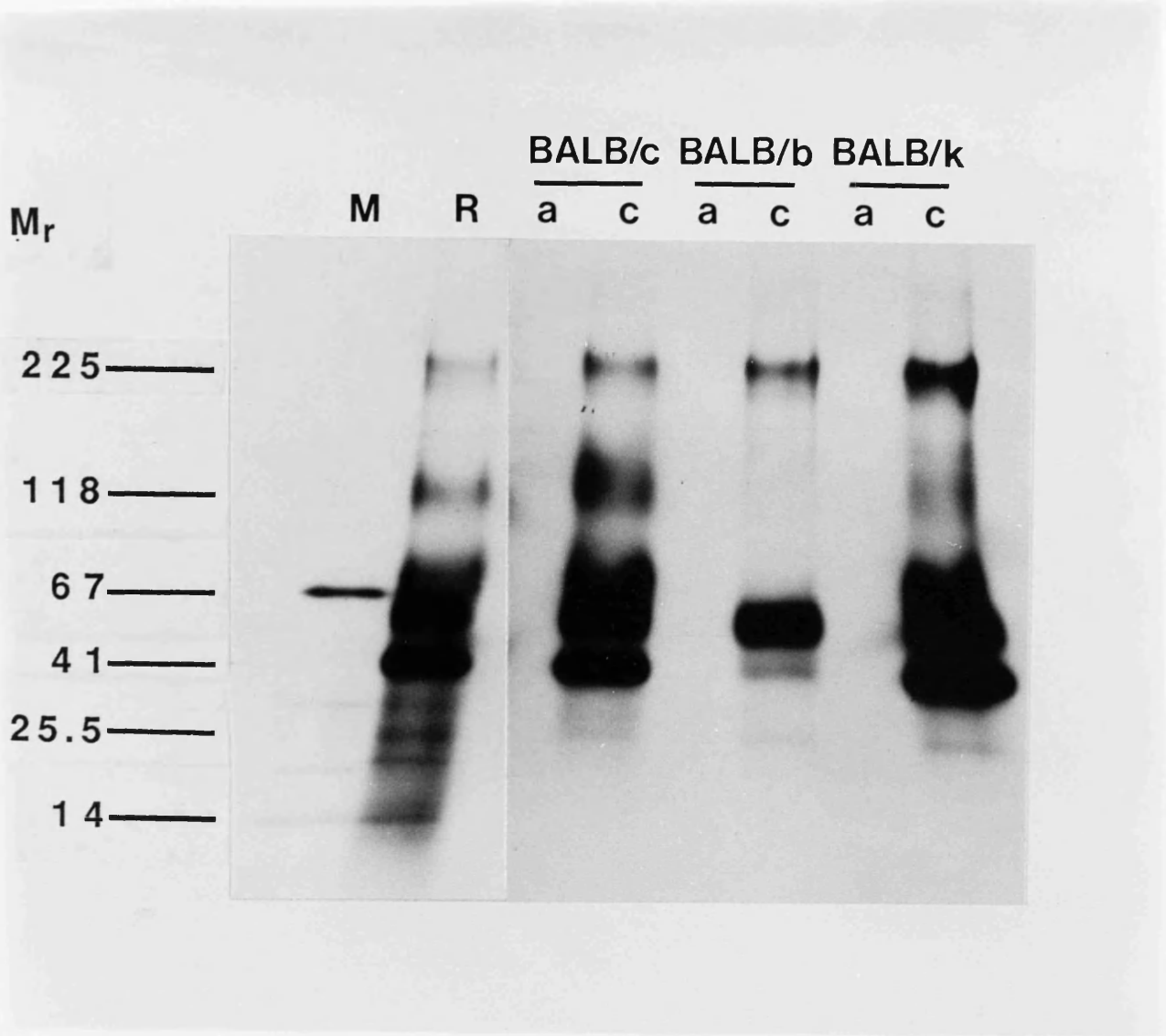
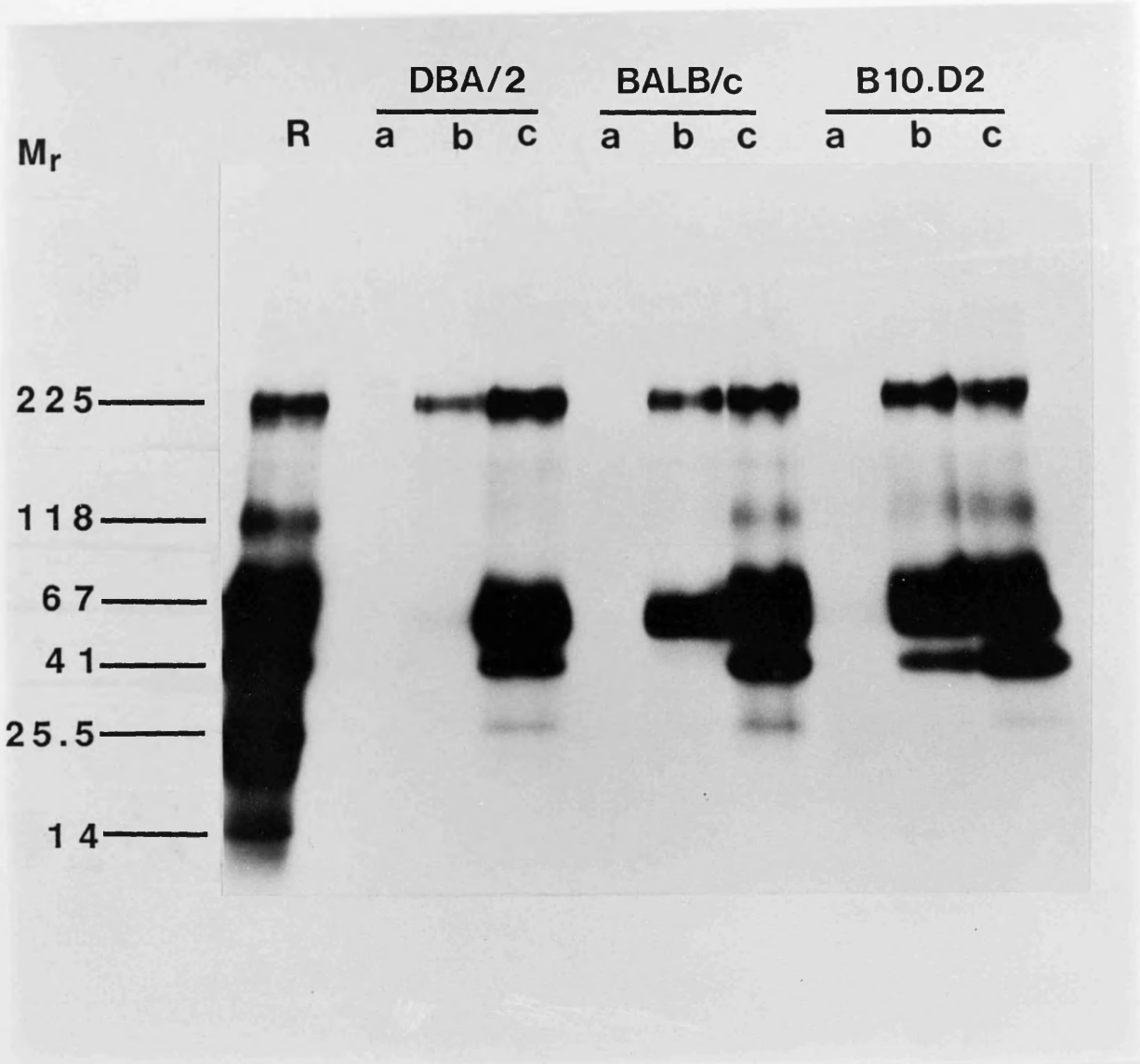

BALB/c
BALB/b
BALB/k
M
R
a
c
a
c
a
c
Mr
225
118
67
41
25.5
14

Figure 20; The recognition profiles of H-2^d strains of mice in response to *Ascaris* infection. Radioiodinated L3/4 ES (R) was immunoprecipitated with normal serum from each strain (a), serum from mice 28 days after a primary infection (b) and 14 days after a tetiary infection (c).


DBA/2
BALB/c
B10.D2
Mr
R
a b c
a b c
a b c
225
118
67
41
25.5
14

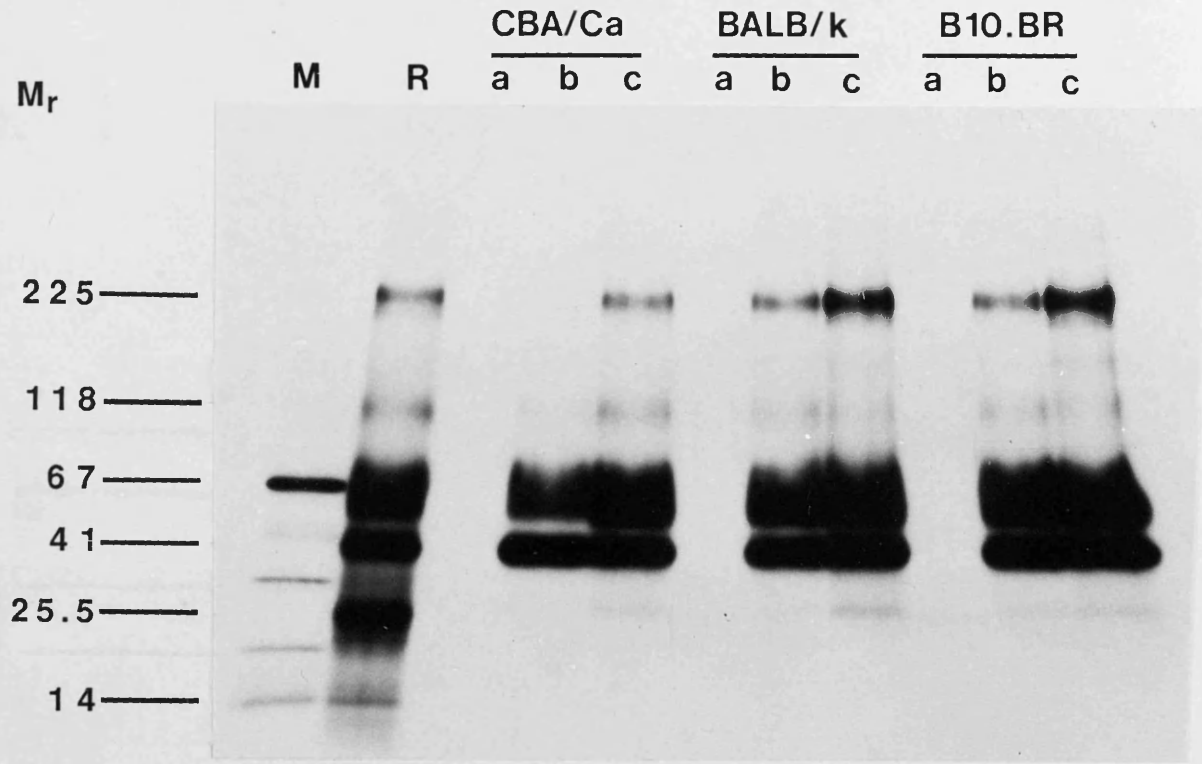

CBA/Ca
BALB/k
B10.BR
M
R
a b c
a b c
a b c
Mr
225
118
67
41
25.5
14

Figure 21; Recognition profiles of H-2^k strains of mice in response to *Ascaris* infection. Radioiodinated L3/4 ES (R) was immunoprecipitated with normal serum from each strain (a), serum taken 28 days after a primary infection (b) and serum from 14 days after a tertiary infection (c).

Figure 22; The recognition profiles of H-2^b haplotype mice infected with *A. suum*. Radio-iodinated ABF (R) was immunoprecipitated with normal mouse serum (a), or tertiary infection serum from BALB/c (b), BALB.B (c), BALB.K (d), C57BL10 (the profile in this track is very faint but the H-2^b specific 16kDa band is visible) (e) or BALB/c anti-14kDa serum (f).

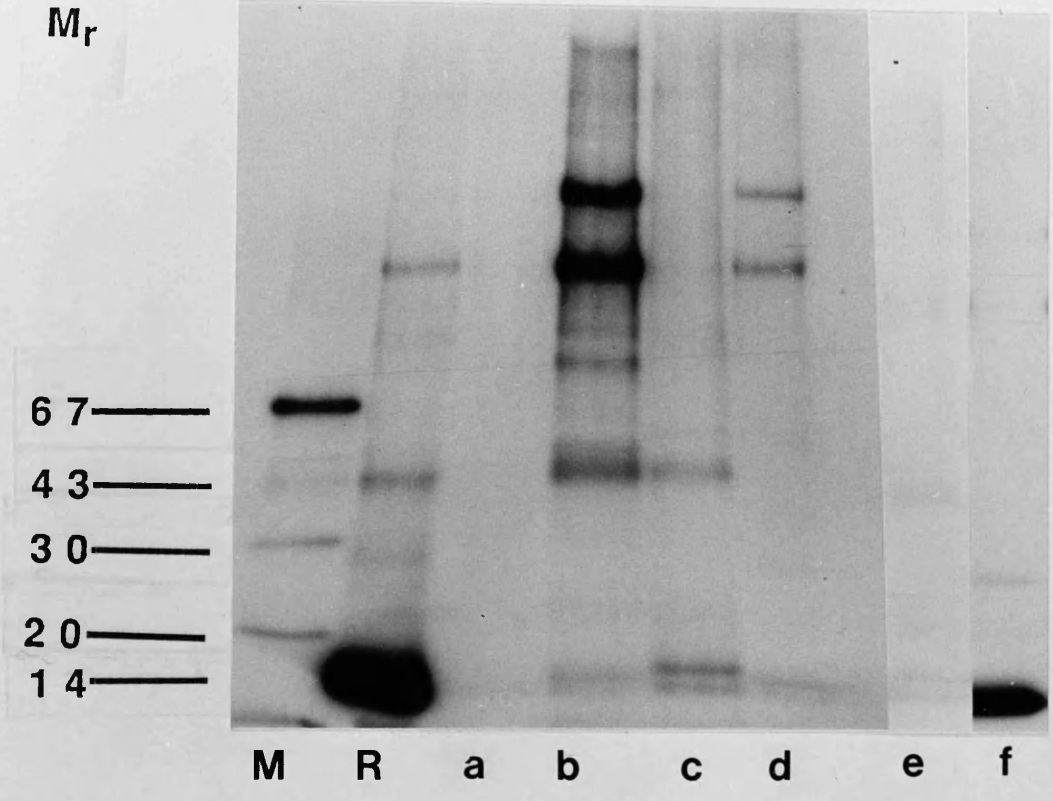

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Figure 23; Recognition of the 14kDa molecule by rats infected with *A. suum*. Radio-iodinated 14kDa was immunoprecipitated with normal rat serum (a), or tertiary infection serum from WLEP (b), AGUS (c), AO (d), PVG (e), PVG-RT1^u (f), PVG-RT1^l (g), (WLEP x PVG)F_1 hybrid (h) or (AGUS x PVG)F_1 hybrid (i).

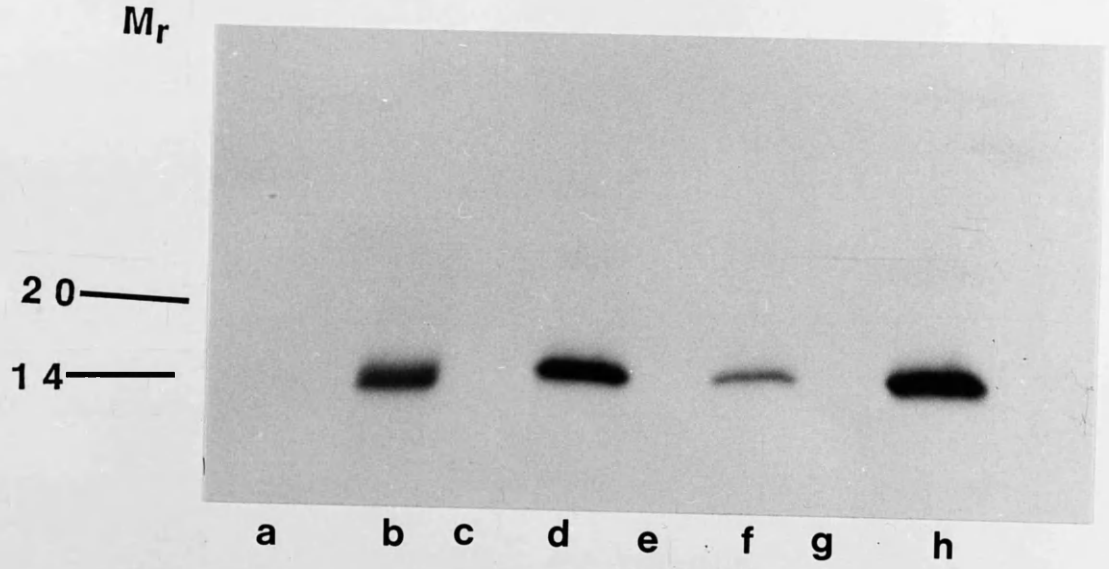
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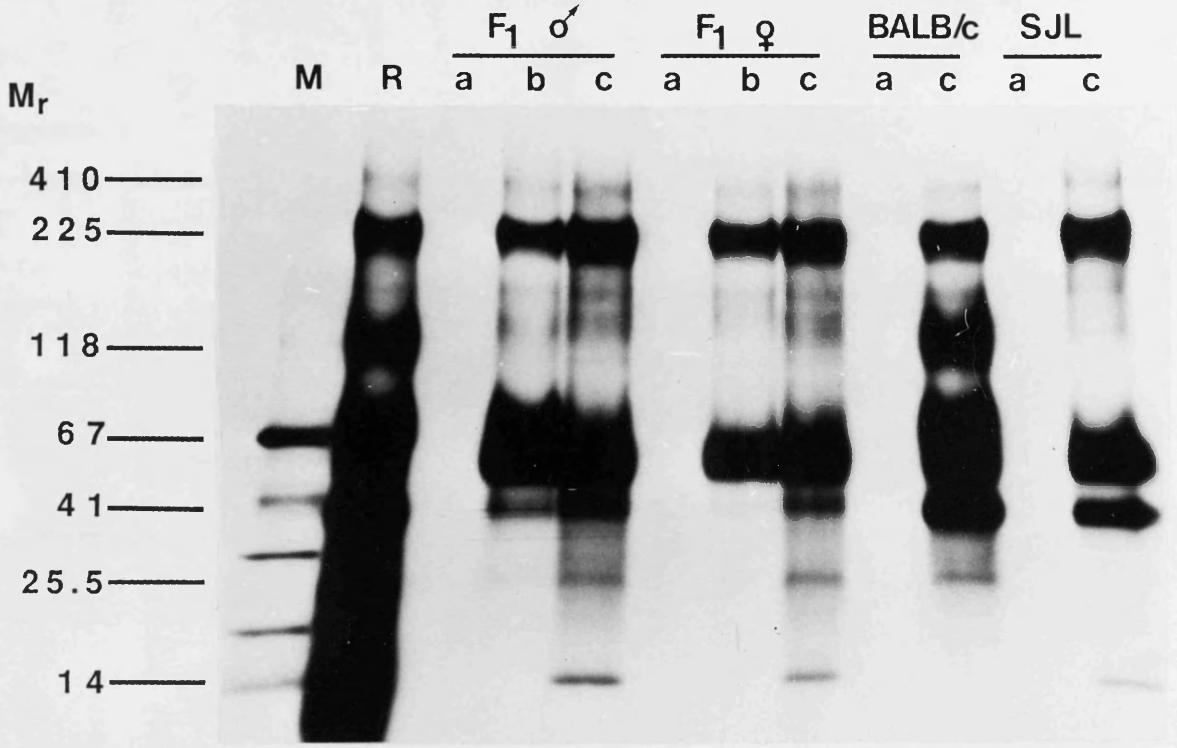
TABLE 5

Immunoprecipitation of infection serum with I^{125} labelled 14kDa.

Sera	% Precipitated
NRS	2.54
WLEP	30.96
AGUS	1.58
AO	21.88
PVG	2.55
PVG-$RT1^u$	20.54
PVG-$RT1^l$	2.40

Figure 24; The recognition profile of (BALB/c x SJL)F_1 hybrids and parental strains infected with *A. suum*. Radio-iodinated L3/4 ES was immunoprecipitated with normal serum from each strain (a), serum from 28 days after a primary infection (b) or serum from 14 days after a tertiary infection (c).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F1 ♀
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BALB/c
a
c
SJL
a
c
410
225
118
67
41
25.5
14

recognised by both the SJL parent and the hybrid, showing a dominant mode of inheritance. The 25.5kDa molecule recognised by the BALB/c parent and the hybrid showed a similar mode of inheritance. The 118kDa molecule, however, was inherited in a recessive manner; the BALB/c parent responded to this molecule but neither the SJL nor the hybrid recognised it.

This (BALB/c x SJL)F_1 hybrid was derived from parental strains that varied at both the MHC and background gene level. To compare the effects of MHC genes in isolation a hybrid between B10.D2 and B10.S mice was produced. This hybrid produced a recognition profile identical to that of the (BALB/c x SJL) hybrid, with the exception of the 118kDa molecule (Figure 25). In this hybrid, there was dominant recognition of the 14kDa and the 25.5kDa molecules supporting the hypothesis that recognition of these molecules is dependent on the correct association of MHC and antigen. It would appear from the strains examined that only the H-2^{ss} haplotype is capable of forming an effective association with the 14kDa molecule, hence, triggering T cell help and antibody production. The 118kDa molecule is recognised by the (B10.D2 x B10.S)F_1 hybrid supporting the hypothesis that the failure of the (BALB/c x SJL)F_1 hybrid to respond to this molecule is due to a resemblance to self.

Of the F_1 hybrids examined, all the mouse hybrids with the ^{s} allele in their H-2 complex, e.g. (BALB/c x SJL)F_1,

Figure 25; The recognition profiles of (B10.D2 x B10.S)F_1 hybrid and parental strains. Radio-iodinated L3/4 ES was immunoprecipitated with normal serum from B10.D2 (a), F_1 (c), B10.S (e) or tertiary infection serum B10.D2 (b), F_1 (d), B10.S (f).

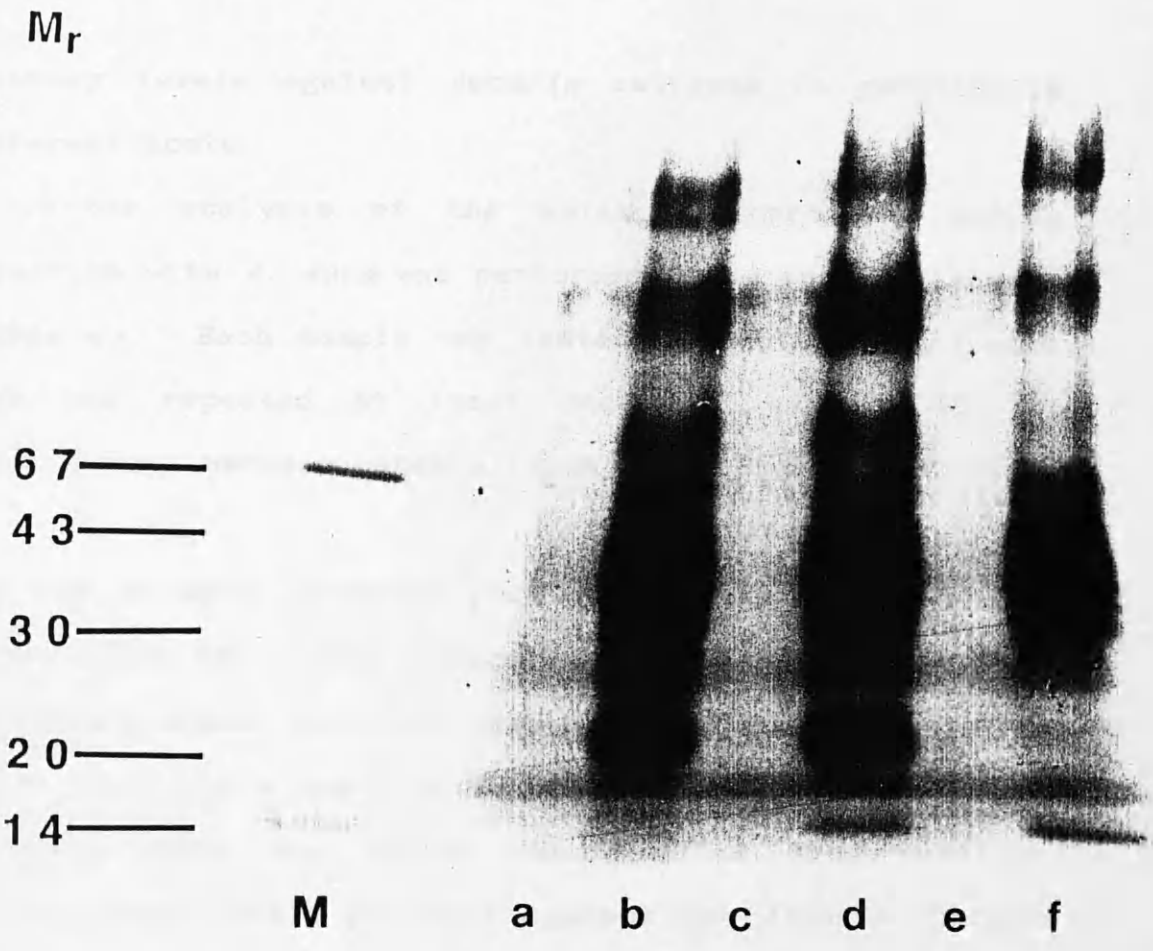
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Figure 25, and (SJL x C57BL10)F_1, Figure 26, and the rat hybrids with the u allele in their RT1 complex responded to the 14kDa molecule (see Figure 23 and Figure 28). While F_1 hybrids lacking these alleles fail to respond to this molecule (e.g. AGUS x PVG, Figure 27).

Antibody levels against *Ascaris* antigens in genetically different hosts.

A further analysis of the antibodies produced during infection with *A. suum* was performed using an ELISA system (Table 6). Each sample was tested in duplicate and each test was repeated at least once. An example of the correlations between repeats is shown in Figure 29.

All the strains screened produce antibodies against ABF (see Figure 30). The recognition of this adult antigen, by animals which have not been exposed to the adult worm, shows that there must be components in adult and larval antigens which are either identical or cross-reactive. The antibody levels produced against the *Ascaris* antigens are on the whole high, with the exception of four strains, BALB.K, DBA2, C3H and CBA. These strains produce much lower levels against these antigens. The low antibody levels of these animals suggest that both background and H-2 genes are regulating antibody production.

The four strains which produce lower levels of antibody during *Ascaris* infection have different background genes and, with the exception of BALB.K, have independent

Figure 26; The recognition profiles of (SJL x C57BL10)F_1 hybrid and parental strains. Radio-iodinated L3/4 ES was immunoprecipitated with normal serum from SJL (a), F_1 (c), C57BL10 (e) or tertiary infection serum SJL (b), F_1 (d), C57BL10 (f).

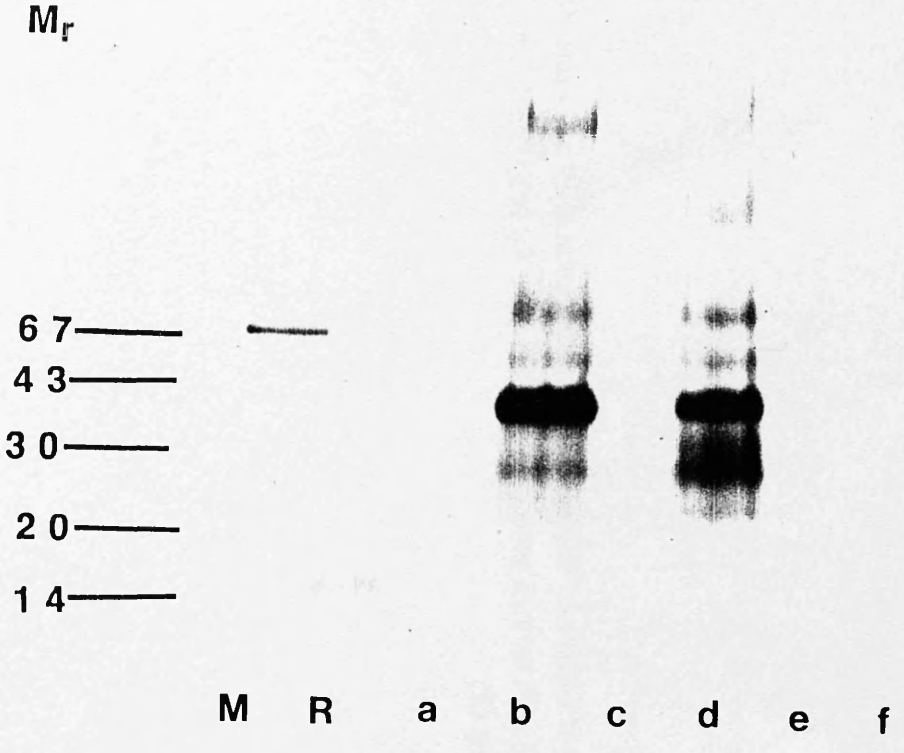

Mr
67
43
30
20
14
M
R
a
b
c
d
e
f

Figure 27; The recognition profiles of (AGUS x PYG)F_1 hybrid and parental strains. Radio-iodinated L3/4 ES (T) was immunoprecipitated with normal serum from AGUS (a), F_1 (c), PYG (e) or tertiary infection serum AGUS (b), F_1 (d), PYG (f).

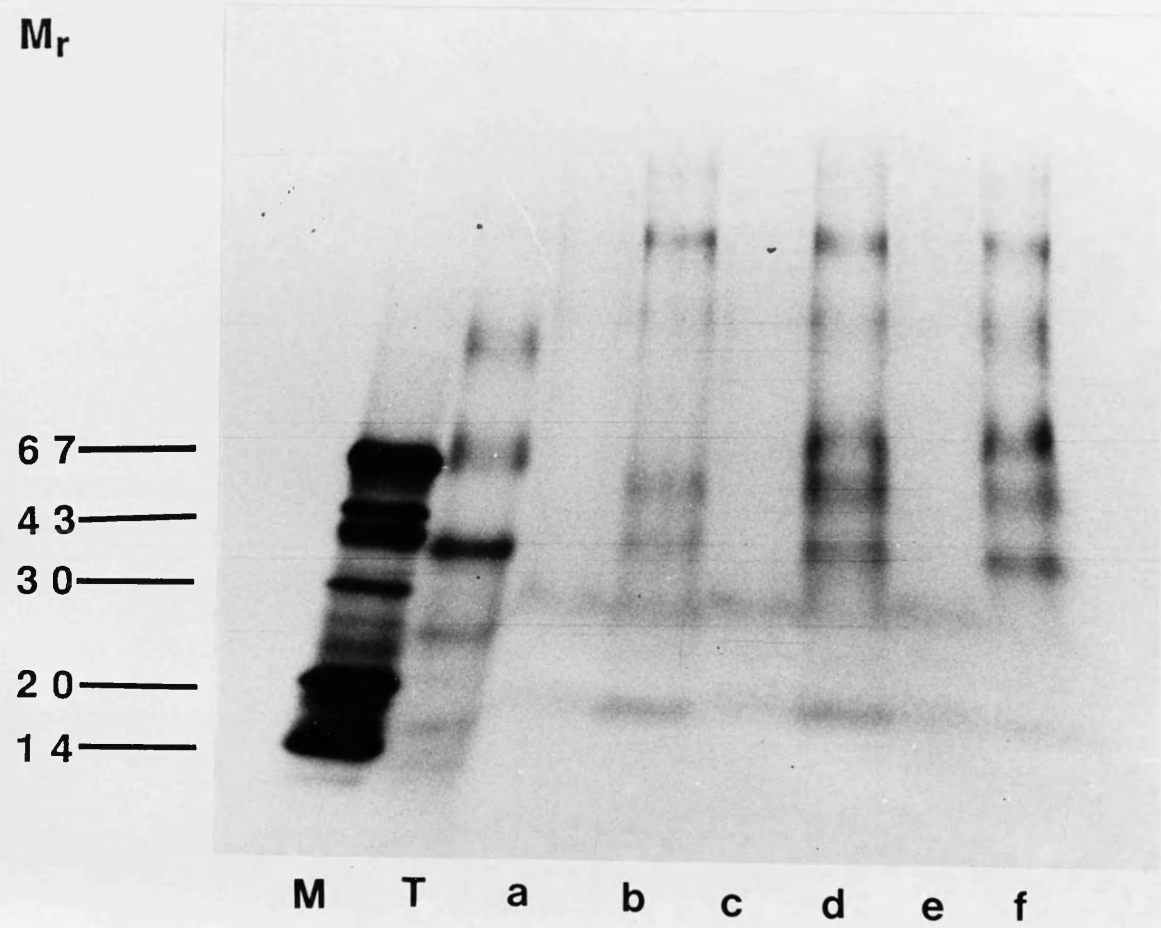

Mr
67
43
30
20
14
M
T
a
b
c
d
e
f

Figure 28; Recogntion of the 14kDa molecule by F_1 hybrid mice infected with *A. suum*. Radio-iodinated 14kDa (T) was immunoprecipitated with normal serum (a) or serum taken 14 days after a quarternary infection from (C57BL x B10.S) (b), (B10.D2 x B10.S) (c), (BALB/c x CBA) (d), blank track (e), (SJL x C57BL) (f), (CBA x SJL) (g), (SJL x B10.D2) (h), (BALB/c x SJL) (i) F_1 hybrids.

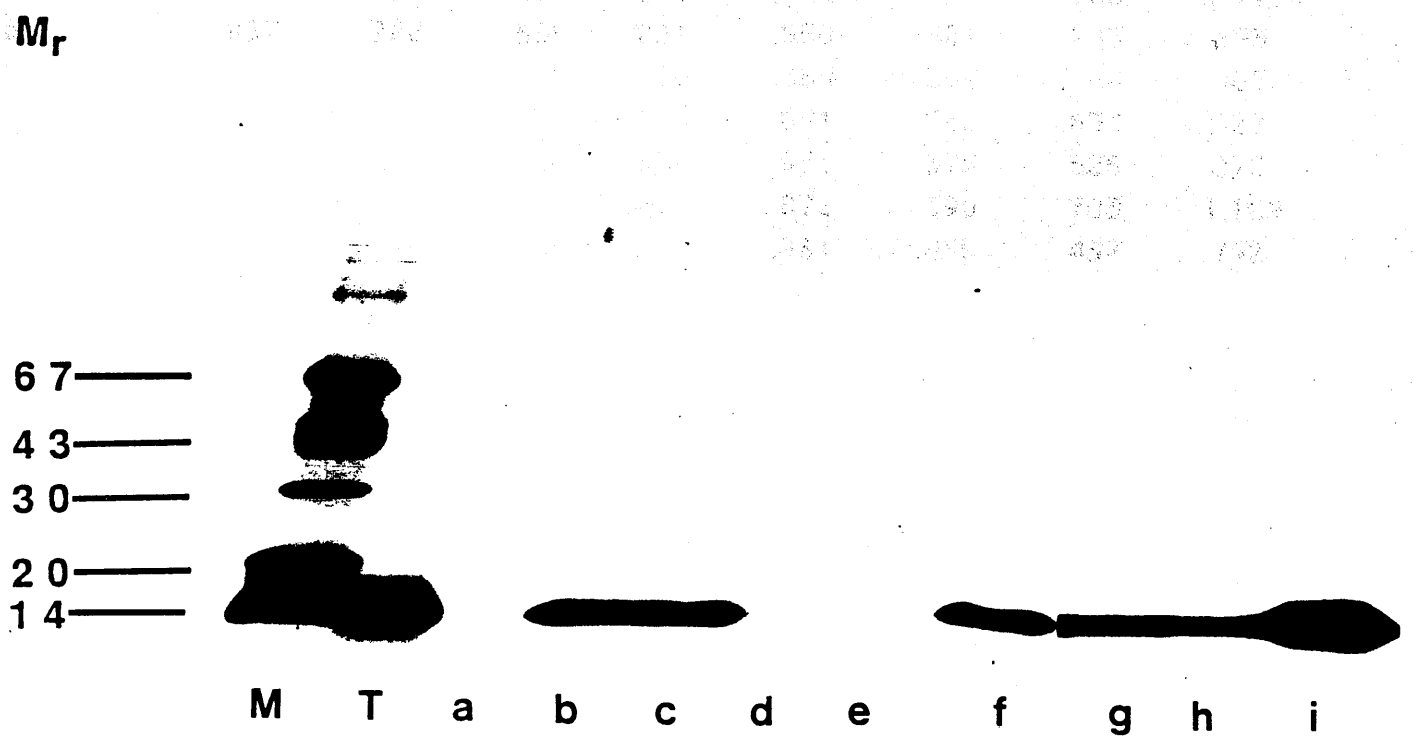

M_r
67
43
30
20
14
M
T
a
b
c
d
e
f
g
h
i

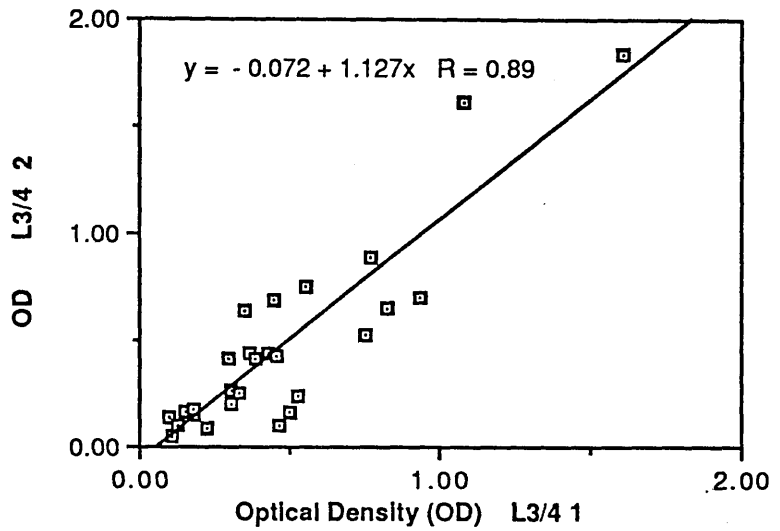
Table 6: Mouse ELISA Data.

Strain	ABF 1	14K 1	L3/4 1	L2 1	ABF 2	14K 2	L3/4 2	L2 2
BALB/c N	.167	.117	.106	.063	.071	.407	.050	.233
BALB/c	1.052	.227	.557	.695	.742	.371	.746	1.111
BALB.B	.967	.220	.535	.221	.550	.401	.243	.673
BALB.K	.425	.136	.467	.090	.384	.364	.104	.408
C57BL10	1.349	.201	.830	.555	.871	.560	.654	.822
B10.D2	1.665	.287	.754	.384	.951	.378	.525	.870
B10.BR	1.046	.246	.941	.492	.872	.390	.703	1.109
B10.G	1.001	.190	.435	.355	.931	.455	.437	.792
DBA2	.658	.223	.502	.162	.283	.417	.167	.262
SJL	1.374	.302	.304	.172	.708	.600	.257	.624
C3H	.455	.189	.307	.154	.125	.419	.206	.376
CBA	.423	.140	.228	.109	.162	.407	.093	.346
NIH	.760	.161	.455	.836	.428	.239	.422	.914
B10.S	.	.347	.779	.962	.647	.970	.891	1.442
BALB/cxSJL				.566	.437	.294	.754	1.420
C57BL10xB10.S	1.089	.286	1.079	1.367	.624	.378	1.610	1.972
B10D2xB10S	1.000	.239	1.607	1.528	.746	.510	1.834	1.904
CBAxBALB/c	.894	.190	.367	.347	.490	.290	.443	.689

Strain	ABF 1	14K 1	L3/4 1	L2 1	ABF 2	14K 2	L3/4 2	L2 2
CBAxSJL	.704	.139	.352	.586	.583	407	.642	1.371
SJLxC57BL10				.700	.744	.404	.937	1.588
anti-14K 1	.316	.224	.152	.136	.165	.386	.164	.411
anti-14K 2						1.923		

Tertiary infection sera from various strains of mice was screened against Ascaris antigens in an ELISA system. Each sera was screened in duplicate in each test and the test repeated twice (ABF1, ABF2).

Figure 29
Correlation between repeats of mouse L3/4 ES ELISA



Correlation between repeats of mouse L2 ES ELISA

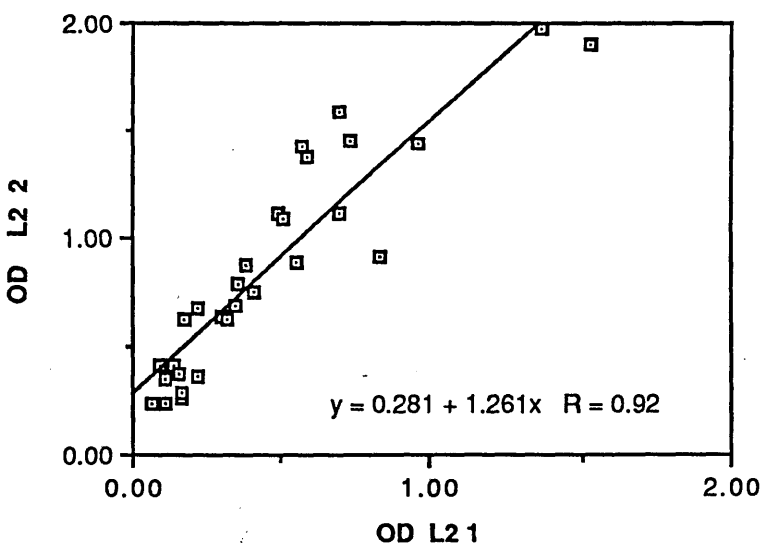


Figure 30
The OD of infection serum against ABF

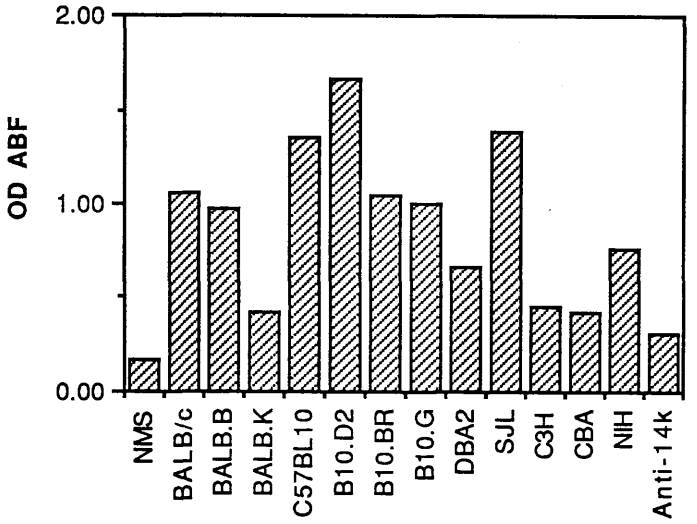
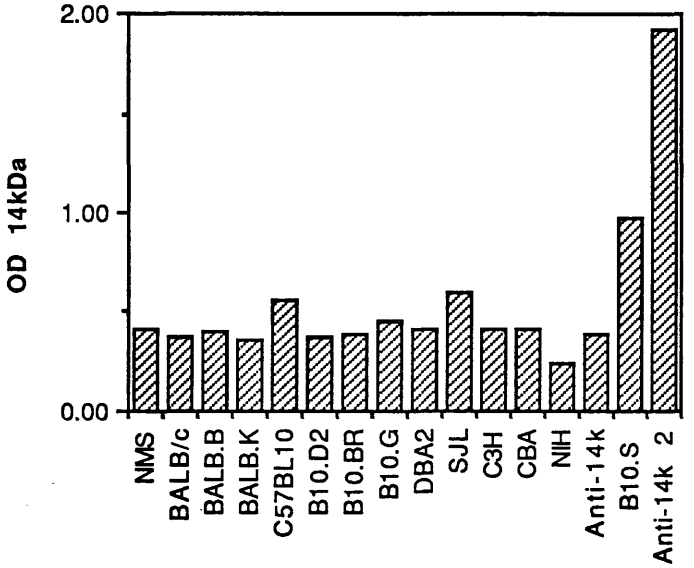


Figure 31
The optical density of infection sera against 14kDa



backgrounds to all the other animals screened. This suggests that the difference may be controlled by background genes and that these three strains have genes in their genetic backgrounds which result either directly or indirectly in low antibody production. This does not, however, explain the low response of BALB.K mice, as the other BALB animals produce high antibody levels. It can be postulated that antibody production is controlled by a relationship between background and MHC-linked genes. The results observed in BALB.K animals can, therefore, be explained in the context of MHC-linked genes limiting the antibody production by controlling the specificity of recognition, from a genetic background capable of synthesising high levels of antibody.

Of the four low responders, three have H-2^{k} haplotypes, which may suggest that animals with this haplotype respond to fewer of the potentially antigenic molecules and hence produce lower antibody levels. Of the animals screened there are four H-2^{k} strains, the other, B10.BR, produces high antibody titres against these antigens. This suggests that the effects of the MHC may be altered by background genes. In BALB.K animals, the MHC haplotype appears to result in lower antibody production by down regulating the background genes. Whereas, in B10.BR animals, the combination of H-2^{k} haplotype and B10 background results in higher antibody levels. It should be noted, however, that most of the B10 animals produce very high antibody titres and the B10.BR production may

have been down regulated as much as the BALB, but due to the higher production this regulation is less obvious.

Antibody levels against the purified 14kDa molecule

The only animals to recognise the 14kDa molecule in ELISA were B10.S mice and BALB/c mice immunised with 14kDa and adjuvant (see Figure 31). The antibody level of B10.S mice was higher than that for SJL mice. This may reflect a background difference in antibody production or may reflect the fact that these two strains of animals may actually recognise different epitopes on the 14kDa molecule, the epitopes favoured by SJL mice being obscured in the ELISA system.

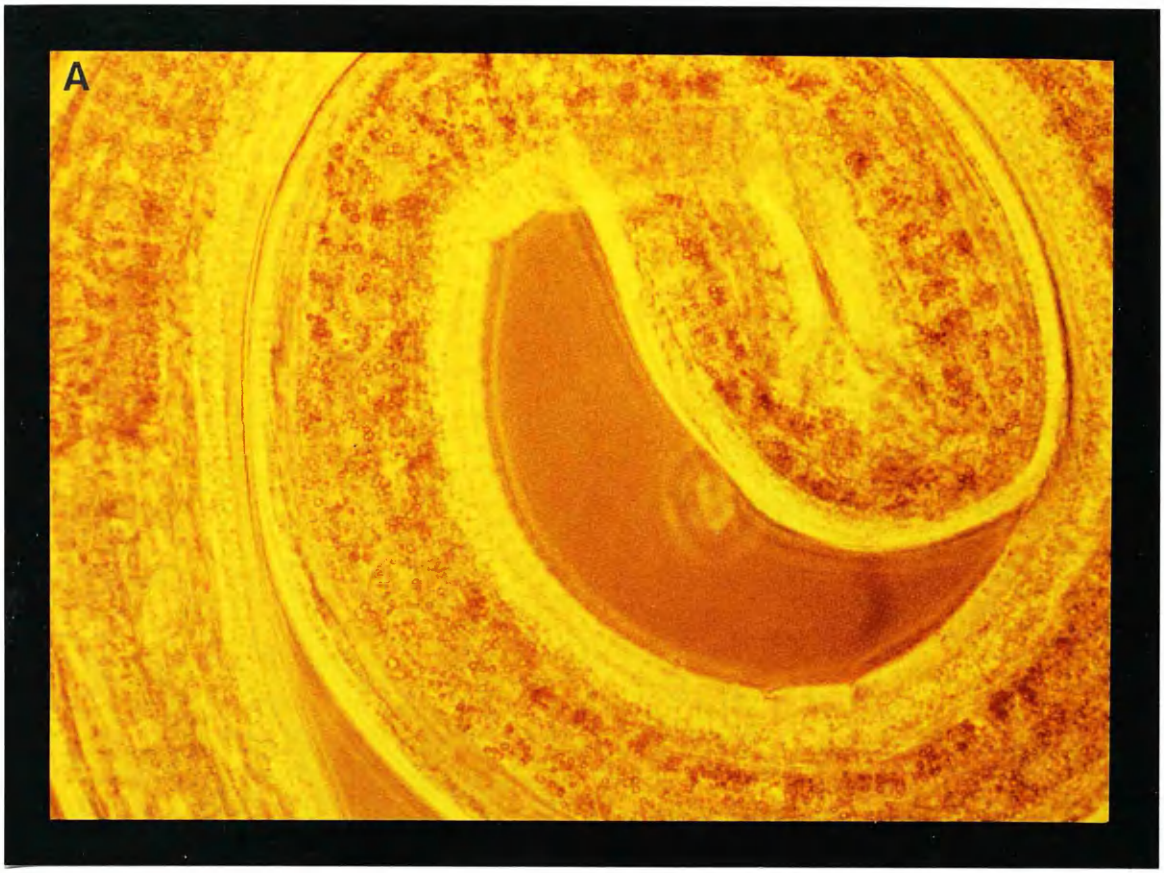
Of the F_1 hybrids screened, none produced significant amounts of antibody against the 14kDa after a tertiary infection. In order to produce significant results against the 14kDa molecule, these animals had to be infected for a fourth time. Why these F_1 hybrids should appear to respond more slowly to the 14kDa molecule is unclear. This delayed response can also be observed in SDS-PAGE analysis of this sera. After a tertiary infection the 14kDa recognition is quite faint but after a subsequent infection the recognition was far more intense (Figure 28).

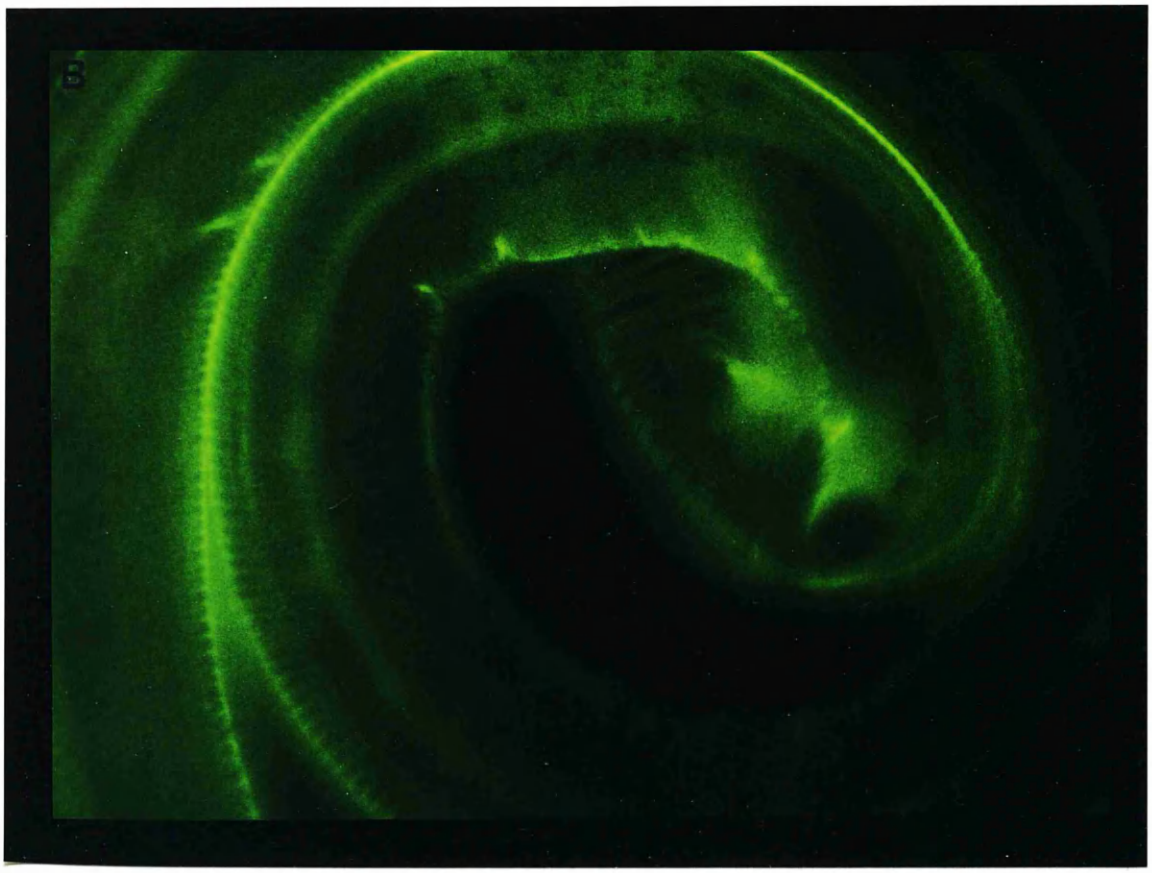
Indirect immunofluorescence on living parasitic larvae.

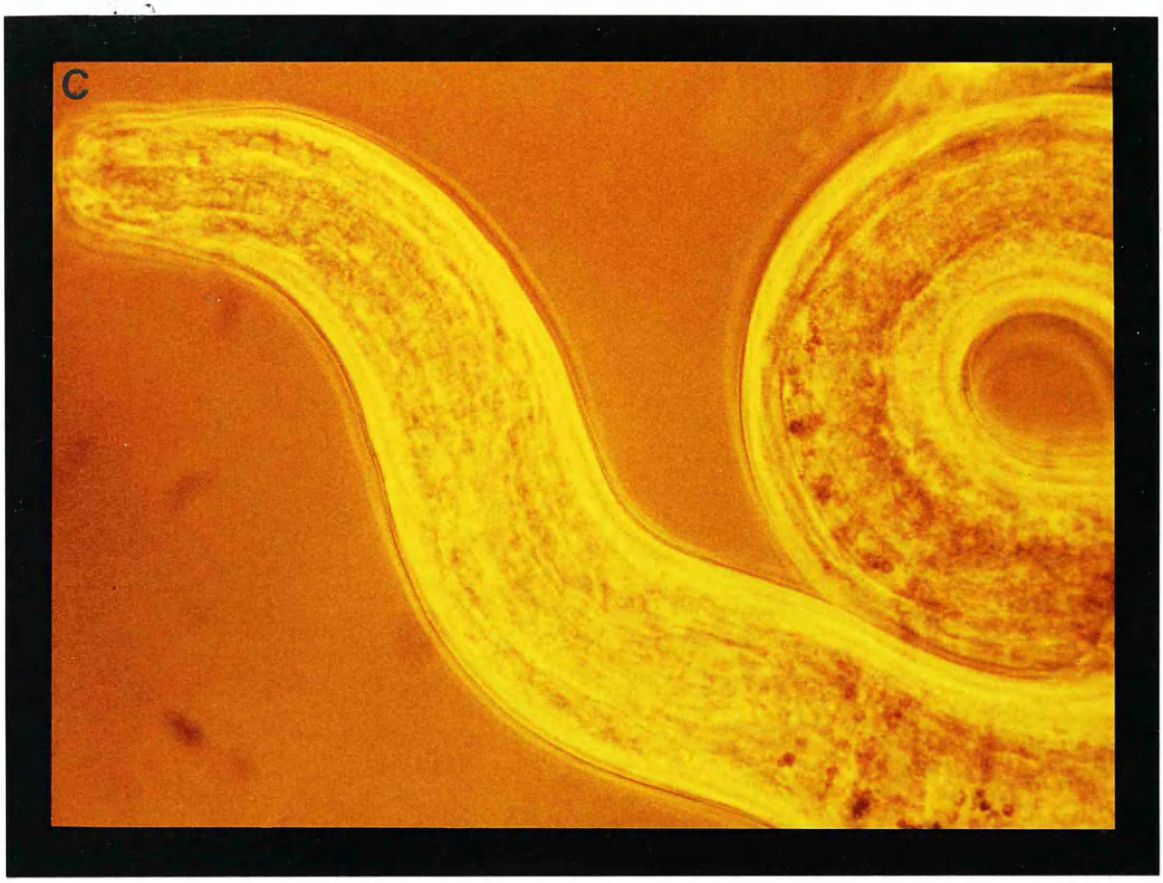
The surface binding antibody produced by several strains of rats and mice was examined using a FITC conjugated antibody and a photometer. A sample of approximately 21 L2 larvae and 21 readings from at least 5 different L3/4 larvae were counted for each serum sample (see Figure 32, a positive serum sample against L3/4 larvae; Figure 33, a positive serum sample against L2 larvae; Figure 34, a negative serum sample). The results show that most of the strains tested produce approximately equal amounts of surface binding antibodies (Figure 35, Mouse strains; Figure 36, Rat strains). The readings against the L3/4 larvae are higher than those against the L2 larvae in relation to a positive control. However, B10.S mice produced lower readings against the L2 larvae, and BALB/c mice and PVG-RT1^{u} rats produced lower readings against L3/4 larvae. These results may be explained by a combination of background and MHC genes resulting in these animals not producing optimal antibody production against the molecules expressed on the surface of living larvae.

This analysis, of the binding capacity of infection sera to the surface of living larvae, shows that the larvae examined consist of a heterogeneous population. This is most clearly seen with respect to the L2 larvae (see Figure 37). From an analysis of this sort, one would normally expect the results for a given sera to be normally distributed around the mean value, but while this can be seen with some of the sera tested it is not the

Figure 32; *A. suum* L3/4 larvae (x 40). Panel A and C, L3/4 larvae seen under bright field . Panel B and D, L3/4 larvae visualised using a surface binding rabbit serum and a FITC conjugate.


A


B



D

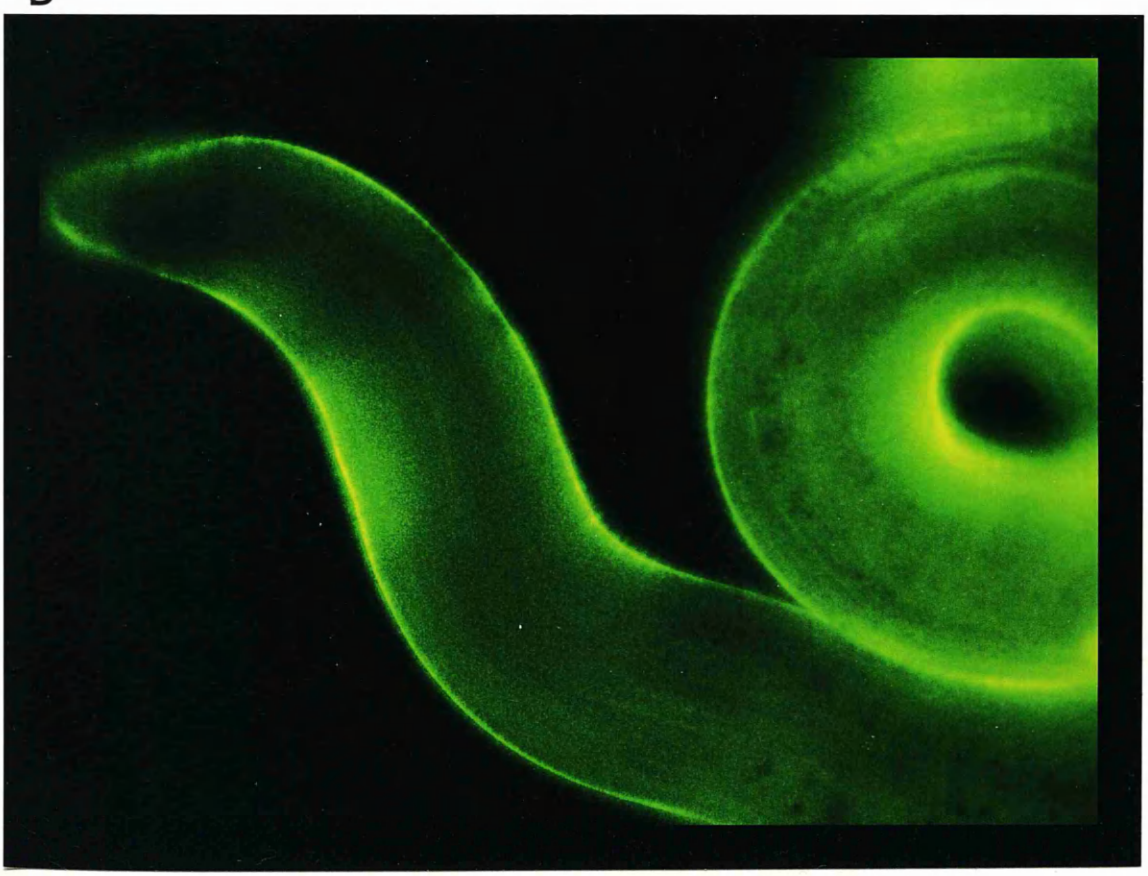
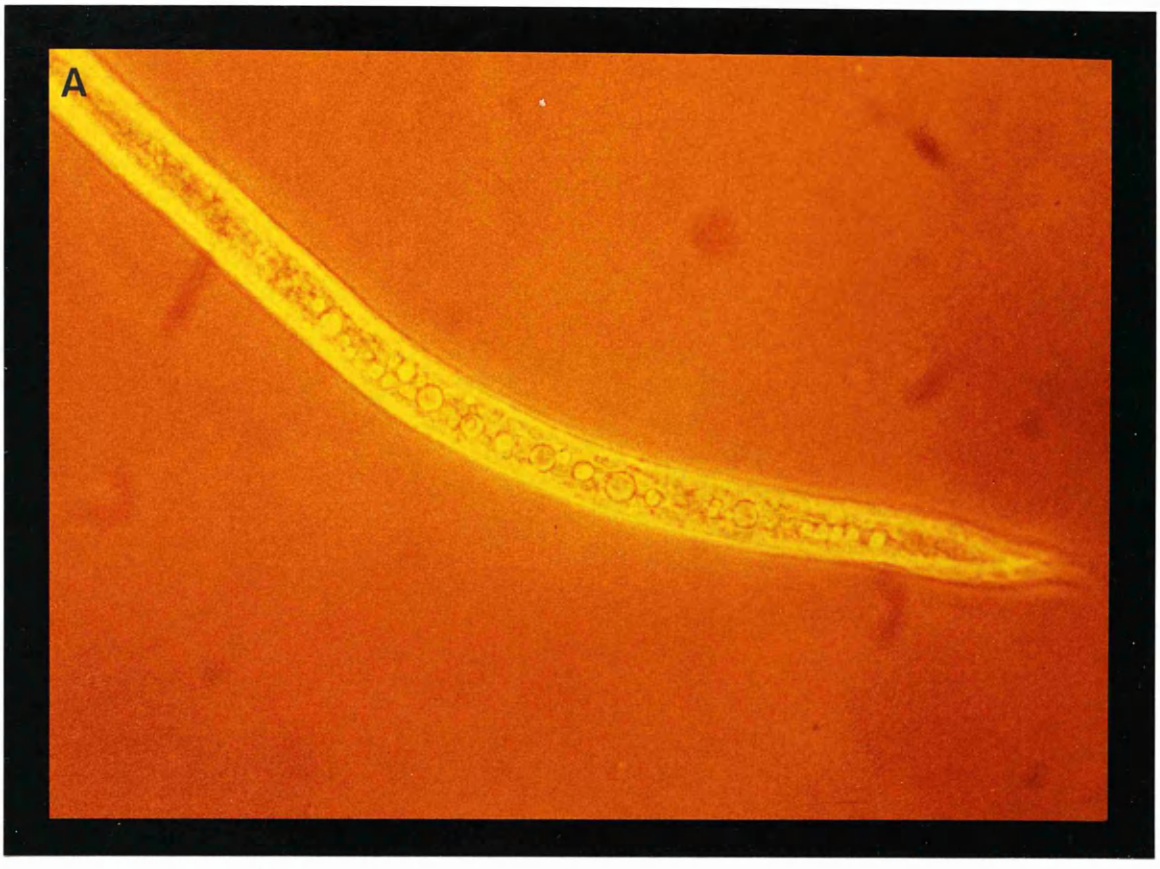


Figure 33; *A. suum* L2 larvae (x 40). Panel A, L2 larvae, bright field. Panel B, L2 larvae visualised using a surface binding rabbit serum and a FITC conjugate.


A

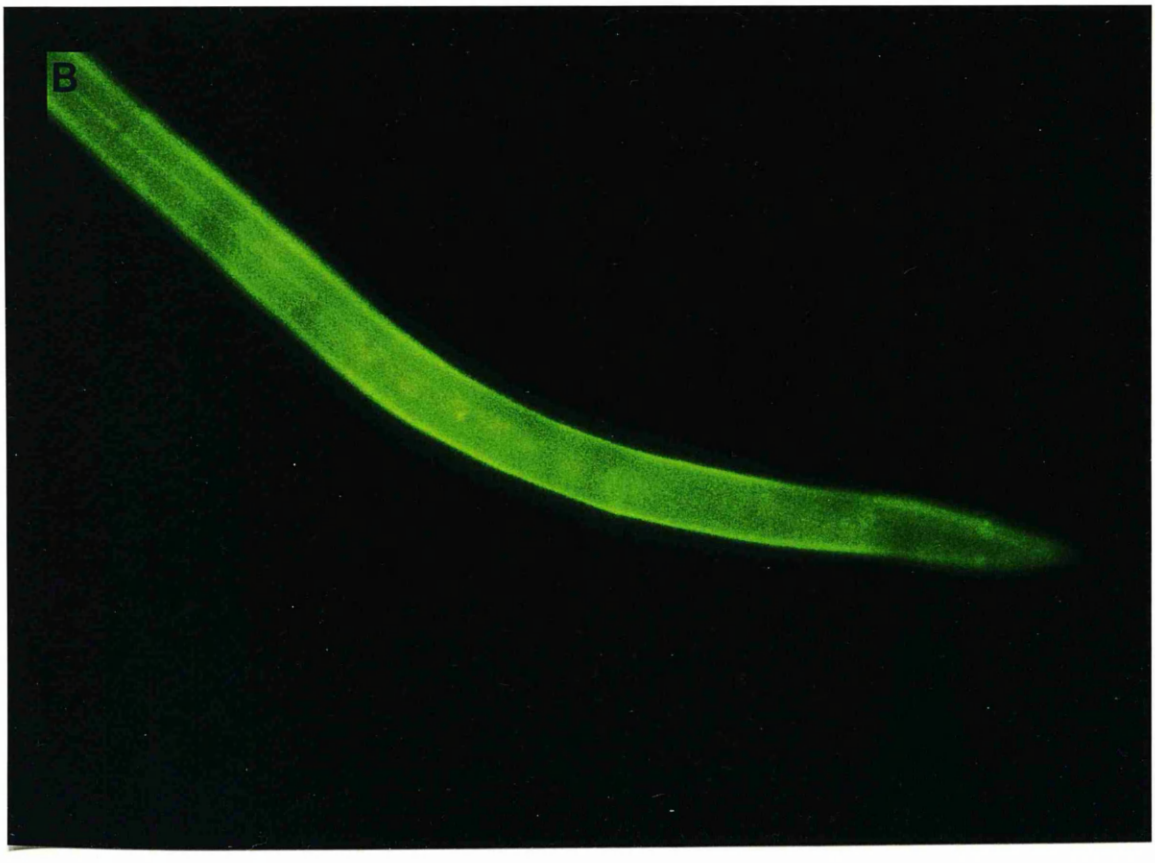
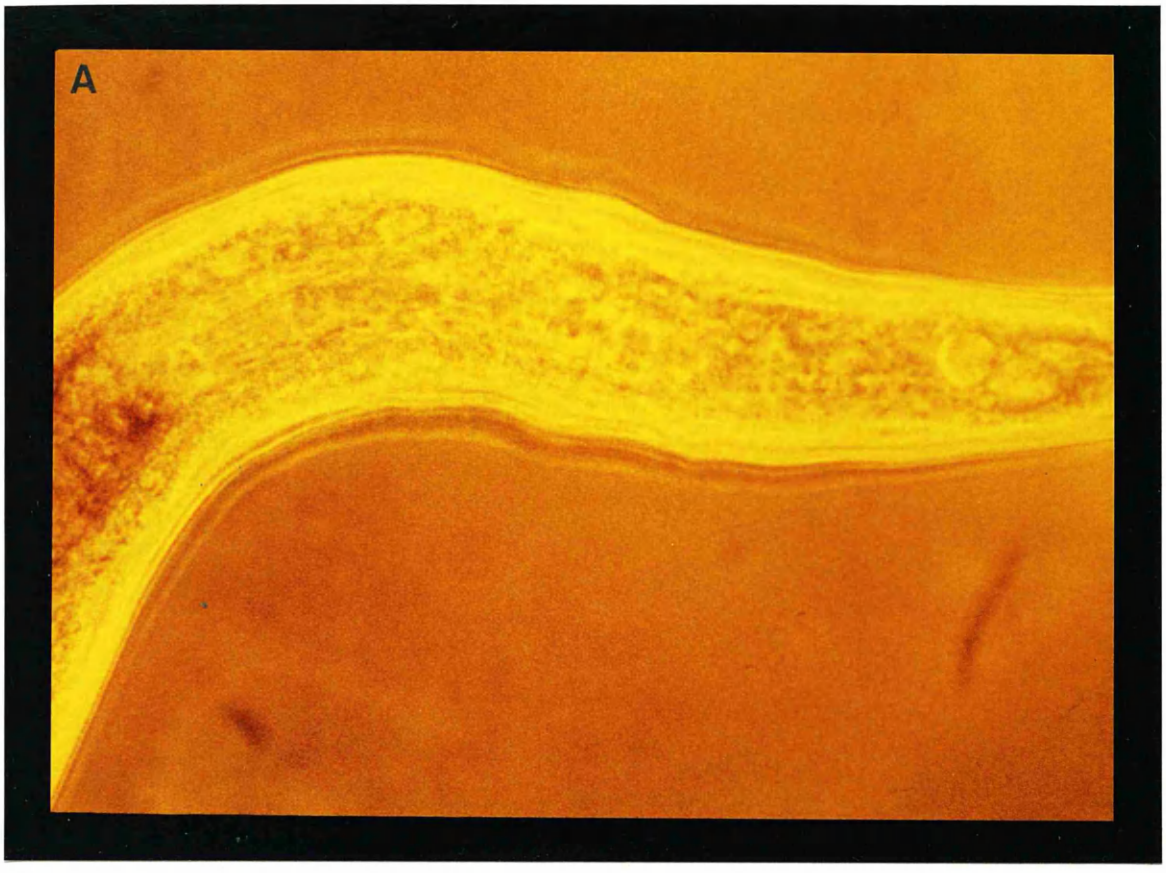

B

Figure 34; *A. suum* L3/4 larvae (x 40). Panel A, L3/4 larvae, bright field. Panel B, L3/4 larvae visualised using normal rabbit serum and a FITC conjugate.



B

Figure 35
Mean fluorescence readings of various strains of mice

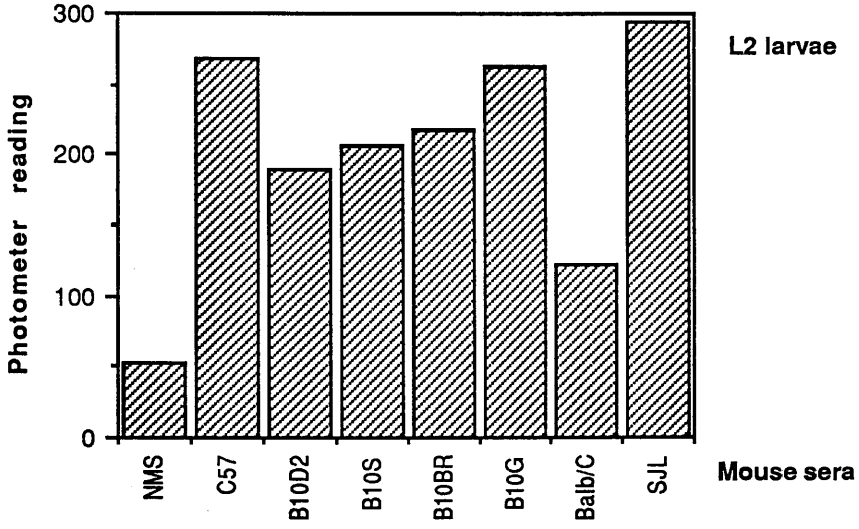


Figure 36
Mean fluorescence readings from various strains of rats

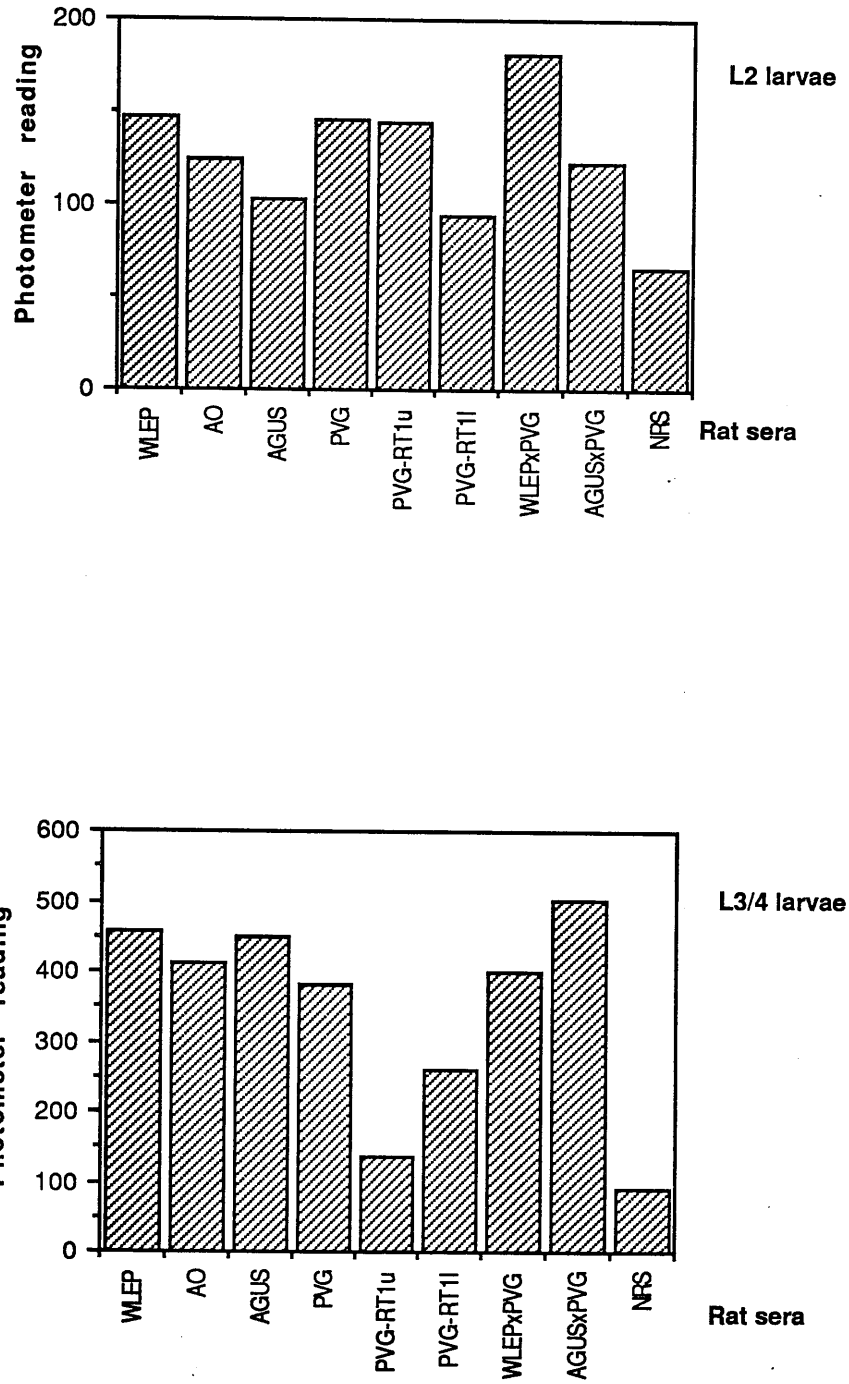
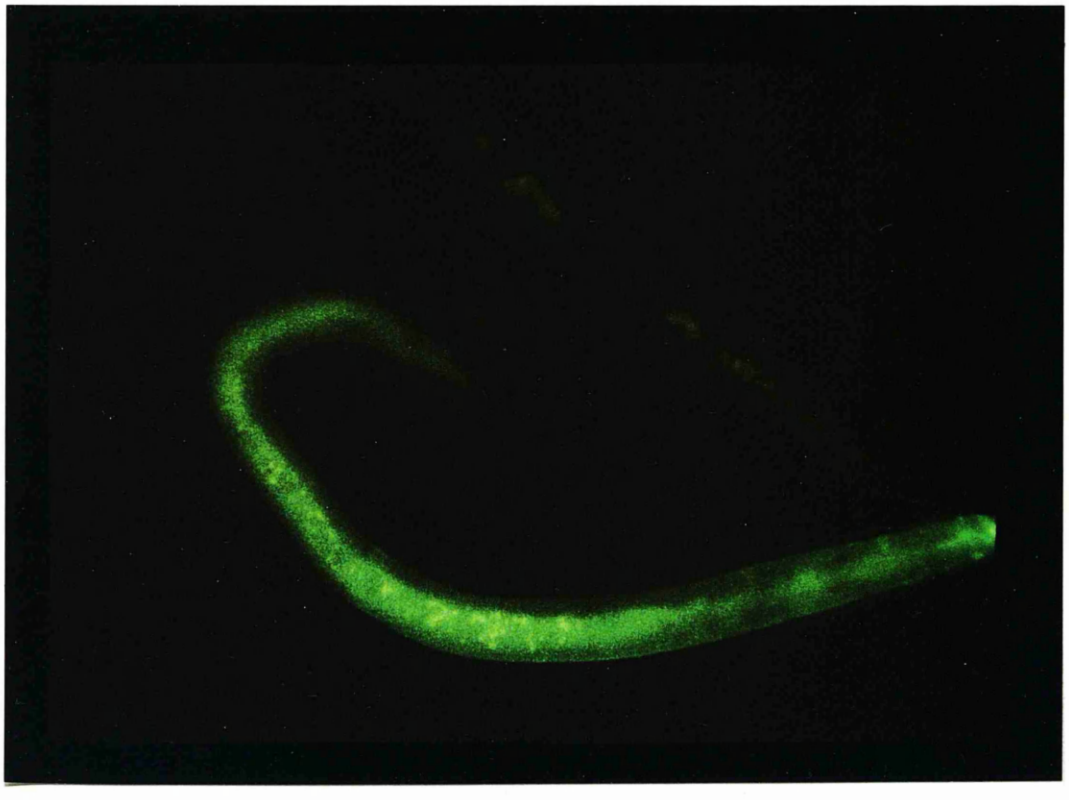


Figure 37; *A. suum* L2 larvae (x 40). Panel A, L2 larvae, bright field. Panel B, L2 larvae visualised using rabbit-anti *A. suum* serum and a FITC conjugate. This panel highlights the heterogeneity seen between individual larvae.

B



case for all of them (Figure 38, Normal rat serum, WLEPxPVG and AGUS frequency distribution of photometer readings).

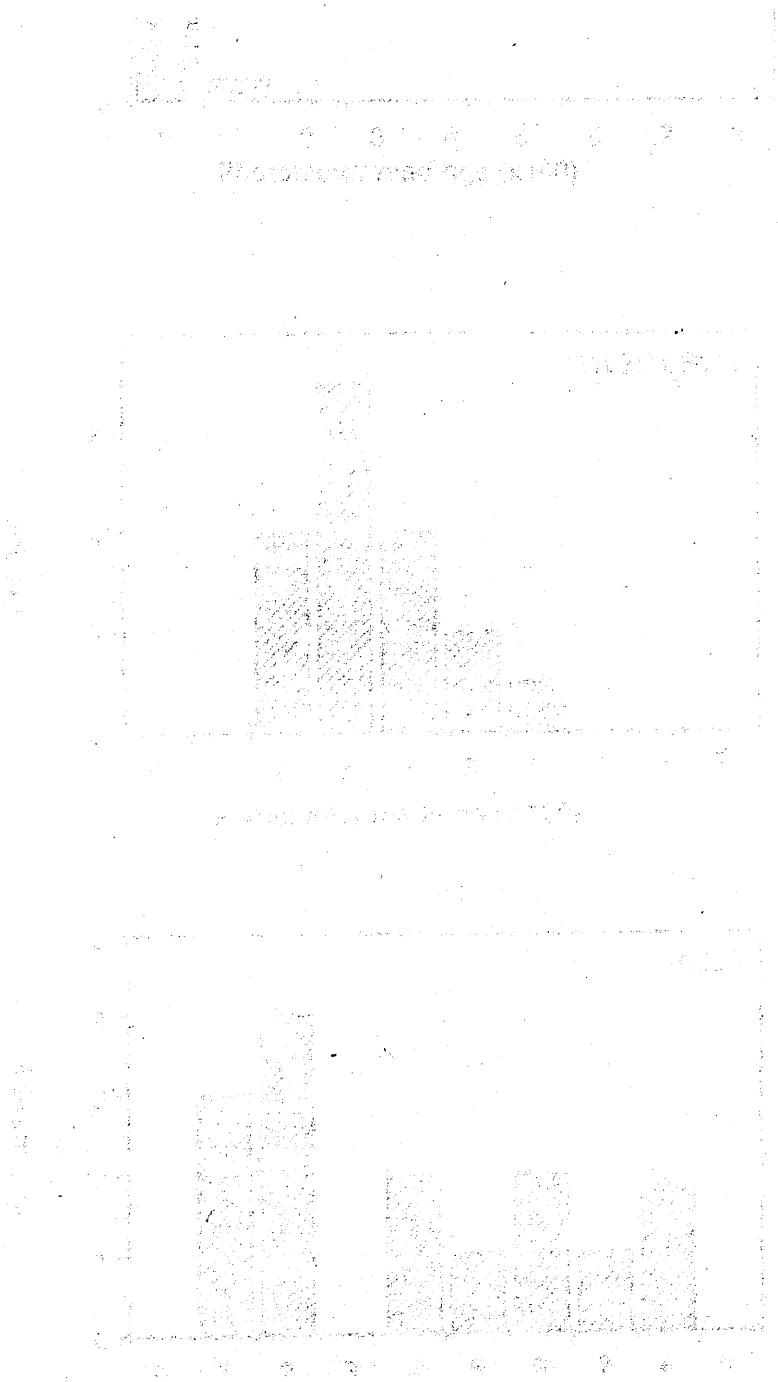
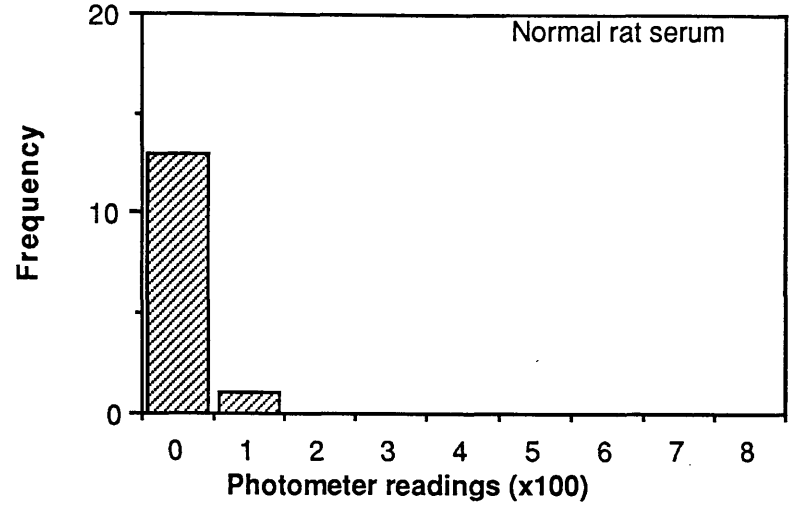
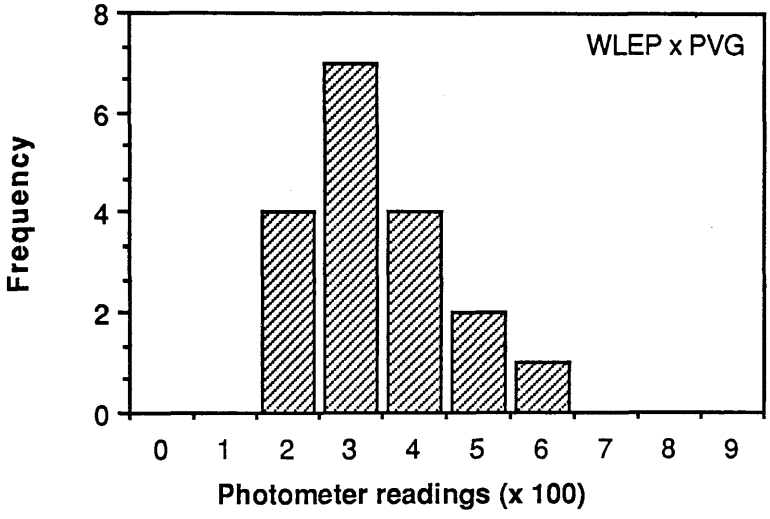
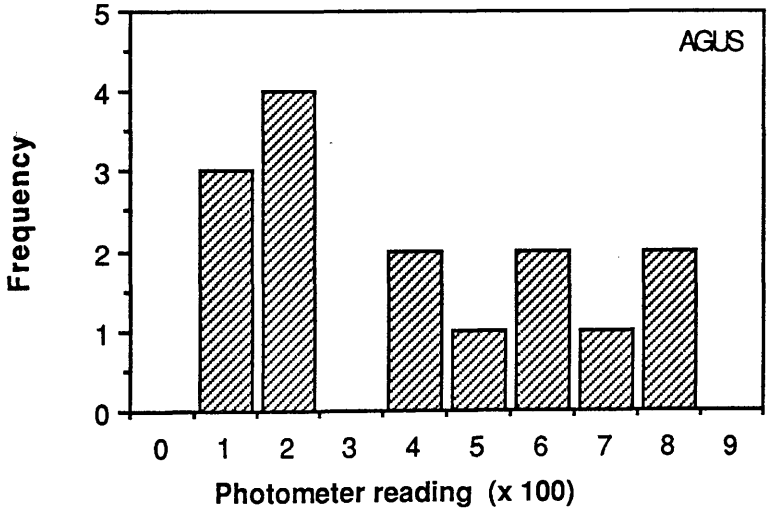


Figure 38
The frequency distribution of photometer readings







Chapter 2

Discussion

The results presented in this chapter suggest that the qualitative and quantitative antibody response of mice infected with *A. suum* is controlled by MHC-linked and non-MHC-linked genes, respectively. This observation has been made in several studies involving a variety of organisms (Wassom *et al*, 1984; Wakelin, 1985; Wakelin and Blackwell, 1988; Else and Wakelin, 1989). Of the inbred and congenic animals examined, none respond to all the potentially antigenic components of the ABF or E/S products. This lack of response to certain components can be explained in the context of antigen presentation to T cells, as the IgG production monitored is T cell dependent.

The immune system has "blind spots" which lead to precisely delineated failures in responsiveness (Benacerraf and Mc Devitt 1972; Schwartz 1986). These "holes" in the repertoire can be caused by three separate gene groups. If the necessary V region gene for either the T cell or B cell receptor is missing from an animal's gene pool, it will be unable to respond to the specific antigen this V region codes for (Blomberg *et al*, 1972; Cohn *et al*, 1974; Epstein *et al*, 1986). This explanation may account for the non-responsiveness to some of the *Ascaris* components (those not recognised by any strain) but cannot explain the selective recognition by some congenics. All congenic animals should have the same background genes. This means that the gene pool encoding the T and B cell

receptors for B10 or BALB congenics is the same. Consequently, the ability of some animals to respond, for example, the H-2^{s} haplotype strains respond to the 14kDa, and H-2^{b} respond to the 16kDa molecule, means that the necessary receptors are present in the repertoire of these congenic animals. The required receptor for 14kDa recognition is also present in BALB/c mice as these animals can respond to this molecule when immunised with purified 14kDa and FCA.

Alternatively, this lack of response may be caused by MHC or Ir genes. The immune response against protein antigens involves the activation of MHC-restricted T cells by peptide fragments of the antigen complexed to Class II (Ia) molecules on the surface of the APC (Heber-Katz *et al*, 1983). As there does not appear to be any differences in how antigen is processed and presented by different strains (Freidman *et al*, 1983) it is suggested that these differences occur due to the association of antigen with Ia molecules. It has been found that polymorphisms in these Ia molecules correlate with responsiveness to many antigens and hence failure to respond represents a failure of the antigenic peptide to bind to the Ia molecule (Janeway *et al*, 1976; Benacerraf, 1978). The products of some MHC alleles cannot form complexes with particular antigens (Babbitt *et al*, 1985; Buus *et al*, 1987). Consequently, individuals bearing these alleles cannot respond to that specific antigen. This hypothesis may

explain the lack of response seen in the *Ascaris* model. It can be postulated that the only Ia molecule which results in T cell activation, when complexed with the 14kDa molecule, is that coded for by the H-2^{s} haplotype, and likewise for H-2^{b} haplotype and the 16kDa molecule. This would also suggest that the Ia molecules encoded by the other H-2 haplotypes do not associate in the correct way with the antigenic peptides of either of these molecules.

The third possibility is that self-tolerance prevents an immune response to particular parasite components. To avoid continuously attacking self-tissue, animals are tolerised to their own body proteins, ie. they are unable to mount a response against them, hence any combination of foreign antigen and Ia that resembles self does not induce a response (Billingham *et al*, 1956; Vidovic and Matzinger, 1988). This hypothesis may also provide an explanation for the results observed in this study. If the complex formed between the Ia molecule and the antigenic peptides resemble self the animals will be tolerant to that determinant of the foreign antigen. Therefore, any combination of 14kDa and H-2 other than 14kDa-H-2^{s} may resemble some self component and the animals would be unable to respond. The same hypothesis could be proposed for recognition of the 16kDa molecule with H-2^{b}. This hypothesis may explain the observations in this system. If there is more than one allele for a given background

gene, then expression of a different allele between congenic strains together with a different H-2 haplotype could result in tolerance in one congenic strain but not the others (McDevitt and Chintz, 1969).

Regulation of the immune system is also mediated by regulatory pathways, for example suppressor cells. One model which links suppressor cells, the MHC and response to infection was proposed by Baxevanis and colleagues (1981). All inbred strains of mice express I-A molecules but some strains, those with the H-2 haplotypes b, s, f and q, do not express I-E molecules (Murphy *et al*, 1980; Jones *et al*, 1981). It has been shown that antigen presented in the context of I-E can induce a response which suppresses the ability of I-A restricted T cells to proliferate (Baxevanis *et al*, 1981; Oliveria *et al*, 1985). This phenomena has been used to explain the fact that mice which express I-E molecules are generally more susceptible to *T. spiralis* and *N. dubius* infections (Wassom *et al*, 1987). If the restricted recognition seen in the *Ascaris* model was controlled by expression, or non-expression of I-E molecules, there would be a direct correlation between I-E expression and antigen recognition. This correlation could be argued to exist in some strains with respect to recognition of the 14kDa and 16kDa molecules; these molecules are recognised by strains of animals which do not express I-E molecules (b and s). However, these molecules are not recognised by all non-

I-E expressing strains (H-2^{q} haplotype mice do not express I-E, and do not recognise either the 14kDa or 16kDa molecules). The F_1 hybrids which respond to the 14kDa molecules will express both I-A and I-E molecules.

All the F_1 hybrids with the s alleles in their H-2 respond to the 14kDa molecule in the context of infection. This further supports the hypothesis that this regulation is mediated by the combination of Ia and antigen, as these hybrids will have the necessary H-2 allele to associate with the 14kDa molecule. In the (BALB/c x SJL)F_1 cross there is recessive inheritance of recognition of an 118kDa molecule. This molecule is recognised by the BALB/c parent but both the SJL and the hybrid failed to respond. This result can be rationalised within the cross-tolerance model (Schwartz, 1978; Matzinger, 1981). This model proposes that in a cross between a responder and a non-responder, which results in a non-responding hybrid, more than one allelic form of the self antigen is available and a different allele is expressed in the hybrid than in the responder parent. Hence, the Ia-antigen complexes expressed in the hybrid mimics self and the hybrid is tolerant of the antigen.

The apparent delay in responding to the 14kDa molecule seen in F_1 hybrids, as compared to parental responses, may be explained in two ways. The F_1 hybrids express

both parental haplotypes in their MHC, consequently, the amount of Ia of either haplotype expressed is lower than in the homozygous parent. This reduction in the expression of the relevant Ia molecule may result in less efficient antigen presentation and consequently less Th activity, resulting in a slower response. The expression of more than one allele at the MHC may produce suppression as well as helper activity. If, for example, the non-responder parent failed to produce antibody against the 14kDa molecule because this molecule, in conjunction with the non-responder Ia resulted in the activation of suppressor cells, then these suppressors would also be activated in the F_1 hybrid. This would result in competition between Th cells triggered by one Ia allele and Ts cells triggered by the other Ia allele, therefore the final response would depend on the balance between these two cell populations.

The heterogeneity seen between individual larvae in the immunofluorescence analysis reflects the fact that these parasites form a genetically diverse population. The differences in readings between certain strains may reflect the fact that the larvae liberated in these animals, when infected with embryonated eggs, had a different genotype to the ones used in the analysis, or that the antigens expressed on the larval surface varied. Alternatively, these differences may reflect the fact that the antigens recognised by the host are

different from those expressed on the surface of the lavae.

The results from the ELISA and immunofluorescence analyses suggest that the differences observed between these congenic strains of animals are qualitative rather than quantitative, ie. no strain of animal is incapable of producing antibody when infected with *Ascaris* and this antibody is capable of binding to the larval surface. However, the ELISA results do show differences in the level and specificity of antibody produced between strains of mice. These differences suggest that background and MHC-linked genes affect antibody production in infection with *Ascaris*. The MHC-linked genes determine the specificity of the response in collaboration with the genetic background and non-MHC-linked genes determine the level of antibody production.

Chapter 3

The IgE response to *Ascaris* infection

Introduction

Helminth infection results in the production of substantial amounts of parasite-specific and non-specific IgE (Johnasson *et al*, 1968; Orr and Blair, 1969; Jarrett and Stewart, 1972; Jarrett and Miller, 1982; Ray and Saha, 1978) which is T cell dependent (Ogilivie, 1967; Jarrett, 1976). The major biological characteristic of reagins from mammals is their prolonged tissue binding capability (Bloch, 1967; Gershwin, 1978). This tissue binding is a function of the Fc end of the heavy chain of this class of immunoglobulin and is damaged by heating at 56°C (Dorrington and Bennich, 1978). When specific antigen binds to cell bound IgE, aggregation of the Fc receptors occurs and vasoreactive components are released from the cell (Ishizaka and Ishizaka, 1978).

These parasite-specific reagins are thought to play a role in the immune response against the parasite, but their efficacy in parasite elimination is controversial (Ogilvie and Parrott, 1977; Musoke *et al*, 1978). For example the self cure reaction seen in infection with certain helminths is thought to be mediated by a local allergic reaction (Stewart, 1953, 1955; Urquhart *et al*, 1965) and IgE antibodies may have other functions, for example the binding of macrophages to the surface of schistosomula (Ogilvie and De Savigny, 1982).

IgE antibodies have been found in the sera of patients infected with *A. lumbricoides* in a number of studies (Tsuji *et al*, 1977; O'Donnell and Mitchell, 1978). This

parasite-specific IgE identified a range of allergens from *Ascaris*. Heterogeneity in the IgE response has also been demonstrated in many other parasitic infections, for example, *S. mansoni* (Harris, 1975; Vannier *et al*, 1974) and *T. taeniaeformis* (Leid and Williams, 1975).

Helminth infection is a potent means of inducing IgE production and a number of parasitic components have been identifed as allergens (Pe.ry and Luffau, 1979). The most extensively studied parasitic allergens are probably those of the ascarids (Jarrett and Miller, 1982). Hogarth-Scott (1967) studied the allergens from *N. brasiliensis, Toxocara, Toxascaris* and *Ascaris*. He found that the allergens from these helminths had molecular weights in the range 10,000-50,000, consistent with allergens from other sources. This allergenic activity tended to be heterogeneous, in that infection serum identified several allergens, and it has been shown that there is cross-reactivity between these allergenic components from various parasites (Turner *et al*, 1980). Allergenic activity has been identified in somatic and ES antigens (Murrell *et al*, 1974; Kobayashi *et al*, 1972; Senft *et al*, 1979; Senft and Maddison, 1975).

The presence of multiple allergens in parasitic components may be explained in two ways; completely separate molecules with allergenic activity, or as demonstrated by Fujita *et al* (1979), the allergen may be

a single entity which aggregates or forms complexes with other molecules in the parasitic extract.

Several groups have identified *Ascaris* allergens (Ambler *et al*, 1974; Bradbury *et al*, 1974; O'Donnell and Mitchell, 1978; Hussain *et al*, 1972; Herzig, 1974; Kuo and Yoo, 1977; Campbell, 1936; Kent, 1963) and several of these molceules have been purified. Allergen A, which is believed to be the principle allergen of *A. suum* (Ambler *et al*, 1972, 1973) has a molecular weight of 14,000Da and is a very stable protein, present in both E/S and extracts of adult worms (Ambler *et al*, 1974). Asc-1 is a negatively charged glycoprotein with a molecular weight of 17,000-19,000Da (Hussain *et al*, 1973), and is present in all stages of the parasite's life cycle. Asc-1 appears to be directly involved in stimulating reagin production during the migratory phase of the parasite's life cycle (Bradbury *et al*, 1974). Allergens other than Asc-1 and allergen A have also been identified in ABF (Kuo and Yoo, 1977).

The specificity of the response against these allergens varies with the route of administration of initial infection (Ambler *et al*, 1973). It is known that parasites release allergens into the medium when maintained in culture (Wilson *et al*, 1967; Ogilvie and Jones, 1969). These fluids, in which worms have been maintained *in vitro*, are often more reactive as allergens than whole worm extracts (Kobayashi *et al*, 1972; Murrell

et al, 1974; Ogilvie and Jones, 1971). Immunisation with parasite extracts induces a relatively low and transient reagin response (Ogilvie, 1967). Some property of helminths more fundamental than association with a particular host tissue is involved in reagin production as neither the stage of the parasite nor the particular location in the host is critical for IgE stimulation.

As well as producing potent allergens, factors have been identified in worm extracts which directly elicit histamine release from mast cells of unsensitised animals (Thompson 1972; Tolone *et al*, 1974; Uvnas *et al*, 1960; Uvnas and Wold, 1967). Mast cell degranulators isolated from *Ascaris* have molecular weights 2000-3000 (Uvnas and Wold, 1967) 8000-9000 (Thompson, 1972) and 25000-40000 (Tolone *et al*, 1974). IgE potentiating and suppressive factors have also been described (Suemara and Ishizaka, 1979; Yodoi *et al*, 1981; Hirashima *et al*, 1980,1981).

In this chapter the IgE produced in response to *Ascaris* infection has been assayed by PCA and Western blotting. The genetic control and specificity of this response will be examined using MHC congenic animals and a purified *Ascaris* allergen. The influence on PCA titre of simple chemical and physical manipulation of the allergens has also been studied.

Chapter 3

Results

In this chapter the reaginic antibodies produced by several strains of rats and mice infected with *A. suum*, have been measured using passive cutaneous anaphylaxis (PCA). PCA in rats has been shown to be specific for the reaginic antibodies of both rats and mice (Prouvost-Danon, 1972; Ogilvie, 1964). PCA analysis can detect both kinds of homocytrotrophic antibodies, the transiently tissue fixing IgG and the heat labile IgE which can bind the Fc receptors on mast cells and basophils for far longer periods. To determine which type of reagin was being measured titres between native sera and heat inactivated sera, challenged after 2h and 24h, were compared (Table 7). This showed that the PCA titre resided in the heat labile component of the sera which bound mast cell receptors for more than 24h. The kinetics of IgE production during a course of *Ascaris* infections are presented in Table 8. This shows that the PCA titre peaks after a tertiary infection and remains constant after subsequent infections.

Reaginic antibody titre against ABF, in homologous PCA.
The PCA titres of several strains of rats against ABF were compared. All strains examined produced IgE against ABF in the course of infection. This implies that there are allergens in ABF which are either identical or cross-reactive with allergens found in the larval parasites, as the rats had only been exposed to the larval stages of infection. All 6 strains of rats produced PCA titres within the same range when

TABLE 7

The PCA titre of heat inactivated serum against ABF.

Sera	Challenged after (h)	Titre
NRS	4	0
WLEP	4	0
NRS	24	0
WLEP	24	0
Untreated sera		
NRS	24	0
WLEP	24	32

WLEP infection serum was heat inactivated by incubating for 2h in a 56^{0}C water bath. After cooling the serum was used in a PCA test and the test animals were challenged with 500µg ABF 4h or 24h after administration of the serum.

TABLE 8

The PCA titre of WLEP rats infected with *A. suum*.

Sera	Titre
NRS	0
WLEP 1°	0
WLEP 3°	32
WLEP 8°	32

Sera from WLEP rats infected with A. suum was tested by PCA. The test rats were challenged after 24h with 500µg ABF and the PCA titre recorded 30 min later.

challenged with ABF (Table 9). An analysis of two F_1 hybrid crosses showed that one cross, (WLEP x PVG)F_1, produced IgE within the expected range. However, the (AGUS x PVG)F_1 hybrid produced lower levels of IgE than either parental strain.

The reaginic antibody response against the 14kDa molecule in homologous PCA.

Given that SDS-PAGE analysis had revealed restricted recognition of the 14kDa molecule, infection sera were assayed for IgE activity against this molecule (Table 10). The concentration of the challenge antigen was reduced due to the restricted availability of this purified molecule, hence the PCA test rats were challenged with 50 microgrammes of purified 14kDA. The results from this assay revealed that the 14kDa is a potent allergen capable of eliciting PCA titres higher than those recorded with the ABF on an activity:weight basis. This analysis also revealed restricted recogniton of the 14kDa molecule; only rats with the $RT1^u$ haplotype produced IgE against this molecule. The (WLEP x PVG)F_1 hybrid, which has an RT1 haplotype of u/c, showed lower levels of IgE production than either parental strain.

PCA titres against chemically modified allergens

In an attempt to gain some information on the chemical structure of the IgE directed epitopes of these parasite allergens, the ABF and 14kDa preparations were

Table 9
PCA titres of sera from various strains of rats against native and modified ABF

Strain	RT1 Haplotype	Native	Autoclaved	Periodated	Reduced/ Alkylated
WLEP	u	16/32	32	32	32
AGUS	l	16/32	0	16	16
AO	u	32	32	32	32/64
PVG	c	16/32	0	0	16
PVG-RT^u	u	32/64	32	32	32
PVG-RT1^l	l	32/64	8	32	64
(AGUSxPVG) F_1	l,c	8	-	-	-
(WLEPxPVG) F_1	u,c	16/32	-	-	-

Sera from various strains of rats infected with *A. suum* were tested in homologous PCA. PCA rats were challenged with 500µg of native, autoclaved, periodated, or reduced/alkylated ABF, in Evans blue. Each serum was tested in duplicate in two separate rats and the end point titre for each animal is shown.

TABLE 10

PCA titre of rat serum in homologous PCA against native and modified 14kDa.

Strain	RT1 Haplotype	Native	Autoclaved	Periodated
WLEP	u	32/64	16	64
AGUS	l	0	-	-
AO	u	64	16/32	64
PVG	c	0	-	-
PVG-RT^u	u	64	16	64
PVG-RT1^l	l	0	-	-
(AGUSxPVG) F_1	l,c	0	-	-
(WLEPxPVG) F_1	u,c	16	-	-

Sera from various strains of rats infected with *A. suum* were tested in homologous PCA. Each serum sample was tested in duplicate in two separate animals. PCA rats were challenged, i.v., with 50µg of native, autoclaved, or periodate treated 14kDa in Evans blue, and the titre of the serum was recorded 30 minutes later. The endpoint titre from each of the duplicate animals is recorded.

chemically or physically treated in one of three ways. Samples were autoclaved at 120°C, or periodate treated to disrupt the carbohydrate epitopes, and ABF was reduced and then alkylated to separate di-sulphide linked dimers. The chemical modification of the ABF did not alter the PCA titres with respect to the animals with the $RT1^{u}$ haplotype (Table 9). However, the titres of some of the other strains tested were altered by chemical manipulation of the antigen. After autoclaving the ABF lost its ability to provoke mast cell degranulation in AGUS and PVG rats and the PCA titre in PVG-$RT1^{1}$ was reduced. Periodate treatment of the ABF rendered the molecule no longer allergenic to PVG rats.

Autoclave treatment of the 14kDa molecule did not render it inactive, however, the PCA titre was reduced (Table 10). Periodate treatment of the 14kDa molecule did not effect its allergenicity (Table 10).

Reaginic antibody response against ABF in heterologous PCA

An analysis of the reaginic antibody produced by several strains of mice as a result of infection with *A. suum* was carried out (Table 11). Unlike rats, mice fell into three groups; low, intermediate and high responders, with respect to the IgE titre produced in response to infection. The level of IgE appeared to be determined by non-MHC-linked genes, as animals with the same genetic background produced comparable IgE titres.

TABLE 11

Antibody titre against ABF in heterologous PCA.

Strain	H-2 Haplotype	End point titre
C3H/He	k	16
SJL	s	16/32
BALB.B	b	32
BALB/c	d	64
DBA/2	d	32
CBA	k	64
NIH	q	32
C57BL/10	b	256
B10.D2	d	256
B10.BR	k	>512
B10.S	s	>512
(C57BL10 x B10.S)F_1	b,s	256
(C57BL10 x SJL)F_1	b,s	64
(B10.D2 x B10.S)F_1	d,s	256
(BALB/c x SJL)F_1	d,s	>512
(CBA x SJL)F_1	k,s	256
(CBA x BALB/c)F_1	k,d	256

Sera from various strains of mice were tested in heterologous PCA, in duplicate and the end point titres are shown above. PCA rats were challenged with 500µg ABF in Evans blue, approximately 24 hours after intradermal injection of the test serum.

An analysis of several F_1 hybrids showed that these animals produced high IgE titres when infected, with one exception, the (C57BL10xSJL)F_1 which fell into the intermediate group.

Reaginic antibody titre, against the 14kDa molecule, in heterologous PCA

The reactivity of the reaginic antibody produced by mice infected with *A. suum* against the 14kDa molecule was analysed by screening against this molecule (Table 12). This revealed that only the B10.S mice produced IgE of this specificity, a result which supports the previous finding that only mice with the H-2^s haplotype respond to the 14kDa molecule. The lack of detectable response by the SJL mice may reflect the very low IgE produced by these animals. The reaginic activity produced as a result of immunisation with the purified 14kDa molecule and adjuvant was also examined. Mice immunised in this way did not produce detectable levels of IgE.

Intranasal immunisation

Several strains of mice, including B10.S, were exposed to approximately 500 microgrammes of ABF/mouse, on 4 occasions over a 7 week period using a nebuliser. This type of exposure failed to produce detectable antibody titres, as measured by immunoprecipitation or PCA.

TABLE 12;
The reaginic antibody titre of various strains of mice against the 14kDa molecule.

STRAIN	H-2 HAPLOTYPE	TITRE
C57BL10	b	0
B10.D2	d	0
B10.BR	k	0
B10.G	q	0
B10.S	s	128
SJL	s	0
BALB/c anti-14k	d	0

Sera from mice infected with *A. suum,* or immunised with 14kDa in adjuvant were tested by PCA in rats. Each serum was tested in two rats and the end point titres are shown above. PCA rats were challenged after approximately 24 hours with 50μg 14kDa in Evans blue.

Reaginic antibody activity against ES molecules

The reaginic antibody activity with specificity for the ES molecules was examined in mice and rats, infected with *Ascaris*. This analysis was limited due to the short supply of ES, and consequently, the PCAs used very small quantities of ES (Table 13). This analysis revealed that ES molecules are potent allergens in the context of infection, in both 14kDa responder and non-responder strains.

Western Blot analysis of serum from rats infected with *A. suum*.

Given that the specificty of IgG produced in response to infection had been shown to be influenced by MHC-linked genes and that there is strain restricted differences in IgE specificity a fuller analysis of the IgE response was warranted. A western blot system was developed to compare the IgG and IgE components of infection sera. This analysis revealed that the profiles produced by SDS-PAGE could be reproduced by Western blotting, and the blot profiles were the same whether visualised using an HRP-conjugate, or a I^{125}-conjugated antibody (Figures 39 and 40). The profiles produced using an Ig epsilon chain-specific monoclonal antibody were the same as those produced using an anti-gamma antibody (Figure 41). This suggests that the IgE antibodies produced in response to infection have the same specificity as the IgG antibodies.

Table 13;
Reaginic antibody activity against E/S material.

STRAIN	MHC haplotype	ANTIGEN	END POINT TITRE
C57BL10	b	L3/4	4
B10.S	s	L3/4	8
WLEP	u	L3/4	4
PVG	c	L3/4	16
WLEP	u	L2	4
PVG	c	L2	4

Sera from two strains of rats and two strains of mice were screened against L2 and L3/4 ES in PCA and the end point titres are shown above. PCA rats were challenged with 7.5µg of L2 ES or 8.5µg of L3/4 ES.

Figure 39; The recognition profiles of serum from *Ascaris* infected rats produced by ELISA on Western blots. Rat infection serum was screened against ABF, which had been run on SDS-PAGE and transferred onto nitrocellulose. Antibodies binding to the transferred protein were visualised using a an anti-gamma globulin-horse radish peroxidase conjugated antibody. Normal serum (a), WLEP (b), AGUS (c), AO (d), PVG (e), PVG-RT1^{u} (f), PVG-RT1^{l} (g), normal rabbit serum (h), rabbbit anti-14kDa serum (i). The transferred protein (ABF) was visualised using amido black stain (R).

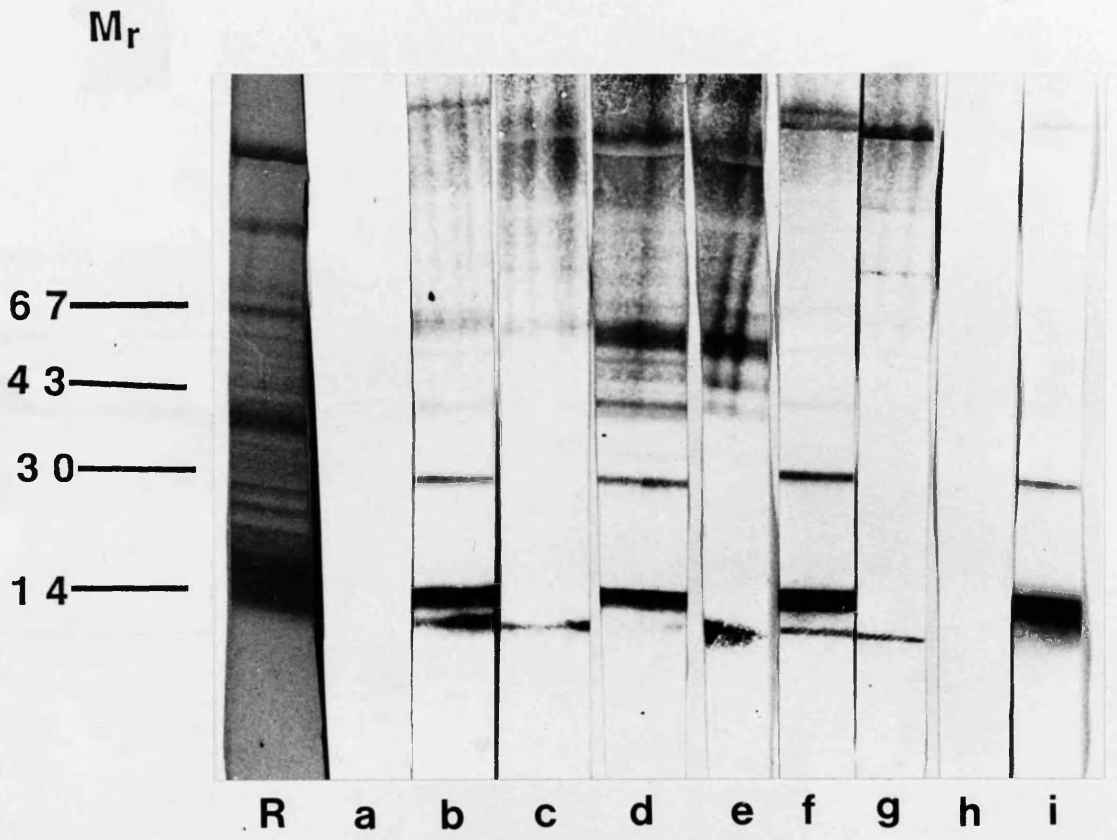

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Figure 40; The recognition profiles of serum from *Ascaris* infected rats produced by ELISA on Western blots. Rat infection serum was screened against ABF, which had been run on SDS-PAGE and transferred onto nitrocellulose. Antibodies binding to the transferred protein were visualised using a I^{125} labelled sheep anti-rat gamma globulin antibody. Negative control, i.e. no primary antibody (a), normal serum (b), WLEP (c), AGUS (d), AO (e), PVG (f), PVG-$RT1^u$ (g), PVG-$RT1^l$ (h), (WLEP x PVG)F_1 hybrid (i), (AGUS x PVG)F_1 hybrid (j).

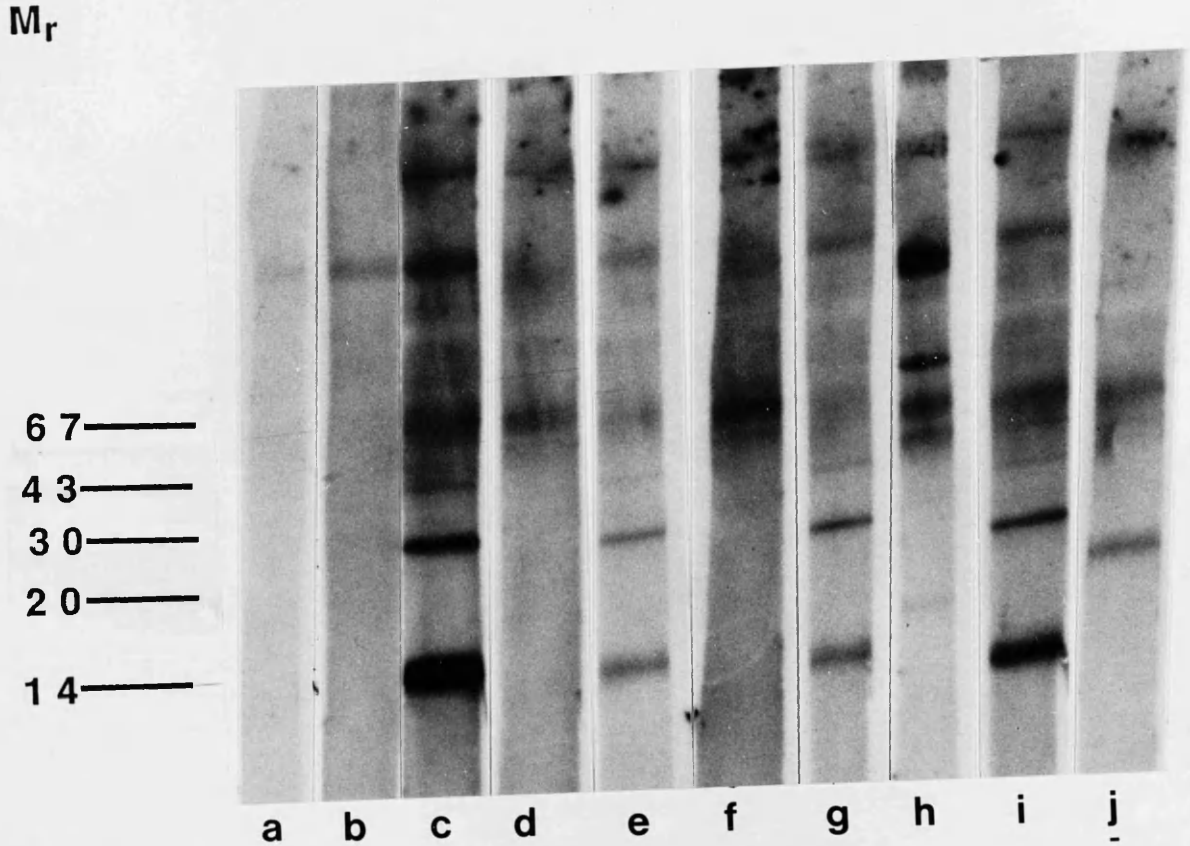
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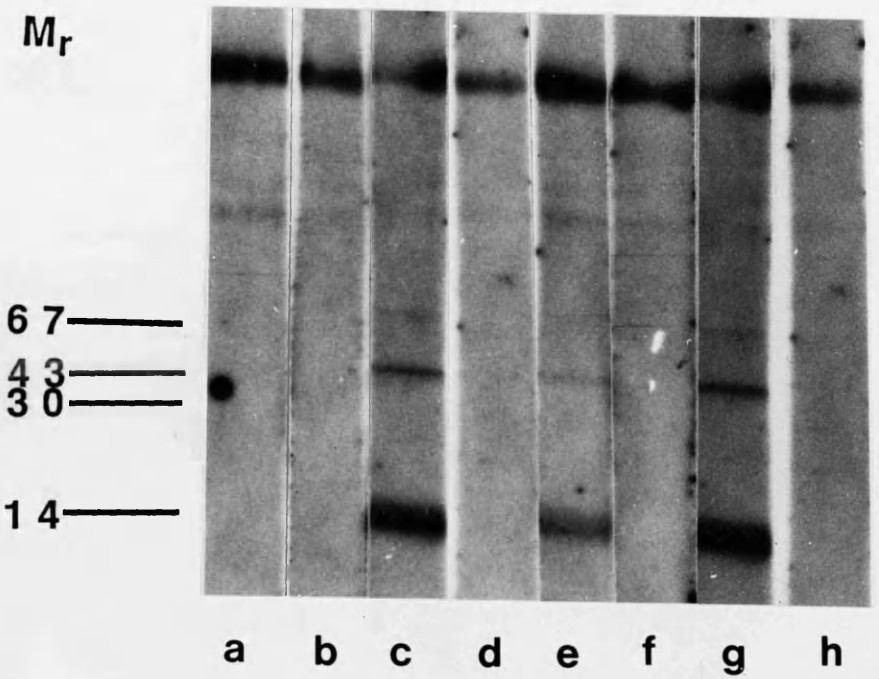
Figure 41; The recognition profiles of serum from *Ascaris* infected rats produced by mouse anti-rat Ig epsilon-chain specific ELISA on Western blots. Rat infection serum was screened against ABF, which had been run on SDS-PAGE and transferred onto nitrocellulose. IgE antibodies binding the transferred protein were visualised using an I^{125} labelled mouse anti-rat Ig epsilon-chain specific monoclonal antibody. Negative control, i.e. no primary antibody (a), normal serum (b), WLEP (c), AGUS (d), AO (e), PVG (f), PVG-$RT1^u$ (g), PVG-$RT1^l$ (h).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Chapter 3

Discussion

The factors which control IgE production have been the subject of several studies over a number of years (Jarrett and Miller, 1982). In parasitic infections, it has been shown that host genetic factors can regulate the amount of IgE produced (Jarrett, 1978; Jarrett and Bazin, 1977). People living in endemic areas have higher IgE levels even when not infected, which may reflect previous or undetected infections, or there may be some genetic difference between populations (Houba and Rowe, 1973; Ray and Saha, 1978; Somorin *et al*, 1977). One observation which suggests that there may be a genetic difference between human populations is that healthy Nigerians who emigrated to America still had substantially higher IgE levels several years later (Somorin *et al*, 1977). In man the manifestation of allergy has been shown to be controlled by genetic factors (Cooke and Van der Veer, 1916; Schwartz, 1952) and Ir genes have been identified which regulate the recogntion of certain allergens (Levine *et al*, 1972; Davie *et al*, 1972). The presence of these Ir genes is necessary, but not sufficient, to cause allergy, that is other factors, some of which are genetic, some enviromental are also involved. This system of dual control of IgE production has also been demonstrated in the mouse.

Studies involving the induction of IgE to small quantities of antigen presented in adjuvant, have demonstrated that IgE production is controlled by genes at more than one locus in the mouse (Vaz *et al*, 1971; Levine and Vaz, 1971;

Vaz *et al*, 1974). These authors demonstrated that different strains of mice produce different amounts of IgE, and the level of IgE produced depended on non-MHC-linked genes. IgE production was controlled by two systems, one of which affected the level of IgE and the other the specificity of the response. The genes controlling specificity were linked to the MHC complex of the host. The work presented in this chapter, on IgE production in response to *Ascaris* infection, agrees with that of the above study; IgE production in the mouse is dependent on genes outside the MHC locus, which regulate the level of the response, and MHC-linked genes which regulates the specificity of the response.

Strain dependent differences have also been identified in rats (Jarrett, 1978) where poor IgE responses were shown to be due to active suppression of the response by non-specific IgE Ts cells (Watanabe *et al*, 1976; Chiorazzi *et al*, 1977; Gollapudi and Kind, 1977). The work presented in this chapter shows that the specificity of the IgE produced by rats infected with *A. suum* is regulated by MHC-linked genes as in mice.

Physical or chemical modification of the antigens altered the PCA titres. After autoclaving the ABF lost its ability to provoke mast cell degranulation in AGUS and PVG rats and the PCA titre in PVG-RT1^{1} was reduced (Table 9). This suggested that these animals may be responding to a structural determinant of the allergens present in ABF

and that autoclave treatment damages this tertiary structure. Peridoate treatment of the ABF removed the allergenic activity when tested in PCA using serum from *Ascaris* infected PVG rats (Table 9). Periodate treatment of the kind used in this study disrupts carbohydrate determinants (Maizels *et al*, 1987) and it is possible that these carbohydrates are involved in the tertiary structure which produces the recognition site for the PVG rats. Alternatively, these carbohydrate determinants may themselves form the IgE epitopes and disruption of the carbohydrate would, therefore, destroy the epitope. Autoclave treatment of the 14kDa molecule reduced the PCA titres (Table 10). This may suggest that this molecule had been damaged by autoclaving but the allergenic epitope was still available. Alternatively, the 14kDa molecule may contain more than one epitope only some of which are susceptible to autoclave treatment.

SJL mice have been shown to produce very low levels of IgE against a wide range of antigenic stimuli (Levine and Vaz, 1971), and this trait has been shown to be due to Ts cells (Ovary *et al*, 1978). The data presented here shows that SJL mice produce a low level of IgE against the multiple components of ABF, but when challenged with the purified 14kDa component no IgE is detected. This result is surprising given that SJL mice have been shown to produce IgG antibodies against this component (Chapter 2.) This lack of IgE against the 14kDa molecule may reflect the fact that the assay system used is not sensitive

enough to detect the very low levels of IgE produced against this isolated molecule, and IgE is detected against the ABF because this preparation presents a larger allergenic challenge.

The sera of BALB/c mice immunised with 14kDa in FCA showed no detectable IgE against this component despite producing a very high IgG response (see Chapter 2). This finding supports work done previously which showed that purified antigens or parasite extracts are often not good allergens in naive animals (Ogilvie, 1967). This highlights one of the problems of purifing parasite allergens; in that a product which results in IgE production when administered with adjuvant is not necessarily the allergen the infected animal is exposed to. In this study the purified 14kDa did bind to either IgE or IgG antibodies elicited as a result of infection with the parasite.

The F_1 hybrids examined fall into the high IgE group, as defined by this system, with the exception of (C57BL x SJL)F_1. This hybrid produced IgE titres in the intermediate group. This result was surprising because the suppression, which is known to cause the low responses of SJL mice, is inherited in a recessive manner (Ovary *et al*, 1978), a result which would be supported by the other SJL crosses. This lower than expected response is not due solely to the MHC haplotype of the hybrid, since animals with the same H-2 haplotype (b/s), produce high IgE levels. One explanation would be cross tolerance in the

hybrid (Schwartz, 1978; Matzinger, 1981) resulting in a more limited repertoire of antibody specificities and hence a lower titre against the ABF.

In a study examining IgE produced by individuals naturally infected with *A. lumbricoides* it was found that this sera identified a whole range of allergens in ABF (Tsuji *et al*, 1977; O'Donnell and Mitchell, 1978). Heterogeneity in the IgE response has also been demonstrated in many other parasitic infections, for example, *S. mansoni* (Harris, 1975; Vannier *et al*, 1974) and *T. taeniaeformis* (Lloyd and Williams, 1975). In this chapter we have shown that rodents infected with *Ascaris* produce IgE against a whole range of parasite allergens. Heterogeneity in IgE responses has also been documented with other parasite systems (Hogarth-Scott, 1967) and one explanation for this response is that a small allergenic component associates with itself to form allergenic dimers, or with other molecules present in the parasite antigen (Fujita *et al*, 1979). When the specificity of IgE from rats infected with *Ascaris* is examined by Western blotting allergens with molecular weights in the region of 14, 28 and 42kDa are detected, by rats with the $RT1^{u}$ haplotype, raising the possibility that these allergens are actually aggregates of the 14kDa molecule.

The IgE and IgG produced as a result of *Ascaris* infection appear to have the same specificity. This dual production of antibodies with the same specificity but of a different

class may be important in the outcome of infection in terms of resistance to the parasite and the presence of clinical allergy.

In the tropics there is a lower than expected prevalence of allergies against parasite antigens in humans (Alcasid *et al*, 1973; Cheah and Khan, 1972; Turner *et al*, 1978). Several hypotheses have been proposed to account for this phenomenom; it has been speculated that the large amounts of non-specific IgE produced as a result of parasitic infection may block the IgE binding sites on mast cells and hence limit the binding of specific IgE (Jarrett *et al*, 1971; Stanworth, 1971). Alternatively, infection in very young animals may alter the development of subsequent allergies (Turner *et al*, 1982). A role for IgG antibodies has also been proposed. In a study on lymphatic filariasis, Hussain and Ottesen (1985), demonstrated that while there were high levels of IgE in the whole population relatively few individuals showed clinical signs of allergy. These authors propose that one means of modulating the effects of IgE is by IgG blocking antibodies. If antibodies of both classes are produced with the same specificity then there will be competition for antigen binding and the response could thus be down regulated.

The model system presented in this chapter has important implications for the study of IgE production in response to helminth infection and for hypersensitivity reactions

in general. The mouse has been shown to control IgE production in a manner very similar to that seen in man (Levine *et al*, 1972). Consequently, this model provides a means of studying that control in the context of infection. The genetically determined restriction of the antibody repertoire in this model provides an opportunity to study the genetic control of IgE production purely in the context of infection, in contrast to some other studies which have tended to look at IgE produced as a result of immunisation with adjuvant. The differences between infection-induced or adjuvant-induced responses can be clearly seen with the lack of IgE antibodies in animals immunised with purified 14kDa and FCA. Therefore this model system provides a means of studying the genetic control of IgE production in different strains of animals and utilising various means of immunisation.

Chapter 4.

The human antibody response to *Ascaris lumbricoides*.

Introduction.

There are numerous reports on the prevalence and intensity of human infection with *A. lumbricoides* from many regions, especially the tropics and subtropics, where ascariasis is endemic (Crompton, Nesheim and Pawlowski, 1985, 1989; Anderson, 1986; Seo *et al*, 1979; Croll *et al*, 1982; Bundy *et al*, 1985; Anderson and May, 1985; Anderson and Schad, 1985; Elkins *et al*, 1986). The results of these studies can be summarised as follows; prevalence of infection is usually greater in the young and decreases with age, giving a convex curve of prevalence against age. The intensity of infection tends to be greater in young children, but varies between individuals of all age classes, so that there is an aggregated dispersion of the parasite within the host population. This overdispersion means that a small percentage of the total population of hosts harbours the majority of the parasite population. This heavily infected group plays a major role in *Ascaris* transmission and the level of contamination of the environment. Overall, there seem to be no differences between the sexes in either intensity or prevalence. Some studies, however, have found sex differences, but these can usually be explained by differing social and behavioural patterns, or differing occupations which means that the exposure rate of these groups is different (Elkins *et al*, 1986). The persistence of high prevalence of infection in some areas can be attributed to poverty and its associated problems of malnutrition, ignorance, inadequate sanitation and overcrowding.

Mass chemotherapy can reduce the long-term levels of infection only if anthelmintics are administered regularly, at least every 4-6 months, and if there is a parallel improvement in sanitation and education to sustain the reduction in transmission and reduce the chances of reinfection. After chemotherapy, people become re-infected rapidly, often reaching their pre-treatment levels within 6-12 months (Anderson and Medley, 1986; Elkins *et al*, 1986; Hlaing *et al*, 1987; Elkins *et al*, 1987). Infection with *Ascaris* (ascariasis) often occurs as part of a multiple infection, particularly common accomplices being *Trichuris trichiura* and hookworm (Kan, 1985; Elkins et al,1987; Welch et al, 1986; Bundy et al, 1987; Robertson *et al*,1989). There has been conflicting data on whether predisposition to heavy infection with one helminth reflects a predisposition to heavy infection with other helminths (Bundy *et al*, 1987; Haswell-Elkins *et al*, 1987; Croll and Ghadirian, 1981). Immunosuppression may also be an important factor in multiple infections, as there is experimental evidence which suggests that infection with *Ascaris* may suppress immune responses against heterologous antigens (Crandall, Crandall and Jones, 1978; WHO, 1981). Parasite-induced suppression against parasite-specific and heterologous antigens has been reported with parasites other than *Ascaris* (Wakelin, 1985), and therefore the outcome of multiple infections will be decided by the combination of parasites involved as well as the host response.

Several studies have demonstrated that the fecundity of female *Ascaris* is inversely related to worm density (Hlaing et al, 1984; Croll et al, 1982; Keymer, 1982), this implies that if the number of worms is reduced, the worm fecundity will increase. This may explain the observation that, as re-infection occurs, after chemotherapy, the egg output from previously heavily infected individuals actually appears to increase, until the previously high number of worms re-establish.

In the past, the differences in intensity of infection with age, and between communities, has been explained on the basis of exposure levels. The hypothesis has been that the more heavily infected groups have been exposed to a greater challenge from infective eggs because of their environments, or because of their high risk behaviour (Kan, 1985; Jones, 1977). While the link between exposure and infection cannot be disputed it is possible that other factors, such as the host's ability to resist infection, may also be relevant in this relationship.

The strongest indications that factors other than exposure are relevant come from studies of predisposition and the reduction of prevalence with age. Several studies have demonstrated a marked predisposition with regard to the intensity of infection (Anderson and May, 1985; Anderson and Medley, 1985; Schad and Anderson, 1985; Hlaing, 1985; Elkins *et al*, 1986; Bundy, 1986; Bensted-Smith *et al*, 1986; Haswell-Elkins *et al*, 1987). This can be aptly

demonstrated by the fact that, after successful chemotherapy, people who previously had heavy worm burdens reacquire these high levels of infection, while those with lower levels of infection reacquire lower levels of infection. Predisposition, which is found in all age groups, suggests that there are differences between individuals, which determines their level of infection and that, these differences cannot be explained purely by exposure.

The observation that older people have lower levels of infection may be explained by altered behaviour and/or some form of acquired immunity. After chemotherapy, however, these people are not re-infected at a significantly slower rate than the younger members of the community and immature larvae are found in adult hosts, suggesting that this group is continuously re-infected (Elkins *et al*, 1986). While these results rule out a role for sterile immunity, they do not exclude the possibility of some level of protection. Reports, from studies using laboratory models, have demonstrated that, if acquired immunity is dependent on past infection then in communities with a high overall intensity of transmission the age intensity profile will be more convex when compared to communities with lower levels of transmission (Anderson and May, 1985; Crombie and Anderson 1985; Keymer, 1985). This appears to be the case when different communities are compared. Therefore, some sort of immunity may be involved. This immunity, although not completely protective, could serve to limit the extent

of the infection. This 'limiting' immunity may explain the differences between wormy individuals and their more lightly infected peers. Heavily infected people may be more susceptible, either because they have a lower level of innate immunity, or because they are less able to mount an effective response. The factors most likely to be responsible for a deficient immune response in these circumstances are diet and host genetic constitution.

There is significant evidence to link ascariasis with malnutrition, which can lead to a deficient immune response (Nesheim, Crompton and Sanjur, 1985; Suskind, 1977; Beisel, 1982; Taren *et al*, 1987; Geefkysen *et al*, 1971; Stephenson, 1987). The importance of genetics and nutrition has been demonstrated in several laboratory models (Wakelin, 1985; Crombie and Anderson, 1985; Behnke and Robinson, 1985; Keymer and Hiorns 1986; Slater and Keymer, 1986). If genetic constraints are part of the answer to predisposition, then one possible area of control would be the major histocompatability complex (MHC), as this is involved in the regulation of the immune response. There are several reports using animal models demonstrating MHC effects on the immune response (Wakelin, 1985; Wakelin and Blackwell, 1988) and a growing number of associations between diseases and HLA alleles or haplotypes have been described in man (Salam *et al*, 1979; Chong-gong *et al*, 1984; Smeraldi *et al*, 1986). There is now enough evidence to warrant an examination of the possible link between

genetic control, immune response and levels of infection in parasitic infections.

In this chapter the serological responses of individuals resident in one of several endemic communities have been examined, and possible links between antibody titre, age and level of infection considered. The antigens used were either from the human roundworm, *A. lumbricoides*, or the closely related pig roundworm, *A. suum* (WHO, 1981). The use of *A. suum* in this analysis can be justified by the high level of homology between the components of *A. suum* and *A. lumbricoides* (Kennedy *et al*, 1987).

Chapter 4

Results.

Serum samples from a total of 285 individuals, living in four endemic areas (Anse Le Ray (A), Dennery (D), Boguis (B) and Canaries (C)), were screened against four different *Ascaris* antigen preparations in an ELISA system, using an anti-IgG conjugate. Each sample was screened in duplicate and each test was repeated at least once. An example of the reproducibility between tests is shown in Figure 42, in which the correlation between optical densities, recorded in two separate tests, from ELISAs involving sera from Anse le Ray (panel A), or Dennery (Panel B), are screened against L3/4 ES.

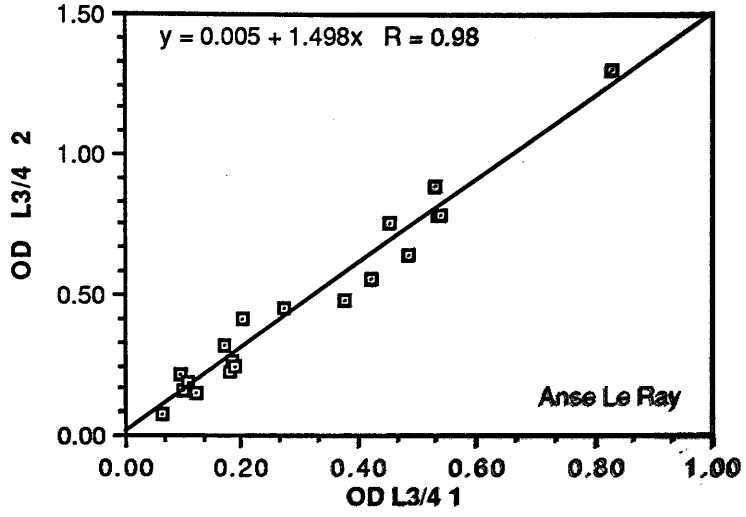
Level of infection and age

The data available on these sera shows that the most heavily infected individuals, in terms of eggs per gram of faeces (epg), or worm burden, i.e. the number of worms passed after anthelminthic treatment, occurred in the under 20 years old age group (Figure 43).

Level of infection and antibody levels

The relationship between antibody level from ELISA and infection in terms of epg was examined (Figure 44). This analysis revealed that there was no simple correlation between infection levels and antibody level, that is, a heavier infection does not necessarily result in higher antibody levels. The widest range of antibody levels occurred in the individuals who had no eggs in their faeces. The antibody level patterns associated with a measure of the infection intensity were very similar for

Figure 42
The correlation between ELISA repeats using human sera



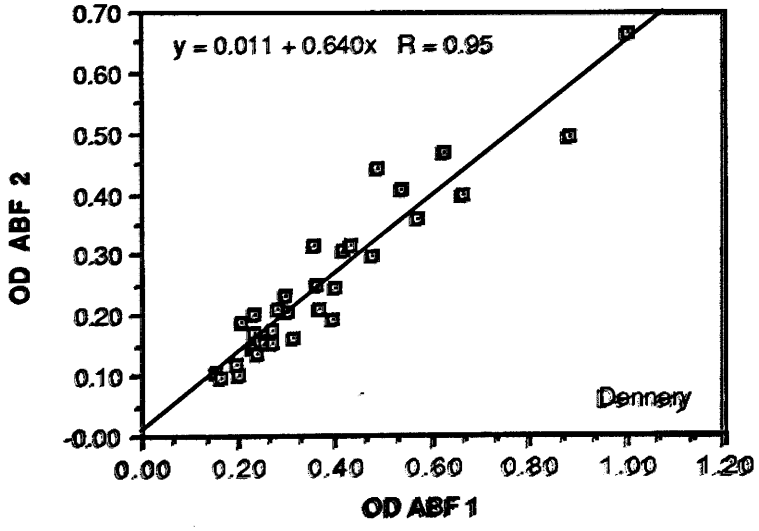
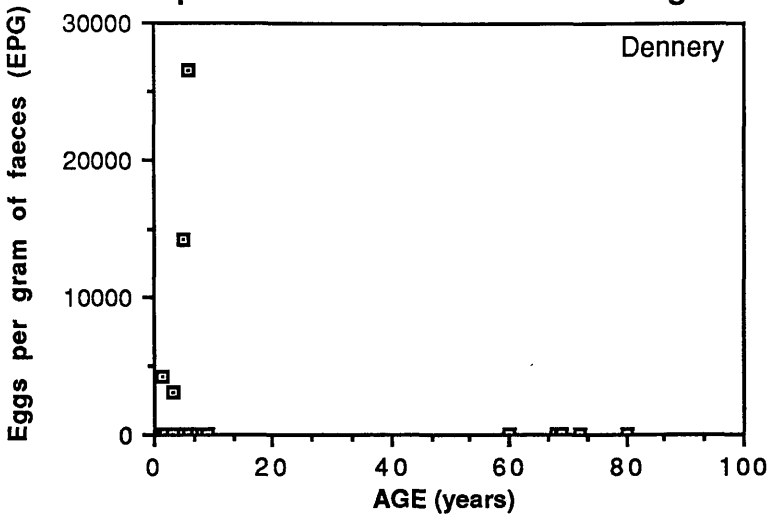
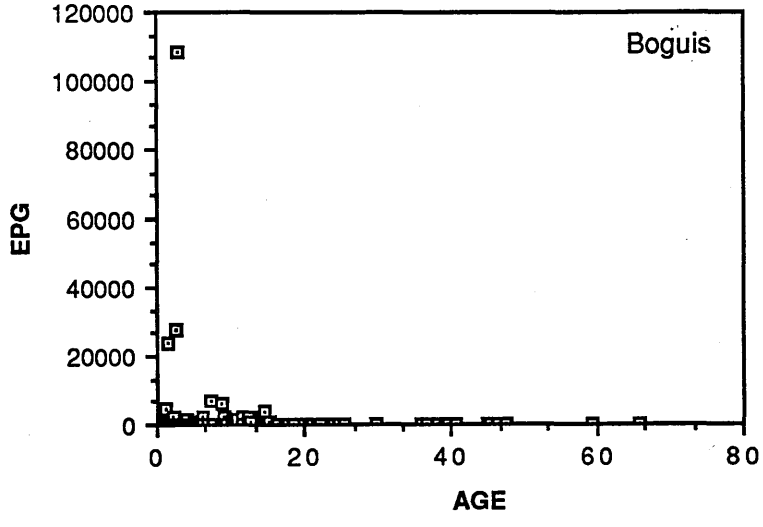


Figure 43
Relationship between EPG of faeces and age





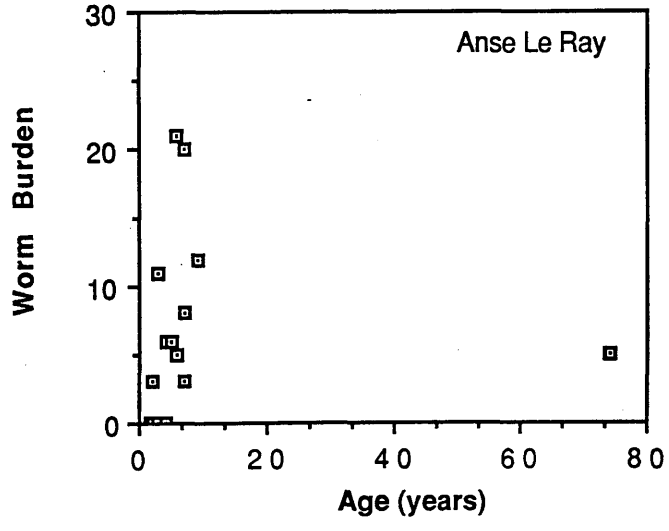
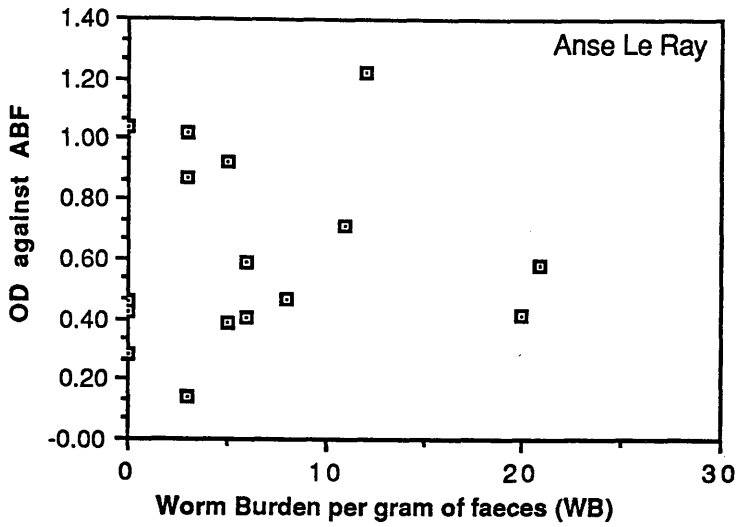
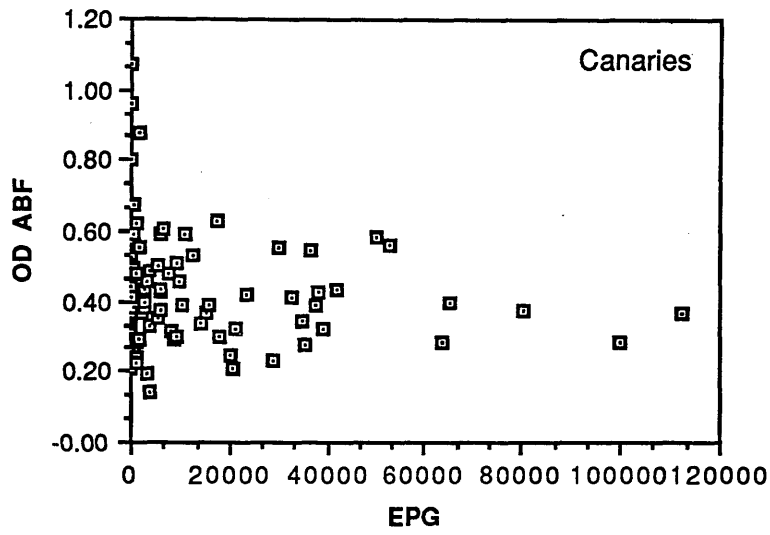
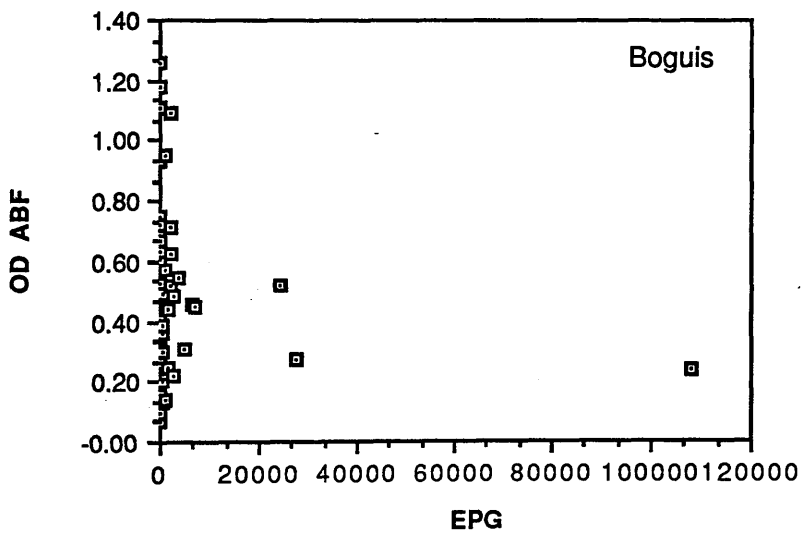


Figure 44
Relationship between level of infection and ABF







all four antigens examined (Figure 45). The majority of the sera tested produced antibody levels which are higher than the control serum but are still at the lower end of the ELISA scale, with a minority of individuals producing higher antibody levels.

Antibody level and age

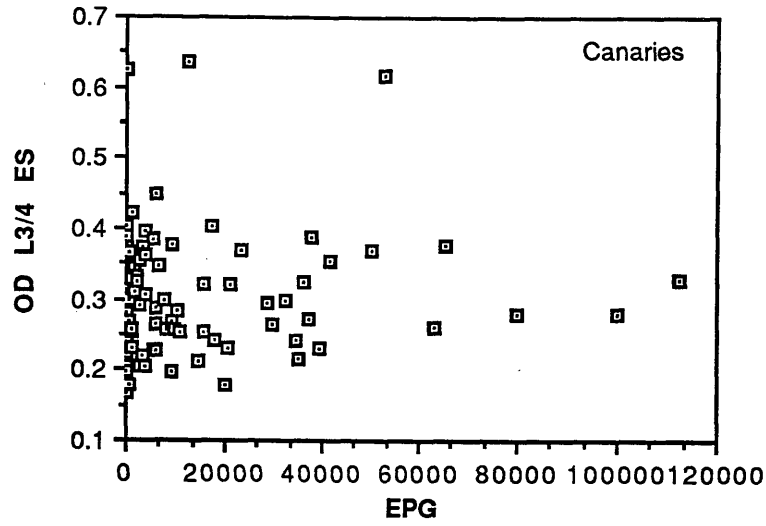
It is possible that immunity develops or fluctuates with age, in response to repeated infection. Accordingly, the correlation between age and antibody level was examined (Figure 46). The antibody levels of older individuals lie within the expected range, as defined by the ELISA readings for the total population, with no significant increase in antibody level with repeated infection.

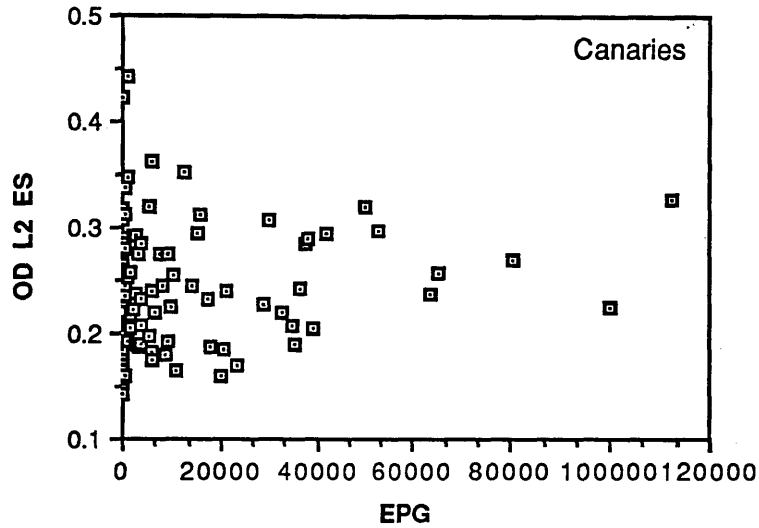
Antibody level against stage specific antigens

Examination of the degree of correlation between antibody levels and each of the antigens used in this analysis was undertaken in order to investigate the relative importance of stage-specific antigens (Figure 47). A high degree of correlation between levels for ABF and 14kDa, L3/4 ES and ABF, and L2 ES and L3/4 ES was detected. No positive correlation between the levels against L2 ES and ABF was observed.

The responses of several individuals against each antigen were studied in greater detail (Figure 48). This approach emphasises the variation in antibody levels

Figure 45
The relationship between EPG and OD





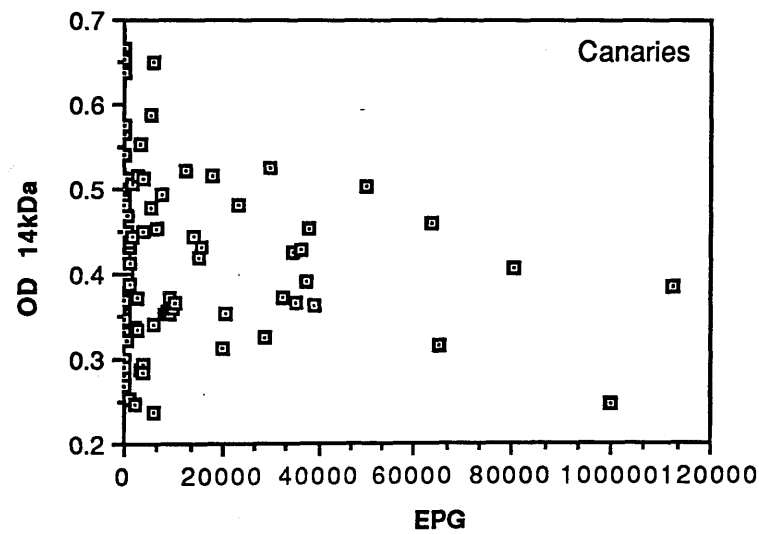
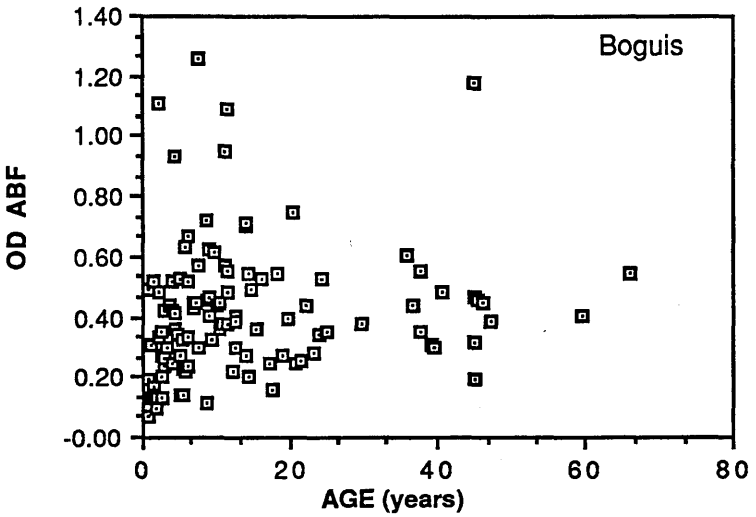
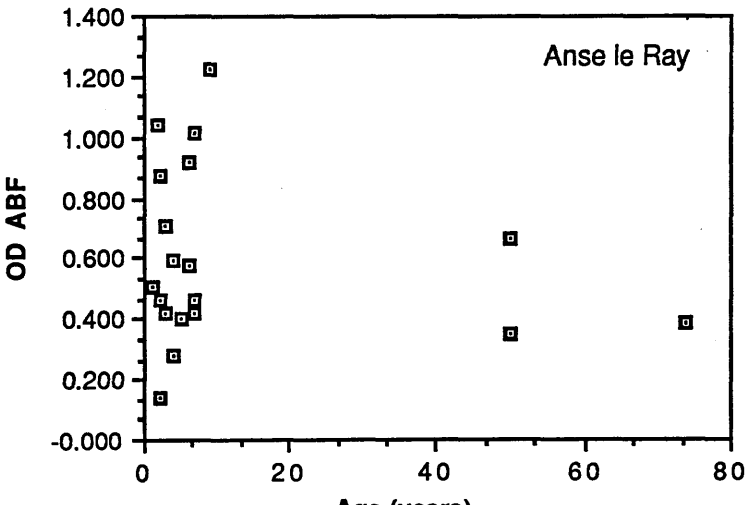


Figure 46
Relationship between age and OD





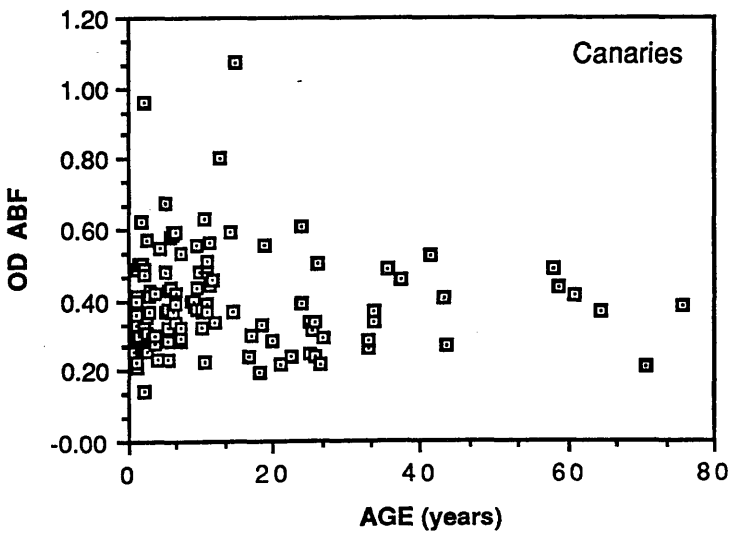
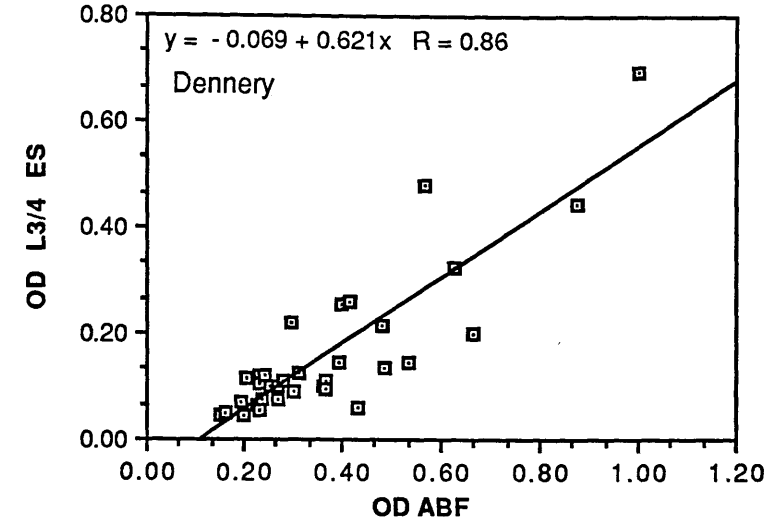
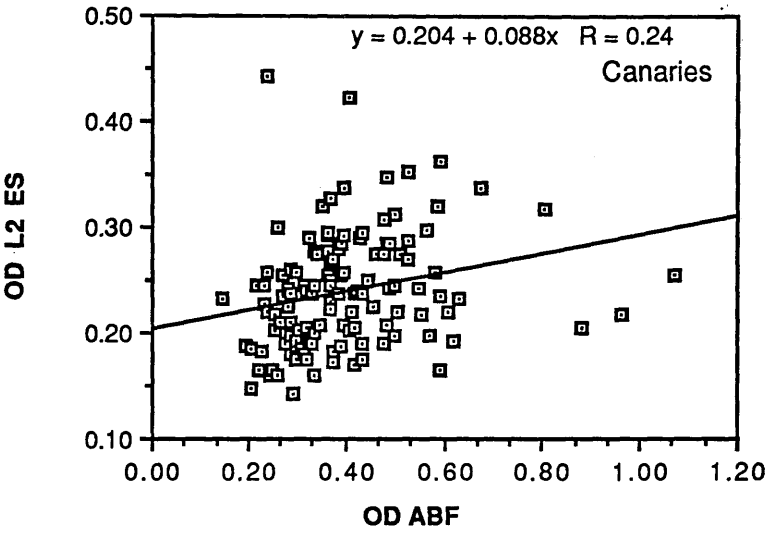


Figure 47
The correlation between ODs of different antigens





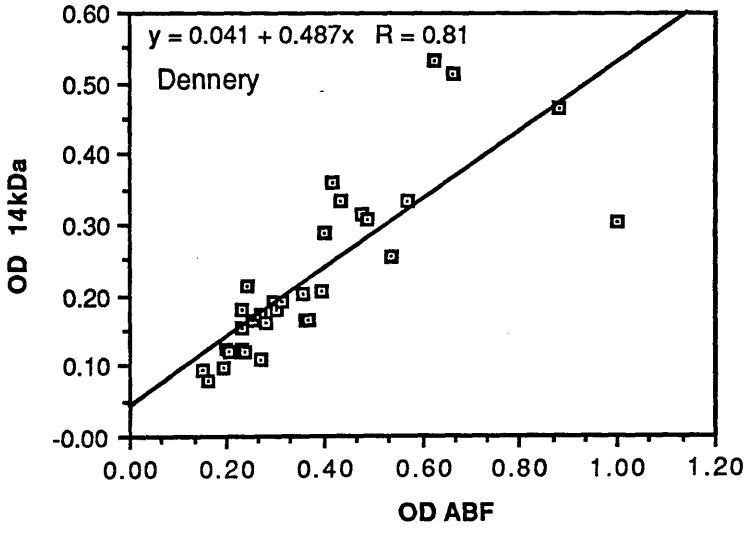


Figure 47 (contd.)

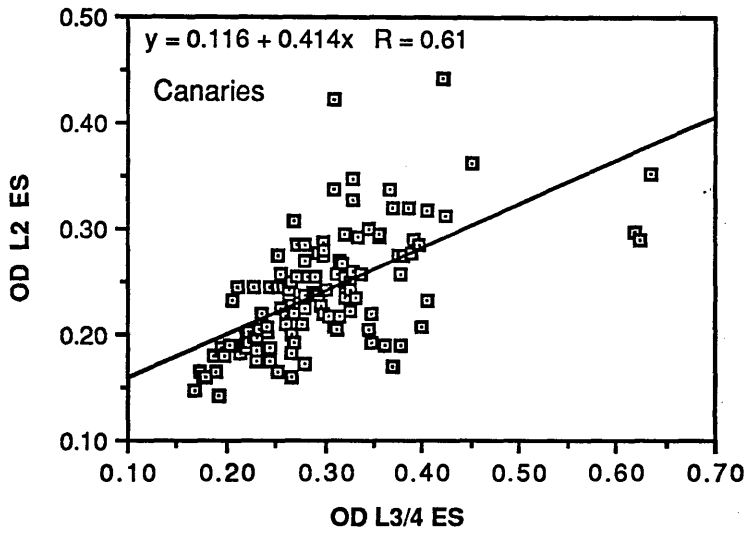
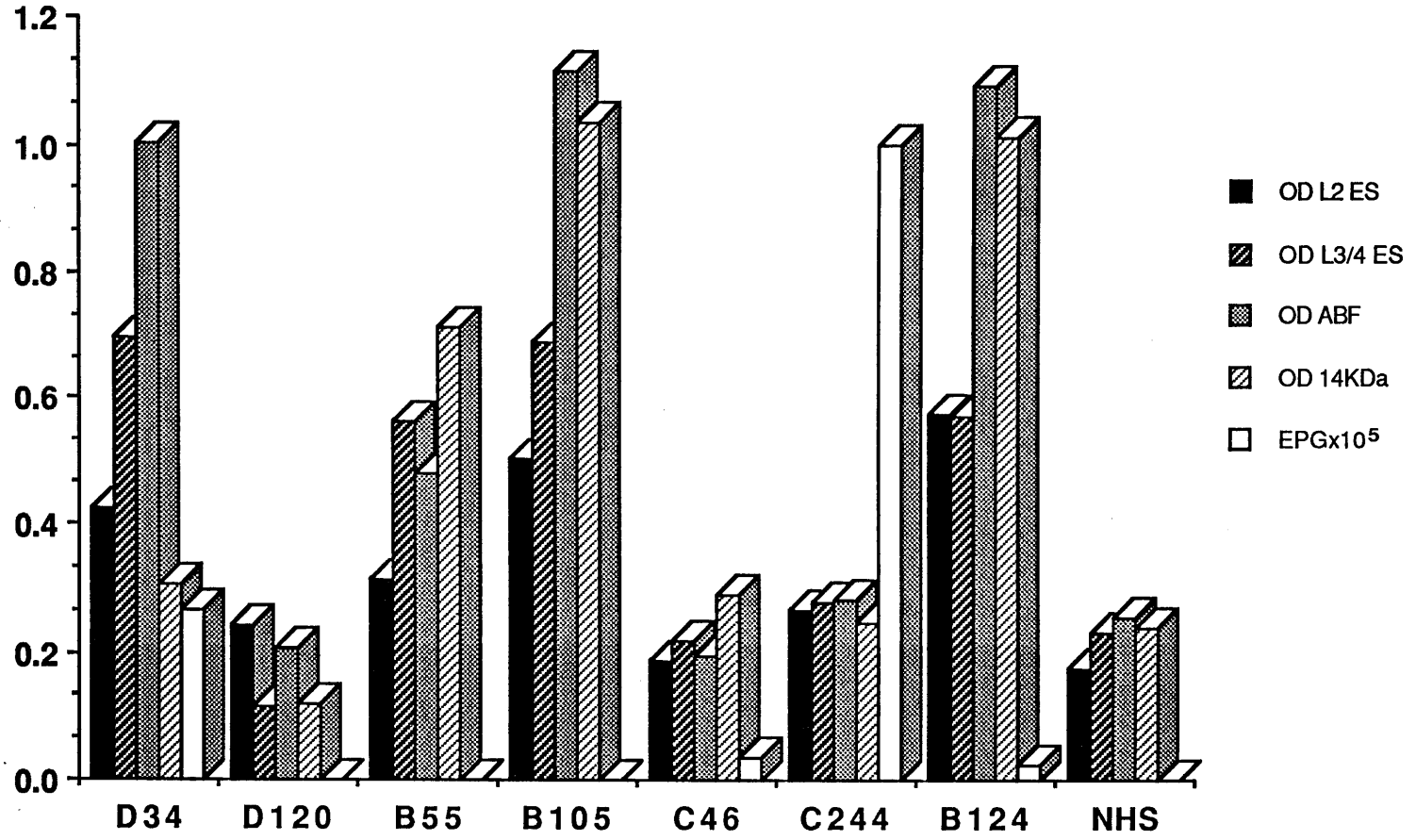


Figure 48
Antibody response and infection level in 7 individuals



within the population. Some individuals produce very low antibody levels, apparently independently of the presence of adult worms, seen, for example in patient 120 from Dennery (D120), aged 3 years. This child was found not to be passing eggs in the faeces and had a low antibody level. Patient 46 from Canaries (C46), aged 18 years, was found to have an infection intensity of 3510 epg and had a low antibody level. Patient C244, aged 5 years, had an infection intensity of 99770 epg and had a low antibody level.

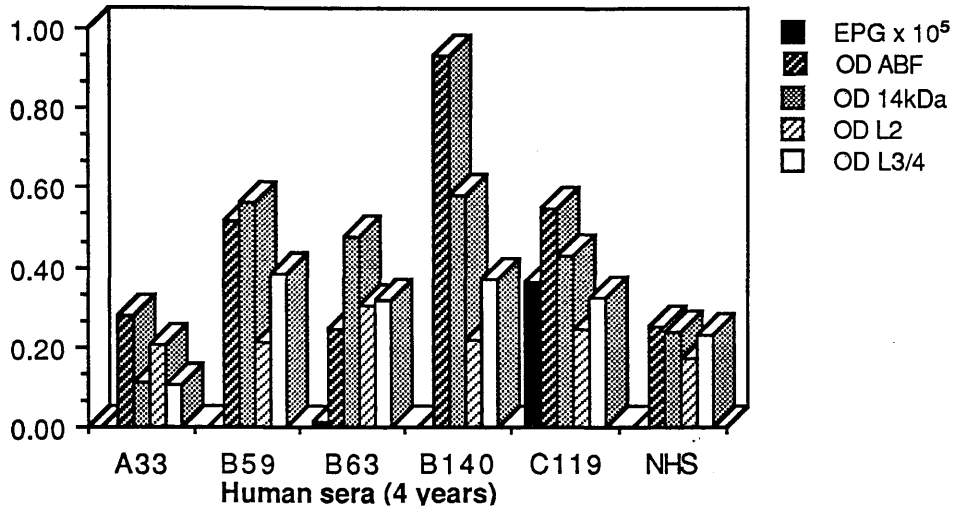
There is also variation in the infection status of patients identified as high antibody responders, for example, B105, 0 epg, B124, 2400 epg and D34, 26540 epg. Within this high responder group there is also variation in the recognition of the 14kDa molecule. B105 and B124 produce high levels against the 14kDa molecule whereas D34, which has a comparable level against ABF, does not produce a significant response against the 14kDa.

This variation in detected antibody levels is also apparent in age matched samples (Figure 49).

Antibody level using a broad spectrum conjugate

The possibility that use of an anti-gamma specific conjugate may have influenced the correlation between antibody level and infection status was investigated by use of a broad spectrum conjugate. The correlation between antibody level and infection status (Figure 50)

Figure 49
Antibody levels in age matched individuals



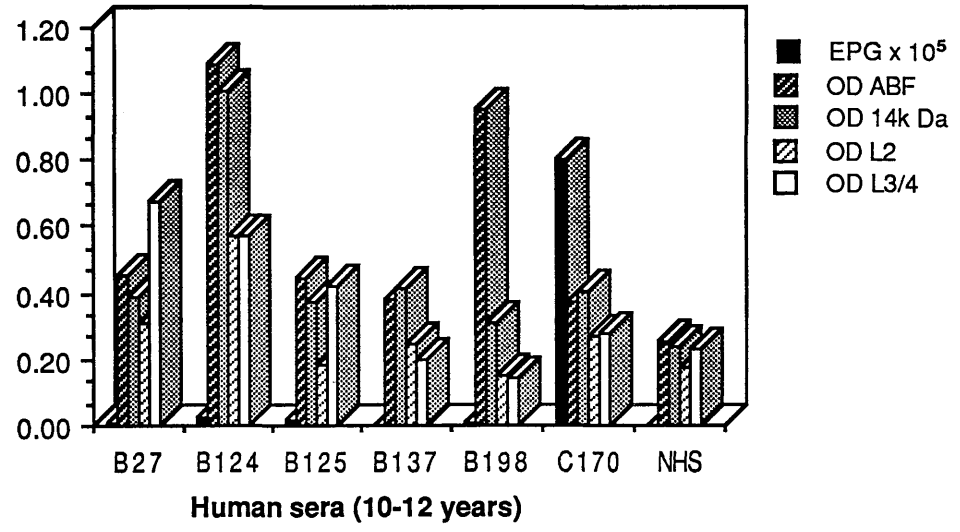
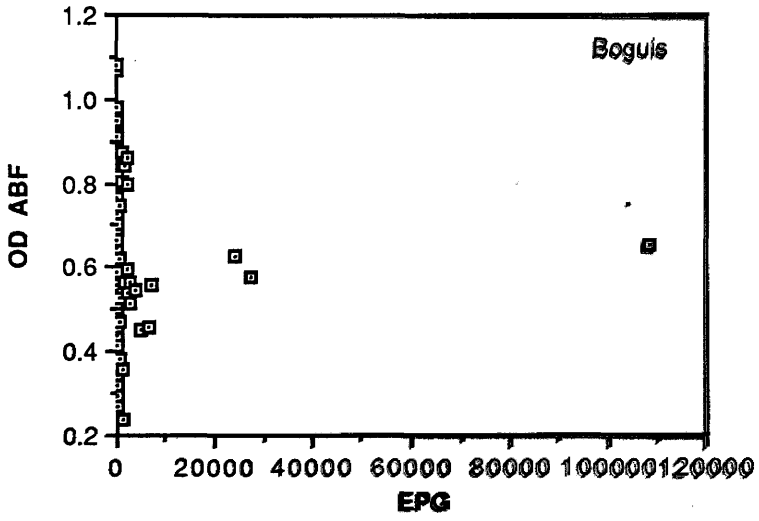
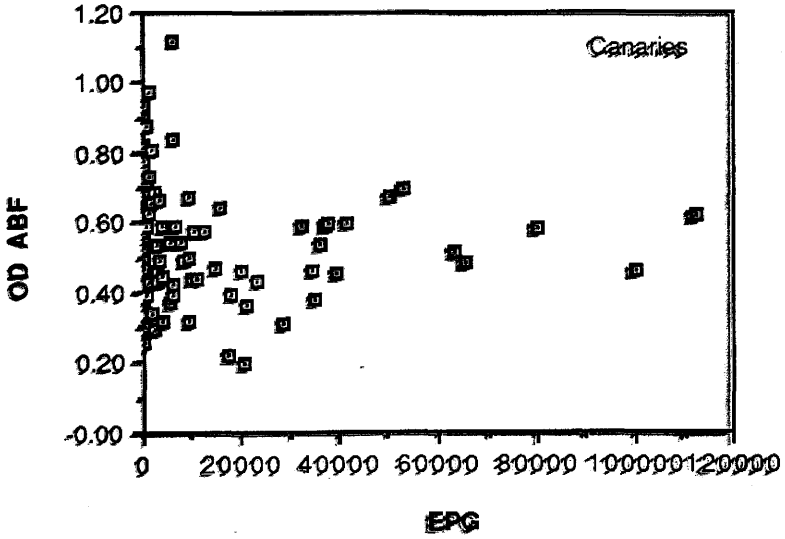


Figure 50
OD against level of infection using anti-gamma globulin





were compared with those produced using the anti-gamma reagent (Figure 44). There was no difference in the patterns produced by ELISA with either of these reagents.

SDS-PAGE analysis of human sera

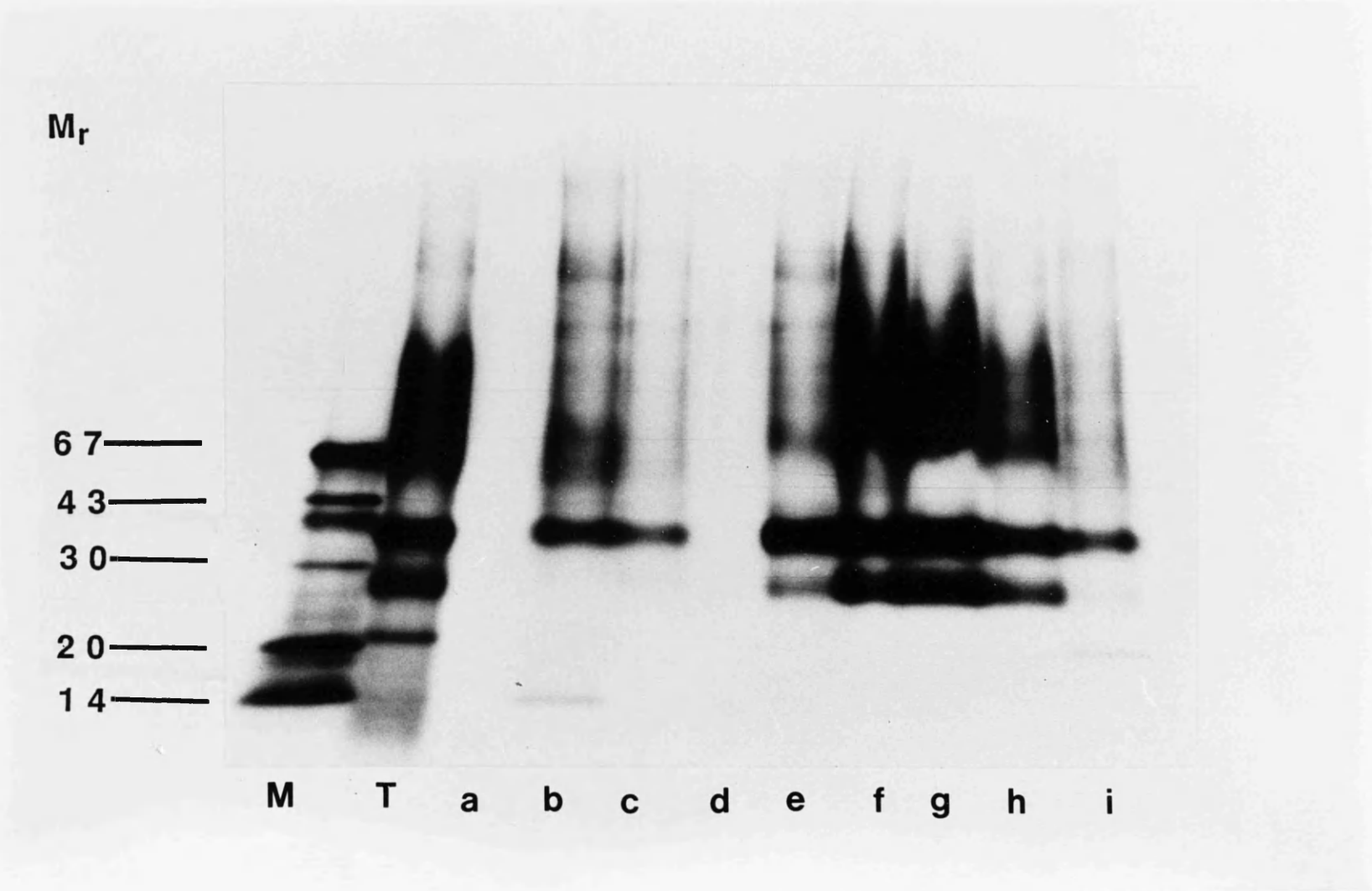
Antigen recognition profiles against A. lumbricoides L3/4 ES

As well as analysis by ELISA, samples of sera were analysed by SDS-PAGE. The antigen recognition profiles produced when sera from 8 individuals living in the Canaries region were immunoprecipitated with *A. lumbricoides* L3/4 ES are shown in Figure 51. This result reveals that there is restricted recognition of the ES; no single serum recognises all the potentially antigenic components. Different recognition profiles are detected for different individuals; most notably only one person, from this group, recognises the 14kDa molecule, only one person recognises the 23kDa molecule and 4 out of the 8 recognise the 28kDa band.

Antigen recognition profiles and age

The possibility that all the potentially antigenic components would be recognised with time and repeated infection was examined by looking at the antigen recognition profiles of serum from 7 individuals over the age of 60 years (see Figure 52). This analysis showed that the recognition profiles did not become less restricted with age. Indeed the same level of restriction and heterogeneity in antigen recognition can

Figure 51; The recognition profiles of 8 individuals from an *Ascaris* endemic area. Radio-iodinated *A. lumbricoides* L3/4 ES (T) was immunoprecipitated with normal human serum, i.e. serum from a non-endemic area (a), or serum from Canaries C58(b), C42 (c), C61 (d), C38 (e), C119 (f), C130 (g), C56 (h), C118 (i). The immunoprecipitated antigen was run on SDS-PAGE and molecular weights were estimated by comparision with marker proteins (M).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14
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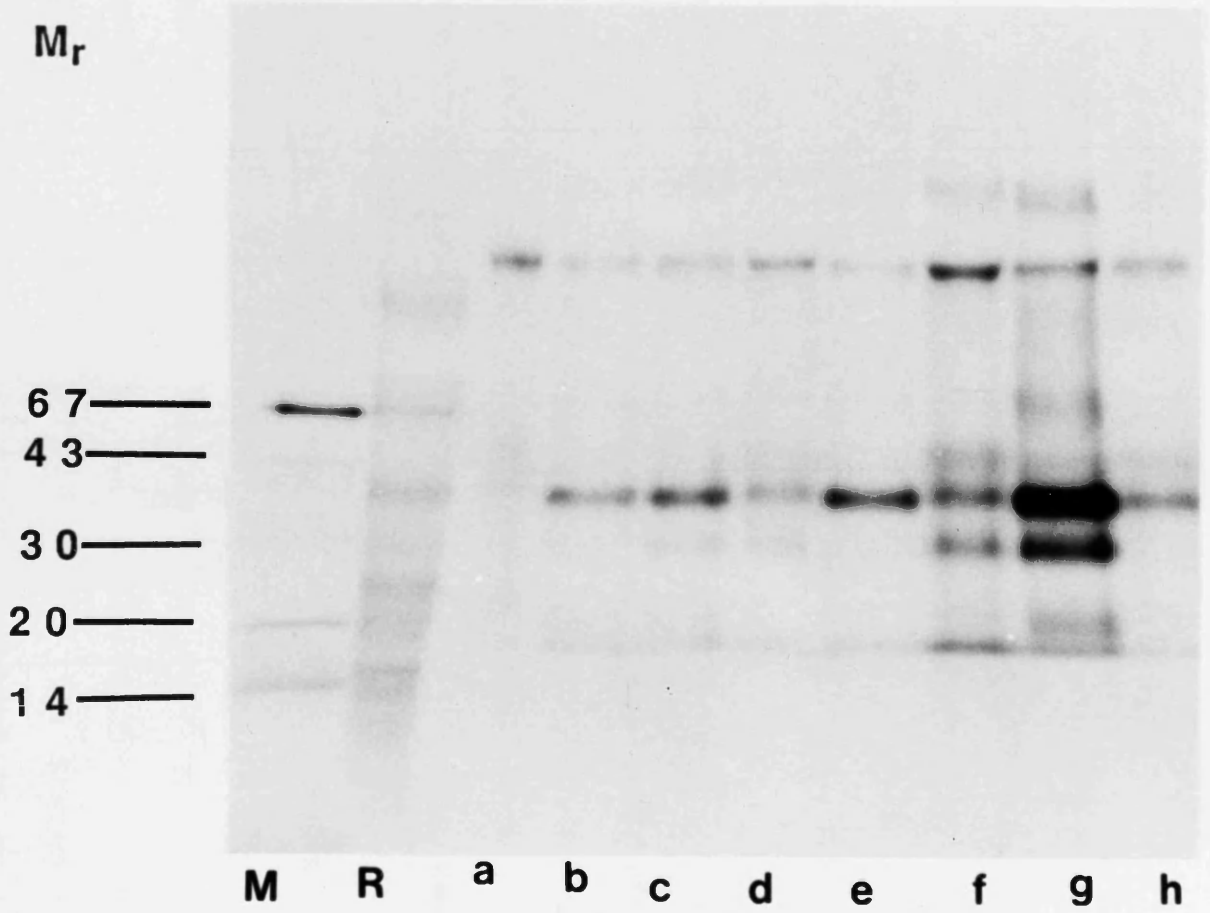
i

Figure 52; The recognition profiles of 7 individuals, over 60 years of age from an *Ascaris* endemic area. Radio-iodinated *A. lumbricoides* L3/4 ES (R) was immunoprecipitated with normal human serum, i.e. serum from a non-endemic area (a), or serum from Dennery D129 (b), D154 (c), D176 (d), D178 (e), Anse Le Ray 201 (f), A214 (g), A270 (h). The immunoprecipitated antigen was run on SDS-PAGE and molecular weights were estimated by comparision with marker proteins (M).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be seen in this age group, for example none of these serum samples recognised the 14kDa molecule.

Restricted recognition of the 14kDa molecule

In view of the restricted recognition of the 14kDa in ES and ABF, serum samples were screened against a purified preparation of this antigen. Restricted recognition of this purified molecule by serum from individuals living in an *Ascaris* endemic area is presented in Figure 53.

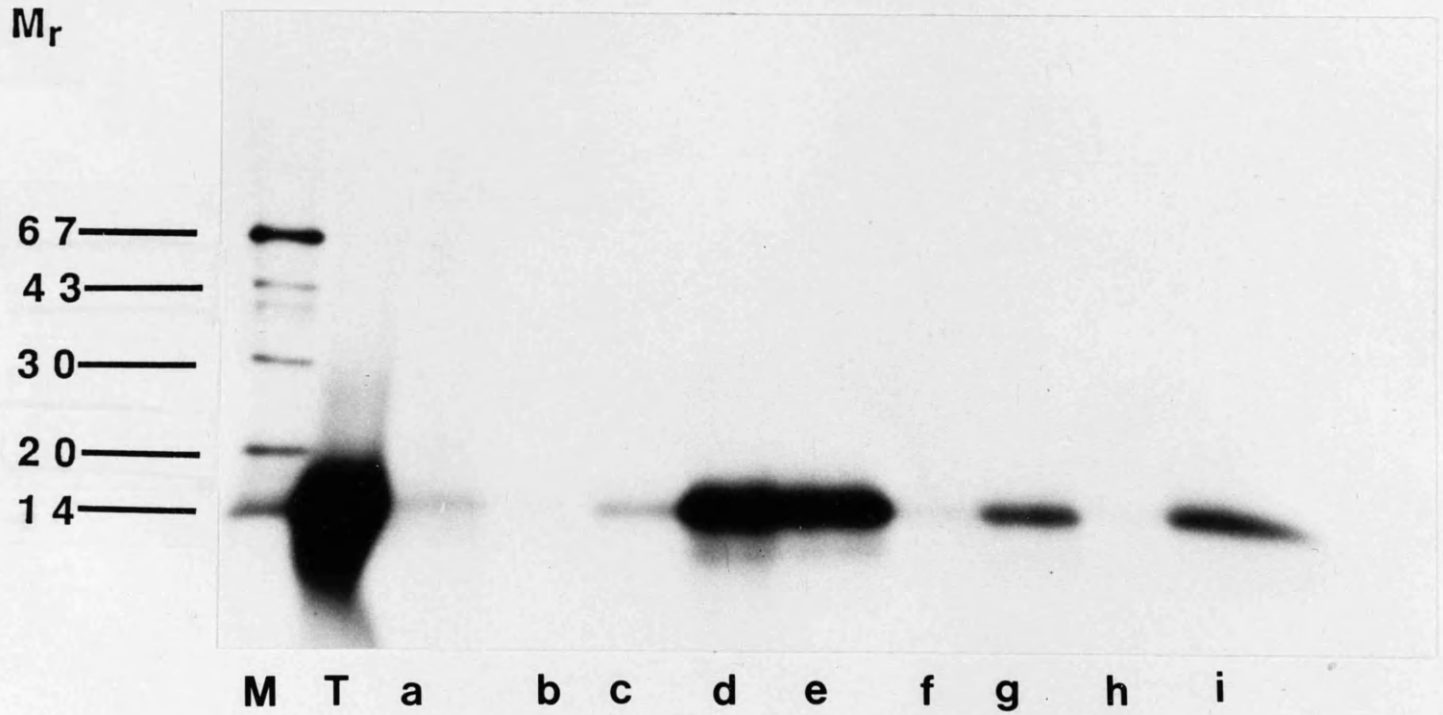
Helminth cross-reactivity

One of the main problems for serodiagnosis under field conditions is the cross-reactivity of certain parasite antigens (Maizels *et al*, 1982; Kennedy *et al*, 1989). The cross-reactivity against *Ascaris* antigens in the animal model is demonstrated in Figure 54. In this study individuals who, on field examination, were thought only to be infected with *Toxocara*, *Trichuris*, or bore no evidence of intestinal helminths were examined. These sera produced strong recognition profiles when screened against *Ascaris* antigens (Figure 55). The presence of antibodies which bind *Ascaris* antigens, in the sera of people who did not appear to be infected, could be explained by previous exposure, or an infection which has not as yet reached the stage of adult worms (day 70). The presence of *Ascaris* -specific antibodies in the *Toxocara* or *Trichuris* only groups maybe due to cross-reactivity or prior *Ascaris* infection.

Figure 53; The recognition profiles of 7 individuals from an *Ascaris* endemic area. Radio-iodinated 14kDa (R) was immunoprecipitated with normal human serum, i.e. serum from a non-endemic area (a), or serum from Boguis blank track(b), B197 (c), B95 (d), B124 (e), B3 (f), B138 (g), B30 (h), B155, (i). The immunoprecipitated antigen was run on SDS-PAGE and molecular weights were estimated by comparision with marker proteins (M).

Figure 54; Cross-reactivity against *Ascaris* ES. Radio-iodinated *A. suum* L3/4 ES was immunoprecipitated with **(Panel A)** normal hamster serum (a), Hamster anti-*Necator* (b), normal mouse serum (c), NIH anti-*A. suum* (d), mouse anti-*T. muris* (e), mouse anti-*N. brasiliensis* (f), mouse anti-*T. spiralis* (g), mouse anti-*T. cati* (h), mouse anti-*T. canis* (i).

(Panel B) Normal rat serum (a), WLEP anti-*A. suum* (b), rat anti-*N. brasiliensis* (c), rat anti-*Strongyloides* immunoprecipitated with I^{125} labelled ABF.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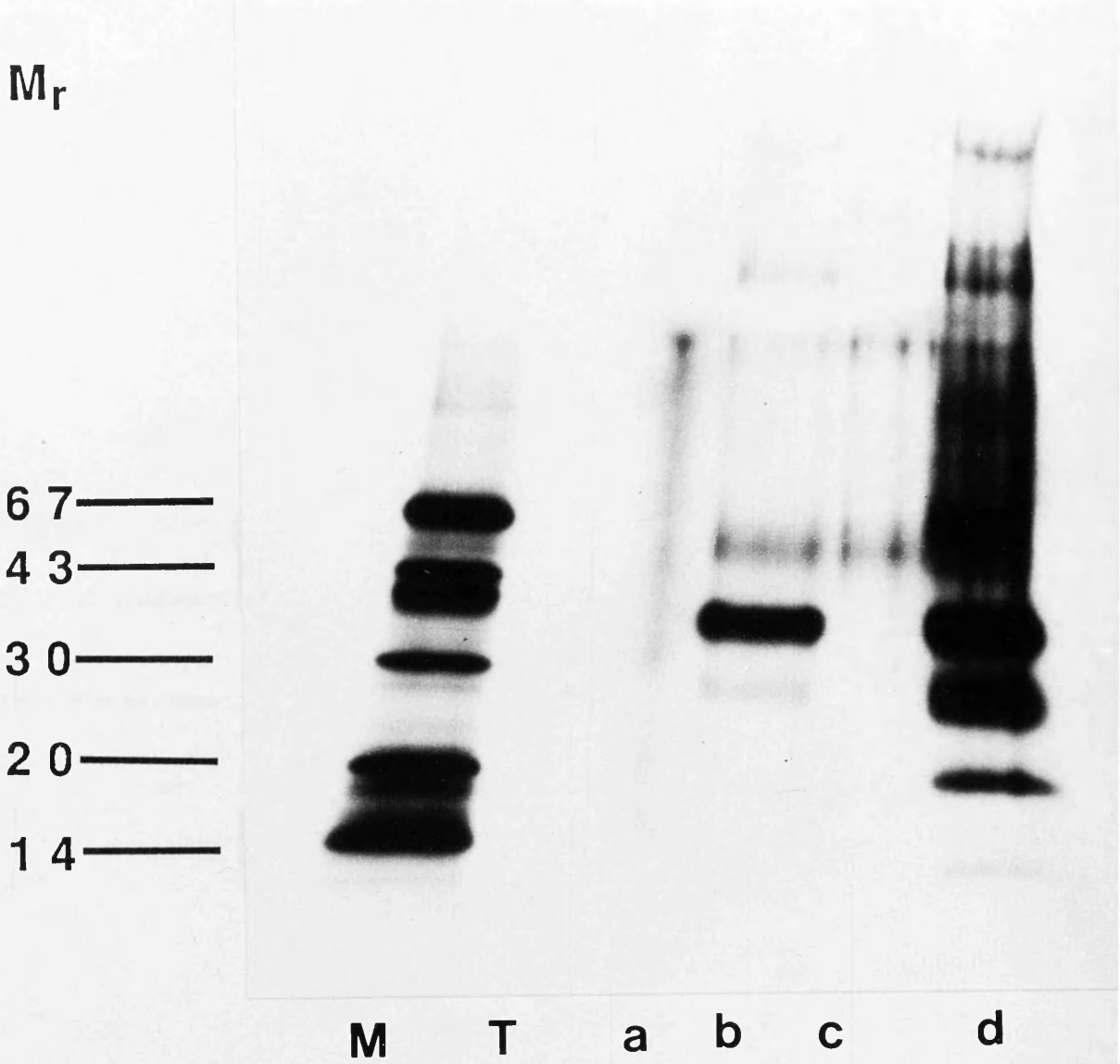
M T a b c d e f g h i

Figure 55; The recogniton profiles produced against *Ascaris* by individuals thought to be infected with *Toxocara* only. Radio-iodinated *A. lumbricoides* L3/4 ES was immunoprecipitated with normal human serum (a), D81 (b), D99 (c), A176 (d).


Mr
67
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Chapter 4

Discussion

The antibody responses of four human populations known to be exposed to *Ascaris lumbricoides* have been examined. Within these populations there is heterogeneity in response against the parasite in terms of antibody titre and in the parasite components recognised. One possible explanation for these results would be a difference in levels of infection, so that more heavily infected people respond to a broader spectrum of antigens or produce higher antibody titres. Alternatively, higher antibody titres may be indicative of a protective response and hence be present in individuals whose worm burdens are lower. This sort of relationship between antibody and anti-parasite immunity was demonstrated by Piessens *et al* (1980), in a detailed study on 90 individuals in a region endemic for *Brugia malayi*. These authors demonstrated a correlation between microfilaraemia and anti-microfilaria antibodies. The higher the microfilaraemia the lower the antibody titre, the lower the parasitaemia the higher the level of antibodies. There were individual variations but at the population level, the relationship held. Patients tended to show either a cell mediated immune (CMI) response against microfilaria or anti-microfilarial antibodies and no CMI. The reasons for this variation between individuals is unclear.

The data presented in this chapter are not commensurate with the view that antibody titre is dependent on level of infection. There is heterogeneity of response in all infection levels studied. The most marked heterogeneity

being present in the group of individuals who do not have eggs in their faeces. This difference between individuals is also evident in all age groups.

It is possible that monitoring infection by measuring epg of faeces does not give a completely accurate picture of *Ascaris* infection because this method only indicates the number of female worms present. The most important stage for triggering antibody production and effective immune intervention may be against the embryonated eggs and tissue penetrating larvae. However, one can assume that individuals within these endemic areas are continually exposed, and that the number of adult worms developing is indicative of the host's susceptibility, which is a combination of egg intake and the host response.

The differences in antigen recognition and antibody titre are between individuals regardless of their infection level or age. Therefore a mechanism other than exposure to the parasite is responsible for these differences. This would suggest that the genetic constitution of these hosts is responsible for this difference.

In the last 60 years there has been a steady accumulation of information demonstrating differences in response to infection, with a variety of organisms, between populations and individuals within that population (Keller *et al*, 1937; Cram 1940; Ackert 1942; Croll and Ghadirian, 1981). The available evidence suggests that there may be

some genetic basis for these differences. Recently, several reports have demonstrated the importance of the MHC in the deveopment of resistance against parasites in animal models (Wakelin and Blackwell, 1988; Wassom *et al*, 1983) and there is growing evidence of the importance of HLA in regulation of the immune system and the response to infection in humans (Tiwari and Terasaki, 1985).

The association of particular HLA alleles or haplotypes with resistance or susceptibility to parasitic infection and the associated pathology has been the subject of several studies. In a study of 2 groups of Egyptian schoolchildren with similar levels of infection with *S. mansoni* a link was established between HLA and pathology. There was a higher frequency of HLA-A1 and -B5 in individuals with hepatomegaly and splenomegaly (Salam *et al*, 1979). It has been proposed that this may explain the heterogeneity seen in the response to this parasite. A correlation has also been found with HLA and response against *S. japonicum* (Sasasuki *et al*, 1980). Work involving infection with *S. japonicum* has linked HLA-A1 and HLA-B13 with susceptibility to the more severe stages of disease (Chong-gong *et al*, 1984).

In 1983, Okta *et al*, suggested the presence of an HLA-linked Immune suppression (Is) gene which determinded responses to post-schistosomal liver cirrhosis. The mechanism was shown to be mediated by an antigen-specific suppressor CD8+ T cell. This led to a study of the roles

of particular HLA encoded products in the expression of high and low responsiveness to *S. japonicum* (Hirayama *et al*, 1987). Using specific T cell lines from high and low responding individuals they demonstrated that HLA-DQw1 plays an important role in determining antigen-specific suppression and HLA-DR2 is required for proliferation of T cells against *S. japonicum* antigen. They concluded that DR2 is the product of an Ir gene, DQw1 the product of an Is gene and that these genes control the response against this parasite.

Studies in areas endemic for ascariasis have now revealed a link between infection status and particular HLA haplotypes (Bundy *et al*, 1988). The evidence for an association between HLA haplotype and level of infection supports the hypothesis that the differences in antibody titre reflect individual differences which are controlled by the genetic constitution of the host. In the rodent model, presented in Chapters 1 and 2, variation in the antibody repertoire produced to *Ascaris* infection has been shown to be controlled by genes linked to the MHC. This work would support the hypothesis that the serological differences between individuals with a similar parasite-exposure rate may be explained by the genetic constitution of the host, and would warrant a study of HLA haplotype and infection status, and HLA haplotype and antibody specificity.

General Discussion

1: The genetic control of the IgG repertoire during infection with *Ascaris*

In this thesis the results of an investigation of the genetic control of antibody production during infection with *Ascaris* have been presented. When rodents and rabbits are used as hosts for *Ascaris* under experimental conditions, infection proceeds to the lung stage, exposing the host's immune system to the infective and tissue penetrating larvae (Mitchell *et al*, 1982). All the evidence shows that *A. lumbricoides* follows the same route in humans (Crompton, 1989). This model system is, therefore, a valid means of studying the response against the larval stages of infection, and there appear to be cross-reactive or identical antigens present in the larval and adult stages of the parasite (Kennedy and Qureshi, 1986).

In an effective immune response against eukaryotic parasites, the importance of both antibody specificity and isotype have been demonstrated in several studies (Mitchell *et al*, 1976; River-Ortiz and Nussenzweig, 1976; Almond and Parkhouse, 1986a, b; Roelants and Pinder, 1987); the specificity may vary between antibody classes (Almond *et al*, 1987). These authors studied the immune response responsible for the clearance of microfilariae from the circulation in mice infected with *D. viteae*. In this host-parasite relationship, elimination of microfilariae is antibody mediated, the protective antibody being IgM. Other antibody classes, most notably

IgA, are also produced but they do not bind to the surface of the intact microlfilariae, and seem to be specific for internal components. However, in the *Ascaris* model no such variation in specificity between antibody isotypes has been detected. The antigen recognition profile produced by IgG, IgE and total immunoglobulin did not vary significantly, and there was restricted recognition of *Ascaris* components in all cases (Chapters 1 and 3). It is possible that a more detailed analysis of isotype specificity, using anti-IgA and anti-IgM reagents, and sera from primary and subsequent infections, might reveal some differences in the class profiles obtained, but as IgG and IgE antibodies are known to be the most relevant in immunity to *Ascaris* (Brown *et al*, 1977; Johansson *et al*, 1968) this study has concentrated on these isotypes.

The genetic control of antibody production has been the subject of several studies (Rivera-Ortiz and Nussenzweig, 1976; Jungery and Ogilvie, 1982; Storey *et al*, 1987) and both MHC and non-MHC linked affects have been described (Deelder *et al*, 1978; Kennedy *et al*, 1986; Kee *et al*, 1986; Ivanyi and Sharp, 1986). When experimental animals are infected with *Ascaris*, the antibody response is affected by both non-MHC-linked and MHC-linked genes. The antibody repertoire produced as a response to this infection is controlled by genes linked to the MHC (Chapter 2). These genes restrict the specificity of the response, during the course of the infection, but this restriction can be overcome if the parasite antigens are

presented with Freund's adjuvant (Chapter 1). The level of antibody production and the kinetics of the response appear to be under the control of non-MHC linked genes.

The antigen recogniton profiles produced by H-2 congenic strains of mice, infected with standard doses of *Ascaris*, suggest that the allele controlling recogniton, at least for the 14kDa molecule, is located at the I-A region since the presence or absence of cell-surface I-E molecules does not seem to be related to recognition of this molecule. This hypothesis could be tested using recombinant strains of mice expressing different alleles at their I-A and I-E loci together with a recombinant strain which expresses different alleles at the A-alpha and A-beta loci which could be used to map this allele.

Antibodies may have a variety of functions in the host response against helminth infection. Some reports link specific antibody with elimination of the worm either through hypersensitivity reactions (Taffs, 1964), or adherence of effector cells (Ogilvie and de Savigny, 1982), and the lack of specific antibody has been associated with less resistant strains of animals (Brown *et al*, 1977; Mitchell *et al*, 1985). The presence of specific antibodies has been linked with immunity in several parasite systems (Thompson *et al*, 1979; Ogilvie and de Savigny, 1982; Storey *et al*, 1985). Parasite specific antibodies have, however, also been associated with susceptibility to infection (Deelder *et al*, 1978;

Pond *et al*, 1988) and in these studies it is thought that these antibodies block effective immune responses. The antibody production during *Ascaris* infection is thought to be under T cell control (Bindseil, 1971) and therefore the MHC control of the specificity of the antibody repertoire will reflect the MHC control of T cell specificity.

ii: The genetic control of the IgE repertoire during infection with *Ascaris*

Some *Ascaris* antigens are known to be potent allergens (Ambler *et al*, 1973; Marsh, 1975; Bradbury *et al*, 1974), and the parasites also produce histamine-releasing factors which can cause degranulation of mast cells in naive hosts (Uvnas and Wold, 1967; Thompson, 1972; Tolone *et al*, 1974). In this study, the production of IgE has been found to be controlled by both MHC-linked and non-MHC genes (Chapter 3). The specificity of the IgE antibodies is controlled by MHC-linked genes, and the IgE repertoire, produced by a given strain, appears to be identical in specificity to the IgG repertoire. The level of IgE produced in response to infection is controlled by non-MHC linked genes as reported in the response to other antigens (Levine and Vaz, 1971). The antibody response against specific allergens may play a crucial role in the clinical manifestations of infection. *Ascaris* can cause potent hypersensitivity reactions in uninfected individuals exposed to the parasite (Pawlowski, 1978; Coles, 1975; 1985) as well as hypersensitivity responses in infected individuals (Pawlowski, 1978). These hypersensitivity

reactions are believed to be an important factor in the clinical manifestations of the infection. The factors controlling this specific IgE production may reflect the control of IgE in allergic individuals and hence provide a valuable model for hypersensitivity reactions in general. This model looks at IgE production in response to infection and may thus represent genetic control against specific allergens more precisely than models involving the use of adjuvants.

iii: The antibody response against *Ascaris* in the course of natural exposure

During natural infections, *Ascaris* induces antibody production, predominantly IgE and IgG, against parasite specific components (Johansson *et al*, 1968). In the analysis of sera from four endemic areas, carried out in the present study, IgG antibodies which react with the larval and intestinal stages of the parasite have been observed (Chapter 4). There is considerable heterogeneity in this antibody response in terms of both quantitative and qualitative analyses. No obvious correlation between worm burden and antibody level was observed; although this type of correlation has been reported elsewhere (Leikina and Guseinov, 1954; Leikina *et al*, 1957). The type of analysis undertaken in the present study can identify the level of antibody produced and its specificity, but does not aim to identify any role in protection or allergy leading to disease. The results show that there is a difference in antibody production in individuals which

cannot be explained purely by exposure to the parasite. Links between antibody titre and susceptibility have been demonstrated in other host-parasite relationships (Else and Wakelin, 1989; Gibbens *et al*, 1986) and in these studies, higher antibody production has been linked with susceptibility to infection.

It is possible that worm burden may more readily correlate with IgE antibody levels as these antibodies may play the major role in elimination of the infection (Miller, 1984). Alternatively, a correlation between cell mediated immunity and protection may occur. A relationship between antibody response and cell mediated immunity has been described in individuals infected with *B. malayi* (Piessens *et al*, 1980). In this study the presence of anti-microfilarial antibodies correlated with low microfilaraemia, and patients appeared to develop either an antibody response or a cell mediated response. During infection with *Ascaris* protection may be due to either an IgE or a cell-mediated reponse; this would account for the apparent lack of correlation between worm burden and IgG. Cross-reactivity of antibodies produced to concommitant infections must also be borne in mind in an analysis of this type. In the sample population analysed, infection with *Toxocara* and *Trichuris* are known to occur (Bundy D.A.P., personal communication) and there is a strong degree of cross-reactivity between *Toxocara* and *Ascaris* components (Kennedy *et al*, 1989; Chapter 4).

The immune response against a complex multicellular organism such as *Ascaris* is inevitably complex, involving several stages, which may function independently (Wassom *et al*, 1984; Wakelin, 1985). Genetic control, therefore, could be mediated at any one of these levels, and the outcome of infection would depend on the inter-relationship between these responses. For example, two of the effector mechanisms associated with the immune response against a helminth infection are IgE and eosinophilia, SJL mice are very poor IgE producers but produce high eosinophilia in response to parasite infection (Sugane and Oshima, 1984). This model system provides a means for studying the MHC control of the antibody repertoire during infection.

Future development of the analytical system used in this work may identify a serological marker, within these various antibody responses, which could be used to predict the level of infection, or the clinical manifestations of that infection. Alternatively, the heterogeneity in the specificity of the host response may reveal protective antigens recognised by resistant hosts. Given that the heterogeneity of antibody response witnessed in the rodent host also appears to occur in natural infection one possible explanation for this heterogeneity in man would be MHC involvement. Interestingly, preliminary studies by two groups suggest that there may be a link between certain HLA alleles and resistance or susceptibility to infection (Bundy, 1988; Holland *et al*, 1989).

APPENDIX

ASCARIS CULTURE MEDIUM

100ml RPMI (Gibco, 041-02409M) supplemented with 240 microgram L-glutamine (Flow, 16-801-49), 100 microgrammes D-glucose (Formachem Ltd. Scotland), 40 microgrammes Tripeptide (Sigma, G-1887), 4 microgrammes glutathione (Sigma, G-4251), 10,000IU penicillin and 10,000 microgrammes streptomycin (Flow, 16-700-49), 1mM sodium pyruvate (Flow, 16-820-49), 1mg sodium bicarbonate (Flow, 16-883-49), 2ml fungizone (Flow, 16-723-46), 0.25ml gentamycin (Sigma, G-7632), 1 tablet Cephalexin selectatab (Mast, MS10), 1 tablet VCNT selectatab (Mast, MS6).

ELISA CARBONATE/BICARBONATE BUFFER

1.5g sodium carbonate (BDH, 10240)

2.93g sodium hydrogen carbonate (BDH, 0164160)

0.2g sodium azide (Sigma, S-2002)

Made up to 1L with deionised water, store at 4°C.

ELISA ENZYME SUBSTRATE AND CHROMAGEN SOLUTIONS

ALKALINE PHOSPHATASE

5mg phosphatase substrate tablet (Sigma, 104-105) per 5ml diethanolamine buffer.

DIETHANOLAMINE BUFFER, pH 9.8

97ml diethanolamine (BDH, 10393)

0.5mM magnesium chloride (May and Baker, 25108-295)

0.2g sodium azide

TRIS/GLYCINE BUFFER

9.09g Tris (BCL, 11450320/44)

43.26g glycine (Sigma, G-7126)

600ml methanol (May and Baker, L868)

3.0g SDS (BDH, 44244)

Made up to 3L with deionised water.

AMIDO BLACK STAIN

0.1% amido black (DIFCO, 8338-12)

45% methanol

10% acetic acid (May and Baker, L723)

AMIDO BLACK DESTAIN

45% methanol

10% acetic acid

TRIS BUFFERED SALINE, pH 7.2

9g sodium chloride (Formachem, 82685)

1.21g Tris

Made up to 1L with deionised water.

PHOSPHATE BUFFERED SALINE, pH 7.2

100g sodium chloride

2.5g potassium chloride (Hopkin and Williams, 692800)

14.37g sodium dihydrogen phosphate (BDH, 30716)

2.5g potassium orthophosphate (anhydrous) (BDH, 10203)

Made up to 10L with deionised water.

Solution N

40% acrilamide (BDH, 44299)

0.54% N,N'-methylene-bis-acrylamide (BDH, 44300)

Stored at 4°C with Amberlite monobed resin (MB-1, BDH, 55007)

SOLUTION AA, PH 9.8

480ml 1M hydrochloric acid (May and Baker, MY0042)

366g Tris

0.8% SDS

0.23% Temed (Sigma, T 8133)

Made up to 1L with deionised water.

SOLUTION S, pH 6.8

121.1g Tris

0.8% SDS

0.4% Temed

Made up to 1L with deionised water

Solution AP

200 microgrammes Ammonium persulphate (BDH, 10031)

2ml deionised H_2O

5%-25% GRADIENT GELS (x 4, 0.75mm thick)

	5%	25%	STACK
H_2O (ml)	36	12	30
Sol N	6	30	5
Sol AA	6	6	0
Sol S	0	0	5
AP (microlitres)	180	100	400

20% HOMOGENEOUS GELS (x 4, 1.5mm thick)

	20%	STACK
H_2O (ml)	72	60
Sol N	96	10
Sol AA	24	0
Sol S	0	10
AP (microlitres)	400	800

BUFFER B (x5)

5.98g Tris

0.46ml Temed

48ml hydrochloric acid

Made up to 100ml with deionised water. Dilute 1:5 to use.

SAMPLE BUFFER, pH 6.8

5g SDS

5ml 1M Tris, pH 7.5

2ml 100mM PMSF in isopropanol (May and Baker, L865)

1ml 100mM EDTA (Sigma, ED255)

10ml Glycerol (May and Baker, L885)

2ml 0.2% Bromophenol blue (BDH, 44305)

Made up to a final volume of 95ml.

ELECTROPHORESIS BUFFER

140g glycine (Sigma, G-7126)

300g Tris

50g SDS

Made up to a final volume of 10L with deionised water.

References

ACKERT, J. E. (1942). Natural resistance to helminthic infections. J. Parasitology **28**, 1, p1.

ALCASID, M.L.S., CHIARAMONTE, L.T., KIM, H.J., ZOHN, B., MULLIN, W. (1973). Bronchial asthma and intestinal parasites. New York St. J. Med. **73**, p1786.

ALMOND, N.R. AND PARKHOUSE, R.M.E. (1986a). Immunoglobulin class specific responses to biochemically active defined antigens of *T. spiralis*. Parasite Immunol. **8**, p391.

ALMOND, N.R. AND PARKHOUSE, R.M.E. (1986b). The immunoglobulin class distribution of anti-phosphoryl choline responses in mice infected with parasitic nematodes. Immunology **59**, p633.

ALMOND, N.M., WORMS, M.J., HARNETT, W. AND PARKHOUSE, R.M.E. (1987). Variation in class-specific humoral immune responses of different mouse strains to microfilariae of *Dipetalonema viteae*. Parasitology **95**, p559.

AMBLER, J. AND ORR, T.S.C. (1972). Studies on an allergenic component extracted from *N. brasiliensis*. Immunochemistry **9**, p263.

AMBLER, J., CROFT, A.R., DOE, J.E., GEMMELL, D.K., MILLER, J.N. AND ORR, T.S.C. (1973). Biological techniques for studying the allergenic components of

nematodes. II. The characterisation of the allergen released by *Ascaris suum* maintained in saline. J. Immunol. Methods 2, p315.

AMBLER, J., MILLER, J.N. AND ORR, T.S.C. (1974). Some properties of *A. suum* allergen A. Int. Archs. Allergy appl. Immunol. **46**, p427.

ANDERSON, R.M. (1986). The population dynamics and epidemiology of intestinal nematode infections. Trans. Roy. Soc. Trop. Med. and Hyg. **80**, p686.

ANDERSON, R.M. AND MAY, R.M. (1985a). Helminth infections of humans: mathematical models, population dynamics and control. Advances in Parasitology **24**, p1.

ANDERSON, R.M. AND MAY, R.M. (1986b). Herd immunity to helminth infection : implications for disease control. Nature **315**, p493.

ANDERSON, R.M. AND MEDLEY, G.F. (1985). Community control of helminth infections in man by mass and selective chemotherapy. Parasitology **90**, p629.

ANDERSON, R.M. AND SCHAD, G.A. (1985). Hookworm burdens and faecal egg counts: an analysis of the biological basis of variation. Trans. Roy. Soc. Trop. Med. and Hyg. **79**, p812.

ANNAN, A., CROMPTON, D.W.T., WALTERS, D.E. AND ARNOLD, S.E. (1986). An investigation of the prevalence of intestinal parasites in pre-school children in Ghana. Parasitology **92**, p209.

ARFAA, F. AND GHADIRIAN, (1977). Epidemiology and mass-treatment of ascariasis in six rural communities in central Iran. Am. J. Trop. Med. Hyg. **26**, p866.

ARNASON, A.N., DICK, T.A. AND WASSOM, D.L. (1986). A model to assess survival mechanisms of parasites in a genetically defined host system. Parasitology **92**, p253.

ASKENASE, P.W. AND VAN LOVEREN, H. (1983). Delayed-type hypersensitivity; activation of mast cells by antigen-specific T cell factors initiates the cascade of cellular interactions. Immunology Today **4**, p259.

BABBIT, B.P., ALLEN, P.M., MATSUEDA, G., HABER, E. AND UNANUE, E.R. (1985). Binding of immunogenic peptides to Ia histocompatibility molecules. Nature **317**, p359.

BAAL, E.J. AND STASTNY, P. (1984). Antigen-specific T-cell lines. II. A GAT-specific T-cell line restricted by a determinant carried by an HLA-DQ molecule. Immunogenetics **20**, p547.

BAXEVANIS, C.N., NAGY, Z.A. AND KLEIN, J. (1981). A novel type of T-T cell interaction removes the

requirement for I-B region in the H-2 complex. Proc. Natl.Acad.Sci. USA **78**, p3809.

BEER, D.J. AND ROCKLIN, R.E. (1984). Histamine-induced suppressor-cell activity. J. Allergy and Clinical Immunol. **73**, p439.

BEFUS, D. (1986). Immunity in intestinal helminth infections: present concepts, future directions. TRANS. Roy. Soc. Trop. Med. and Hyg. **80**, p735.

BEFUS, A.D. AND BIENENSTOCK, J. (1984). Induction and expression of mucosal immune responses and inflammation to parasitic infections. In: Contemporary Topics in Immunobiology: Immunobiology of Parasites and Parasitic Infections. (ED. MARCHALANIS, J.J.) Plenum Press p71.

BEHNKE, J.M., HANNAH, J. AND PRITCHARD, D.I. (1983). *Nematospiroides dubius* in the mouse: evidence that adult worms depress the expression on homologous immunity. Parasite Immunol. **5**, p397.

BEHNKE, J.M. AND ROBINSON, M. (1985). Genetic control of immunity to *N. dubius*; a nine day anthelmintic abbreviated immunising regime which separates weak and strong responder strains of mice. Parasite Immunol. **7**, p235.

BEISEL, W.R. (1982). Single nutrients and immunity. Am. J. of Clinical Nutrition **35**, p417.

BELL, R.G., ADAMS, L.S. AND OGDEN, R.W. (1984). A single gene determines rapid expulsion of *T. spiralis* in mice. Infect. Immunol. **45**, p273.

BENACERRAF, B. (1978). A hypothesis to relate the specificity of T lymphocytes and the activity of I-region specific Ir genes in macrophages and B lymphocytes. J. Immunol. **120**, p1809.

BENACERRAF, B. AND MC DEVITT, H.O. (1972). Histocompatibility-linked immune response genes. Science **175**, p273.

BENKOVA, M. (1982). The immunising effect and dynamics of ciculating antibodies after treating pigs with antigens from *A. suum*. Helminthologia **19**, p47.

BENSTEAD-SMITH, R., ANDERSON, R.M., BUTTERWORTH, A.E., DALTRON, P.R., KAIRIUKI, H.C., KOERCH, D., MUGAMBI, M., OUMA, J.H., SIONGOK, T.K. AND STURROCK, R.F. (1986). Evidence for predisposition of individual patients to reinfection with S. mansoni after treatment. Trans. Roy. Soc. Trop. Med. Hyg. **81**. 651.

BILLINGHAM, R.E., BRENT, L. AND MEDAWAR, P.B. (1956). Quantitative studies on tissue transplantation immunity.

III. Actively acquired tolerance. Phil. Trans. Roy. Soc. Lond. **239**, p357.

BINDSEIL, E. (1969). Immunity to *A. suum* 2. Investigations of the fate of larvae in immune and non-immune mice. Acta Pathologica and Microbiologica Scandinavia **77**, p223.

BINDSEIL, E. (1970). Immunity to *A. suum* 3. The importance of the gut for immunity in mice. ACTA Pathologica et Microbiologica Scandinavia **78**, p183.

BINDSEIL, E. (1971). Preliminary studies on the immunity to *A .suum* in pigs. Acta Vet. Scandinavia **12**, p128.

BLACKWELL, J.M. (1983). *Leishmania donovani* infection in heterozygous and recombinant H-2 haplotype mice. Immunogenetics **18**, p101.

BLACKWELL, J.M. (1985). A murine model of genetically controlled host responses to leishmaniasis. In Ecology and Genetics of Host-Parasite Interactions. The Linnean Soc. of Lond.

BLOCH, K.J. (1967). The anaphylactic antibodies of mammals including man. Prog. Allergy **10**, p84.

BLOCH, K.J., BLOCH, D.B., STEARNS, M. AND WALKER, W.A. (1979). Intestinal uptake of macromolecules. VI. Uptake

of protein antigens *in vivo* in normal rats infected with *N. brasiliensis* or subjected to mild systemic anaphylaxis. Gastroenterology **77**, p1039.

BLOMBERG, B., GECKELER, W.R. AND WEIGERT, M. (1972). Genetics of the antibody response to dextran in mice. Science **177**, p178.

BRADBURY, S.M., PERCY, D.H. AND STREJAN, G.H. (1974). Immunology of *A suum* infection. I. Production of reaginic antibodies to worm components in rats. Int. Arch. Allergy **46**, p498.

BRADLEY, D.J. (1979). Regulation of *Leishmania* populations within the host. IV. Parasite and host cell kinetics studied by radioisotope labelling. Acta Tropica **36**. p171.

BROWN, A.R., CRANDALL, C.A. AND CRANDALL, R.B. (1977). The immune response and acquired resistance to *A. suum* infection in mice with an X-linked B lymphocyte defect. J. Parasitology **63**, p950.

BROWN, I.N., GLYNN, A.A. AND PLANT, J. (1982). Inbred mouse strain resistance to *Mycobacterium lepraemurium* follows the *Ity/Lsh* pattern. Immunology **47**, p149.

BROWN, J.H., JARDETZKY, T., SAPER, M.A., SAMRAOUI, B., BJORKMAN, P.J. AND WILEY, D.C. (1988). A hypothetical

model of the foreign antigen binding site of CLII histocompatibility molecules. Nature **332**, p845.

BUNDY, D.A.P. (1986). Epidemiological aspects of *Trichuris* and trichuriasis in Caribbean communities. Trans. Roy. Soc. Trop. MED and Hyg. **80**. p706.

BUNDY, D.A.P. (1988). Population ecology of intestinal helminth infections in human comunities. Phil. Trans. Roy. Soc. Lond. **321**, p405.

BUNDY, D.A.P., COOPER, E.S., THOMPSON, D.G., DIDIER, J.M. AND SIMMONS, I. (1987). Epidemiology and population dynamics of *A. lumbricoides* and *T. trichiura* infection in the same community. Trans. Roy. Soc. Trop. Med. Hyg. **81**, p987.

BUNDY, D.A.P., THOMPSON, P.E., GOLDEN, M.H.N., COOPER, E.S., ANDERSON, R.M. AND HARLAND, P.S.E. (1985). Population distribution of *Trichuris trichuria* in a community of Jamaican children. Trans. Roy. Soc. Trop. Med. and Hyg. **79**, p232.

BUUS, S., SETTE, A., COLON, S.M., MILES, C. AND GREY, H.M. (1987). The relations between MHC restriction and the capacity of Ia to bind immunogenic peptides. Science **235**, p1353.

CABRERA, B.D. (1984). *Ascaris*; most popular worm. World Health.

CAMPBELL, D.H. (1936). An antigenic polysaccharide fraction of *A. lumbricoides* (from Hog). J. Infect. Dis. **59**, p266.

CASTRO, G.A., ROY, S.A. AND SCHANBACHER, L.M. (1975). Lethacity of disrupted intestinal lamina propria cells for *T. spiralis in vitro*. J. Parasitology **61**, p1053.

CHEAH, J.S. AND KHAN, S.P. (1972). Lack of association between helminthic infestations and bronchial asthma in Singapore. Aust. NZ. J. Med. **4**, p383.

CHEEVER, A.W., DUVALL, R.H. AND HALLACK, T.A. (1983). Hepatic fibrosis in schistosoma haematobium-infected mice. Trans. Roy. Soc. Trop. Med. Hyg. **77**, p673.

CHIORAZZI, N., FOX, D.A. AND KATZ, D.H. (1977). Hapten specific IgE responses in mice. VII. Conversion of IgE 'Non-responder' strains to IgE 'Responders' by elimination of suppressor T cell activity. J. Immunol. **118**. p48.

CHONG-GONG, W., QIU-YING, Z., PAN-YU, H., YI-WEN, Z., JIAN-UW, W., YI-PING, S., SHENG-JIE, Y. AND JING-YU, C. (1984). HLA and schistosomiasis japonica. Chinese Med. Journal **97**, p603.

CIVIL, R.H. AND MAHMOUD, A.A.F. (1978). Genetic differences in BCG-induced resistance to *S. mansoni* are not controlled by genes within the MHC of the mouse. J. Immunol. **120**, p1070.

CLAAS, F.H.J. AND DELDER, A.M. (1979). H-2 linked immune response to murine experimental *S. mansoni* infections. J. Immunogenetics **6**, p167.

COBDEN, I., ROTHWELL, J. AND AXON, A.T.R. (1979). Intestinal permeability in rats infected by *N. brasiliensis*. Gut **20**, p716.

COHEN, A. AND WARREN, K.S. (1982). Immunology of Parasitic Infections. Blackwell Scientific Publications, Oxford.

COHN, M. (1974). In The Immune System, Genes, Receptors, Signals (EDS. SERCARZ, E.E., WILLIAMSON, A.R. AND FOX, C.F.) p89, (Academic Press, New York).

COLES, G.C. (1975). Gastro-intestinal allergy to nematodes. Trans. Roy. Soc. Trop. Med. and Hyg. **69**. p362.

COLES, G.C. (1985). IN Ascariasis and its public health significance. (ED. CROMPTON, D.W.T., NESHEIM, M.C. AND PAWLOWSKI, Z.S.) p167. (Taylor and Francis).

COOKE, R.A. AND VAN DER VEER, A. (1916). Human sensitisation. J. Immunol. **1**, p201.

CRAM, E.B. (1940). Studies on oxyuriasis. XXIV. Comparative findings in the white and negro races. Proc. of the Helminthological Soc. of Washington **7**, p31.

CRANDALL, C.A. (1976). *A. suum*: Homocytotropic antibody in mice. Exp. Parasitology **39**, p69.

CRANDALL, C.A. AND AREAN, V.M. (1965). The protective effect of viable and non-viable *A. suum* larvae and egg preparations in mice. Am. J. Trop. Med. and Hyg. **14**, p765.

CRANDALL, C.A. AND CRANDALL, R.B. (1971). A. suum: Immunoglobulin responses in mice. Exp. Parasitology **30**, p426.

CRANDALL, C.A. AND CRANDALL, R.B. (1976). *A. suum*: Immunosuppression in mice during acute infection. Exp. Parasitology **40**, p363.

CRANDALL, R.B., CRANDALL, C.A. AND JONES, J.F. (1978). Analysis of immunosuppression during early acute infection of mice with *A. suum*. Clin. Exp. Immunol. **33**, p30.

CROLL, N.A. AND GHADIRIAN, E. (1981). Wormy persons; contributions to the nature and patterns of

overdispersion with *A. lumbricoides*, *Ancylostoma duodenale* and *Trichuris trichiura*. Trop. and Geographic Med. **33**, p241.

CROLL, N.A., ANDERSON, R.M., GYORKOS, T.W. AND GHADIRIAN, E. (1982). The population biology and control of *A. lumbricoides* in a rural community in Iran. Trans. Roy. Soc. Trop. Med. and Hyg. **78**, p187.

CROMBIE, J.A. AND ANDERSON, R.M. (1985). Population dynamics of *S. mansoni* in mice repeatedly exposed to infection. Nature **315**, p491.

CROMPTON, D.W.T., NESHEIM, M.C. AND PAWLOWSKI, Z. S. (1985). Ascariasis and its public health significance. (Taylor and Francis).

CROMPTON, D.W.T., NESHEIM, M.C. AND PAWLOWSKI, Z. S. (1989). Ascariasis and its prevention and control. (Taylor and Francis).

DAVIE, J.M., PAUL, W.E., KATZ, D.H. AND BENACERRAF, B. (1972). Hapten-specific tolerance, preferential depression of the high affinity antibody response. J. Exp. Med. **136**, p426.

DAVIS, M.M. AND BJORKMAN, P. (1988). T-cell antigen receptor genes and T-cell recognition. Nature **334**, p395.

DEAN, D.A., BUKOWSKI, M.A. AND CHEEVER, A.W. (1987). Relationship between acquired resistance, portal hypertension and lung granulomas in ten strains of mice infected with *S. mansoni*. Am. J. Trop. and Hyg. **30**, p806.

DEELDER, A.M., CLAAS, F.H.J. AND DE VRIES, R.R.P. (1978). Influence of the mouse H-2 gene complex on experimental infections witn *S. mansoni*. Trans. Roy. Soc. Trop. Med. and Hyg. **72**, p321.

DESPOMMIER, D.D., MC GREGOR, D.D., CRUM, E.D. AND CARTER, P.B. (1977). Immunity to *T. spiralis*. II. Expression of immunity against adult worms. Immunology **33**, p797.

DE VRIES, R.R.P., LAI, A., FAT, R.F.M., NIJENHUIS, L.E. AND VAN ROOD J.J. (1976). HLA-linked control of host response to *Mycobacterium leprae*. Lancet **2**, p1328.

DE VRIES, R.R.P., VAN EDEN, W. AND VAN ROOD, J.J. (1981). HLA-linked control of the course of *M. leprae* infections. Leprosy Rev. Suppl. 1. **52**, p109.

DORF, M.E. (1981). Genetic control of immune responsiveness p221. In the Role of the MHC in Immunobiology (ED. M.E. DORF). Garland STPM Press, New York.

DORRINGTON, K.J. AND BENNICH, H.H. (1978). Structure-function relationships in human IgE. Immunol. Rev. **41**. p3.

DUNCAN, J.L., SMITH, W.D. AND DARGIE, J.D. (1978). Vet. Parasitology **4**, p21.

ELKINS, D.B., HASWELL-ELKINS, M. AND ANDERSON, R.M. (1986). The epidemiology and control of intestinal helminths in the Publicat Lake region of southern India. I. Study design and pre-and post-treatment observations on *A. lumbricoides* infection. Trans. Roy. Soc. Trop. Med and Hyg. **80**, p774.

ELLNER, J.J., OLDS, G.R., OSMAN, G.S. KHOLY, A.E. AND MAHMOUD, A.A.F. (1981). Dichotomies in the reactivity to worm antigen in human schistosomiasis mansoni. J. Immunol. **126**, p309.

ELSE, K. AND WAKELIN, D. (1989). Genetic variation in the humoral immune responses of mice to the nematode *Trichuris muris*. Parasite Immunol. **11**, p77.

EPSTEIN, R., SHAM, G., WOMACK, J. YAGUE, J., PALMER, E. AND COHN, M. (1986). The cytotoxic T cell response to the male-specific histocompatibility antigen (H-Y) is controlled by two dominant Ir genes, one in the MHC, the other in the Tar alpha-locus. J. Exp. Med. **163**, p759.

FANNING, M.M. AND KAZURA, J.W. (1985). Further studies on genetic variation of hepatosplenic disease and modulation in murine schistosomiasis mansoni. Parasite Immunol. **7**, p213.

FAUBERT, G. AND TANNER, C.E. (1975). Leucoagglutination and cytoxicity of the serum of infected mice and of extracts of *T. spiralis* larvae and the capacity of infected mouse sera to prolong skin allografts. Immunology **28**, p1041.

FERGUSON, A. AND JARRETT, E.E.E. (1975). Hypersensitivity reactions in small intestine. I. Thymus dependence of experimental 'partial villous atrophy'. Gut **16**, p114.

FERNANDO, S.T. (1968). Immunological responses of rabbits to *Toxocara* infection. Parasitology **58**, p91.

FINE, P.E.M., WOLF, E., PRITCHARD, J., WATSON, B., BRADLEY, D.J., FESTENSTEIN, H. AND CHOCKO, C.J.G. (1979). HLA-linked genes and leprosy: a family study in Karigin, South India. J. Inf. Dis. **140**, p152.

FREIDMAN, A., ZERUBAVEL, R., GITLER, C. AND COHEN, I.R. (1983). Molecular events in the processing of avidin by antigen presenting cells (APC). II. Identical processing by APC of H-2 high- and low-responder mouse strains. Immunogenetics **18**, p277.

FUJITA, K., IKEDA, T. AND TSUKIDATE, S. (1979). Immunological and physio-chemical properties of a highly purified allergen from *Dirofilaria immitis*. Int. Archs. Allergy appl. Immunol. **60**, p121.

GEEFHUYSEN, J., ROSEN, E.V., KATZ, J., IPP, T. AND METZ, J. (1971). Impaired cellular immunity in kwashiorkor with improvement after therapy. British Med. J. **IV**, p527.

GERSHWIN, L. (1978). The phylogenetic development of anaphylactic activity and homocytotropic antibodies. Dev. Comp. Immunol. **2**. p595.

GIBBENS, J.C., HARRISON, L.J.S. AND PARKHOUSE, R.M.E. (1986). Immunoglobulin class responses to *Taenia taeniaeformis* in susceptible and resistant mice. Parasite Immunol. **8**, p491.

GLEICH, G.J. AND JACOB, G.L. (1975). IgE antibodies to pollen allergens account for high percentages of total IgE protein. Science **190**, p1106.

GOLLAPUDI, S.U.S. AND KIND, L.S. (1977). Inhibition of IgE production in mice by non specific suppressor T cells. Int. Arch. Allergy **53**, p395.

GROVE, D.I., MAHMOUD, A.A.F. AND WARREN, K.S. (1977). Eosinophils and resistance to *T. spiralis*. J. Exp. Med. **145**, p755.

GUNTHER, E., RUDE, E., MEYER-DELIUS, M. AND STARK, O. (1973). Immune-response genes linked to the MHC in the rat. Transplant. Proc. **5**, p1467.

GUPTA, M.C., ARORA, K.L., MITHAL, S. AND TANDON, B.N. (1977). Effect of periodic deworming on nutritional status of *Ascaris* infested preschool children recieving supplementary food. Lancet **ii**, p108

HAQUE, A., WORMS, M.J., OGILVIE, B.M. AND CAPRON, A. (1980). *Dipetalonema viteae*: microfilariae production in various mouse strains and in nude mice. Exp. Parasitology **47**, p398.

HARRIS, W.G. (1975). The allegens of *S. mansoni*. II. Further separation by Sephadex G200 and ion-exchange chromatography. Immunology **29**, p835.

HASWELL-ELKINS, M., ELKINS, D.B. AND ANDERSON, R.M. (1987). Evidence for predisposition in humans to infection with *Ascaris*, hookworm, *Enterobius* and *Trichuris* in a South Indian fishing community. Parasitology **95**, p323.

HEBER-KATZ, E., HANSBERG, D. AND SCHWARTZ, R.A. (1983). The Ia molecule of the APC plays a critical role in immune response gene regulation of T cell activation. J. Mol. Cell. Immunol. **1**. p3.

HERZIG, D.J. (1974). *Ascaris* sensitivity in the dog. I. Isolation and characterisation of an antigen. J. Immunol. Methods **5**, p219.

HIRASHIMA, M., YODOI, J. AND ISHIZAKA, K. (1980). Regulatory role of IgE binding factors from rat T lymphocytes. III. IgE-specific suppressive factor with IgE binding activity. J. Immunol. **125**, p1442.

HIRASHIMA, M., YODOI, J. AND ISHIZAKA, K. (1981). Regulatory role of IgE-binding factors from rat T lymphocytes. V. Formation of IgE-potentiating factors by T lymphocytes from rats treated with *Bordetella pertussis* vaccine. J. Immunol. **126**, p838.

HIRAYAMA, K., MATSUSHITA, S., KIKUCHI, I., IUCHI, M., OHTA, N. AND SASAZUKI, T. (1987). HLA-DQ is epistatic to HLA-DR in controlling the immune response to shistosoma antigen in humans. Nature **327**. p426.

HLAING, T. (1985). A. lumbricoides infections in Burma. In Ascariasis and its public health significance. (ED. CROMPTON D.W.T., NESHEIM M.C. AND PAWLOWSKI Z.S.) (Taylor and Francis).

HLAING, T., SAW, T., AYE, H.H., LWIN, M. AND MYINT, T.M. (1984). Epidemiology and transmission dynamics of *A. lumbricoides* in Okpo village rural Burma. Trans. Roy. Soc. Trop. Med. Hyg. **78**, p497.

HLAING, T., SAW, T. AND LWIN, M. (1987). Reinfection of people with *A. lumbricoides* following single, 6-month and 12-month interval mass chemotherapy in Okpo village, rural Burma. Trans. Roy. Soc. Trop. Med. and Hyg. **81**, p140.

HOGARTH-SCOTT, R.S. (1967). The molecular weight of nematode allergens. Immunology **13**, p535.

HOLLAND, C.V., ASAOLU, S.O., CRICHTON, W.B., CROMPTON, D.W.T. AND TORIMIRO, S.E.A. (1989). Factors influencing predisposition to A. lumbricoides in humans. Manuscript in Preparation.

HORMAECHE, C.E., HARRINGTON, K.A. AND JOYSEY, H.S. (1985). Natural resistance to *Salmonellae* in mice: Control by genes within the MHC. J. Inf. Dis. **152**, p1050.

HOUBA, C. AND ROWE, D.S. (1973). A comparision of African and European serum levels of IgE. Bull. Wld. Hlth. Org. **49**, p539.

HOWARD, J.G., HALE, C. AND LIEW, F.Y. (1980). Immunological regulation of experimental cutaneous

leishmaniasis. III. Nature and significance of specific suppression of cell mediated immunity in mice highly susceptible to *Leishmania tropica*. J. Exp. Med. **152**, p594.

HUDSON, L. AND HAY, F.C. (1980). Practical Immunology p188-190. Blackwell Scientific Publications.

HUSSAIN, R., STREJAN, G. AND CAMPBELL, D.H. (1972). Hypersensitivity to *Ascaris* antigens. VII. Isolation and partial characterisation of an allergen. J. Immunol. **109**, p638.

HUSSAIN, R., BRADBURY, S.M. AND STREJAN, G. (1973). *Ascaris* antigens. VIII. Characterisation of a highly purified allergen. J. Immunol. **111**, p260.

HUSSAIN, R. AND OTTESEN, E.A. (1985). IgE responses in human filariasis. III. Specificities of IgE and IgG antibodies compared by immunoblot analysis. J. Immunol. **135**, p1415.

INOMATA, T., FUJIMOTO, Y., NATORI, T. AND AIZAWA, M. (1983). The genetic control of the immune response to insulins in the rat. Transplant Proc. **15**, p1593.

ISHIZAKA, K. AND ISHIZAKA, T. (1978). Mechanisms of reaginic hypersensitivity and IgE antibody response. Immunol. Rev. **41**. p109.

IVANYI, J. AND SHARP, K. (1986). Control by H-2 genes of murine antibody responses to protein antigens of *Mycobacterium tuberculosis*. Immunology **59**, p329.

JANEWAY, C.A., BINZ, H. AND WIGZELL, H. (1976). Two different V_H gene products make up the T-cell receptor. Scand. J. Immunol. **5**, p993.

JARRETT E.E.E. (1976). Production of IgE and reaginic antibody in relation to worm infection. In Molecular and biological aspects of the acute allergic reaction. (ED. JOHANSSON, STRANDBERG, AND UVNAS) p105. Plenum Press.

JARRETT, E.E.E. (1978). Stimuli for the production and control of IgE in rats. Immunol. Rev. **41**, p52.

JARRETT, E.E.E. AND BAZIN, H. (1977). Serum immunoglobulin levels in *N. brasiliensis* infection. Clin. Exp. Immunol. **30**, p330.

JARRETT, E.E.E. AND HAIG, D.M. (1984). Mucosal mast cells *in vivo* and *in vitro*. Immunology Today **5**, p115.

JARRETT, E.E.E. AND MILLER, H.R.P. (1982). Production and activities of IgE in helminth infection. Prog. Allergy **31**, p178.

JARRETT, E.E.E., ORR, T.S.C. AND RILEY, P. (1971). Inhibition of allergic reactions due to competition for

mast cell sensitisation sites by two reagins. Clin. Exp. Immunol. **9**, p585.

JARRETT, E.E.E. AND STEWART, D. (1972). Potentiation of rat reaginic (IgE) antibody by helminth infection. Simultaneous potentiation of separate reagins. Immunology **23**, p749.

JARRETT, E.E.E., URQUHART, G.M. AND DOUTHWAITE R.M. (1969). Immunological unresponsiveness to helminth parasites. II. Antibody response and hypersensitivity reaction associated with *N. brasiliensis* infection in young rats. Exp. Parasitology **24**, p270.

JOHANSSON, S.G.O., MELLBIN, T. AND VAHLQUIST, B. (1968). Immunoglobulin levels in Ethiopian pre-school children with special reference to high concentrations of IgE (IgND). Lancet **i**, p1118.

JONE, H.I. (1977). Haemagglutination tests in the study of Ascaris epidemiology. Annals Trop. Med. **71**, p219.

JONES, V.E., EDWARDS, A.J. AND OGILVIE, B.M. (1970). The circulating immunoglobulins in protective immunity to the intestinal stage of *N. brasiliensis* in the rat. Immunology **18**, p621.

JONES, P.P., MURPHY, D.B. AND MC DEVITT, H.O. (1981). Variable synthesis and expression of $E_{\alpha 1}$ and Ae

(E_{beta}) Ia polpeptide chains in mice of different H-2 haplotypes. Immunogenetics **12**, p321.

JONES, V.E. AND OGILVIE, B.M. (1971). Protective immunity to N. brasiliensis; the sequence of events which expels worms from the rat intestine. Immunology **20**, p549.

JUNGERY, M. AND OGILVIE, B.M. (1982). Antibody response to stage-specific *T. spiralis* surface antigens in strong and weak responder mouse strains. J. Immunol. **129**, p839.

KAJI, R., KAMIJO, T., YANO, A. AND KOJIMA, S. (1983). Genetic control of immune responses to *S. japonicum* antigen. Parasite Immunol. **5**, p217.

KAYES, S.G., OMHOLT, P.E. AND GRIEVE, R.B. (1985). Immune responses of CBA/J mice to graded infections with *T. canis*. Infection and Immunity **48**, p697.

KEE, K.C., TAYLOR, D.W., CORDINGLEY, J.S., BUTTERWORTH, A.E. AND MUNRO, A.J. (1986). Genetic influence on the antibody response to antigens of *S. mansoni* in chronically infected mice. Parasite Immunol. **8**, p565.

KELLER, A.E., LEATHERS, W.S. AND KNOX, J.C. (1937). The present status of hookworm infestation in North Carolina. Am. J. of Hyg. **26**, p437.

KELLEY, G.W AND NAYAK, D.P. (1964). Acquired immunity to migrating larvae of *A. suum* induced in pigs by repeated oral inoculation of infective eggs. J. Parasitology **50**, p499.

KELLEY, G.W. AND NAYAK, D.P. (1965a). Passive immunity to migrating *A. suum* transmitted by parenterally administered immune serum or immune globulins. Cornell Vet. **55**, p607.

KELLEY, G.W. AND NAYAK, D.P. (1965b). Passive immunity to *A. suum* transferred in colostrum from sows to their offspring. Am. J. Vet. Res. **26**, p948.

KENNEDY, M.W., GORDON, A.M.S., TOMLINSON, L.A. AND QURESHI, F. (1986).Genetic (MHC?) control of the antibody repertoire to the secreted antigens of *Ascaris*. Parasite Immunol. **9**, p269.

KENNEDY, M.W. AND QURESHI, F. (1986). Stage-specific and secreted antigens of the parasitic larval stages of the nematode *Ascaris*. Immunology **58**, p515.

KENNEDY, M.W., QURESHI, F., HASWELL-ELKINS, M. AND ELKINS, D.B. (1987). Homology and heterology between the secreted antigens of the parasitic larval stages of *A. lumbricoides* and *A. suum*. Clin. Exp. Immunol. **67**, p20.

KENNEDY, M.W., QURESHI, F., FRASER, E.M., HASWELL-ELKINS, M., ELKINS, D.B. AND SMITH, H.V. (1989). Antigenic relationships between the surface-exposed, secreted and somatic materials of the nematode parasites *A. lumbricoides, A. suum* and *T. canis*. Clin. exp. Immunol. **75**, p493.

KENT, N.H. (1963). Seminar on immunity to parasitic helminths. V. Antigens. Exp. Para. **13**, p45.

KERR, K.B. (1938). The cellular response in acquired resistance in guinea pigs to an infection with pig *Ascaris*. Am. J. Hyg. **20**, p28.

KESSLER, S.W. (1975). Rapid isolation of antigens from cells with a staphylococcal protein A-antibody absorbent: parameters of the interaction of antibody-antigen complexes with protein A. J. Immunol. **115**, p1617.

KEYMER, A.E. (1985). Experimental epidemiology; *N. dubius* and the laboratory mouse. (ED. ROLLINSON, D. AND ANDERSON, R.M.) P55. In Ecology and Genetics of Host Parasite Interactions. (Academic Press, London).

KEYMER, A.E. AND HIORNS, R.W. (1986). *Heligmosoides polygyrus* (Nematoda); the dynamics of primary and repeated infection in outbred mice. Proc. Roy. Soc. Lond. Series B.

KHOURY, P.B., STROMBERG, B.F. AND SOULSBY, E.J.L. (1977). Immune mechanisms to *A. suum* in inbred guinea piga. I. Passive transfer of immunity by cells or serum. Immunology 32, p405.

KLEIN, J. (1982). In Immunology the Science of Self-Nonself Discrimination. (John Wiley and Sons).

KOBAYASHI, A., KUMADA, M. AND ISHIZAKI, T. (1972). Evaluation of somatic and ES antigens causing immunological injury of mast cells in mice infected with *Anisakis* larvae. Jap. J. Med. Sci. Biol. **25**, p335.

KUO, C.Y. AND YOO, T.J. (1977). A new allergen from the perienteric fluid of *A. suum* with repect to charges. Int. Archs. Allergy appl. Immunol. **54**, p308.

KWA, B.H. AND LIEW, F.Y. (1977). Immunity in Taeniasis-cysticercosis. I. Vaccination against *T. taeniaeformis* in rats using purified antigen. J. Exp. Med. **146**, p118.

LEE, G.B. AND OGILVIE, B.M. (1981). The mucus layer of the small intestine-its protective effect in rats immune to *T. spiralis*. In Trichinellosis p91. (ED. KIM, C.W., RUITENBERG, E.J. AND TEPPEMA, J.S.).

LEID, R.W. AND WILLIAMS, J.F. (1975). Immunological response of the rat to infection with *T. taeniaeformis*.

II. Characterisation of reaginic antibody and an allergen associated with the larval stage. Immunology **27**, p209.

LEIKINA, E.S. AND GUSEINOV, G.A. (1954). Results of application of serological reactions in determination of the stage of ascariasis. Medicinskaja Parazitologica I Parasitarnye Bolenzi **23**, p79.

LEIKINA, E.S., GEFTER, V.A. AND ZORIKHINA, V.I. (1957). Application of agglutination with carmine for early diagnosis of ascaridiasis in mass examination of the population. Med. Para. Parazitarnye Bolenzi **23**, p612.

LEJINKA, E.S. (1965). Research on ascarisis immunity and immunodiagnosis. Bull. Wld. Hlth. Org. **32**, p699.

LEVINE, B.B. AND VAZ, N.M. (1971). Genetic control of reagin production in mice. Federation Proc. **30**, p469.

LEVINE, B.B., STREMBER, R.H. AND FOTINO, M. (1972). Ragweed hay fever: Genetic control and linkage to HL-A haplotypes. Science **178**, p1201.

LLOYD, S. AND SOULSBY, E.J.L. (1985). Ascariasis in animals. In *Ascaris* and its public health significance (ED. CROMPTON, D.W.T., NESHEIM, M.C. AND PAWLOWSKI, Z.S.) p25. (Taylor and Francis).

MAIZELS, R.M., KENNEDY, M.W.K., MEGHJI, M., ROBERTSON, B.D. AND SMITH, H.V. (1987). **Shared carbohydrate epitopes on distinct surface and secreted antigens of the parasitic nematode *Toxocara canis*.** J. IMMUNOLOGY **139**, p207.

LUBRINIECKI, A.S. AND CYPESS, R.H. (1975). Immunological sequelae of *T. spiralis* infection in mice. Effect on the antibody responses of sheep erythrocytes and Japenese B encephalitis virus. Infection and Immunity **11**, p1306.

MAIZELS, R.M., PHILIPP, M. AND OGILVIE, B.M. (1982). Molecules on the surface of parasitic nematodes as probes of the immune response in infection. Immunological Rev. **61**, p109.

MANSON-SMITH, D.F., BRUCE, R.G., ROSE, M.L. AND PARROTT, D.M.V. (1979). Migration of lymphoblasts to the small intestine. VII. Strain sifferences and relationship to distribution and duration of *T. spiralis* infection. Clin. Exp. Immunol. **38**, p475.

MARKWELL, M.A.K. AND FOX, C.F. (1978). Surface-specific iodination of membrane proteins of viruses and eucaryotic cells using 1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril. Biochemistry **17**, p4807.

MARSH, D.G. (1975). In the antigens (ED. SELA, M.) **3**, p271. (Academic Press, New York).

MATHIS, D.J., BENOIST, C., WILLIAMS, V.E., KANTER, M. AND MC DEVITT, H.O. (1983). Several mechanisms can account for defective E_{alpha} gene expression in diferent mouse haplotypes. Proc. Natl. Acad. Sci. USA **80**, p273.

MATZINGER, P. (1981). A one-receptor view of T-cell behaviour. Nature **292**, p497.

MC DEVITT, H.O. AND CHINTZ, A. (1969). Genetic control of the antibody response; relationship between immune response and histocompatibility (H-2) type. Science **163**, p1207.

MILLER, H.R.P. (1981). Immune exclusion and mucus trapping during rapid expulsion of *N. brasiliensis* from primed rats. Immunology **44**, p419.

MILLER, H.R.P. (1984). The protective mucosal response against gastrointestinal nematodes in ruminants and laboratory animals. Vet. Immunol. Immunopath. **6**, p167.

MITCHELL, G.F., HOGARTH-SCOTT, R.S., EDWARDS, R.D., LEWERS, H.M., COUSINS, G. AND MOORE, I. (1976). I. *A. suum* larvae numbers and antiphosphorylcholine responses in infected mice of various strains and in hypothymic nu/nu mice. Int. Archs. Allergy appl. Immunol. **52**, p64.

MITCHELL, G.F., RAJASEKARIAH, G.R. AND RICKARD, M.D. (1980). A mechanism to account for mouse strain variation in resistance to the larval cestode, *T. taeniaeformis*. Immunology **39**, p481.

MITCHELL, G.F., ANDERS, R.F., BROWN, G.V., HANDMAN, E., ROBERTS-THOMSON, C.R., CHAPMAN, C.B., FORSYTH, K.P., KAHL,

L.P. AND CRUISE, K.M. (1982). Analysis of infection characteristics and antiparasite immune responses in resistant compared with susceptible hosts. Immunol. Rev. **61**, p137.

MITCHELL, G.F., BEALL, J.A., CRUISE, K.M., TIU, W.U. AND GARCIA, E.G. (1985). Antibody responses to the antigen Sj26 of *S. japonicum* worms that is recognised by genetically resistant 129/J mice. Parasite Immunol. **7**, p165.

MURPHY, D.B., JONES, P.P., LOKEN, M.R. AND MC DEVITT, H.O. (1980). Interaction between I region loci influences the expression of a cell surface Ia antigen. Proc. Natl. Acad. Sci. USA **77**, p5404.

MURRELL, K.D. AND CLAY, B. (1972). *In vitro* detection of cytotoxic antibodies to *S. mansoni* schistosomules. Am. J. Trop. Med. Hyg. **21**, p569.

MURRELL, K.D., VANNIER, W.E. AND AHMED, A. (1974). *S. mansoni*: antigenic heterogeneity of excretions and secretions. Exp. Parasitology **36**, p316.

MUSOKE, A.J., WILLIAMS, J.F. AND LEID, R.W. (1978). Immunological response of the rat to infection with T. taeniaformis. VI. The role of immediate hypersensitivity in resistance to reinfection. Immunology **34**, p565.

NAUMYCHEVA, M.I. AND MALAKHOVA, E.I. (1958). Experiments with the active immunisation of pigs against ascaridosis with multiple doses of invasive ascarid eggs. Tezisv Nauch. Kont VOG p91.

NESHEIM, M.C., CROMPTON, D.W.T. AND SANJUR, D. (1985). Nutritional aspects of ascariasis. Report on a community study carried out in Chiriqui Province, Republic of Panama, during 1983 to 1984. Div. of Nutritional Sciences, Cornell University.

NIYAMA, T., KOJIMA, K., MIZUNO, K., MATSUNO, Y., FUJII, H., MISONOU, J., NATORI, T., AIZAWA, M. AND OIKAWA, K. (1987). Genetic control of the immune responsiveness to *Streptococcous mutans* by the MHC of the rat (RT1). Infection and Immunity **55**, p3137.

O'DONNELL, I.J. AND MITCHELL, G.F. (1978). An investigation of the allergens of *A. lumbricoides* using a radioallergosorbent test (RAST) and sera from naturally infected humans; comparision with an allergen for mice identified by passive cutaneous anaphylaxis test. Aust. J. Biol. Sci. **31**, p459.

O'DONNELL, I.J. AND MITCHELL, G.F. (1980). An investigation of the antigens of *A. lumbricoides* using a radio immunoassay and sera of naturally infected humans. Int. Arch. Allergy appl. Immunol. **61**, p213.

OGILVIE, B.M. (1964). Reagin-like antibodies in animals to helminth parasites. Nature **204**, p91.

OGILVIE, B.M. (1967). Reagin-like antibodies in rats infected with the nematode *N. brasiliensis*. Immunology **12**, p113.

OGILVIE, B.M., ASKENASE, P.W. AND ROSE, M.E. (1980). Basophils and eosinophils in three strains of rats and in athymic (nude) rats following infection with the nematodes *N. brasiliensis* or *T. spiralis*. Immunology **39**, p385.

OGILVIE, B.M. AND DE SAVIGNY, D. (1982). Immune responses to nematodes p715. In Immunology of Parasitic Infections (ED. COHEN, S. AND WARREN, K.S.). Blackwell Scientific Publications.

OGILVIE, B.M. AND JONES, V.E. (1971). Reaginic antibodies and immunity to N. brasiliensis in the rat. I. The effect of thymectomy, neonatal infections and splenectomy. Parasitology **57**, p335.

OGILVIE, B.M. AND PARROTT, D.M.V. (1977). The immunological consequences of nematode infection. In Immunology of the Gut, CIBA Foundation Symposium **46**, p183. (Associated Sientific Publishers; Amsterdam).

OGILVIE, B.M., PHILIPP,M., JUNGERY, M., MAIZELS, R.M., WORMS, M.J. AND PARKHOUSE, R.M.E. (1980). The surface of

nematodes and the immune response of the host p99. In The host-invader interplay. (ED. VAN DEN BOSSCHE).

OHTA, N., MINAI, M. AND SASASUKI, T. (1983). Antigen-specific suppressor T lymphocytes (Leu-2a+3a-) in human schistosomiasis japonica. J. Immunol. **131**, p2524.

OLIVERIA, D.B.G., BLACKWELL, N., VIRCHIS, A.E. AND AXELROD, R.A. (1985). T helper and T suppressor cells are restricted by the A and E molecules, respectively, in the F antigen system. Immunogenetics **22**, p169.

ORR, T.S.C. AND BLAIR, A.M.J. (1969). Potentiated reagin response to egg-albumin and conalbumin in *N. brasiliensis* infected rats. Life Sci. **8**, p1073.

OVARY, Z., TAYA, I., WATANABE, N. AND KOJIMA, S. (1978). Regulation of IgE in mice. Immunol. Rev. **41**, p26.

PAWLOWSKI, Z.A. (1978). Ascariasis. Clinics in Gastroenterology **7**, p157.

PAWLOWSKI, Z.S. (1982). Ascariasis; Host-pathogen biology. Rev. Inf. Dis. **4**, p806.

PAWLOWSKI, Z.S. AND ARFAA, F. (1984). In Ascariasis in Trop. and Geographical Medicine, (ED. WARREN, K.S. AND MAHMOUD, A.A.F.) p347. Mc Graw-Hill, New York.

PERRY, P. AND LUFFAU, G. (1979). Antigens of helminths p83. In the Antigens, (ED. SELA, M.). Academic Press, New York.

PIESSENS, W.F., MC GREEVY, P.B., RATIWAYANTO, S., MC GREEVY, M., PIESSENS, P.W., KOIMAN, I., SAROSOS, J.S. AND DENNIS, D.T. (1980). Immune responses in human infections with *B. malayi*; correlation of cellular and humoral reactions to microfilarial antigens with clinical status. Am. J. Trop. Med. **29**, p563.

PHILLS, J.A., HARROLD, A.J., WHITEMAN, G.V. AND PERELMUTTER, L. (1972). Pulmonary infiltrates, asthma and eosinophilia due to *A. suum* infestation in man. New England J. Med. **286**, p965.

POND, L., WASSOM, D.L. AND HAYES, C.E. (1988). Disparate humoral responses to *T. spiralis* infection in resistant and susceptible mice. FASEB J. **2**, A677.

PRITCHARD, D.I., ALI, N.M.H. AND BEHNKE, J.M. (1984). Analysis of the mechanism of immunodepression following heterologous antigenic stimulation during concurrent infection with *N. dubius*. Immunology **51**, p633.

PROUVOST-DANON, A. (1972). Characterisation of mouse IgE and mast cell reactions, p271.

RANSOM, B.H. AND FOSTER, W.D. (1919). Recent discoveries concerning the life history of *A. lumbricoides*. J. Parasitology 5, p93.

RAY, D. AND SAHA, K. (1978). Serum immunoglobulin and complement levels in tropical pulmonary eosinophilia and their correlation with primary and relapsing stages of the illness. Am. J. Trop. Med. Hyg. **27**, p503.

REIS, K.J., HANSEN, H.F. AND BJORCK, L. (1984). Amersham research news 1. J. Immunology **133**, p961.

RICHARDS, A.J., BRYANT, C., KELLY, J.D., WINDON, R.G. AND DINEEN, J.K. (1977). The metabolic lesion in N. brasiliensis induced by prostaglandin E, *in vitro*. Int. J. Para. **7**, p153.

RICKARD, M.D. AND ADOLPH, A.J. (1977). Vaccination of lambs against infection with *Taenia ovis* using antigens collected during short term *in vitro* incubation of activated *T. ovis* oncospheres. Parasitology **75**, p183.

RIVERA-ORITIZ, C.I. AND NUSSENZWEIG, R. (1976). *T. spiralis*; Anaphylactic antibody formation and susceptibility in strains of inbred mice. Exp. Parasitology **39**, p7.

ROBERTS, F.H.S. (1934). The large roundworm of pigs *A. lumbricoides* L.1758. Its life history in Queensland,

economic importance and control. Bull. Anim. Hlth. Yeerongpilly **1**, p81.

ROBERTSON, L.J., CROMPTON, D.W.T., WALTERS, D.E., NESHEIM, M.C., SANJUR, D. AND WALSH, E.A. (1989). Soil-transmitted helminth infections in school children from Cocle Province, Republic of Panama. Parasitology (in press).

ROELANTS, G.E. AND PINDER, M. (1987). The virulence of *T. congolyense* can be determined by the antibody response of inbred strains of mice. Parasite Immunol. **9**, p379.

ROSENTHAL, A.S. AND SCHEVACH, E.M. (1973). Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirements for histocompatible macrophages and lymphocytes. J. Exp. Med. **138**, p1194.

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. Int. J. Parasite **4**. p293.

ROTHWELL, T.L.W., PRICHARD, R.K. AND LOVE, R.J. (1974). Studies on the role of histamine and 5-hydroxytryptamine in immunity against the nematode *Trichostrongylus colubriformis*. Int. Archs. Allergy appl. Immunol. **46**, p1.

SALAM, E.A., ISHAAC, S. AND MAHMOUD, A.F. (1979). Histocompatibility-linked susceptibility for hepato-splenomegaly in human schostosomiasis mansoni. J. Immunol. **123**, p1829.

SASAZUKI, T., OHTA, N., KANEOKA, R. AND KOJIMA, S. (1980). Association between an HLA haplotype and low responsiveness to schistosomal worm antigen in man. J. Exp. Med. **152**, p314.

SASAZUKI, T., KANEOKA, H., NISHIMURA, Y., KANEOKA, R., HAYAMA, M. AND OHKURI, H. (1985). An HLA-linked immune suppression gene in man. J. Exp. Med. **152**, p297.

SCHAD, G.A. AND ANDERSON, R.M. (1985). Predisposition to hookworm infection in humans. Science **228**, p1537.

SCHWARTZ, M. (1952). Hereditary in bronchial asthma. (Muksgaard, Copenhagen).

SCHWARTZ, R.H. (1978). A clonal deletion model for Ir gene control of the immune response. Scand. J. Immunol. **7**, p3.

SCHWARTZ, R.H. (1986). Immune response (Ir) genes of the murine MHC. Advances in Immunol. **38**, p31.

SCHWARTZ, R.H. (1985). T lymphocyte recognition of antigen in association with gene products of the MHC. In Annual

Review of Immunology p237. (ED. PAUL, W.E., FATHMAN, C.G. AND METZGER, H.)

SCOTT, M.E. (1988). Predisposition of mice to *Heligmosomoides polygyrus* and *Aspiculuris tetraptera* (Nematoda). Parasitology **97**, p101.

SENFT, A.W. AND MADDISON, S.E. (1975). Hypersensitivity to parasitic proteolytic enzyme in schistosomiasis. Am. J. Trop. Med. Hyg. **24**. p83.

SENFT, A.W., WELTMAN, J.K., GOLDGRABER, M.B., EGYUD, L.G. AND KUNTZ, R.E. (1979). Species specificity of the immediate hypersensitivity to schistosomal proteolytic enzyme. Parasite Immunol. **1**, p79.

SEO, B. AND CHAI, J. (1979). Frequency distribution of *A. lumbricoides* in rural Koreans with special reference on the effect of changing endemicity. Korean J. Parasitology **17**, p105.

SHER, A., HEINY, S. AND JAMES, S. (1984). Mechanisms of protective immunity against *S. mansoni* infection in mice vaccinated with irradiated cercariae. VI. Influence of the MHC. Parasite Immunol. **6**, p319.

SHER, A. AND SCOTT, D.A. (1982). Clin. Immunol. Allergy **2**, p489.

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. J. Parasitology **48**, p562.

SINSKI, E. AND HOLMES, P.H. (1977). *N. brasilensis*: Systemic and local IgA and IgG immunoglobulin responses in parsitised rats. Exp. Para. **43**, p382.

SKAMENE, E., GROS, P., FORGET, A., PATEL, P.J. AND NESBITT, M.N. (1984). Regulation of resistance to leprosy by chromosome 1 locus in the mouse. Immunogenetics **19**, p117.

SLATER, A.F.G. AND KEYMER, A.E. (1986). *Heligmosomoides polygyrus* (Nematoda); the influence of dietary protein on the dynamics of repeated infection. Proc. Roy. Soc. Lond., Series B.

SMERALDI, R.S., FABIO, G., LAZZARIN, A., EISERA, N.B., MORONI, M. AND ZANUSSI, C. (1986). HLA-associated susceptibility to acquired immunodeficiency syndrome in italian patients with human-immunodeficiency-virus infection. Lancet p1187.

SOMORIN, A.O., HEINER, D.E. AND AJUGWO, R.E. (1977). IgE in Nigerian onchocerciasis. Am. J. Trop. Med. Hyg. **26**, p872.

SPILLMAN, R.K. (1975). Pulmonary ascariasis in tropical communities. Am. J. Trop. Med. Hyg. **24**, p791.

SPRENT, J.F.A. (1952). On the migratory behaviour of the larvae of various ascarid species in white mice. I. Distribution of larvae in tissues. J. Infect. Dis. **90**, p165.

SPRENT, J.F.A. AND CHEN, H.H. (1949). Immunological studies in mice infected with larvae of A. lumbricoides. I. Criteria of immunity and immunising effect of isolated worm tissues. J. Infect. Dis. **84**, p111.

STANWORTH, D.R. (1971). The experimental inhibition of reagin mediated reactions. Clin. Allergy **1**, p25.

STEPHENSON, L.S. (1987). The impact of helminth infections on human nutrition. (Taylor and Francis, London).

STEPHENSON, L.S., POND, W.G., NESHEIM, M.C., KROOK, L.P. AND CROMPTON, D.W.T. (1980). *A. suum*; nutrient absorption, growth and intestinal pathology in young pigs experimentally infected with 15-day-old larvae. Exp. Parasitology **49**, p15.

STEWART, D.F. (1953). Studies on resistance of sheep to infestation with *Haemonchus contortus* and *Trichostrongylus* species and in the immunological reactions of sheep

exposed to infection. V. The nature of the 'self cure' phenomenon. Aust. J. Agric. Res. **4**, p100.

STEWART, D.F. (1955). Self cure in nematode infestations of sheep. Nature **176**, p1273.

STOREY, N., WAKELIN, D. AND BEHNKE, J.M. (1985). The genetic control of host responses to *D. viteae* (Filariodea) infections in mice. Parasite Immunol. **7**, p349.

STOREY, N., BEHNKE, J.M. AND WAKELIN, D. (1987). Immunity to *D. viteae* (Filariodea) infections in resistant and susceptible mice. Acta Tropica **44**, p43.

STRASSMAN, G., ESHAR, Z. AND MOZES, E. (1980). Genetic regulation of delayed-type hypersensitivity responses to poly(LTyr, LGlu)-poly(DL Ala)-poly(L Lys). II. Evidence for a T-T cell collaboration in delayed type hypersensitivity responses and for a T-cell defect at the efferent phase in non-responder H-2^{k} mice. J. Exp. Med. **151**, p628.

STROMBERG, B.E. (1979a). The isolation and partial characterisation of a protective antigen from developing larvae of *A. suum*. Int. J. Para. **9**, p307.

STROMBERG, B.E. (1979b). IgE and IgG antibody production by a soluble product of *A. suum* in the guinea pig. Immunology **38**, p489.

STROMBERG, B.E., KHOURY, P.B. AND SOULSBY, E.J.L. (1977). Development of larvae of *A. suum* from the third to the fourth stage in a chemically defined medium. Int. J. Para. **7**, p149.

SUEMARA, M. AND ISHIZAKA, K. (1979). Potentiation of IgE response *in vitro* by T cells from rats infected with *N. brasiliensis*. J. Immunol. **123**, p918.

SUGANE, K. AND OSHIMA, T. (1984). Interrelationship of eosinophilia and IgE antibody production to larval ES antigen in *Toxocara canis* infected mice. Parasite Immunol. **6**, p409.

SUSKIND, (1977). Malnutrition and the immune responses. Raven Press, New York.

TAFFS, (1964). Immunological studies on experimental infection of pigs with *A. suum* Goeze 1782. III. The antibody response and acquired immunity. J. Helminthology **38**, p129.

TAREN, D.L., NESHEIM, M.C., CROMPTON, D.W.T., HOLLAND, C., BARBEAU, I., RIVERA, G., SANJUR, D., TIFFANY, J. AND TUCKER, K. (1987). Contributions of ascariasis to poor

nutritional status in children from Chiriqui Province, Republic of Panama. Parasitology **95**, p603.

THOMPSON, A. (1972). Isolation and characterisation of a mast cell degranulating substance from *A. suum*. Biochem. Biophys. Acta **261**, p245.

THOMPSON, J.P., CRANDALL, R.B., CRANDALL, C.A. AND NEILSON, J.T. (1979). Clearance of microfilariae of *D. viteae* in CBA/n and CBA/H mice. J. Parasitology **65**, p966.

TIWARI, J.L. AND TERASAKI, P.I. (1985). HLA and disease associations. In Gastroenterology p232.

TOLONE, G., BONASERA, M., BRAI, M., FERINA, F. AND PONTIERI, G.M. (1974). Isolation of mast cell degranulators from the coelomatic fluid of *Parascaris equorum*. Pathol. Microbiol. **41**, p41.

TSUJI, M., HAYASHI, T., YAMAMOTO, S. AND TOSHIBA, T. (1977). IgE type antibodies to *Ascaris* antigens in man. Int. Archs. Allergy appl. Immunol. **55**, p78.

TURNER, K.J., QUINN, E.H. AND ANDERSON, H.R. (1978). Regulation of asthma by intestinal parasites. Investigation of possible mechanisms. Immunology **35**, p281.

TURNER, K.J., FEDDEMA, L. AND QUINN, E.H. (1979). Non-specific potentiation of IgE by parasitic infections in man. Int. Archs. Allergy appl. Immunol. **58**, p232.

TURNER, K.J., FISHER, E.H. AND MC WILLIAM, A.S. (1980). Homology between roundworm (*Ascaris*) and hookworm (*N. Americanus*) antigens detected by human IgE antibodies. AJEBAK **58**, p249.

TURNER, K.J., FISHER, E.H. AND HOLT, P.G. (1982). Host age determines the effects of helminthic parasite infestation upon expression of allergic reactivity in rats. Aust. J. Exp. Biol. Med. Sci. **60**, p147.

URBAN, J.F. AND DOUVES, F.W. (1984). Culture requirements of *A. suum* larvae using a stationary multi-cell system: increased survival, development and growth with cholesterol. Vet. Para. **14**, p33.

URQUHART, G.M., MULLIGAN, W., EADIE, R.M. AND JENNINGS, F.W. (1965). Immunological studies on *N. brasiliensis* infection in the rat: the role of local anaphylaxis. Exp. Para. **17**, p210.

UVNAS, B., DIAMANT, B., HOGBERG, B. AND THAN, I.L. (1960). Mechanisms of mast cell disruption induced by a principle extracted from *A. suis*. Am. J. Physiol. **199**, p575.

UVNAS, B. AND WOLD, J.K. (1967). Isolation of a mast cell-degranulating polypeptide from *A. suum*. Acta Physiol. Scand. **70**, p269.

VADAS, M.A. (1980). Parasite immunity and the MHC. Immunogenetics **11**, p215.

VAN EDEN, W., DE VRIES, R.R.P., MEHRA, N.H., VAIDYA, M.C. D'AMARO, J. AND VAN ROOD, J.J. (1980). HLA segregation of tuberculoid leprosy: confirmation of the DR2 marker. J. Inf. Dis. **141**, p693.

VAN EDEN, W., GONZALES, N.M., DE VRIES, R.R.P., CONVIT, J. AND VAN ROOD, J.J. (1985). HLA-linked control of predisposition to lepramotous leprosy. J. Inf. Dis. **151**, p9.

VANNIER, W.E., FIREMAN, B.I., CHESTNUT, R.Y. AND MURRELL, K.D. (1974). *S. mansoni*: immediate hypersensitivity to adult antigens in the rat. Exp. Parasitology **36**, p45.

VAZ, N.M., PHILLIPS-QUAGLIATA, J.M., LEVINE, B.B. AND VAZ, E.M, (1971). H-2 linked genetic control of immune responsiveness to ovalbumin and ovomucoid. J. Exp. Med. **134**, p1335.

VAZ, N.M., DE SOUZA, C.M. AND MARX, L.C.S. (1974). Genetic control of immune responsiveness in mice

responsiveness to ovalbumin in (C57BL x DBA/2)F_1 mice. Int. Archs. Allergy appl. Immunol. **46**, p275.

VIDOVIC, D. AND MATZINGER, P. (1988). Unresponsiveness to a foreign antigen can be caused by self-tolerance. Nature **336**, p222.

WAKELIN, D. (1978). Immunity to intestinal parasites. Nature **273**, p617.

WAKELIN, D. (1985). Genetic control of immunity to helminth infections. Parasitology Today **1**, p17.

WAKELIN, D. (1988). Helminth infections p153. In Genetics of Resistance to Bacterial and Parasitic Infections. (ED. WAKELIN, D. AND BLACKWELL, J.M.). Taylor and Francis.

WAKELIN, D. AND DONNACHIE, A.M. (1980). Genetic control of immunity to parasites: adoptive transfer of immunity between inbred strains of mice characterised by rapid and slow immune expulsion of *T. spiralis*. Para. Immunol. **2**, p249.

WAKELIN, D. AND DONNACHIE, A.M. (1981). Genetic control of immunity to *T. spiralis*. Donor bone marrow cells determine responses to infection in mouse radiation chimaeras. Immunology **43**, p787.

WAKELIN, D. AND DONACHIE, A.M. (1983). Genetic control of immunity to *T. spiralis*: influence of H-2 linked genes on immunity to the intestinal phase of infection. Immunology **48**, p343.

WASSOM, D.L., BROOKS, B.O., BABISCH, J.G. AND DAVID, C.S. (1983). A gene mapping between the S and D regions of the H-2 complex influences resistance to *T. spiralis* infections in mice. J. Immunogenetics. **10**, p371.

WASSOM, D.L., DAVID, C.S. AND GLEICH, G.J., (1979). Genes with in the MHC influence susceptibility to *T. spiralis* in the mouse. Immunogenetics **9**, p491.

WASSOM, D.L., DE WITT, C.W. AND GRUNDMANN, A.W. (1974). Immunity to *Hymenolepis citelli* by *Peromyscus maniculatus* genetic control and ecological implications. J. Parasitology **60**, p47.

WASSOM, D.L., DOUGHERTY, D.A., KRCO, C.J. AND DAVID, C.S. (1984). H-2 controlled, dose-dependent suppression of the response that expels adult *T. spiralis* from the small intestine of mice. Immunology **53**, p811.

WASSOM, D.L., KRCO, C.J. AND DAVID, C.S. (1987). I-E expression and susceptibility to parasite infection. Immunology Today **8**, p39.

WASSOM, D.L., WAKELIN, D., BROOKS, B.O., KRCO, C.J. AND DAVID, C.S. (1984). Genetic control of immunity to *T. spiralis* infections of mice. Hypothesis to explain the role of H-2 genes in primary and challenge infections. Immunology **51**, p625.

WATANABE, N., KOJIMA, S. AND OVARY, Z. (1976). IgE antibody production in SJL mice I. Nonspecific suppressor T cells. J. Exp. Med. **143**. p833.

WELCH, J.S., DOBSON, C. AND CHOPRA, S. (1986). Immune diagnosis of *Entamoeba histolytica* and *A. lumbricoides* infections in caucasian and aboriginal Australians. Trans. Roy. Soc. Trop. Med. Hyg. **80**, p240.

WILLIAMSON, A.R. AND TURNER, M.W. (1987). Essential Immunogenetics. Blackwell Scientific Publications.

WILSON, R.J.M. (1967). Homocytotropic antibody response to the nematode *N. brasiliensis* in the rat. Studies on the worm antigen. J. Parasitology **53**, p752.

WORLD HEALTH ORGANISATION (1981). Intestinal protozoan and helminthic infections. WHO Technical Report Series **666**, WHO, Geneva.

YODOI, J., HIRASHIMA, M. AND ISHIZAKA, K. (1981). Lymphocytes bearing Fc receptors for IgE. V. Effect of tunicamycin on the formation of IgE-potentiating factor

and IgE suppressive factor by Con A-activated lymphocytes. J. Immunol. **126**, p877.