DYNAMICS OF CELLULAR AND HUMORAL RESPONSES TO SCHISTOSOMA MANSONI IN MICE EXPOSED TO REPEATED INFECTIONS

by

RICHARD ALAN WEBBER B.Sc., M.Sc.

Thesis submitted for the degree of Doctor of Philosophy
and Diploma of Imperial College in the Faculty of Science
of the University of London.

July, 1990.

Department of Biology,
Imperial College of Science, Technology and Medicine,
Prince Consort Road,
London. SW7 2BB.
England.

Abstract

This investigation examines epidemiological and immunological parameters associated with the acquisition and persistence of resistance to Schistosoma mansoni infection in the laboratory mouse. These parameters were measured following the exposure of the host to repeated low level doses of S. mansoni cercariae. It was considered that this pattern of exposure might approximate the conditions experienced by humans living in endemic communities.

Serial sampling techniques were used to monitor temporal changes in mean worm burden and immune responsiveness. Patterns of infection and cellular and humoral reactivity were dependent upon the intensity of cercarial exposure. Thus, mice exposed to the two highest densities of cercariae gradually developed an acquired resistance to infection with increasing duration of exposure. In contrast, mice exposed to a low dose of cercariae failed to express significant levels of resistance to infection. Splenocytes derived from the more heavily exposed animals responded to stimulation with egg and larval antigenic preparations as determined by the in vitro lymphocyte proliferation assay. Conversely, lymphocytes from mice receiving the lowest dose of infection did not consistently respond to antigenic stimulation. Mice from all experimental groups developed humoral responses to S. mansoni antigens as determined by ELISA. Antibody titres varied directly with the intensity of cercarial exposure.

Chemotherapeutic treatment of existing S. mansoni infection led to the eventual abrogation of resistance to challenge exposure. Mice receiving a primary trickle exposure of 10 weeks duration expressed higher levels of residual resistance than mice which experienced an 18 week trickle infection. Patterns of cellular and humoral reactivity post-treatment, did not appear to correlate with the persistence of resistance.

The patterns of infection and immune responsiveness observed in the present study are considered in relation to those derived from human studies. The limitations and future applications of this experimental model are also discussed.

Contents

List of Figures

Chapter 1

Introduction

1.1 Biological Background

Schistosomiasis affects an estimated 250 million people in 75 countries worldwide, while 600 million people inhabit regions where the disease is endemic (DBL Report, 1987). The disease is caused by helminths belonging to the genus Schistosoma. Of the species known to parasitize humans, S. mansoni, S. haematobium and S. japonicum are of the greatest importance while S. mekongi and S. intercalatum are less widespread (Rollinson and Southgate, 1987). S. mansoni occurs in Arabia, Africa, South America and the Carribean; S. haematobium in Africa and the Middle East; and S. japonicum in Southeast Asia (Warren, 1982). S. mekongi is widespread in the lower Mekong basin while S. intercalatum is most prevalent in Northwest Africa (Hillyer, 1982).

Species of the S. mansoni, S. haematobium and S. japonicum groups commonly develop in the snails Biomphalaria, Bulinus and Oncomelania respectively (Rollinson and Southgate, 1987). Cercariae emerge from the snail intermediate host in response to light (Asch, 1972) with one peak emergence every 24 hours (Jourdane and Theron, 1987). Contact with a suitable mammalian host leads to the penetration of the host's skin and loss of the tail. The head of the penetrated cercaria is called the schistosomulum (Cioli, 1983). A series of structural, physiological and biochemical changes are initiated to facilitate the parasite's survival within it's new environment. Migration through the epidermis and dermis is followed by penetration into

blood vessels and the general circulation where the larvae are carried to the lungs (Miller and Wilson, 1978; 1980). They then migrate from the lungs to the hepatic portal system by a route which has yet to be elucidated (Georgi et al., 1986). The parasites reach maturity in the liver about 4-7 weeks post-infection depending on the species. Male and female worms couple and then migrate against the blood flow to the mesenteric venules of the lower intestine (S. mansoni and S. japonicum) or bladder (S. haematobium) where they produce large numbers of eggs. Continuation of the life cycle involves the passage of ova to the external environment. However, many become trapped in tissues or are swept back to the liver via the bloodstream (Cioli, 1983). Deposition of eggs in the liver elicits a host response which can lead to the development of pathological symptoms characteristic of schistosome infection. Those eggs passed via the faeces or urine which enter water, hatch and produce ciliated free-living miracidia which actively penetrate the snail host. Asexual reproduction culminating in the production of large numbers of infective cercariae facilitates transmission to the mammalian host and completion of the life cycle.

Epidemiological studies of schistosomiasis in human communities are typically based on quantitative measures of prevalence and intensity of infection (Anderson, 1987). The prevalence of infection refers to the proportion or percentage of individuals in a defined community, harbouring the parasite, while infection intensity is a direct or indirect measure of the mean worm burden in the total population. As it is impossible to conduct worm counts in field studies, both parameters must be determined using faecal or urine egg counts. There exists however, some controversy as to the precise relationship between adult worm burden and egg output. Post mortem examinations of S. mansoni (Cheever, 1968) and S. haematobium (Cheever et al., 1977) infected patients, revealed that faecal and urine egg

outputs respectively, could be considered to be proportional to the number of adult worm pairs recovered by perfusion. Conversely, a number of experimental (Anderson, 1978; Keymer, 1982) and field studies (Croll et al., 1982; Anderson and Schad, 1985) have indicated an inverse relationship between helminth fecundity and the density of worms within the host. Recent analyses of the data presented by Cheever and his colleagues have yet to clarify the problem and the controversy still exists (Medley and Anderson, 1985; Wertheimer et al., 1987). A further limitation of the egg counting technique is it's lack of sensitivity, particularly in areas of low endemicity (Warren et al., 1973a). This can lead to the incorrect labelling of true positives as negatives (Teesdale et al., 1985; Kamel et al., 1977) although repeated examinations can reliably identify infected individuals (McCullough and Bradley, 1973; Wilkins and Scott, 1978; Barreto et al., 1978). Despite these problems, the egg counting technique remains the most practical and dependable epidemiological tool for monitoring infection intensities.

An alternative method for the diagnosis of schistosomiasis is the use of immunodiagnostic techniques which are sensitive to either antibody or cell-mediated reactions. Epidemiologically, however, their value is limited due to a lack of specificity, sensitivity and inability to provide quantitative information concerning infection intensities (Smithers and Doenhoff, 1982; Hillyer, 1982; Wilkins, 1987). Warren et al., (1973a,b) observed a large number of false negatives among St. Lucian patients using immediate and delayed intradermal tests to measure antibody and cell-mediated reactions respectively. This was particularly evident in younger subjects, suggesting that the development of hypersensitivity might be dependent upon the intensity or duration of infection (McKay et al., 1973). Recent investigations have employed more sophisticated techniques for the immunodiagnosis of

schistosomiasis, including the Enzyme Linked Immunosorbant Assay (ELISA) for serological analyses and the in vitro Lymphocyte Proliferation Assay to measure cell-mediated responses. The sensitivity of serological testing appears to decline as subject age increases and infection intensity decreases (Hillyer et al., 1979; Nash et al., 1978), while T-cell responses, particularly to Soluble Egg Antigen (SEA), decrease dramatically as the infection progresses from the acute to chronic phase (Colley et al., 1977; Ottesen et al., 1978). Together, these results suggest that a certain period and/or intensity of exposure to schistosome antigens is required before infection can be diagnosed by immunological techniques and that these responses peak during the acute phase of infection. Thus, the apparent inability of younger subjects to respond immunologically in the St. Lucian study, may have reflected the low level doses of infection encountered by the inhabitants (Warren et al., 1973b).

Attempts to correlate infection intensity with immune responsiveness have met with some success, at least qualitatively. Feldmeier et al., (1985a) have observed a positive relationship between egg output levels and IgG and IgE responses. Hillyer et al., (1979) and Roberts et al., (1987) have also observed increasing humoral responses with infection intensity. In contrast it appears that cellular responses vary inversely with infection intensity, with heavily infected individuals exhibiting depressed reactivity towards worm antigens (Ellner et al., 1981; Feldmeier et al., 1985b). This observation is supported by studies showing that individuals harbouring heavy worm burdens have lower than normal T helper/T suppressor cell ratios (Colley, 1983; Feldmeier et al., 1985a). Thus, a paradoxical relationship between humoral and cellular responses would appear to exist in individuals with heavy infections.

At present, the ELISA and lymphocyte proliferation assays lack a high degree of specificity. A number of reports have demonstrated cross reactivity between antigens of different schistosome species using the ELISA (Hillyer et al., 1979; Barral-Netto et al., 1983; Dunne et al., 1984) or cellular proliferation techniques (Barral-Netto et al., 1983).

It is apparent that intensity or prevalence of infection data derived from either egg counting or immunodiagnostic techniques must be interpreted cautiously and that there is a definite need for more sensitive methods of assessing levels of infection in human communities. In the case of immunodiagnosis, this may include better purification and immunochemical characterization of schistosome antigens (Hillyer, 1982).

In endemic communities prevalence and intensity of schistosome infection rise rapidly in childhood, peak during the teenage years and then decline in older age classes (Anderson, 1987; Mott, 1987). The parasites tend to be aggregated in their distributions, with most individuals harbouring few worms and a small number of subjects having heavy worm burdens (Warren, 1982).

Two factors are most frequently suggested to explain the age-related profiles of parasite acquisition and loss. These are changes in patterns of water contact with increasing age (Warren, 1973; 1982) and the gradual development of acquired immunity to infection (Butterworth et al., 1985; Wilkins et al., 1984a,b; 1987). Data in support of the former argument has been collected by Dalton and Pole (1978) from a Ghanian community endemic for S. haematobium. Furthermore, Smith et al., (1979) have observed that in fishing communities on Lake Victoria, where older individuals are continually exposed to infective stages, the intensity of S. mansoni infection does not decline but remains stable or increases throughout adult life. Wilkins (1987), however, argues that this situation

may involve the exposure of individuals to high densities of cercariae and that immune responses to the parasite help to maintain the parasite population at a stable and tolerable level. There is a growing body of evidence to support the view that water contact patterns are insufficient in explaining age related changes in infection intensity.

Kloetzel and da Silva (1967) investigated a population of immigrants to an endemic community in Brazil who were first exposed to S. mansoni in adulthood. These individuals exhibited a similar rise and decline in prevalence and intensity of infection to those who had lived in the area since childhood. This suggested that resistance to reinfection depends on the duration of exposure to infection and not directly on age. As exposure to infection is clearly dependent on age (Jordan, 1985), it is unlikely that the observed age-related patterns in egg output resulted from changes in exposure. In a St. Lucian study, water contact patterns were carefully monitored, and although significant changes in water contact occurred with age, acquired immunity to infection did become apparent in a cohort of women in the 20-29 year old age group (Jordan, 1985).

A more definite role for acquired immunity in reducing infection intensities was proposed by Bradley and McCullough (1973). In a Tanzanian community endemic for S. haematobium, the authors suggested that the development of concomitant immunity rendered older individuals completely immune to infection and that the loss of immunity would only occur with the death of existing worm burdens.

More recent studies on S. haematobium in the Gambia (Wilkins et al., 1984a,b; 1987) and S. mansoni in Kenya (Butterworth et al., 1984; 1985) have provided a somewhat clearer picture of the dynamics of parasite acquisition and loss, and the factors associated with the development of acquired immunity.

Wilkins and his colleagues (1984a,b) compared the rates of parasite acquisition and loss between two communities where S. haematobium transmission was either interrupted with molluscicide or allowed to continue unabated. In the treated area, egg output fell dramatically in all age classes while in the untreated area, infection intensity rose rapidly in younger individuals and tended to remain stable or fall slowly in older individuals. This suggested that worm burdens in the younger subjects were in a dynamic state controlled by rates of acquisition and loss. There was also evidence of continued parasite acquisition by older age subjects. While older individuals were observed at transmission sites less frequently than younger subjects, it was not by an amount which could obviously account for the differences in parasite acquisition.

More recently, Wilkins et al., (1987) observed that among individuals with similar intensities of exposure, intensities of reinfection after chemotherapeutic treatment appeared to be related to age. A similar relationship between duration of exposure and resistance to reinfection was observed by Butterworth et al., (1985), who observed that older school children were more resistant to reinfection following treatment of S. mansoni infection, than younger subjects.

Overall these results suggest that the gradual development of protective immunity combined with reduced exposure to transmission sites, reduces the rate of parasite acquisition to a level at which parasite mortality exceeds immigration of new worms. Thus, as individuals age, the intensity of infection falls from peak levels until a new equilibrium is reached, where the rate of acquisition and loss of infection are again similar (Wilkins, 1987). This, combined with the observation that resistance to infection can occur in the absence of an active infection (Wilkins et al., 1984b; Dessein et al., 1988) supports an epidemiological role for acquired immunity, but not as

suggested by Bradley and McCullough (1973). Continued exposure to cercariae may however be necessary for the maintenance of a protective immune response (Wilkins et al., 1984b; Dessein et al., 1988).

The mechanisms responsible for the development of acquired resistance remains to be elucidated. Recent examination of samples collected from the Gambia (Hagan et al., 1985) and Kenya (Sturrock et al., 1983) have shown a correlation between resistance to reinfection after treatment and peripheral blood eosinophil counts. However, a direct causal relationship between antibody-dependent killing of schistosomula by eosinophils and protective immunity has yet to be demonstrated (Hagan et al., 1987). Indeed, no relationship between antibody titre and resistance to reinfection could be established from serum samples collected from the Kenyan school children (Roberts et al., 1987). A recent study on S. mansoni in a Brazilian community has revealed a relationship between the proportion of sera reacting with a 37-kDa schistosomula antigen and the expression of resistance post-treatment (Dessein et al., 1988). This suggests that there may be a relationship between resistance and protective IgG antibody responses against the schistosomule.

A role for cell-mediated delayed hypersensitivity in immunity to human schistosomiasis has been suggested by the results of a recent Egyptian study (Colley et al., 1986). Patients who became reinfected with S. mansoni after chemotherapeutic cure had significantly lower peripheral blood mononuclear cell (PBMN) proliferative responses to parasite antigens than individuals who failed to become reinfected. However, the authors were careful not to stress a cause and effect relationship.

1.2 The Schistosoma mansoni/Mouse Model

Since the early 1900s scientists have relied on animal models to facilitate their understanding of host responses to schistosome infection. The lack of specificity shown by schistosomes for their definitive hosts has allowed the researcher to examine a variety of host-parasite relationships, each having its own advantages or disadvantages.

Most studies have been carried out using S. mansoni because it is easy to maintain under laboratory conditions. (Smithers and Doenoff, 1982). Economics and availability have favoured the use of rodent models although a large number of primate studies have been carried out. The most frequently used rodent models have been the mouse and the rat. Like man, the mouse host is permissive to S. mansoni infection and allows the parasite to mature and produce eggs over an extended period of time (Smithers and Miller, 1980). Similarly, hamsters (Smith and Clegg, 1976) and guinea pigs (Pearce and McLaren, 1983) can also be classified as permissive hosts. Of the primates, baboons (Damian et al., 1974; Sturrock et al., 1984), rhesus monkeys (Foster and Broomfield, 1971), grivet monkeys (Cheever and Duvall, 1974) and chimpanzees (Sadun et al., 1970) will also permit the maturation of S. mansoni. Conversely, it has been shown that rats are non-permissive hosts and that infections in this host are eliminated by the fourth week of infection (Phillips et al., 1977).

A further advantage of the mouse host is its ability to withstand mild infections for long periods of time (Warren, 1963) while exhibiting similar symptoms of egg-induced pathology to those observed in humans (Warren, 1966). The mouse also provides a suitable model for studying acquired resistance to infection, as like humans, it develops partial immunity to

reinfection (Dean, 1983). The system would therefore appear to be very suitable for studying immunological and epidemiological aspects of chronic S. mansoni infection.

An important point to consider prior to discussing the relationship between immune mechanisms and resistance to schistosome infection in the murine model, relates to an observation made by Smithers and Terry (1967). These authors observed that rhesus monkeys harbouring an active infection were able to resist a challenge infection without harming the adult S. mansoni worms derived from the primary exposure. This resistance to reinfection was termed "concomitant immunity" (Smithers and Terry, 1969) and was assumed to result from specific immune mechanisms. However, the pathology resulting from egg deposition in the liver and subsequent host anti-egg responses contribute non-specifically to resistance to reinfection (Wilson et al., 1983). Thus, migrating worms are prevented from reaching their normal site of sequestration in the liver and accumulate in areas unsuitable for further development (McHugh et al., 1987).

There are however compelling lines of evidence which demonstrate that a part of the resistance exhibited by infected mice is immunologically based. P/J mice, defective in macrophage function, are incapable of developing significant levels of concomitant immunity although their anti-egg responses remain normal (James et al., 1983a; James and Cheever, 1985). Furthermore, McLaren et al., (1987) have shown that a monoclonal antibody, which destroys mouse neutrophils and abrogates the anamnestic dermal inflammatory response to challenge schistosomula, suppresses concomitant immunity.

It is therefore likely, that both specific and non-specific mechanisms act synergistically to determine observed levels of resistance (Colley and Freeman, 1980). Indeed, Pons et al., (1989) have demonstrated that both

immune and non-specific mechanisms can independently confer partial resistance to reinfection in the chronically infected mouse. Therefore, this system may still provide useful information concerning the role of immune mechanisms in resistance to S. mansoni infection in humans.

1.3 The Lymphocyte Proliferation Assay

For the present study, the role of T-cells in immunity to S. mansoni infection was determined using an in vitro lymphocyte proliferation assay. In response to a stimulus lymphocytes proliferate and may mature to effector function as judged by interleukin production (Severinson and Larson, 1986). This suggests that lymphocyte function can be assessed by evaluating lymphocyte blast transformation. However, conditions which are optimal for lymphocyte proliferation may not be optimal for lymphokine production and stimulation of effector mechanisms (Gajewski et al., 1989). The use of in vitro assays as a correlate to in vivo mechanisms of cell-mediated immunity has also been questioned. Phillips et al., (1977) argue that as immunity results from a complex series of interactions between cellular and humoral mechanisms, no single in vitro determination has been shown to accurately reflect the in vivo situation. Indeed, responses detected in vitro may be directed against non-functional parasite antigens and have no involvement in protective immunity (Lloyd and Soulsby, 1988). Furthermore, the effects of regulatory mechanisms may result in responses in vitro not fully expressed in vivo (Colley, 1981).

Despite these doubts, Colley (1981) has proposed that even in the absence of a positive correlation between immune responses to parasite antigens and resistance to infection, such responses may still contribute to host protection. The lack of a correlation may only indicate that they are not

of themselves, sufficient for its expression. Thus, in the absence of suitable alternatives, in vitro techniques remain a viable method of evaluating cell-mediated immunity (Butterworth, 1984).

Cell-mediated immunity can be evaluated by other methods including skin tests for delayed hypersensitivity reactions. However, lymphocyte transformation assays provide a simple, reproducible and quantitative measure for comparison with other methods (Oppenheim and Schecter, 1980). It also provides a means of monitoring immunological defects, assessing immunosuppressive or immunoenhancing treatments and diagnostic testing for detection of previous exposure to the relevant antigen (Maluish and Strong, 1986).

Lymphocyte activation may be classified as either non-specific or specific. Non-specific mitogens stimulate lymphocytes from individuals independent of past exposure, while specific antigens stimulate lymphocytes from sensitized donors with previous exposure (Oppenheim and Schecter, 1980). Specific antigenic challenge appears to measure only T-cell function while non-specific activation can measure T-cell and/or B-cell function, depending on the nature of the mitogen (Rocklin, 1986). The mechanisms of lymphocyte transformation are considered to be the same regardless of the stimulant or source of lymphocytes used (Waithe and Hirschorn, 1978). Therefore, studies on mitogen-activated proliferation can be regarded as comparable to studies on antigen-specific proliferation.

In this assay, dividing cells incorporate radioactive isotope into their DNA. The amount of isotope taken up is measured and this provides an estimate of the degree of transformation (Olivotto et al., 1982; Oppenheim and Schecter, 1980). Levels of proliferation are dependent upon a number of factors including cell density, length of culture, and source of culture medium (Knight, 1987). Variations in tissue culture techniques as well as

uncontrollable environmental influences on a donor's lymphocyte reactivity can also modify proliferative values. It is therefore essential to incorporate age and sex matched controls into the experimental protocol and to demonstrate the reproducibility of the technique (Oppenheim and Schecter, 1980).

Unfortunately, there is no agreed standard method for the representation of proliferation data and the variability inherent in this particular assay. This frequently imposes problems when comparing results between laboratories, as the chosen method of data presentation can greatly influence interpretation of the results.

The three most common methods of expressing lymphocyte proliferation data are as follows:

1) Raw counts per minute (CPM). This method is particularly difficult to analyze during longitudinal studies. This results from occasional spontaneous proliferation of unstimulated cultures (background counts) which may be accompanied by parallel increases in experimental incorporation (Colley et al., 1977). Enhanced proliferation of cultures without antigen may be caused by certain batches of foetal calf serum (Corradin et al., 1977) or carryover of antigenic material from cells of the donor organ (James et al., 1983b). If this occurs, stimulated CPM values may exceed the actual lymphocyte blastogenic reactivity of the host.

2) Stimulation Index (SI). The SI is calculated as CPM of stimulated culture/unstimulated CPM. While this may help to normalize proliferation values, it is based on the incorrect assumption that the degree of reactivity of background and experimental cultures will be affected by variables in a proportional manner (Oppenheim and Schecter, 1980). In reality, factors may stimulate baseline values while experimental responses remain stable. This will lower the SI to a value which fails to reflect the proliferative response

of experimental cultures.

3) Delta CPM- Delta CPM values are calculated as CPM stimulated cultures - unstimulated cultures. This method appears to be an acceptable means of analyzing lymphocyte proliferation data when background values are elevated. In this way, the effects of spontaneous proliferation may be considered without dramatically altering the degree of host reactivity.

1.4 Experimental Studies

Many studies of host infection by schistosomiasis have focused on the factors involved in the acquisition of resistance to infection. Emphasis has been placed on determining immune effector mechanisms responsible for host protection, with a view towards the development of effective vaccines. At present, the conditions and effector mechanisms responsible for the development of resistance to reinfection remain unclear. This is partially due to the complex nature of the host response to infection and our incomplete understanding of the factors responsible for the development of host protection.

As there exists a plethora of information regarding the factors which might affect the acquisition of resistance to schistosome infection, this review will concern itself with the literature thought to be most relevant to the present study.

Smithers and Terry (1969) demonstrated that hosts infected with schistosomes could resist secondary or challenge infections without harming worms derived from the primary exposure (concomitant immunity). This suggested that protective immune responses are directed against challenge schistosomulae. Consequently, resistance to reinfection is frequently

determined by comparing the number of worms recovered from mice given both primary and challenge infections, with age-matched controls given only the primary or challenge infections alone. As the complete elimination of challenge worms is rarely achieved, resistance data is often expressed as per cent reduction in challenge establishment (Colley and Colley, 1989). Worms may be harvested from the host either before or after the maturation of challenge schistosomulae. Studies using the former technique have corroborated the hypothesis of concomitant immunity, in that the number of adult worms remains unchanged following a challenge infection (Doenhoff et al., 1978; Moloney et al., 1984).

Long et al., (1978) observed that resistance to S. mansoni increases to a maximum at about 6 weeks following a primary infection. Significant resistance to S. japonicum was also observed at 6 weeks post-infection, although peak resistance to challenge was not detected until 14 weeks after primary exposure (Moloney et al., 1984). A feature common to both studies is the dependency of the development of maximum resistance upon the primary infection becoming patent. Thus, a number of studies have suggested that the level of host protection varies proportionally with the number of adult worms established from the primary exposure (Colley and Freeman, 1980; Long et al., 1980; Moloney et al., 1984). However, unisexual S. mansoni (Dean et al., 1978a; Bickle et al., 1979a) and S. japonicum (Moloney et al., 1986) infections fail to confer significant levels of resistance. This suggests that it is the presence of eggs and not worms which is necessary for the acquisition of host protection. Indeed, resistance developed to patent primary infection has been correlated with the size of the tissue egg burden (Dean et al., 1978b; Long et al., 1980; Harrison et al., 1982; Moloney et al., 1984). Despite this, attempts to induce protection by injecting S. mansoni (Bickle et al., 1979a; Long et al., 1980; Doenhoff et al.,

1980) or S. japonicum (Moloney et al., 1987a) eggs into the lungs or liver of mice have been largely unsuccessful. One exception is that recorded by Dean et al., (1978b) who induced an average of 57% resistance by injecting S. mansoni eggs into the lungs of NIH mice 9 days before challenge. Recently, Moloney et al., (1987a) were able to show that sensitization of mice with soluble egg antigen (SEA) prior to injecting into the lungs or liver was necessary to induce high levels of resistance. This would appear to support the suggestion that the release of SEA and immunogens common with the schistosomula , may serve to boost the low levels of resistance induced by the earlier stages of the life cycle (Long et al., 1978).

Multiple immunizing infections do not appear to induce greater resistance than single infections of the same duration or intensity (Dean, 1983). This has been observed for S. mansoni in baboons (Sturrock et al., 1984) and mice (Long et al., 1980) and for S. japonicum in mice (Moloney et al., 1984).

Another method of inducing resistance to schistosome infection involves the vaccination of hosts with highly irradiated cercariae (Bickle et al., 1979b; Minard et al., 1978). Attenuation of cercariae with high doses of radiation inhibits larval migration from the skin to the lungs and liver (Miller and Smithers, 1980). In this way, protective immunity can be induced in the absence of patent infections and egg associated pathology. A period of 2 weeks between immunization and challenge appears to be necessary for significant levels of resistance to develop (Dean, 1983). Multiple exposures do not generally enhance resistance although Hsu et al., (1981) did observe enhanced levels of protection when the number of exposures were increased from 1 to 5. The same authors detected maximum levels of resistance of 91% at 30 days and 75% at 545 days after the final immunization. Generally, the levels of protection generated using irradiated

cercariae approach 75%, similar to that observed in the concomitant immunity model (Miller and Smithers, 1980). Resistance to reinfection with schistosomes can also be evaluated following chemotherapeutic cure of existing worm burdens. In this way, the importance of concomitant immunity for the maintenance of host resistance can be determined. This is particularly relevant in endemic areas where mass treatment of human populations could possibly render subjects completely susceptible to harmful doses of infection. Results obtained from studies using the murine model have been inconclusive, with significant levels of residual resistance observed in some instances (Warren et al., 1977; Doenhoff et al., 1980; Andrade and de Brito, 1982; Tawfik and Colley, 1986; Moloney et al., 1987b; Sithithaworn, 1987) and not others (Cheever et al., 1965; Gold and Lengy, 1975; Crombie and Anderson, 1985). On those occasions when significant levels of host protection have been demonstrated, the duration of immunological memory has varied. Thus, Andrade and de Brito (1982) observed significant levels of resistance up to 2 months post-treatment but not at 6 months, while in mice treated for S. japonicum, levels of partial protection declined to 10% by 4 weeks post-treatment (Moloney et al., 1987b). In any event, levels of resistance are generally observed to be higher and of longer duration in infected non-treated mice than treated animals (Doenhoff et al., 1980; Andrade and de Brito, 1982; Tawfik and Colley, 1986). This would appear to support the view that elimination of the primary worm burden, ultimately leads to abrogation of host resistance. While this may be true of the S. mansoni or S. japonicum mouse models, Bushara and his colleagues (1983b) have shown that cattle treated for S. bovis infection, exhibit similar levels of resistance to untreated controls at 7 weeks post-treatment.

Clearly an important factor which must be considered when evaluating resistance data is the genetic background of the host. Thus, the number of challenge schistosomula which mature to patency following a primary exposure has been shown to vary with host strain (Bickle et al., 1980; Dean et al., 1981; Colley and Freeman, 1983). Similarly, the ability of a primary infection to become established (Fanning and Kazura, 1984) and the acquisition of resistance following vaccination with irradiated cercariae (Murrell et al., 1979; Sher et al., 1984) have been shown to be influenced by the mouse strain used. Furthermore, pathological responses to S. mansoni infection will vary between mouse strains (Cheever et al., 1987). Variations in responses of different host strains may explain the contradictory data encountered when comparing results from different studies.

At this time it is difficult to select the host-parasite relationship which most resembles the human situation. Nevertheless, investigations into genetic factors responsible for the development of immunity and pathology in the murine model may help to determine the factors associated with susceptibility to infection and disease in genetically diverse human populations.

Smithers and Gammage (1980) demonstrated that resistance is directed against the migrating schistosomula as indicated by decreased larval recovery from primarily infected animals. The authors further suggested that parasite attrition occurred in the skin and lungs of the protected host with the latter site being of greater importance. Studies using the autoradiographic tracking technique have confirmed that in infected mice, approximately 35% of challenge worms are lost during the skin or pre-lung phase and between 3.5% and 32.9% in the lungs or between the lungs and liver (Delgado and McLaren, 1989). A liver phase of immune killing has been observed using this technique although its significance remains uncertain (Dean and

Mangold, 1984; Delgado and McLaren, 1989). These results indicate that migrating larvae are susceptible to immune killing at a number of sites along the migration route to the hepatic portal system. This would appear to contradict results from in vitro studies which have shown that soon after penetrating the skin, schistosomula are able to escape antibody-dependent damage (Sher and Moser, 1981). Evasion of the host's immune system is thought to result from either the masking of the target by acquisition of host molecules (McLaren et al., 1975; Sher et al., 1982b) or shedding of parasite surface antigens recognized by antibody (Samuelson et al., 1980; Pearce et al., 1986). If these in vitro studies reflect the in vivo situation, more than one immune mechanism of attrition must be operating in the murine chronic infection model. Similarly, a skin and lung phase of parasite attrition has been described in mice vaccinated with irradiated cercariae (Miller and Smithers, 1980). Much controversy exists as to the major site of larval elimination in this model of resistance (Wilson and Coulson, 1989; McLaren, 1989). Thus, some workers have observed the skin to be the major site of challenge death (Kamiya et al., 1987) while others favor the lung or post-lung sites (Mastin et al., 1983; Wilson et al., 1986). Ward and McLaren (1988) have postulated that in both models of resistance, effector mechanisms acting at the skin phase damage schistosmula such that death ultimately occurs at a future site. Thus, although parasite death may occur in the lung or post-lung site, immune damage is actually inflicted in the skin.

Strong similarities in immune responses between infected and vaccinated mice have been observed. These comparable responses in the two experimental models of host resistance to schistosome infection, have led to the suggestion that there may exist a common immune basis for host protection (James and Cheever, 1985; McLaren et al., 1987). Therefore, in considering potential effector mechanisms against schistosomes, results from

studies using both experimental models will be considered.

1.4.1 Role of T Lymphocytes in Immunity

The requirement of T-cells in the generation of resistance to infection with S. mansoni, is illustrated by the inability of athymic mice to develop significant levels of immunity in both the chronic infection (Doenhoff and Long, 1979) and vaccine (Sher et al., 1982a) models. Similarly, T-cell deprived mice are unable to eliminate S. bovis infections within the normal self-cure period (Murare and Doenhoff, 1987). More specifically depletion of CD4+ helper T-cells with monoclonal antibody has been shown to significantly reduce the ability of mice to resist S. mansoni challenge infection (Ducan and Colley, 1986; Kelly and Colley, 1988; Vignali et al., 1989b).

Helper T-lymphocytes may play a number of roles in schistosome immunity, including stimulation of the production of protective antibodies and the recruitment or activation of effector cells (Smithers and Doenhoff, 1982). Ellner et al., (1982) have also shown that T-lymphocytes activated by mitogens or soluble antigens can express non-specific schistosomulicidal effects.

A role for T-helper cells in antibody production has been suggested by the inability of mice treated with an anti-CD4+ monoclonal antibody, to elicit T-dependent antibody responses (Coulie et al., 1985). This treatment abrogates host IgG and IgM responses to soluble antigens but only if applied within 48 hours of immunization (Wofsky et al., 1985). It is possible that after a critical period, sufficient memory helper/effector cells have been activated such that stimulation of antigen-specific CD4+ cells is no longer required for antibody production (Kelly and Colley, 1988).

Proliferating T-cells, in the absence of specific antibody, may produce lymphokines which activate macrophages to kill schistosomula in vitro (Bout et al., 1981; James et al., 1983b). Inhibition of proliferative activity, by treatment with anti-CD4+ monoclonal antibody, reduces macrophage activation in vivo and abrogates host resistance (Vignali et al., 1989b). The importance of helper cell activity has been emphasized by Mendlovic et al., (1987) who suggest that the non-permissiveness of rats to schistosome infection, relative to mice, is reflected by the greater ability of rat lymphocytes to respond to schistosome antigenic stimulation.

That the degree of cell-mediated stimulation might correlate with protective immunity has been suggested by James (1985). This is particularly evident when considering the pattern of T-cell responses to S. mansoni antigens during the course of infection. Thus, during the first 2-3 weeks of infection, T-cell responses to schistosomule antigens become detectable and peak by 8 weeks post-infection (James, 1981). This pattern of T-cell responsiveness has been shown to correlate with the level of macrophage activation (James et al., 1982) and may in part explain, the low levels of resistance detected 14 days post-infection, prior to the deposition of eggs by patent worm pairs (Long et al., 1978). Although cellular responses to schistosome preparations may remain elevated due to repeated stimulation from established worm pairs (James et al., 1982), proliferative values and other parameters of cell-mediated immunity (CMI) generally decline with increasing chronicity of infection (Colley, 1972; 1975; Boros et al., 1975; James, 1981; Tawfik et al., 1986). This modulation of the immune response may represent a general decline in CMI, as blastogenic responses to non-specific T-cell mitogens also decrease soon after infection (Pelley et al., 1976; Diab et al., 1989). Olds and Ellner (1984) have observed that this decline in immune function coincides with a decline in macrophage

activation and host resistance to reinfection by 24 weeks post-infection. It is important to note, however, that these authors and others (Warren et al., 1977), have observed detectable levels of resistance during the chronic stage of infection, suggesting that declines in antigen stimulated blastogenesis may not necessarily reflect an inability to resist infection. This is particularly true of the vaccine model of resistance, where levels of T-cell reactivity can decline to background values by 9 weeks post-immunization even though host resistance to challenge remains intact (James et al., 1981).

1.4.2 Role of Macrophages

Macrophages appear to play a vital role in immunity to schistosomes. Thus, P strain mice, which exhibit a defect in macrophage activation, fail to show significant levels of resistance to challenge in the concomitant immunity (James et al., 1983a) and vaccine (James and Sher, 1983) models. Conversely, in normal mice, the pattern of concomitant immunity correlates with the pattern of induction of activated macrophages (James et al., 1983a).

Histological studies have shown that macrophages accumulate around challenge parasites in both the skin (Incani and McLaren, 1984; Ward and McLaren, 1988) and lungs (Vignali et al., 1989a) of mice. In vitro, macrophages can damage both skin (Mahmoud et al., 1979; James et al., 1983a) and liver phase schistosomula (Pearce and James, 1986). Lung stage larvae however remain refractory (Sher et al., 1982b). Wilson and Coulson (1989) suggest that macrophages may still participate in the lung phase of parasite attrition by accumulating around migrating larvae and inhibiting further larval migration and development.

Although anti-larval activity may be enhanced by the presence of specific antibody, macrophages can still demonstrate larvicidal activity in the

absence of antibody-containing sera (James et al., 1983b). Thus, macrophages can be activated non-specifically by T-lymphocyte derived lymphokines.

The non-specific nature of macrophage killing is illustrated by the ability of mice treated with Bacillus Calmette-Guerin (BCG) to develop partial resistance to S. mansoni (Civil et al., 1978) and that macrophages derived from these mice can damage schistosomulae in vitro (Mahmoud et al., 1979). Likewise, macrophages from S. mansoni infected mice are able to kill tumour cells in vitro (James et al., 1982), which is coincidental with the development of cytotoxicity against skin stage schistosomula (James et al., 1983a). More recently, a macrophage derived cytotoxin, tumour necrosis factor (TNF), which can mediate tumour cell killing by macrophages, has been shown to be toxic to schistosomula in vitro (James et al., 1990). Cell-target contact is required for macrophages to elicit their larvicidal effects (James and Glaven, 1987). However, it is clear that damage is subtegumental, as mitochondria and muscle cell damage is apparent while the outer tegument remains intact (McLaren and James, 1985). The type of damage inflicted on skin stage schistosomula is similar in the presence of antibody (McLaren and James, 1985).

1.4.3 Role of Eosinophils and Neutrophils

In vitro and in vivo evidence suggest that eosinophils and neutrophils play an important role in resistance to schistosome infection. Thus, these granulocytes can effectively kill skin stage schistosomula opsonized with either antibody or complement in vitro (Butterworth et al., 1977; Incani and McLaren, 1981). Attachment of the effector cell to the surface of the parasite is followed by the release of granules containing cationic proteins,

toxic for schistosomula (Butterworth et al., 1979; McLaren et al., 1981). In vivo, depletion of eosinophils and neutrophils with monoclonal antibody has been shown to significantly reduce levels of resistance in both the infected and vaccine models (McLaren et al., 1987). Furthermore, resistance is abrogated by treatment of mice with anti-eosinophil serum before challenge (Mahmoud et al., 1975).

Within 6 hours of challenge infection, neutrophils accumulate around challenge schistosomula (Incani and McLaren, 1984; Ward and McLaren 1988). By 48 hours, these authors have observed that infiltrates become enhanced with eosinophils and macrophages. Evidence exists to suggest that eosinophils in contact with pre-lung stage larvae, degranulate and inflict characteristic surface damage in vivo (McLaren and James, 1985). This can occur immediately after macrophages detach from the larvae suggesting that granulocytes and macrophages work in concert to damage skin stage schistosomula (McLaren and James, 1985).

Mice and rats exposed to sublethal doses of radiation exhibit reduced levels of circulating granulocytes in the lungs. Yet such irradiated animals demonstrate similar levels of vaccine induced resistance to unirradiated controls (Vignali et al., 1988; 1989a). Furthermore, these cells are all but absent from inflammatory trapping foci in the lungs, suggesting that granulocytes do not play a major role in this phase of parasite attrition.

1.4.4 Role of Antibody

Hypergammaglobulinemia is a characteristic of helminth infection in murine hosts (Chapman et al., 1979). More specifically, high concentrations of IgG and IgM isotypes are detected with IgG1 being the dominant subclass (Sher et al., 1977; Abraham and Teale, 1987). During the course of

a chronic S. mansoni infection, specific IgG1 and IgM antibody titres rise gradually to a peak by 12-14 weeks post-infection and then plateau (Ramalho-Pinto et al., 1979; Bout et al., 1980). Similarly, following vaccination with irradiated cercariae, antibody levels remain elevated many weeks after immunization (James et al., 1981).

Serum transferred from infected or vaccinated animals to naive recipients can confer detectable levels resistance (Sher et al., 1975; 1977; Mangold and Dean, 1986). The IgG1 isotype has been shown to be responsible for the resistance achieved in both models (Sher et al., 1977; Delgado and McLaren, 1990). However, sera derived from chronically mice does not necessarily confer resistance on naive recipients (Maddison and Kagan, 1979a). That the passive transfer of such serum does not universally confer protection may be due to the presence of insufficient titres of protective antibody (Vignali et al., 1989c). Indeed, Butterworth (1984) has suggested that high-titre antibodies may be necessary to bind sufficient neutrophils and eosinophils to the target and elicit killing. In the vaccine model, resistance is only conferred by sera from polyvaccinated mice (Dean and Mangold, 1986). This is not surprising, as anti-SWAP antibody titres are significantly higher in 5X vaccinated mice than one time sensitized animals (Kelly and Colley, 1988).

A second explanation suggests that blocking antibodies of the IgM isotype, which recognize antigens common to eggs and schistosomula , bind to the larval target and inhibit the attachment of potentially protective IgG isotypes (Yi et al., 1986). Thus, demonstrable immunity may depend on the balance between protective and blocking antibodies (Omer-Ali et al., 1988). This may explain why, in the absence of egg deposition, that serum from vaccinated mice can consistently confer protection.

The results of these immunological studies indicate that the invading schistosomula must evade a plethora of potential effector mechanisms before it can reach the hepatic portal system.

Overall, it would appear that eosinophils and neutrophils, along with macrophages, represent the first line of host defence against the parasite. As the parasite migrates towards the lungs and liver, macrophages are likely to assume the greatest protective role.

As it has been suggested that the quantity of protective antibody in the serum is vital for the expression of immunity (Vignali et al. 1989c; Butterworth, 1984) it is likely that cell-mediated mechanisms assume a crucial role in host protection during the early stages of infection when antibody titres are low, while antibody-dependent mechanisms become dominant during the chronic phase of infection.

Despite this vast array of potential anti-schistosome devices, resistance to schistosome infection is only partially effective at best. As much of the research on schistosomiasis is conducted with a view towards the development of an effective vaccine, it appears likely that such an agent will be required to trigger as complex an array of effector mechanisms as possible if it is to achieve complete protection of the host.

1.5 Aims of thesis.

Results derived from human studies can provide vital information regarding the immunological and infection status of the host. Nevertheless, a number of potentially important factors, which may contribute to the generation of protective immune responses to S. mansoni infection, cannot be assessed under field conditions. These include, the duration of experience of infection, patterns of exposure to transmission stages and the intensity of infection as determined by total worm burden.

Crombie and Anderson (1985) have described a model which includes the exposure of the murine host to a "trickle infection", the term used for repeated low level exposure to infective stages. These authors have observed that mice exposed to weekly doses of S. mansoni cercariae, generate age-intensity profiles which may be similar to those observed in endemic situations. Thus, mice exposed to high densities of cercariae develop an acquired resistance to infection which leads to an eventual decline in infection intensity with the increasing age of the host. The first objective of the work reported in Chapter 3 of this thesis is to determine a possible immune basis for the generation of this acquired resistance to schistosome infection. This is attempted by relating temporal changes in cellular and humoral reactivity, by the in vitro lymphocyte proliferation assay and ELISA techniques respectively, with changes in worm burden. The experimental protocol to be used in this study will preclude any influence of age-related changes in water contact on the age-intensity profiles generated.

It is possible that the persistence of resistance following the chemotherapeutic elimination of an existing worm burden, is dependent upon

the duration of previous exposure to infection. This conclusion has been drawn from studies conducted both in the laboratory (Tawfik and Colley, 1986) and under field conditions (Butterworth et al., 1984; 1985; Wilkins et al., 1987). The immune mechanisms responsible for the persistence of resistance remain undefined. Therefore, the second aim of this thesis as described in Chapter 4 is to examine the relationship between the duration of a primary trickle exposure and the persistence of residual resistance. The immunological parameters described above will also be measured to determine the possible relationship between cellular and humoral reactivity and the persistence of resistance.

Chapter 2

Materials and Methods

2.1 Intermediate Host and Parasite

The albino strain of Biomphalaria glabrata and the Puerto Rican strain of Schistosoma mansoni used in this study, were originally supplied by Dr. R.A. Wilson from the University of York and have been maintained in the Department of Biology at Imperial College for the past 12 years.

2.2 Snail Maintenance:

Uninfected and infected snails were housed in separate locations within the Animal Unit in the Department of Biology. Stock snails were maintained in plastic trays (50x20x4cm) filled with aerated tap water. Trays were kept in incubators which provided a constant temperature of 25°C±0.5°C. Snails were fed dried lettuce ad libitum which was supplemented with a source of calcium carbonate in the form of white non-toxic chalk. Polystyrene rafts were floated at the surface to facilitate the collection of eggs. Rafts were regularly transferred to clean trays where young snails were hatched. Parasitized snails were maintained in a control temperature room at 25°C±0.5°C. These snails were housed in plastic fish aquaria (30x18x20cm) and forty to sixty snails were maintained in each aquarium. While they were also fed dried lettuce ad libitum, no chalk was

added to the tank. A population of Daphnia species was found to be necessary for the maintenance of water quality in the aquaria.

2.3 Mouse Maintenance:

Male, CBA/Ca mice were purchased from Harlan Olac (Bicester, UK), Bantin and Kingman (Hull, UK) and Tuck (Battlesbridge, UK), or bred on site in the Department of Biology. Mice were housed in cages of 10 animals each and provided with food pellets (Ovoid, no. 41B) and water ad libitum. Cages were kept in air conditioned isolators (Vickers Medical and Isotech, Bicester, UK) which provided relatively constant environmental conditions.

2.4 Infection of Snail Intermediate Host:

Mice harbouring 6-8 week old S. mansoni infections were killed by cervical dislocation. Intestines and livers were removed and sliced into four sections and homogenized by an MSE blender in normal saline for 5 minutes. The homogenate was filtered through Endocott metal sieves (pore size of 180 and 300um) in an excess quantity of normal saline. After 5 minutes the supernatant fluid was carefully removed so as not to disturb the eggs in the sediment. Dechlorinated tap water was then used to wash the sediment into a wide mouth beaker. Excess water was removed and the eggs exposed to light for 15 minutes to initiate hatching of the miracidia. Snails (40-60 in number), 4-6mm in length were infected individually with 10 miracidia in an infecting chamber (35mm in diameter x 10mm in height, 6 chambers/plate). The chambers were then placed in an incubator at 25°C for 24 hours. Snails were then transferred to aquaria in the control temperature room and maintained as described previously.

At six weeks post-infection snails were placed in individual glass pots with aerated tap water and exposed to a light source in order to confirm the presence of cercarial infection.

2.5 Infection of Mouse Definitive Host:

2.5.1 Stock mice:

One hour prior to infection of the definitive host, 40-60 snails harbouring recently patent infections were placed in a glass jar containing 60ml of aerated tap water and exposed to a 15 watt light source to induce cercarial emergence. Ten, 6-8 week old CBA/Ca stock mice were anaesthetized by intraperitoneal injection of 1% body weight with a solution of 10% phenolpentabarbitone (Sagatal) in distilled water. This dose ensured that mice would remain immobile for approximately 2 hours. Mice were then infected percutaneously using the ring method as described by Smithers and Terry (1965). Abdominal fur was removed using an electric razor and mice were placed on their backs between wooden strips (3cm apart) supported by a base board. Shaved areas were then moistened with aerated tap water and metal rings sealed over the wetted area. Sixty freshly emerged cercariae in a 1ml suspension were placed in the ring using a pasteur pipette and left undisturbed for 1/2 hour. Following the completion of the infection period, mice were returned to their cages and left under a heat source in the form of a 15 watt table lamp until recovery from the anaesthetic.

2.5.2 Experimental mice:

Mice were exposed to repeated low level doses of S. mansoni cercariae using the paddling technique described by Crombie (1985).

Cercarial emergence was induced as described previously. Two hundred millilitres of aerated tap water at 25°C±0.5 was added to a plastic

aquarium to a depth of 0.4 cm. A predetermined number of freshly emerged cercariae was added to the aquarium. Ten mice were then placed in the tank and left in contact with the cercariae for 30 minutes. After this exposure period, water containing excess cercariae was drained into a large bucket through a rubber hose fitted to the base of the tank. Mice were than returned to their appropriate cage and isolator.

2.6 Mouse Chemotherapy:

The chemotherapeutic agent used in this study was praziquantel (EMBAY 8400, Biltricide). It was kindly donated by Dr. P. Andrews, Institute for Chemotherapy, Bayer, Germany. The drug was suspended in 2% Cremophor EL (Sigma Ltd., UK) according to Gonnert (1977) and administered in 3 oral doses of 250mg/kg on alternate days.

2.7 Blood Collection:

One day prior to sacrifice, blood samples were collected from mice using the tail bleeding technique.

Mice were placed in a restrainer so that only the tail was exposed. The tip of the tail was sliced off using a razor blade and the cut area emersed in a 1.5ml microtube (Treff Lab, Switzerland). The tail was gently massaged to encourage blood flow and 0.3-0.4ml of blood was collected. The mouse was returned to its cage and the blood sample allowed to clot for 1 hour at room temperature and then left overnight at 4°C. It was then centrifuged at 2000rpm for 5 minutes at 4°C. The serum was pipetted off, placed in a clean, labelled 1.5ml microtube and stored at -20°C until needed.

2.8 Perfusion of Experimental Host:

Portal perfusion of infected mice was conducted as described by Doenhoff et al. (1978) adopted from Smithers and Terry (1965). Animals were injected with 30mg/mouse phenolpentobarbitone (Sagatal) supplemented with 25 units of Heparin. The thoracic and abdominal cavities were dissected and the rib cage carefully removed. The tail and foreleg were then suspended from clothes pegs attached to vertical perspex board. A funnel leading into a 25ml universal container (Sterilin Ltd., UK) was placed under the liver. The intestines were suspended vertically to expose the hepatic portal vein which was then slit open at the junction with the liver. Twenty millilitres of cold perfusion fluid (8.5g sodium chloride and 15g trisodium citrate (BDH Ltd., UK) per liter of distilled water) was then slowly injected into the right ventricle of the heart with a 20ml syringe (Arnold Horwell, UK) and a 19 gauge needle (BDH Ltd., UK). The perfusion was completed when the mesenteric veins were cleared of blood. The contents of the universal container were then rinsed repeatedly into a conical jar (250ml) with tap water and allowed to settle. Excess water was decanted and sedimented worms were counted and sexed as mature (male or female) or juvenile stages. Sexually immature worms were categorized as juvenile. The mouse carcass was then examined carefully under a dissecting microscope for any worms trapped in the mesenteric veins and visceral organs. Spleens were removed and weighed. The liver was then dissected from the body and lobes not encompassing the gall bladder (approximately 65% by weight) were stored at -20°C for tissue egg determination at a later stage.

2.9 Liver Egg counts:

Livers were allowed to thaw at room temperature and then digested in a universal container with 20ml 4% potassium hydroxide at 37°C for 12 hours (Cheever, 1968). Three aliquots of 50ul were taken from each sample, placed on a microscope slide and covered with a coverslip. Counts were made using a compound microscope at 40X magnification and mean values were multiplied by 400 to give the number of eggs in 20ml and thus, the total number of eggs in the tissue sample.

2.10 Antigen Preparation:

2.10.1 Soluble Egg Antigen:

Soluble Egg Antigen (SEA) was generously supplied by Dr. M.J. Doenhoff, London School of Hygiene and Tropical Medicine and prepared according to Boros and Warren (1970). Eggs suspended in a 0.9% sodium chloride solution were placed in an ice bath and then disrupted by sonication using an ultrasonic probe. This was done for 15 minutes, after which time only 0.3% of the eggs remained intact.

2.10.2 Schistosomule Membrane Preparation:

Cercariae were mechanically transformed into schistosomula as described by Colley and Wikel (1974) and modified by James and Taylor (1976).

Recently emerged cercariae were concentrated in a Millipore apparatus (Millipore Corp., USA) using an 8um Millipore filter. Cercariae were induced to aggregate at the surface of the apparatus by means of an artificial light and water from below allowed to drip through the filter. 5-

10ml of the suspension was rinsed with sterile RPMI 1640 medium (Gibco Ltd., UK) and transferred to a 25ml universal container. Cercariae were then exposed to shearing forces in the form of 10-14 passages through a 21 gauge needle attached to a 10ml syringe. A 100ul aliquot was examined to ensure that tails had been sheared from all cercariae. The universal containing schistosomula was then incubated for 3 hours at 37°C. The RPMI 1640 was pipetted off and the schistosomulae rinsed with sterile phosphate buffered saline (PBS) into a 1.5ml microtube. The microtube was then stored at -70°C until a sufficient quantity of schistosomulae was available for pooling and completion of the antigen preparation. Supernatant fractions were pooled in 1.5ml microtubes, placed on ice and sonicated at 14 microns for 10 seconds with 30 second intervals for 5 repetitions. A sample was examined under a compound microscope to ensure that no intact schistosomulae remained. The suspension was then centrifuged at 10,000g in a MSE microfuge for 20 minutes at 4°C and the resulting supernatant transferred to a fresh microtube. This fluid was considered to contain schistosomula tegument material and was stored at -70°C until future use.

2.10.3 Adult Tegumental Membrane:

An adult tegumental membrane antigen was kindly donated by Dr. A.J.G. Simpson and Dr. S.R. Smithers, National Institute of Medical Research and was prepared as described by Simpson et al. (1981). Worms were perfused from 6-week infected hamsters, washed in cold PBS and incubated for 30 minutes in warm (37°C) PBS containing Ca2+ and Mg2+. Worms were again resuspended in 2ml warm PBS containing Ca2+ and Mg2+ and vortexed for 1 minute. Supernatant was then centrifuged at 100,000g for 1 hour at 4°C. The pellet containing the membrane fraction was resuspended in PBS and stored in liquid nitrogen.

2.11 Protein Determination:

The protein concentration of each antigen was determined by a Bradford Protein Assay (Maizels and Robertson, 1988).

2.12 Serological Analysis by ELISA:

Mouse sera were analyzed for antibodies against S. mansoni derived antigens using the Enzyme Linked Immunosorbant Assay (ELISA) as described by Lillywhite et al. (LSHTM int. pub.). Optimal antigen and serum concentrations were predetermined using a chequer-board titration with a reference positive serum (pooled sera collected from mice with 12 week old patent infections) and a reference negative serum (pooled sera collected from naive age-matched controls). Consequently, SEA, schistosomula and adult membrane antigens were diluted to appropriate concentrations in carbonate coating buffer (For details, see appendix A2.1). Diluted antigen (0.1ml) was dispensed into each well of a 96 well micro-titre plate (Linbro, Flow Ltd., UK) and incubated overnight at 4°C. Plates were then washed 3 times with washing buffer (For details, see appendix A2.1) to remove unattached antigen. Individual serum samples diluted to a concentration of 1/200 in incubation buffer (For details, see appendix A2.1) were then added in volumes of 100ul to each well and allowed to incubate for 3 hours at room temperature. The unattached serum was removed by washing. IgG or IgM immunoglobulin activity was measured by adding a 1/1000 dilution of the appropriate anti-mouse peroxidase conjugate (Sigma Ltd., UK) in 100ul of incubation buffer to each well. The plate was then incubated for 3 hours at room temperature. Unattached conjugate was washed from the plate and 100ul of substrate buffer (For details, see appendix A2.1) with orthophenyldiamine added to each well. The reaction was then stopped after 30 minutes by the addition of 50ul of 2M sulphuric

acid to each well. Optical densities were read at 492nm by an automated ELISA reader (Flow Ltd., UK).

2.13 Lymphocyte Proliferation Assay:

The method used for the measurement of in vitro lymphocyte proliferation has been described previously by Corradin et al. (1977) with modifications suggested by Mishell and Shiigi (1980). Aseptic conditions were maintained throughout the procedure to ensure that cell cultures did not become contaminated.

The spleen was used as a source of lymphocytes in this study. It was removed from mice killed by cervical dislocation and placed in a 5ml bijou (Sterilin Ltd., UK) containing 5ml of culture medium (For details of culture medium, see appendix A2.2). Spleens were then poured onto a stainless steel gauze (mesh size 1 x 1 mm, Locker Wire Weavers, UK) situated in a 90mm petri dish (Sterilin Ltd., UK). Single cell suspensions were prepared by forcing the contents of the organ through the mesh using the plunger of a 5ml syringe (BDH Ltd., UK). The gauze was then rinsed with a small amount of medium and the suspension left for 5 minutes to sediment large particulate material. The cells were then pipetted into a 13.5ml conical bottom test tube (Sterilin Ltd., UK), topped off with culture medium and centrifuged at 200g for 5 minutes.

The cells were then resuspended in 2ml of hypotonic lysis solution (For details, see appendix A2.2) for 5 minutes in order to lyse red blood cells and hence facilitate the counting of white blood cells later on in the procedure. Ten millilitres of medium was then added and the cells washed twice in medium. After the second wash, the cells were finally resuspended

in 5ml of medium. The suspension was mixed thoroughly and a 100ul aliquot withdrawn in order to determine the concentration and viability of the cells. This was done by mixing the aliquot with an equal volume of 0.4% Trypan Blue Solution (Flow, UK) and examining a sample of the suspension in an Improved Neubauer Haemacytometer (BDH Ltd., UK).

The cell concentration was then adjusted to the required value and the cells pipetted in 100ul aliquots into the wells of a flat-bottomed microtitre plate (Nunc, 96F, Gibco, UK). Previously added to each well was 100ul of antigen, mitogen or culture medium alone with each treatment being carried out in triplicate.

The plates were covered and incubated in a humidified atmosphere of 95% air, 5% CO_2 at 37°C for a 72 hour pre-pulse period. After this time 50ul of medium containing 0.1uCi of the isotope 5-Iodo-2-deoxyuridine-I-125 (Amersham Laboratories, UK) was added to each well. The cells were then incubated for a further 18 hours (pulse period) and then harvested using an automated cell harvester (Multimash 2000, Dynatech, UK). Cells were harvested onto glass fibre filter paper by sequential washing with 0.85% NaCl, 5% trichloracetic acid and absolute methanol. The paper was allowed to dry and discs corresponding to individual wells placed in LP3 tubes (Lucklam Ltd., UK). The amount of radioactive iododeoxyuridine incorporated was determined by counting the radioactive disintegrations in a gamma counter (1275 Minigamma, LKB Ltd., UK) and expressed as counts per minute (CPM).

Chapter 3

Dynamics of Immune Responses During Repeated Exposure to Infection

3.1 Introduction

It is generally believed that in areas endemic for schistosomiasis, the total worm burden of an individual is acquired as a result of multiple small cercarial exposures over long periods of time (Foster, 1967; Lewis et al., 1987). The age-intensity profile generated by this pattern of exposure is frequently convex in form, with parasite burdens gradually increasing to a maximum in young adults and slowly declining thereafter (Anderson, 1987; Mott, 1987). Results derived from human studies have indicated that this decline in infection intensity may be partially due to the gradual acquisition of protective immunity which is dependent upon the duration of previous exposure to infection (Butterworth et al., 1984; 1985; Wilkins et al., 1987). However, other studies (Dalton and Pole, 1978) show age related changes in exposure to infection, based on patterns of water contact, that could also explain the observed convexity in the age-intensity profiles. It is probable that both age-related exposure to infection and the slow build up of resistance dependent on exposure intensity determine the shape of the profile (Wilkins, 1987).

Ideally, longitudinal studies of epidemiological and immune parameters during the course of infection, should be conducted to provide a better understanding of the relationship between the immunity, infection and exposure status of the host. In this way, immune responses associated with the acquisition of resistance might be determined. This is difficult to do in human situations where the dynamics of parasite acquisition cannot be readily determined. However, detailed studies by Sturrock et al., 1983; Hagan, 1985; and Dessein et al., 1988; for S. haematobium have begun to elucidate immune indicators with resistance exposure and infection status.

Alternatively, experimental models can allow for the simultaneous determination of the host's immune and infection status. Recently, Crombie and Anderson (1985) have shown that mice exposed to repeated low level doses of S. mansoni cercariae, exhibit age-intensity profiles similar to those observed in human communities. Knowledge of the immune responses generated during the course of such infections may provide some insight into the role of immunity in the gradual acquisition of resistance to human infection.

The aim of the present study is to generate age-intensity profiles similar to those observed in endemic situations and to characterize the dynamics of cellular and humoral immune responses of mice, during the course of a trickle S. mansoni infection.

3.2 Methods

In the present study, experimental mice were infected in groups of ten, using the paddling technique described by Crombie (1985). Groups were exposed to either 1, 3 or 10 cercariae/mouse/week. At pre-determined intervals after the initial exposure, blood was collected for serological

analysis and animals killed the following day. Interpretation of the results from this study has been slightly complicated by a problem encountered during the tail-bleeding procedure. On certain occasions mice were unable to survive the procedure and died immediately upon removal from the restrainer. This stress-related mortality occurred irrespective of infection status and was likely induced by the unusually warm conditions encountered in the procedure room of the Animal Unit. On such occasions the procedure was abandoned to ensure that a sufficient number of animals was available for other epidemiological and immunological studies. Consequently, serum samples for analysis of immunoglobulin levels were not always available. On the day of sacrifice, animals were randomly selected for either determination of worm and liver egg burdens or examination of cellular responses to S. mansoni antigens and the T-cell mitogen, concanavalin A (ConA). The significance of these responses was determined by comparison with similarly incubated lymphocytes of naive age/sex matched control animals. As positive (inter-experimental) controls, spleen cells from 6-10 week old male CBA/Ca mice were incubated with a range of concentrations of ConA (50ug/ml double diluted through to 3.125ug/ml).

The duration of each experiment was dependent upon the number of mice made available for infection and the survival of mice at each infection intensity. On those occasions when less than 6 mice were available for study, the in vitro lymphocyte proliferation assay was omitted and all remaining animals perfused.

3.3 Results

3.3.1 Adult Worm Burdens:

The number of adult worms recovered by perfusion at each time period is shown in Fig. 3.1. At the lowest intensity of exposure, the average worm burdens appeared to rise monotonically throughout the 35 week period. This differs slightly from the results of Crombie and Anderson (1985) who observed that worm burdens recovered from mice receiving 1 cercaria/mouse/week, began to plateau by 25 weeks post-initial infection. The observed patterns of worm recovery following higher intensities of cercarial exposure were however similar to those reported by Crombie and Anderson (1985). Thus, as the infection intensity increased to 3 and 10 cercariae/mouse/week, worm burdens rose more rapidly and to higher peak levels. At the moderate dose, worm burdens peaked by 20 weeks and declined slightly thereafter, while at the highest intensity of exposure, worm burdens reached a stable plateau by 15 weeks.

3.3.2 Juvenile Worm Burdens:

The recovery of juvenile worms through time is shown in Fig. 3.2 and their proportion as a percentage of total worm burden in Fig. 3.3. Juvenile worms were recovered at most of the sampling points, indicating a constant immigration of new parasites. At the two lower intensities of exposure (1 and 3 cercariae/mouse/week), the number of juveniles remained stable throughout the experimental period (ANOVA, $p>0.05$). Conversely, at the highest level of exposure, the number of juveniles recovered by perfusion, declined significantly from the peak value attained at 6 weeks

Figure 3.1

Recovery of adult worms following a trickle infection

Data points represent the mean worm burdens.

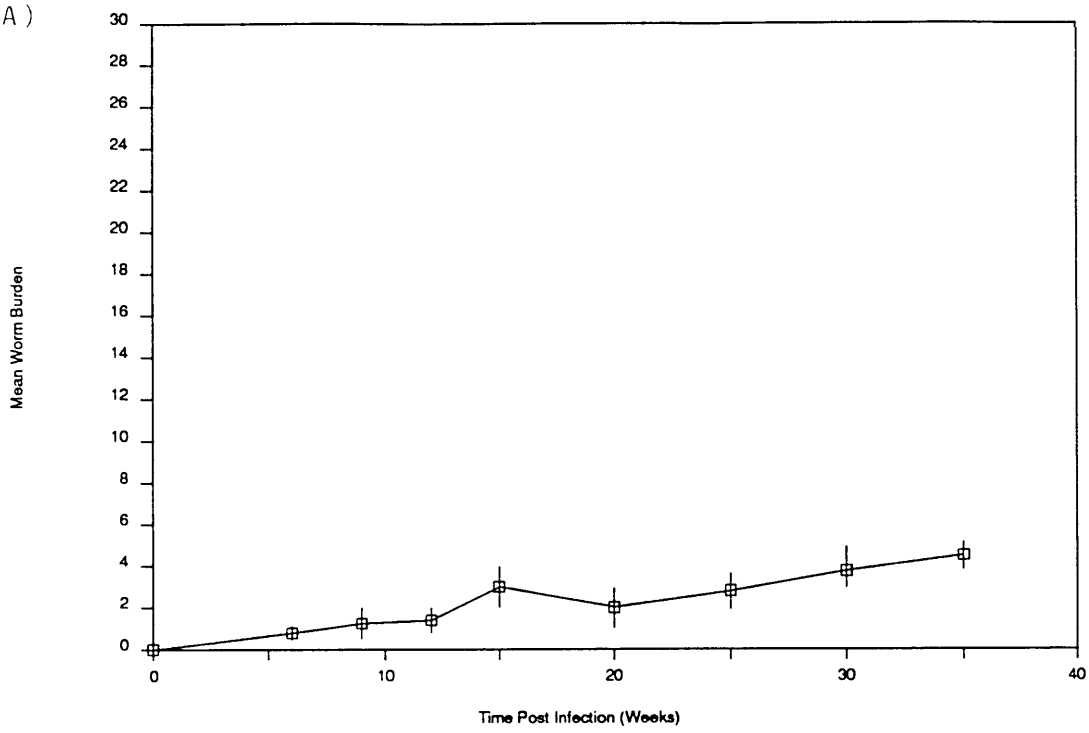
Error bars represent the standard error of the means.

A) 1 cercaria/mouse/week

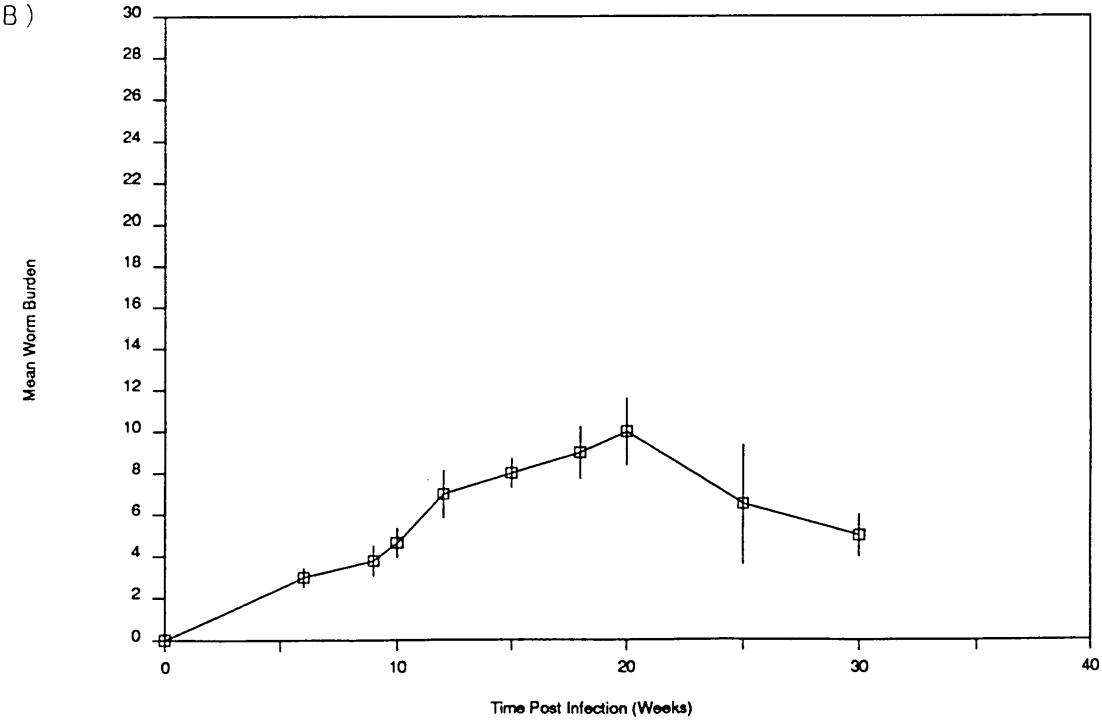
B) 3 cercariae/mouse/week

C) 10 cercariae/mouse/week

A)


Mean Worm Burden
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
10
20
30
40
Time Post Infection (Weeks)

B)


Mean Worm Burden
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
10
20
30
40
Time Post Infection (Weeks)

C)

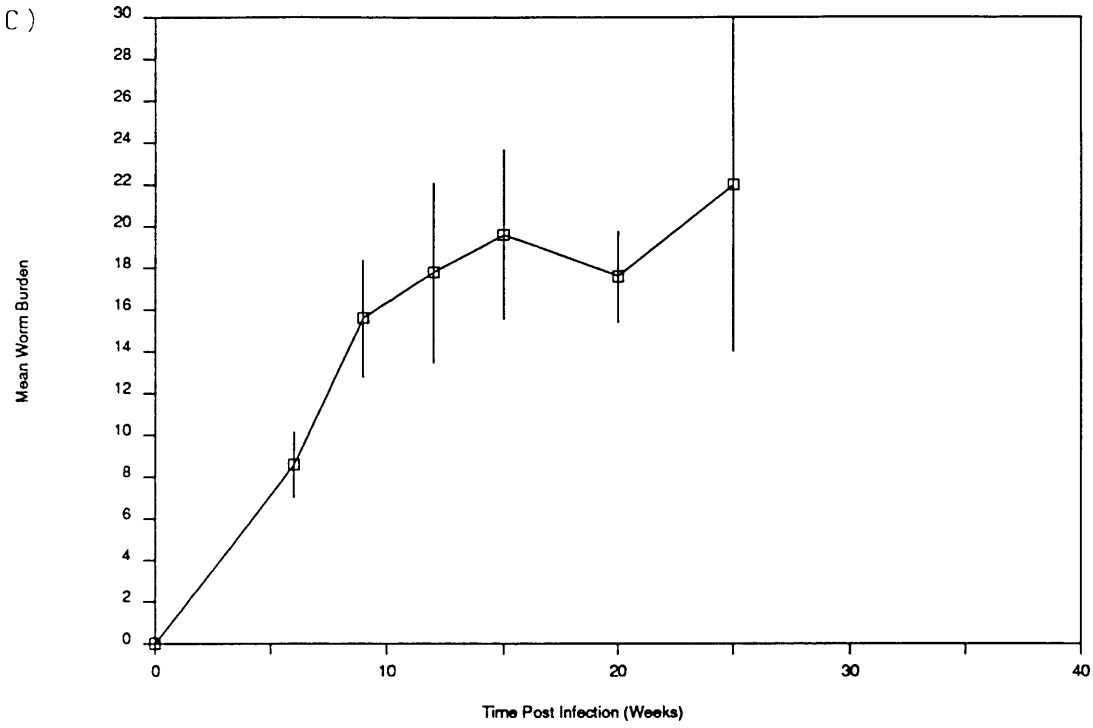

Mean Worm Burden
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
10
20
30
40
Time Post Infection (Weeks)

Figure 3.2

Recovery of juvenile worms following a trickle infection

Data points represent the mean worm burdens.

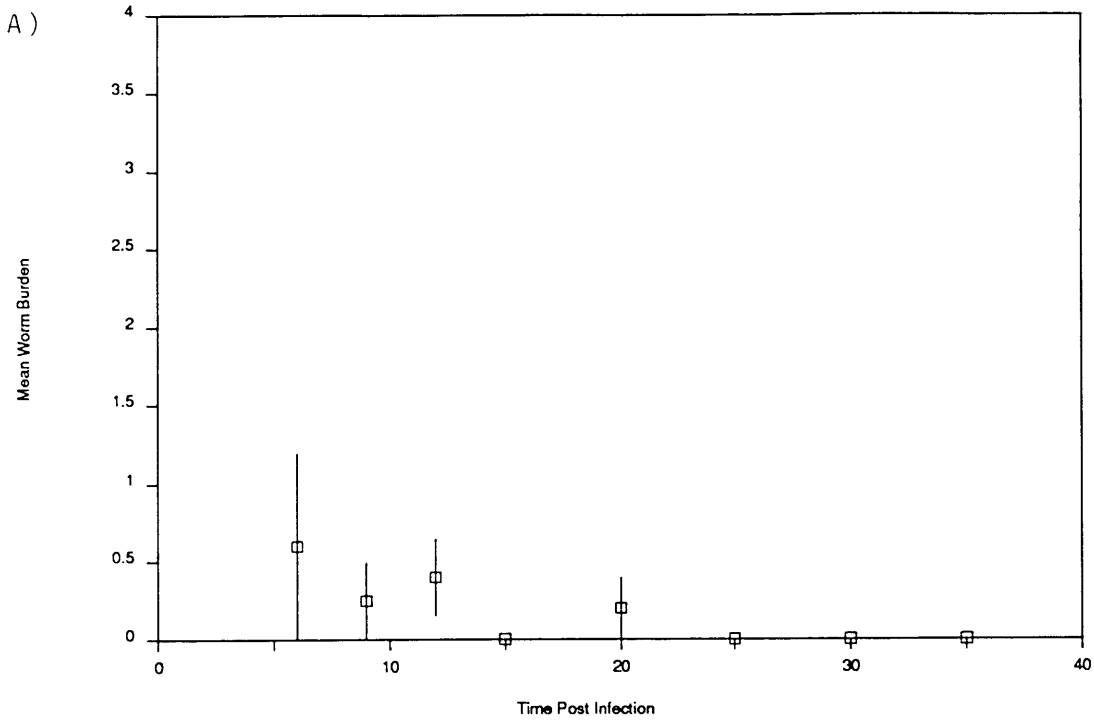
Error bars are the standard error of the means.

A) 1 cercaria/mouse/week

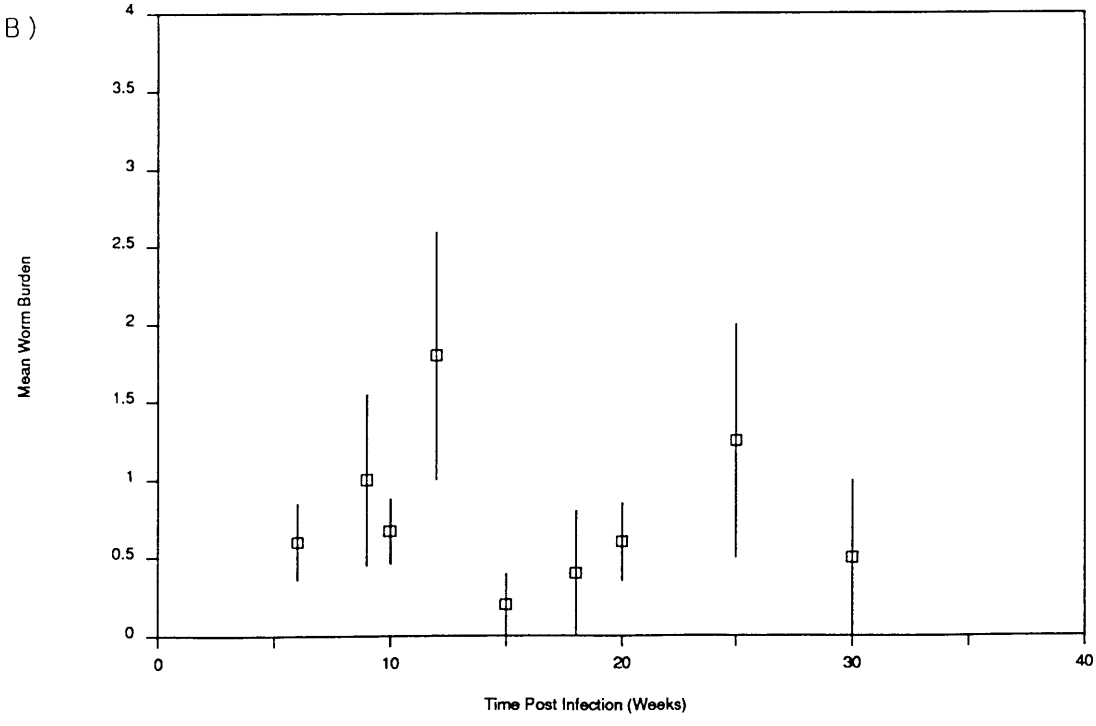
B) 3 cercariae/mouse/week

C) 10 cercariae/mouse/week

A)


Mean Worm Burden
4
3.5
3
2.5
2
1.5
1
0.5
0
0
10
20
30
40
Time Post Infection

B)


Mean Worm Burden
4
3.5
3
2.5
2
1.5
1
0.5
0
0
10
20
30
40
Time Post Infection (Weeks)

C)

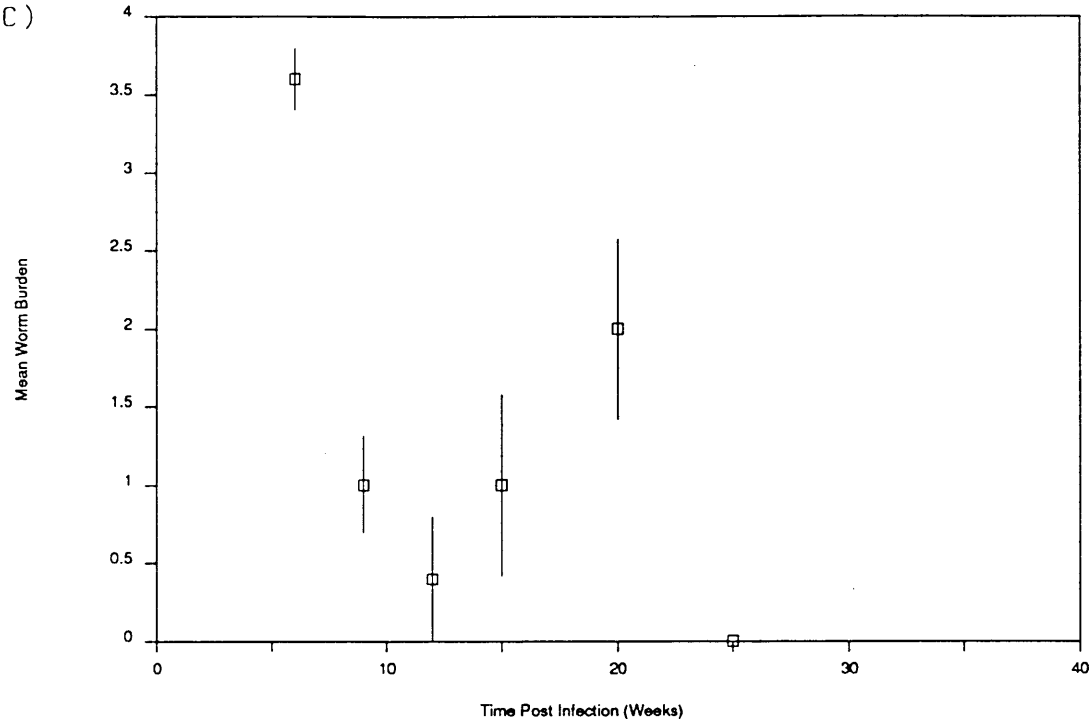

Mean Worm Burden
4
3.5
3
2.5
2
1.5
1
0.5
0
0
10
20
30
40
Time Post Infection (Weeks)

Figure 3.3

Relative number of juvenile worms recovered following a trickle infection.

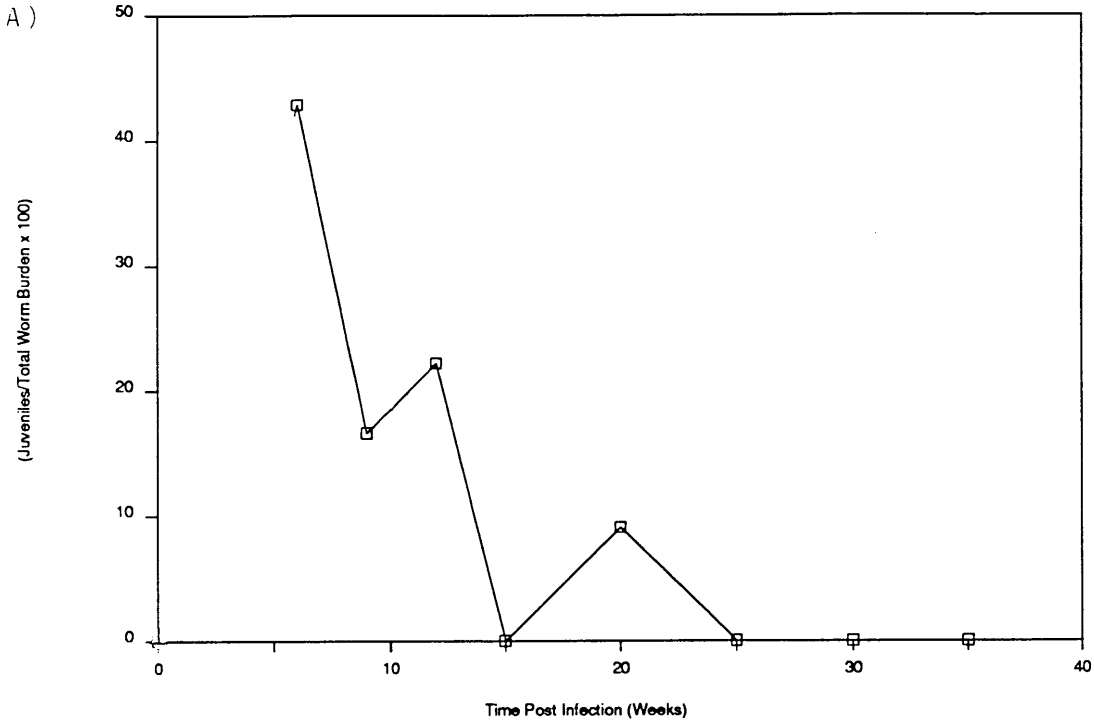
Data points represent number of juveniles as a percentage of total worm burden.

A) 1 cercaria/mouse/week

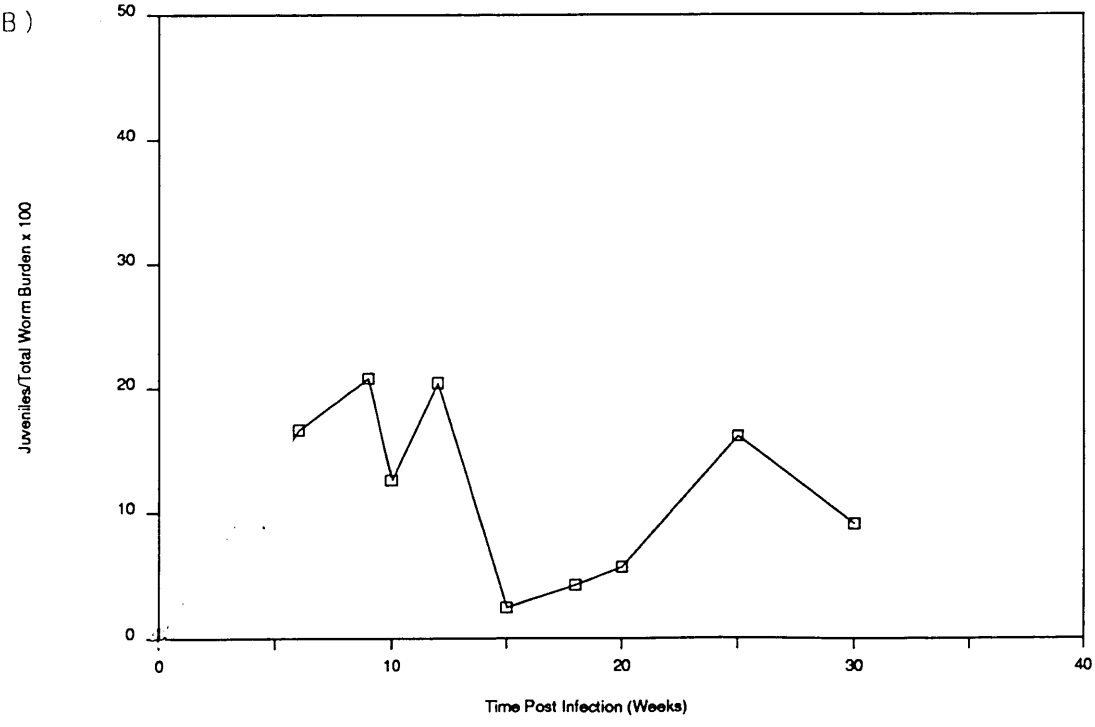
B) 3 cercariae/mouse/week

C) 10 cercariae/mouse/week

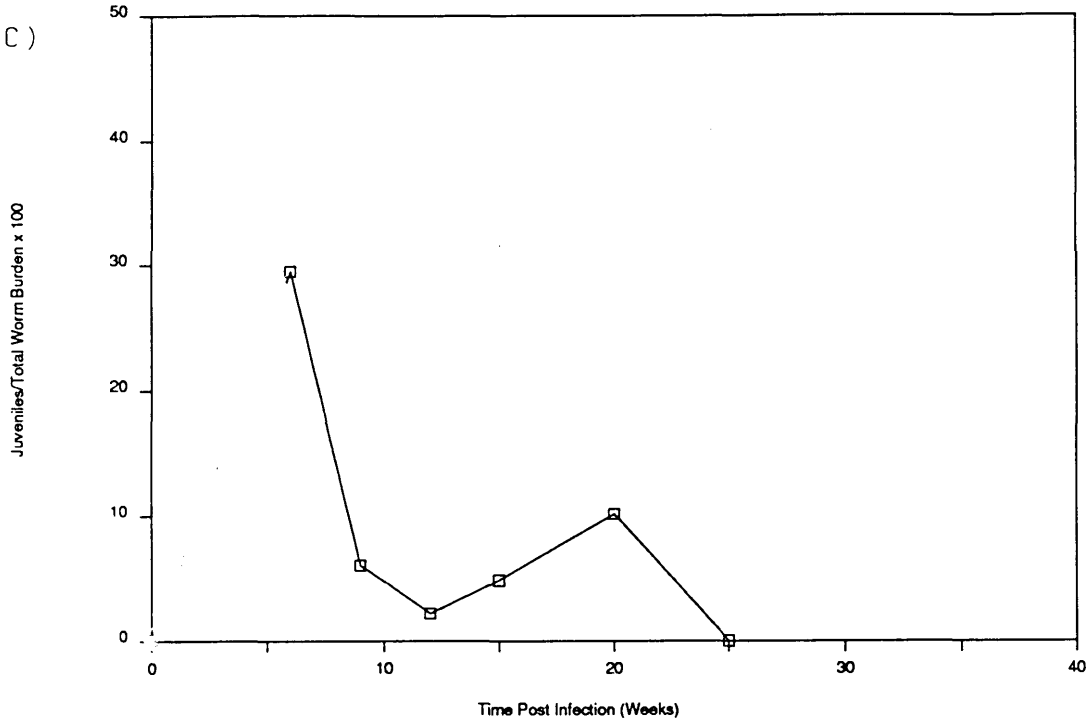
A)


50
40
30
20
10
0
(Juveniles/Total Worm Burden x 100)
0
10
20
30
40
Time Post Infection (Weeks)

B)


50
40
30
20
10
0
Juveniles/Total Worm Burden x 100
0
10
20
30
40
Time Post Infection (Weeks)

C)


50
40
30
20
10
0
Juveniles/Total Worm Burden x 100
0
10
20
30
40
Time Post Infection (Weeks)

post initial infection ($F=7.53$, $p<0.01$).

3.3.3 Liver Egg Burdens:

The number of eggs per worm pair recovered from the livers of experimental mice is shown in Fig. 3.4. With the exception of a slight rise at 25 weeks in mice receiving 1 cercaria/mouse/week, liver egg burdens gradually increased to a plateau irrespective of the intensity of exposure. Furthermore, egg counts did not differ significantly between exposure groups at corresponding times post-initial exposure (t-test, $p>0.05$).

3.3.4 Results of the in vitro Lymphocyte Proliferation Assay.

Baseline Proliferative Values:

Preliminary examination of the results revealed interesting patterns in the blastogenic responses of mouse lymphocytes cultured in the absence of antigenic or mitogenic stimulation (Fig. 3.5). Irrespective of the dose or infection status, baseline values were not stable and fluctuated significantly through time. Statistical analyses are provided in Table A3.1. However, levels of spontaneous proliferation exhibited by cells derived from infected mice, exceeded those of naive controls.

Elevated background responses may result from properties characteristic of the foetal calf serum used (FCS) (Corradin et al., 1977). As a number of FCS preparations were employed during the course of this study, it is possible that certain batches were able to support unstimulated blastogenic activity better than others. In vivo stimulation by parasite extract or antigen carryover into culture by splenic phagocytes may also contribute to the enhancement of baseline proliferative values (James et al., 1983b;

Figure 3.4

Liver egg burdens following a trickle infection

Data points represent mean number of eggs/worm pair.

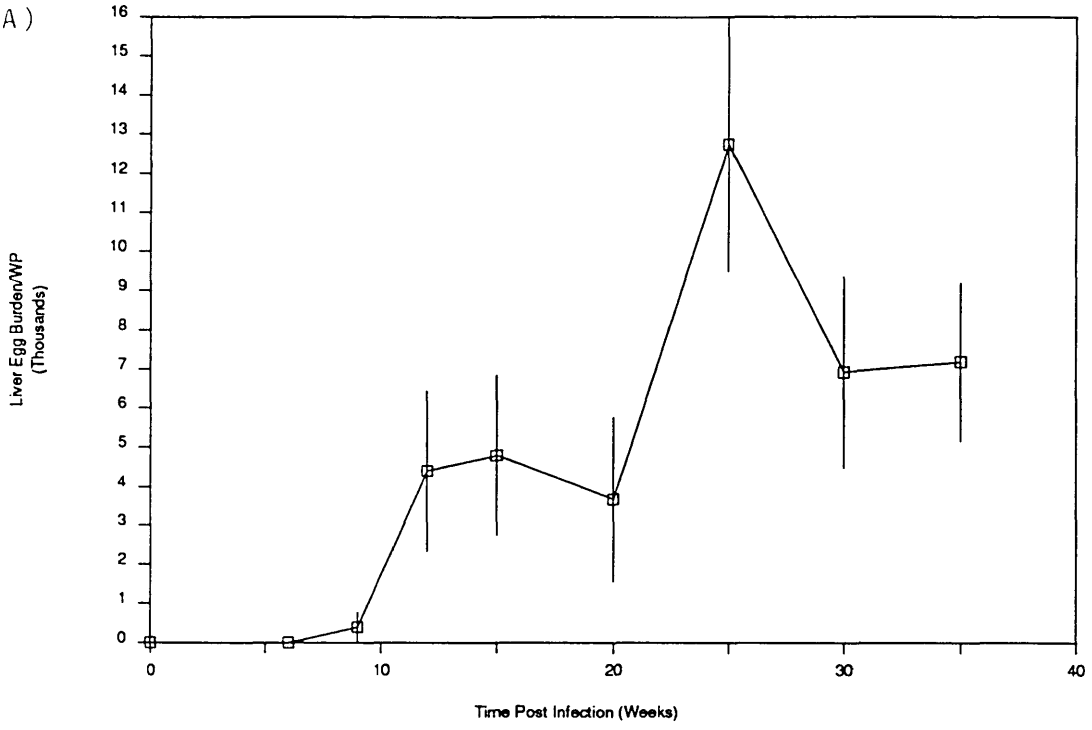
Error bars are the standard error of the means.

A) 1 cercaria/mouse/week

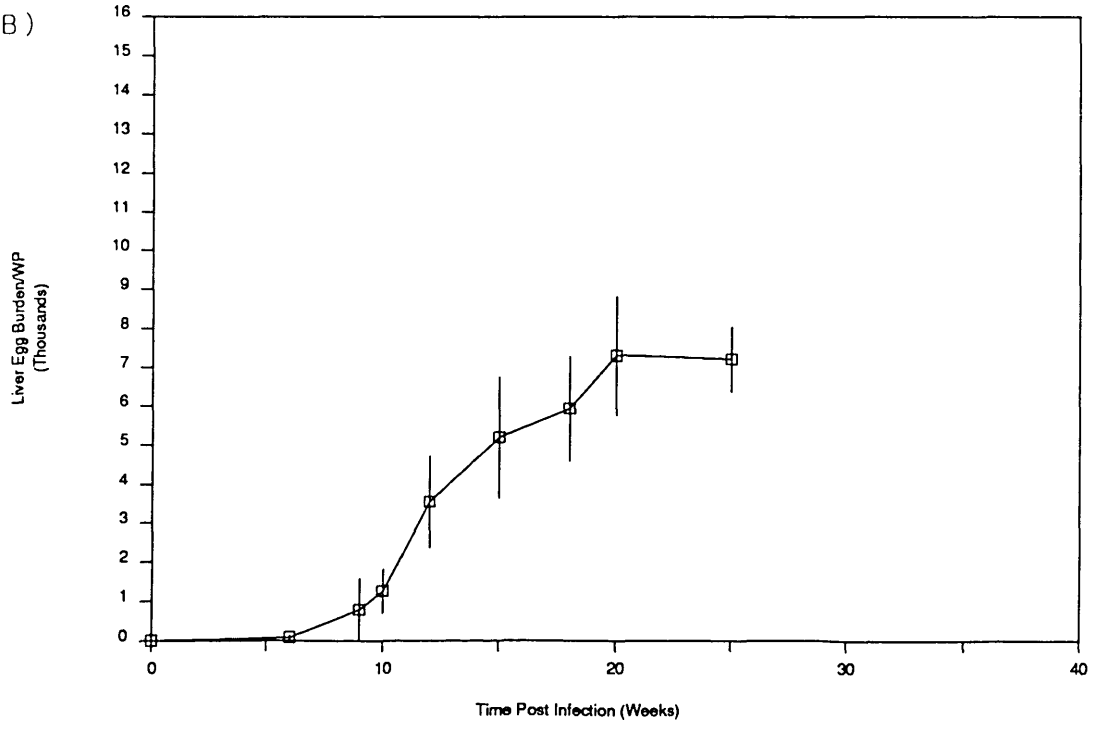
B) 3 cercariae/mouse/week

C) 10 cercariae/mouse/week

A)


Liver Egg Burden/WP
(Thousands)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
0
10
20
30
40
Time Post Infection (Weeks)

B)


Liver Egg Burden/WP
(Thousands)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
0
10
20
30
40
Time Post Infection (Weeks)

C)

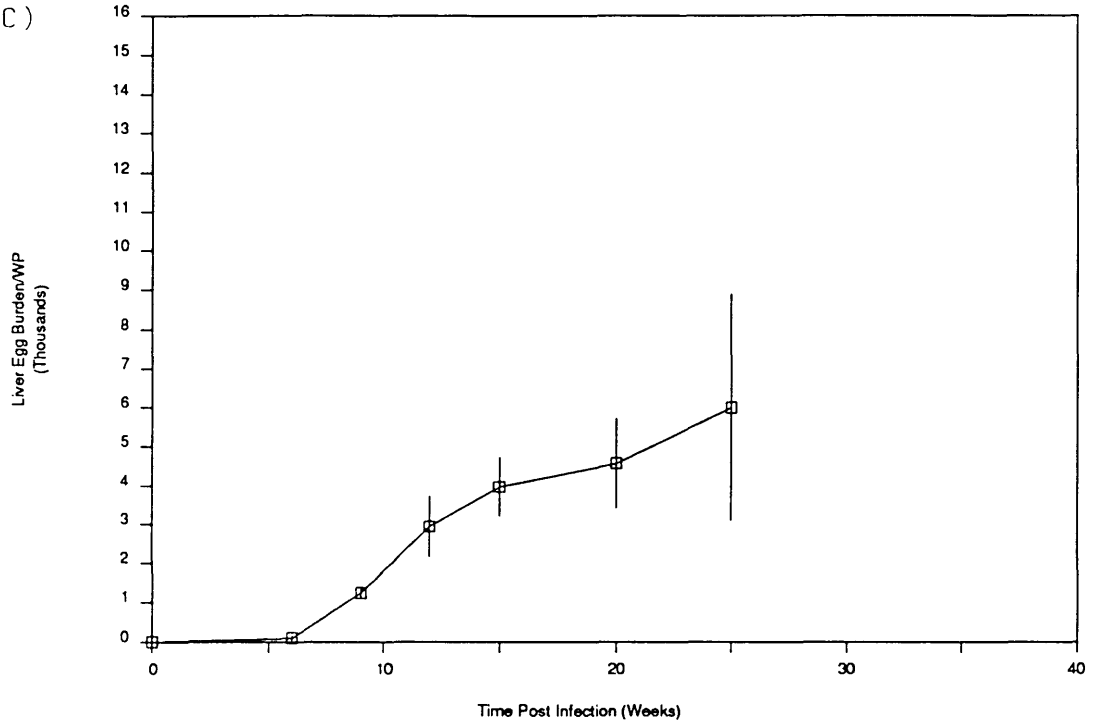

Liver Egg Burden/WP
(Thousands)
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
0
10
20
30
40
Time Post Infection (Weeks)

Figure 3.5

Levels of spontaneous lymphocyte proliferation following a trickle infection

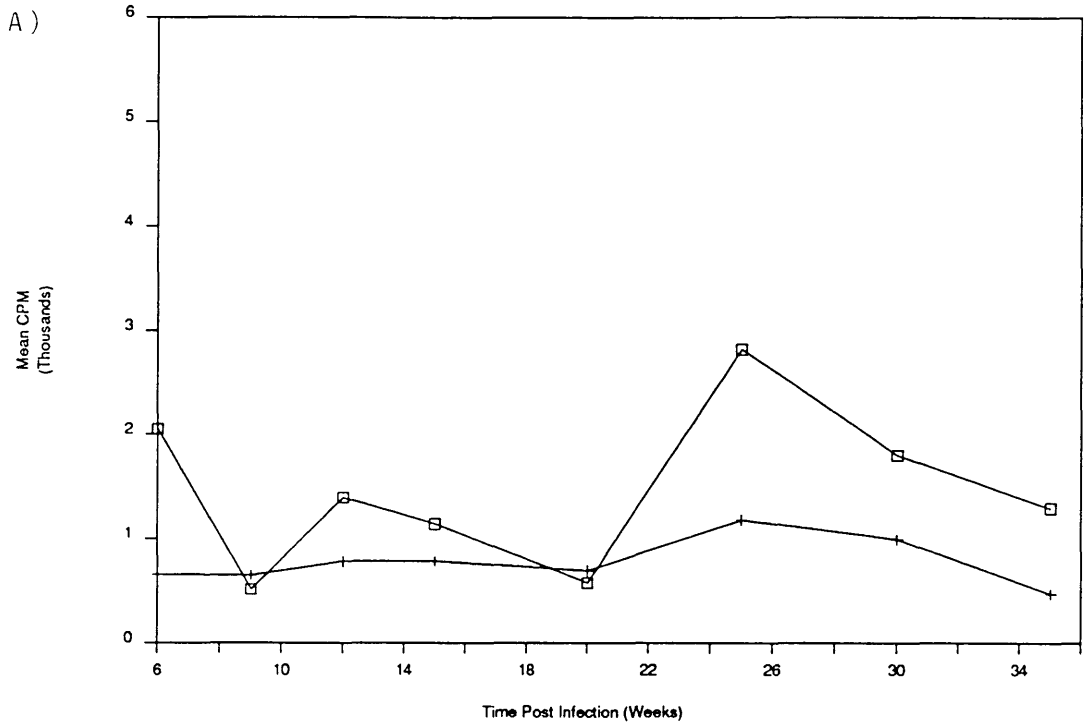
Data points represent baseline proliferative responses of experimental (squares) and naive age/sex matched control mice (crosses).

A) 1 cercaria/mouse/week

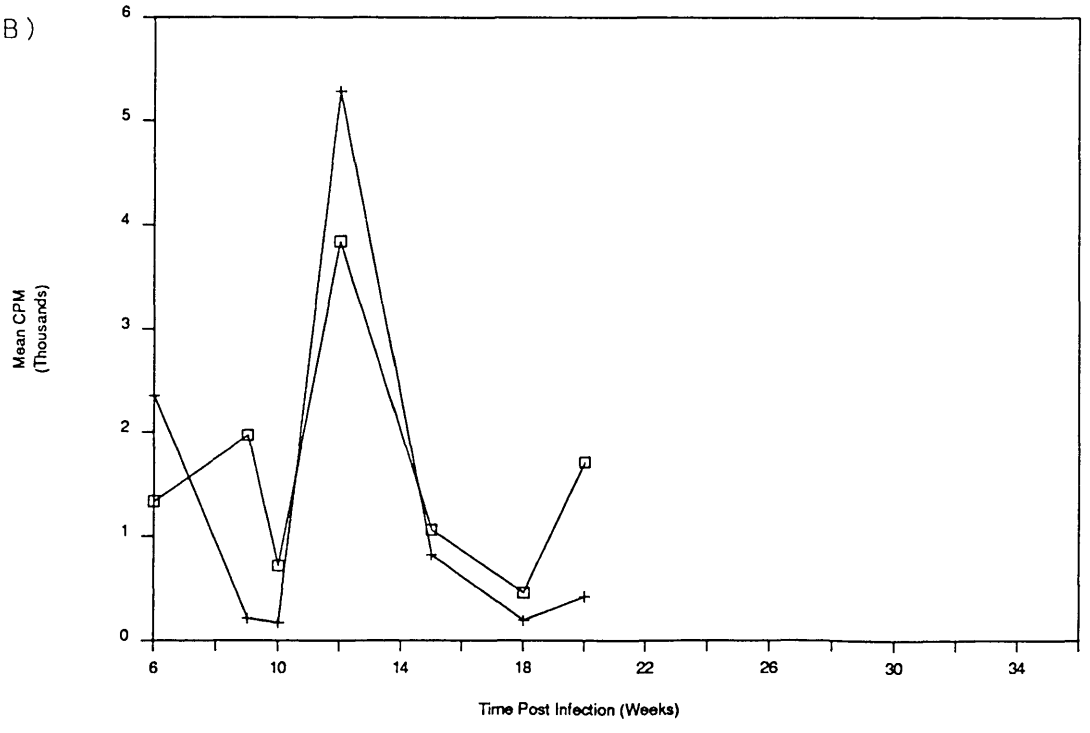
B) 3 cercariae/mouse/week

C) 10 cercariae/mouse/week

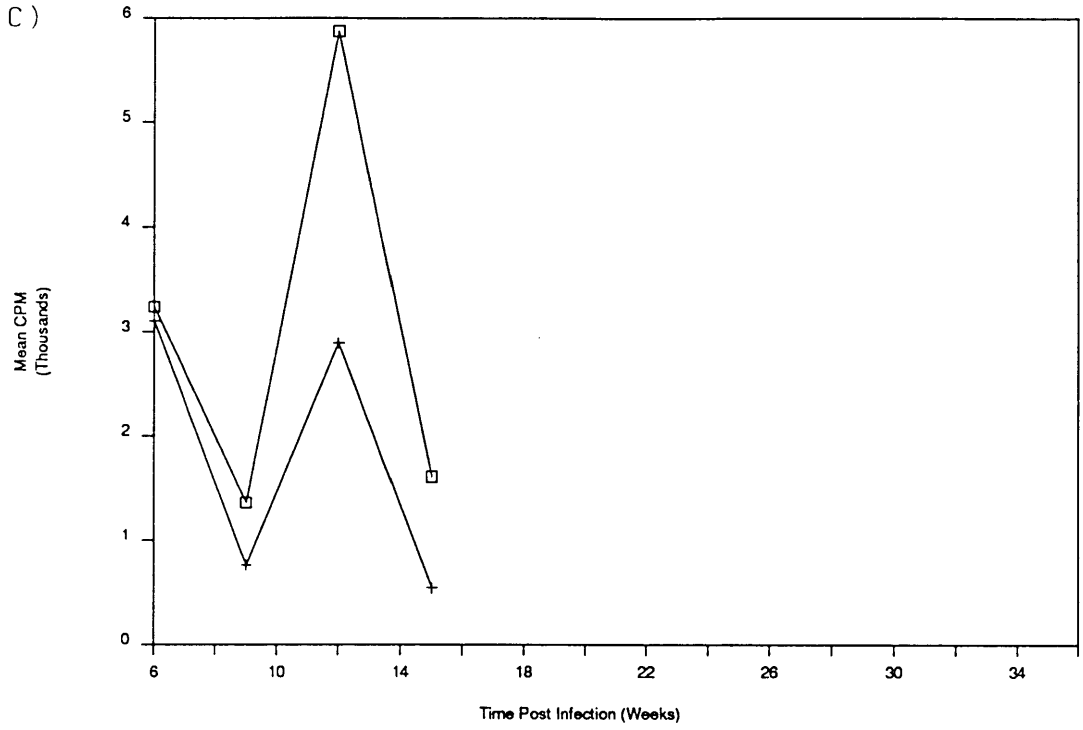
A)


Mean CPM (Thousands)
0
1
2
3
4
5
6
6
10
14
18
22
26
30
34
Time Post Infection (Weeks)

B)


Mean CPM (Thousands)
0
1
2
3
4
5
6
6
10
14
18
22
26
30
34
Time Post Infection (Weeks)

C)


Mean CPM
(Thousands)
0
1
2
3
4
5
6
6
10
14
18
22
26
30
34
Time Post Infection (Weeks)

Maluish and Strong, 1986). This is supported by observations implicating the splenic macrophage derived from S. mansoni (Kayes and Colley, 1984) or Trichinella spiralis (Ljungstrom and Sundquist, 1979) infected mice as a stimulant of baseline DNA synthesis. This would also explain the enhanced levels of spontaneous proliferation expressed by lymphocytes derived from infected animals over naive controls in the present study.

The presence of inconsistent baseline values can complicate the interpretation of the results by masking part of the proliferative response induced by antigen or mitogen (Corradin et al., 1977). It is therefore necessary to compensate for the influence of these enhanced baseline values so that the "true" antigenic or mitogenic effects can be evaluated. Various ways of expressing data derived from lymphocyte proliferation assays have been discussed previously (see Chapter 1). For the purposes of this study, proliferative responses will be expressed as Delta CPM, calculated as counts per minute (CPM) in stimulated cultures minus CPM in unstimulated cultures.

3.3.5 Mitogenic Responses to S. mansoni Antigens:

The lymphoproliferative responses of naive, age/sex matched control mice to S. mansoni antigens are shown in Fig. 3.6. Statistical analyses are provided in Table A3.2.

In the present study, it was observed that S. mansoni antigenic preparations were capable of stimulating splenic lymphocytes derived from naive animals. Levels of non-specific proliferation were observed to vary with time and the antigenic stimulus used in culture. Thus, cells incubated with schistosomula or adult worm antigens, but not SEA, exhibited statistically significant higher levels of cellular reactivity than cells cultured

Figure 3.6

Non-specific lymphoproliferative responses to S. mansoni antigens

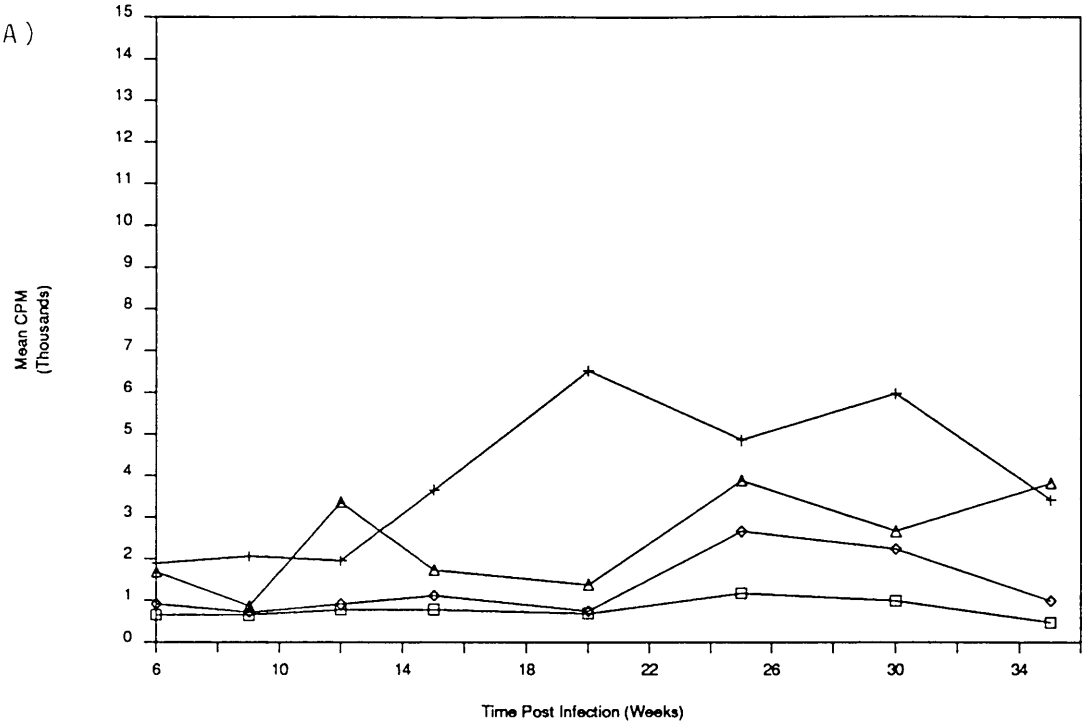
Data points represent mean lymphoproliferative responses of naive age/sex matched control mice to schistosomula antigen (crosses), SEA (diamonds), adult worm antigen (triangles) and in the absence of antigenic stimulation (squares).

A) 1 cercaria/mouse/week

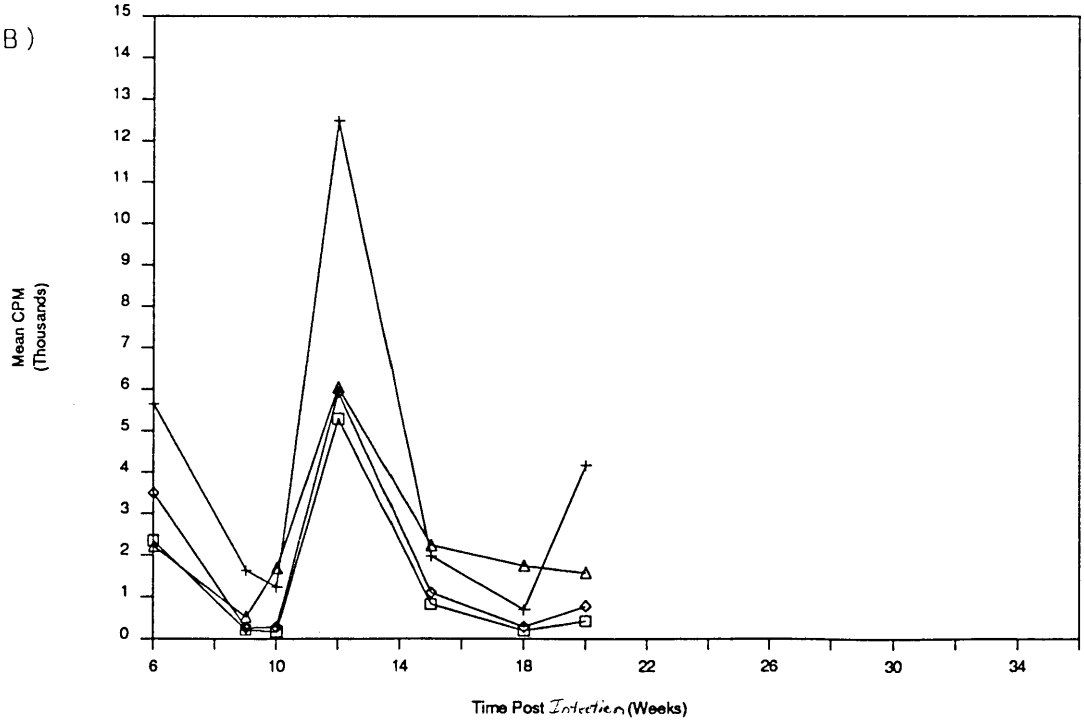
B) 3 cercaraie/mouse/week

C) 10 cercariae/mouse/week

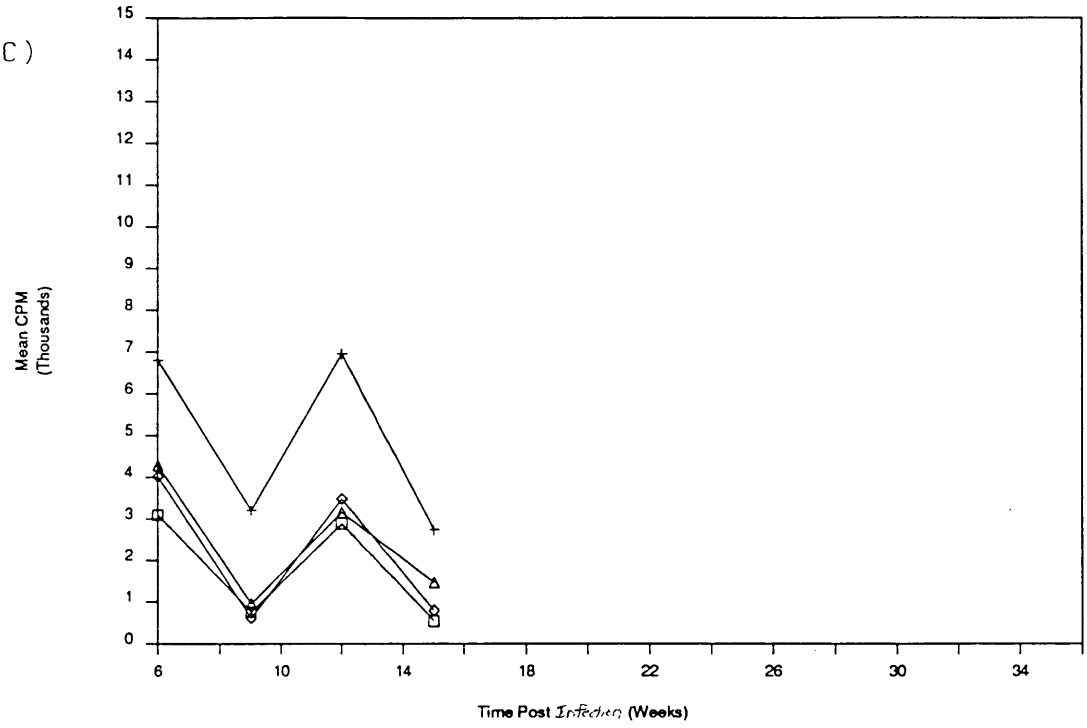
A)


Mean CPM
(Thousands)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
6
10
14
18
22
26
30
34
Time Post Infection (Weeks)

B)


Mean CPM
(Thousands)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
6
10
14
18
22
26
30
34
Time Post Infection (Weeks)

C)


Mean CPM
(Thousands)
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
6
10
14
18
22
26
30
34
Time Post Infection (Weeks)

without antigen. Lymphoproliferative responses to the larval antigen were generally higher than those to the adult worm preparation.

A number of previous studies have also considered the mitogenic effects of parasite antigens. Indeed, S. mansoni (Colley et al., 1977), S. japonicum (Lewart et al., 1979), Trypanosoma brucei (Esuruoso, 1976; Mansfield et al., 1976) and Plasmodium falciparum (Golenser et al., 1985) antigens have all been shown on occasion, to produce in vitro mitogenic effects on host lymphocytes.

Although some antigens, by virtue of their molecular complexity, can be mitogenic (Chapman et al., 1979), extrinsic factors may also contribute to levels of non-specific proliferation. These may include the source, treatment and concentration of foetal calf serum as well as the method of antigen preparation (Esuruoso, 1976). These extrinsic factors may explain the occasional fluctuations in mitogenic activity with time, observed in the present study.

Of immediate concern was the possibility that in vitro mitogenic effects, obscured potential immunogenic responses to the schistosomula and adult worm antigens. As a result, the significance of a response by experimental animals was determined by statistical comparison with responses of similarly treated lymphocytes from naive, age/sex matched control animals.

3.3.6 Lymphoproliferative Responses to S. mansoni Antigens Following a Trickle Infection:

Blastogenic responses of lymphocytes from experimental mice to the three S. mansoni antigens (i.e. egg, schistosomula and adult worm) are shown in Fig. 3.7. Statistical analyses are provided in Table A3.3.

Figure 3.7

Lymphoproliferative responses to S. mansoni antigens following a trickle infection

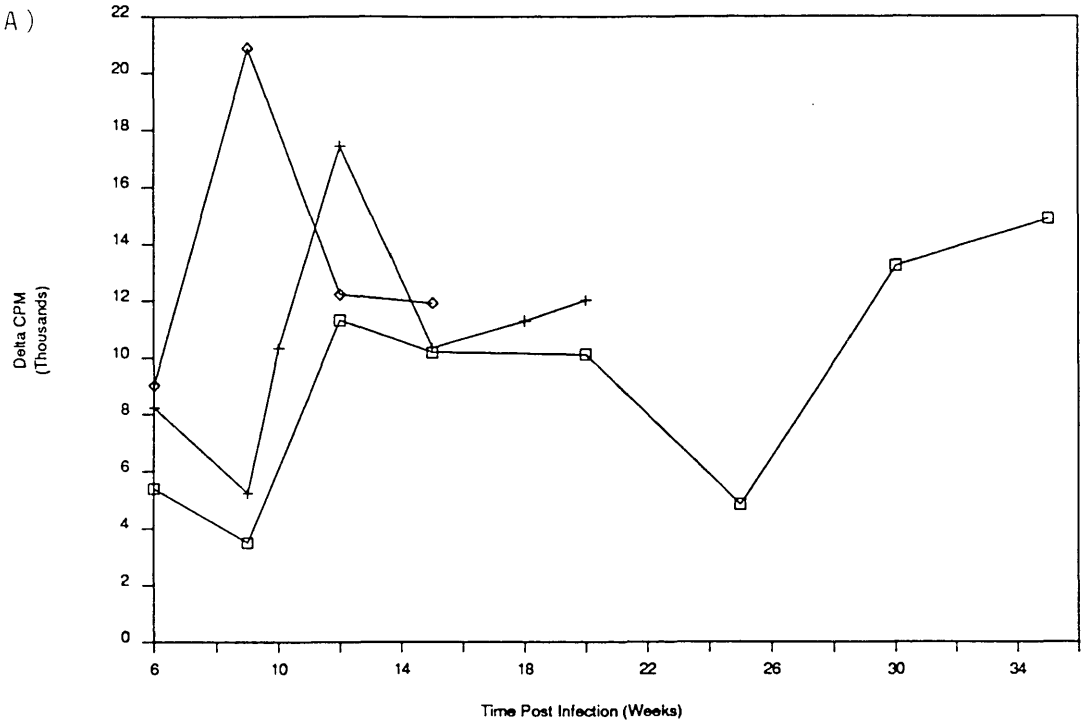
Data points represent lymphoproliferative responses of mice to S. mansoni antigens following exposure to 1 cercaria/mouse/week (squares), 3 cercariae/mouse/week (crosses) and 10 cercariae/mouse/week (diamonds).

A) Responses to schistosomula antigen

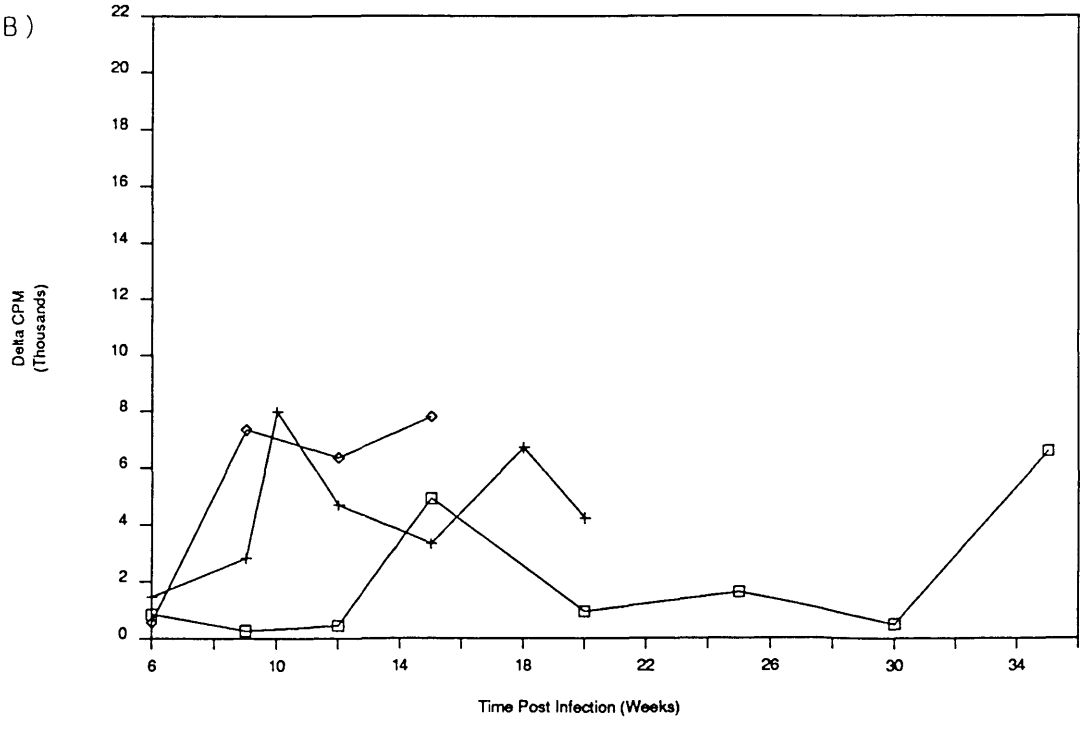
B) Responses to SEA

C) Responses to adult worm antigen

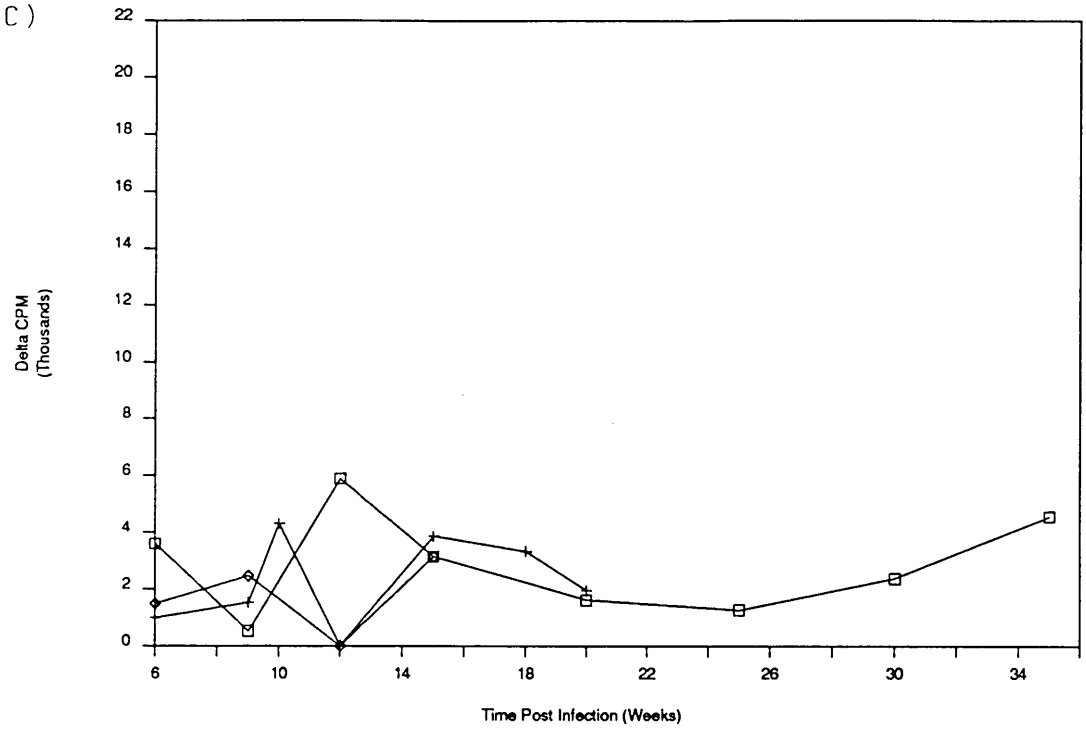
A)


Delta CPM
(Thousands)
0
2
4
6
8
10
12
14
16
18
20
22
6
10
14
18
22
26
30
34
Time Post Infection (Weeks)

B)


Delta CPM
(Thousands)
0
2
4
6
8
10
12
14
16
18
20
22
6
10
14
18
22
26
30
34
Time Post Infection (Weeks)

C)


Delta CPM
(Thousands)
22
20
18
16
14
12
10
8
6
4
2
0
6
10
14
18
22
26
30
34
Time Post Infection (Weeks)

Overall, lymphoproliferative responses to the schistosomula antigen were superior to those elicited by the egg and adult worm antigens. The latter preparation proved to elicit the lowest responses. Indeed, responses of experimental mice to the adult antigen rarely exceeded control values, irrespective of infection dose. In contrast, the pattern of cellular reactivity after stimulation with larval and egg antigens through time, varied with the intensity of parasite exposure. Thus, at the lowest cercarial dose, significant responses to the schistosomula antigen and SEA were not detected before the 12 and 35 week sampling periods, respectively. Overall, blastogenic responses to these antigens did not show any significant increases or decreases with time, at the lowest intensity of exposure. Conversely, anti-larval responses of mice exposed to 3 and 10 cercariae/mouse/week were significantly elevated by 6 weeks post-initial exposure. At the moderate dose of infection, responses to this antigen peaked by 12 weeks and then declined slightly to a stable plateau, remaining significantly elevated for the duration of the study. Anti-larval responses of heavily exposed mice were significantly higher than control values throughout the experimental period and peaked by 9 weeks post-initial exposure before declining significantly to a stable plateau for the two remaining sampling periods. Of significant interest, was the observation that anti-larval responses of moderately and heavily exposed mice had plateaued at similar levels by 15 weeks.

Interestingly, significant lymphoproliferative responses to SEA by splenocytes of moderately and heavily exposed mice were not detected until 9 and 10 weeks respectively. Anti-egg responses of mice receiving 10 cercariae/mouse/week remained unchanged and significantly elevated over control values for the duration of the study. Conversely, in response to SEA, blastogenic responses of moderately exposed mice fell to insignificant levels at 15 and 20 weeks post-initial exposure.

3.3.7 Lymphoproliferative Responses to Concanavalin A:

Blastogenic responses of experimental and naive control mice to the T-cell mitogen, concanavalin A (ConA) are compared in Fig. 3.8. Statistical analyses are provided in Table A3.4.

Overall, the lymphoproliferative responses of cells from experimental mice to ConA, were lower than responses of cells derived from corresponding age-matched control animals. The significance of this difference in reactivity, expressed by the two groups of animals, was most apparent at the higher intensities of cercarial exposure. Thus, at the moderate intensity of exposure, lymphoproliferative responses displayed by cells from naive mice became significantly elevated over responses of experimental animals by 10 weeks post-initial exposure. ConA-driven responses of splenocytes from infected mice remained significantly depressed until 20 weeks when delta CPM values for the two groups became similar. Interestingly, lymphocytes derived from 9-week exposed mice were hyper-reactive relative to those of naive controls. In contrast, splenocytes derived from mice exposed to 10 cercariae/mouse/week were hyporesponsive to ConA by 6 weeks and remained depressed relative to naive controls until 15 weeks, when the response of cells from the two groups became similar.

Figure 3.8

Lymphoproliferative responses to concanavilin A

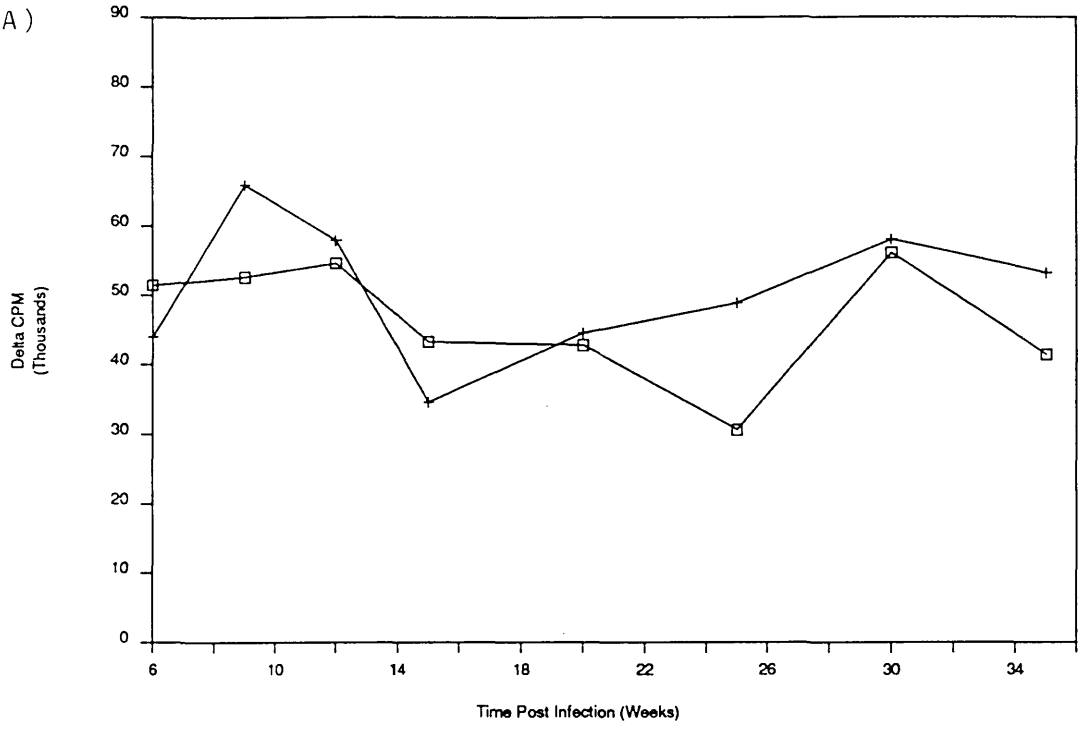
Data points represent lymphoproliferative responses of experimental (squares) and naive age/sex matched control mice (crosses) to the T-cell mitogen, concanavalin A, following a trickle infection.

A) 1 cercaria/mouse/week

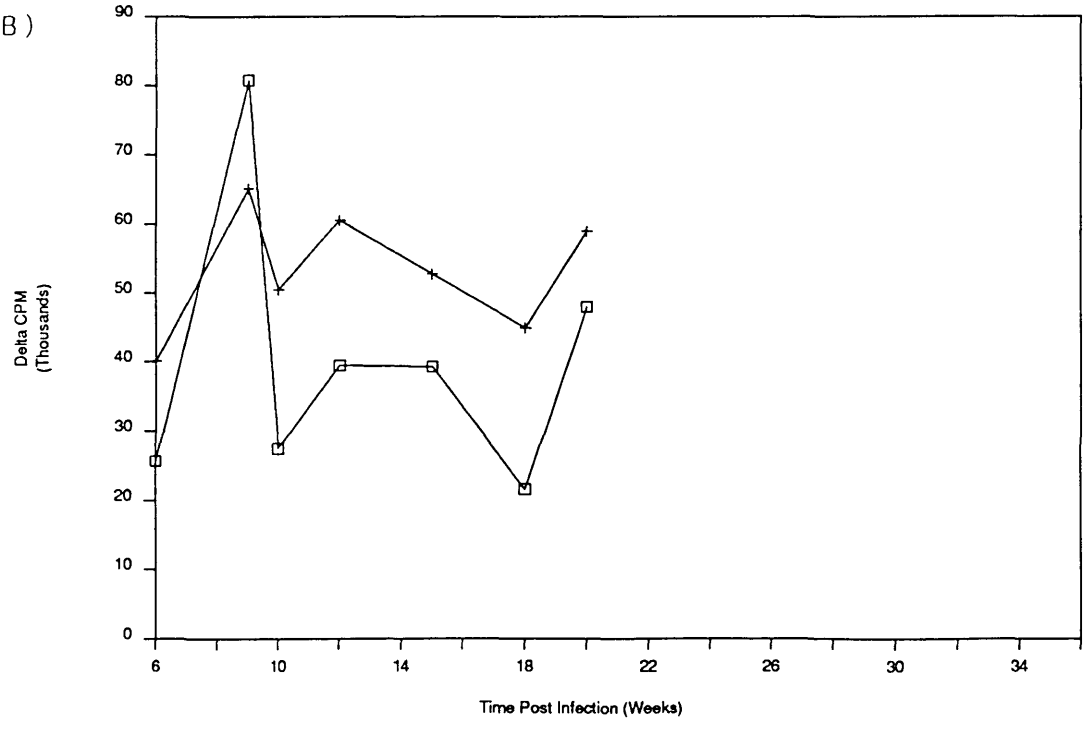
B) 3 cercariae/mouse/week

C) 10 cercariae/mouse/week

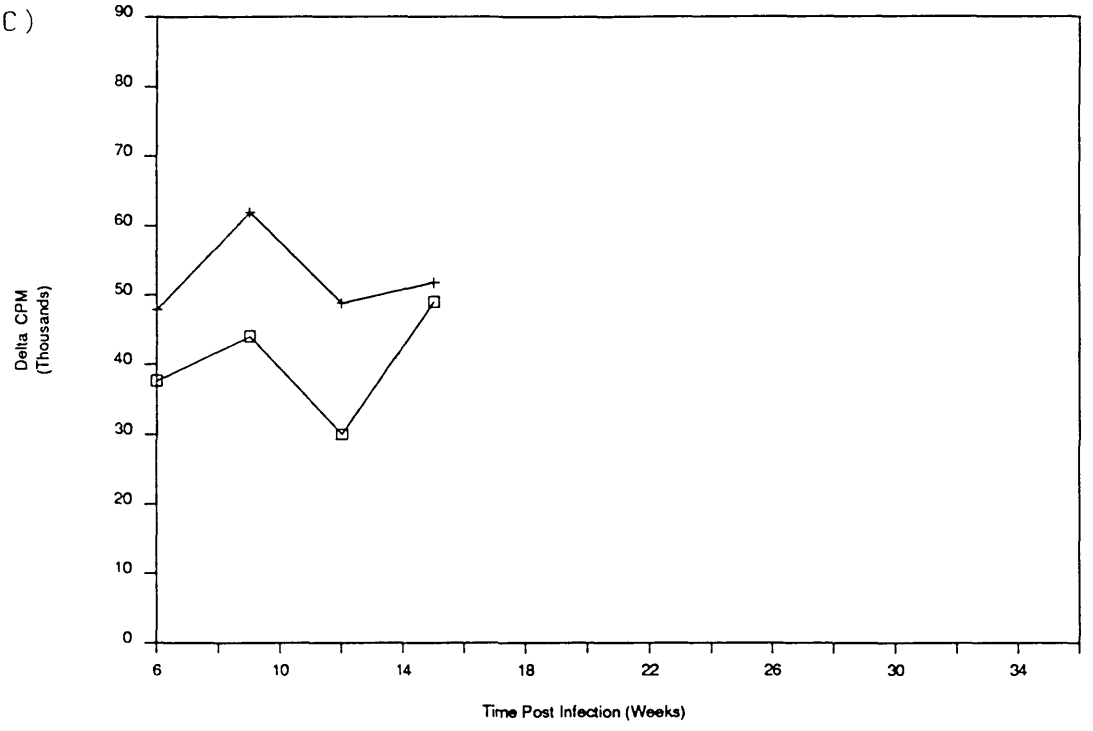
A)


90
80
70
60
50
40
30
20
10
0
Delta CPM
(Thousands)
6
10
14
18
22
26
30
34
Time Post Infection (Weeks)

B)


90
80
70
60
50
40
30
20
10
0
Delta CPM
(Thousands)
6
10
14
18
22
26
30
34
Time Post Infection (Weeks)

C)


90
80
70
60
50
40
30
20
10
0
Delta CPM
(Thousands)
6
10
14
18
22
26
30
34
Time Post Infection (Weeks)

3.3.8 Immunoglobulin Levels Following a Trickle S. mansoni Infection.

IgG:

The levels of IgG antibody specific to the S. mansoni antigens as determined by ELISA, are shown in Fig. 3.9. Statistical analyses are provided in Table A3.5.

The amount of IgG antibody present in the sera of mice that was directed towards each S. mansoni antigen varied directly with the cercarial dose experienced by the experimental animals. Within each dose of infection, IgG responses to the adult worm preparation exceeded those to the larval antigen and SEA, which in turn did not differ from each other. Irrespective of the antigen preparation considered, significant increases in IgG concentrations were not detected until 12 weeks in mice receiving the two lowest cercarial doses, while IgG levels became significantly elevated by 9 weeks in mice exposed to 10 cercariae/mouse/week. In response to all antigen preparations, IgG levels of mice exposed to 1 cercaria/mouse/week increased gradually throughout the 35-week trickle exposure period. In contrast, at the two higher doses of cercarial exposure, IgG antibody responses to the S. mansoni antigens appeared to peak by 15 or 20 weeks and remained unchanged thereafter.

IgM:

The levels of IgM antibody specific to the S. mansoni antigens as determined by ELISA are shown in Fig. 3.10.

Irrespective of the S. mansoni antigen considered, IgM levels present in the sera of mice varied directly with the intensity of cercarial exposure.

Figure 3.9

Immunoglobulin levels (IgG) following a trickle infection

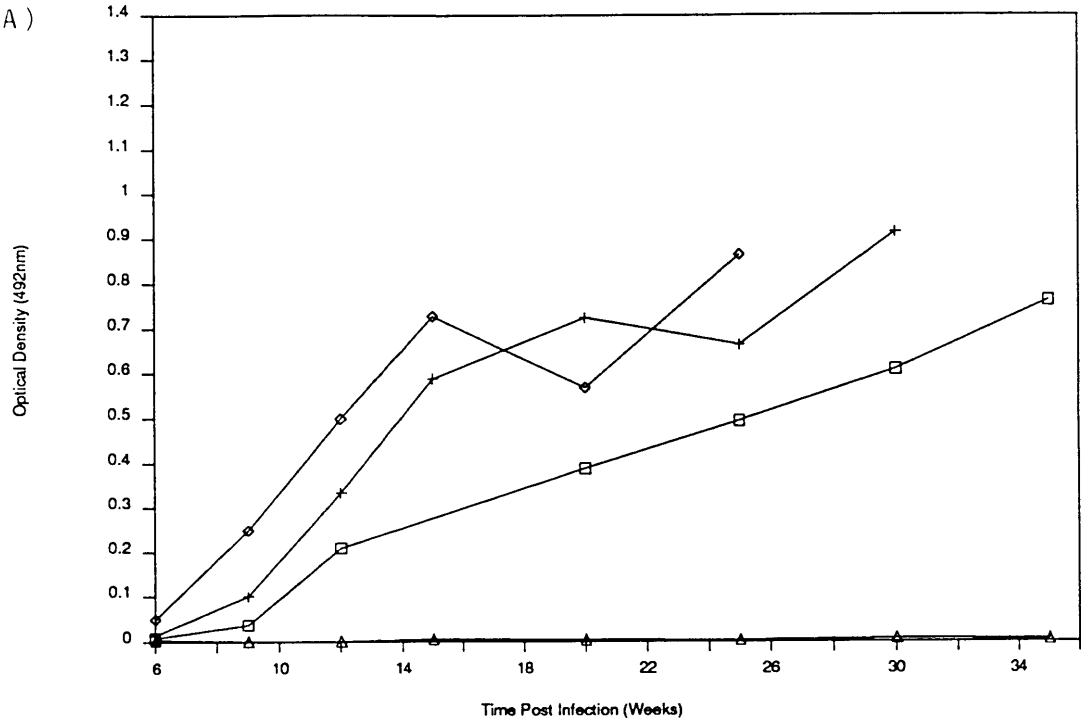
Data points represent mean IgG responses of mice to S. mansoni antigens following exposure to 1 cercaria/mouse/week (squares), 3 cercariae/mouse/week (crosses) and 10 cercariae/mouse/week (diamonds). Responses of age/sex matched control mice are also shown (triangles).

A) Responses to schistosomula antigen

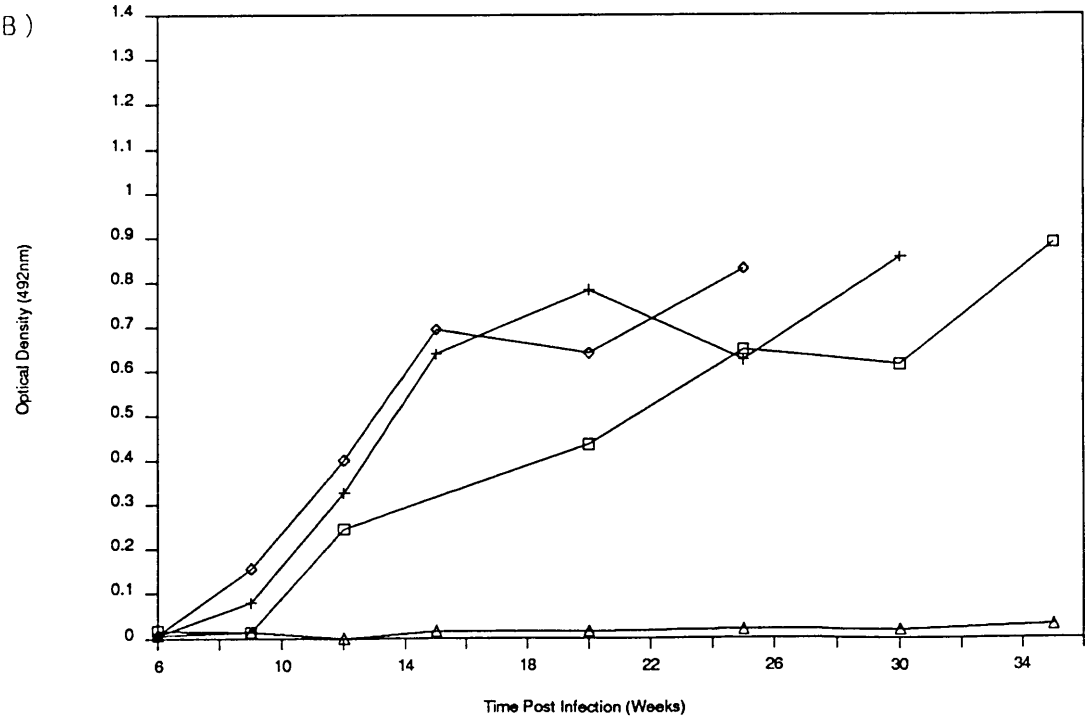
B) Responses to SEA

C) Responses to adult worm antigen

A)


Optical Density (492nm)
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
6
10
14
18
22
26
30
34
Time Post Infection (Weeks)

B)


Optical Density (492nm)
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
6
10
14
18
22
26
30
34
Time Post Infection (Weeks)

C)

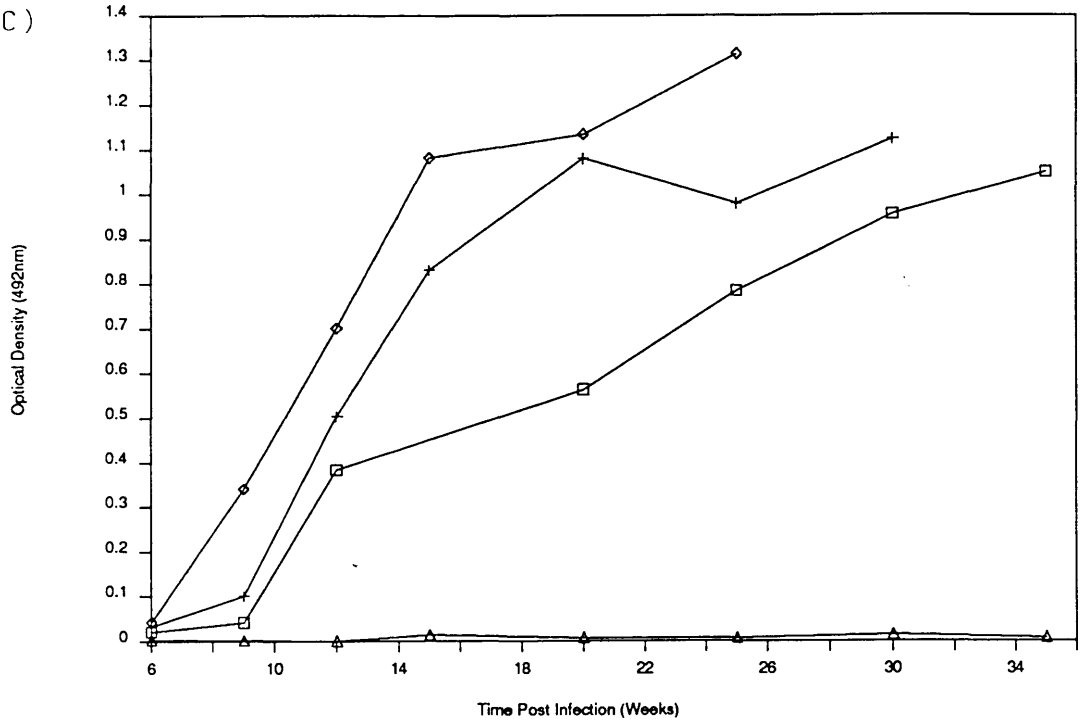

Optical Density (492nm)
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
6
10
14
18
22
26
30
34
Time Post Infection (Weeks)

Figure 3.10

Immunoglobulin levels (IgM) following a trickle infection

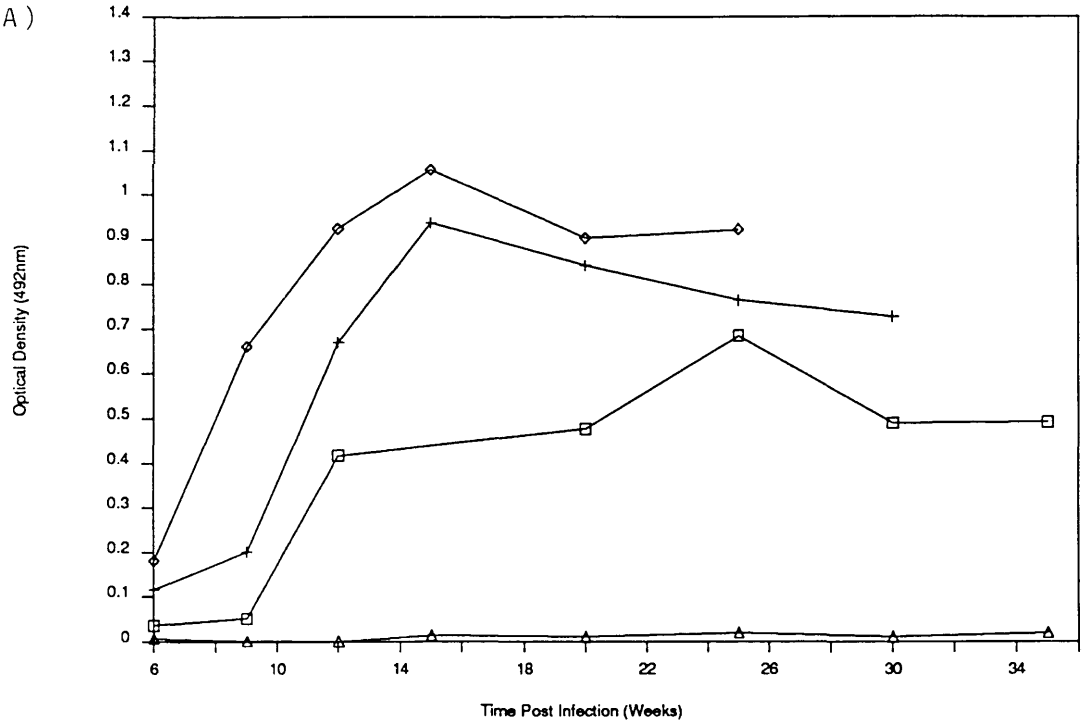
Data points represent mean IgM responses of mice to S. mansoni antigens following exposure to 1 cercaria/mouse/week (squares), 3 cercariae/mouse/week (crosses) and 10 cercariae/mouse/week (diamonds). Responses of age/sex matched control mice are also shown (triangles).

A) Responses to schistosomula antigen

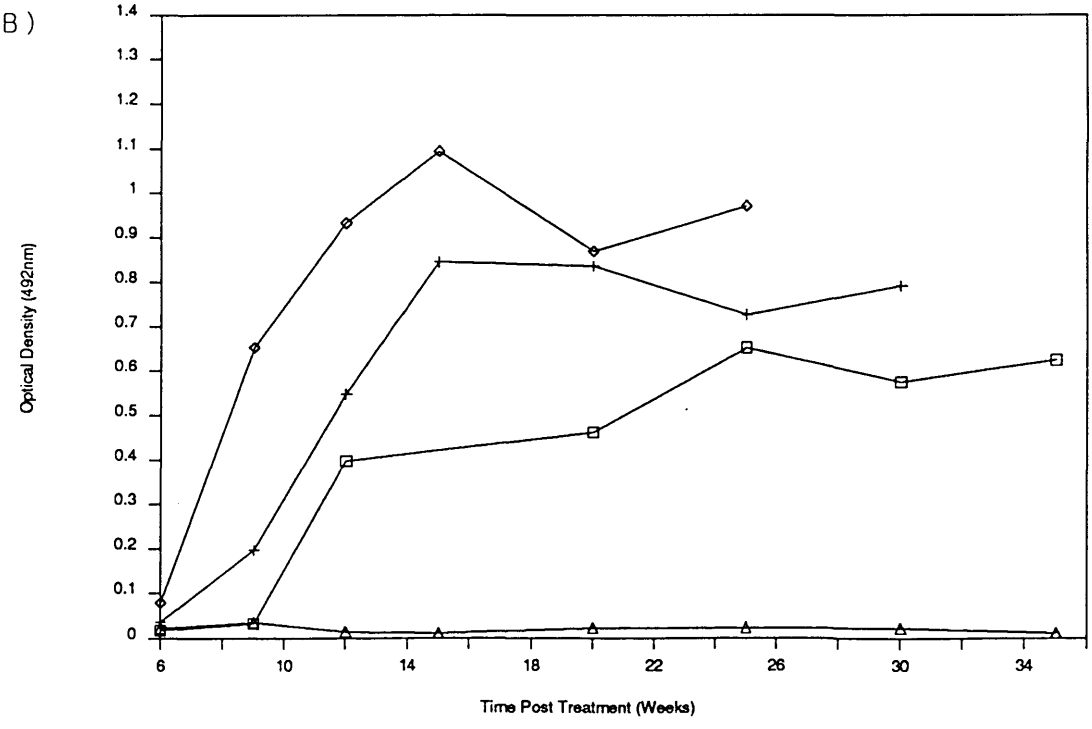
B) Responses to SEA

C) Responses to adult worm antigen

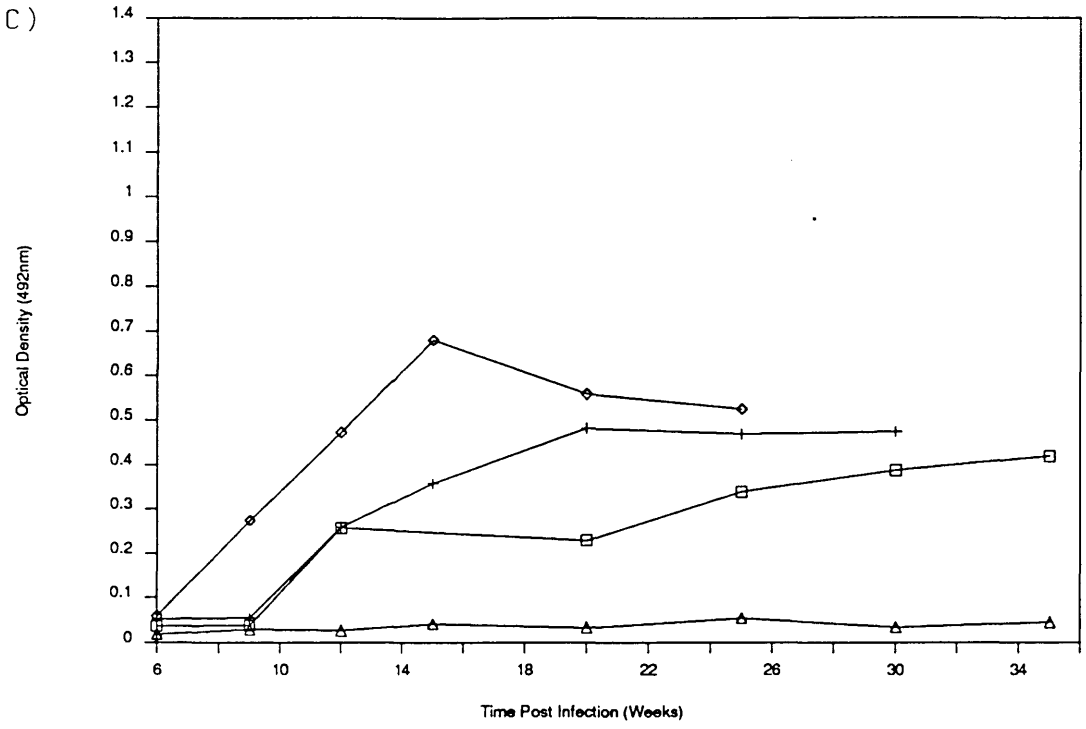
A)


Optical Density (492nm)
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
6
10
14
18
22
26
30
34
Time Post Infection (Weeks)

B)


Optical Density (492nm)
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
6
10
14
18
22
26
30
34
Time Post Treatment (Weeks)

C)


Optical Density (492nm)
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
6
10
14
18
22
26
30
34
Time Post Infection (Weeks)

In contrast to the pattern observed for levels of IgG antibody, anti-schistosomula and anti-SEA IgM antibody levels exceeded anti-adult worm activity. However, IgM responses to the two former antigens were again similar. Mice exposed to 1 cercaria/mouse/week, first expressed significant IgM responses to all antigens at 12 weeks post-initial exposure. This peak response than remained unchanged for the duration of the study. At the two higher intensities of exposure, IgM activity to all antigens peaked by 15 or 20 weeks and remained at that level for the remainder of the experimental period.

3.4 Discussion

In the present study, male CBA/Ca mice were exposed to repeated low level doses of S. mansoni cercariae over an extended period of time. The age-intensity profile generated by the trickle infection was dependent upon the intensity of cercarial exposure as previously described by Crombie and Anderson (1985). Parallel studies of immune responsiveness revealed that the pattern of cellular and humoral responses was also dependent upon the intensity of S. mansoni infection.

Mice exposed to the lowest dose of 1 cercaria/mouse/week, exhibited a gradual monotonic rise in mean adult worm burden. However, had the duration of the study been extended beyond 35 weeks, the total mean worm burden may have reached a stable plateau. In contrast, at the moderate intensity of exposure, mean worm burdens increased gradually to a plateau and then declined slightly by 25 weeks post-initial exposure. As this occurred in the presence of continued exposure, it would appear that animals of this experimental group developed an acquired resistance to infection. The term "resistance" rather than "immunity" must be applied to the present

study as no attempt was made to consider the effects of pathological changes on adult worm establishment (see Wilson et al., 1983). At the highest intensity of exposure, worm burdens rose to peak levels by 12 weeks post-initial exposure but failed to decline thereafter. Nevertheless, the rapid decline in juvenile worm recovery at this dose of infection suggests that animals had developed some resistance to infection.

The age-intensity profiles generated in the present study appear similar to infection patterns frequently observed in endemic situations. The age-intensity profile generated by mice exposed to the moderate dose of cercarial exposure is characteristic of the convex form frequently encountered in human communities, where there is a gradual acquisition of resistance with increasing experience of infection (Anderson, 1987). Interestingly, at the highest intensity of exposure, worm burdens reached a stable plateau but did not become convex in form. This may parallel situations encountered in areas of high endemicity where egg output remains stable or tends to increase throughout adult life (Smith et al., 1979). This pattern is not likely to result from the absence of immunity, but rather from the very intense levels of exposure with the immune system serving to maintain parasite burdens at levels which are tolerable to the host (Wilkins, 1987). That parasite populations may be limited by the level of transmission as well as host immunity is also reflected by the infection pattern generated by mice exposed to low intensities of cercarial exposure. Thus, Smithers and Terry (1969) suggest that in areas of low endemicity years of exposure may be required before host resistance to infection becomes apparent.

In the present study, juvenile worms were recovered from mice, despite relatively long durations of previous exposure to infection and irrespective of the infection level. It is likely that the observed patterns of infection reflect the incomplete nature of host resistance to S. mansoni

infection (Colley and Colley, 1989) and the dynamic interplay between parasite establishment and host mortality (Crombie, 1985). This may be similar to human communities where S. mansoni is endemic. As people age, the gradual build up of immunity and a gradual reduction in water contact leads to a reduction in the rate of parasite acquisition. The lowered rate of parasite recruitment in older individuals does not adequately compensate for the mortality of existing worms, with the result that the intensity of infection declines to a lower level until a new equilibrium is reached, in which the rates of acquisition and loss of infection are again similar (Wilkins, 1987).

The relationship between the age-intensity profiles and patterns of immune responsiveness observed in the present study may also be similar to that observed in human communities. Thus, at the lowest intensity of exposure, significant blastogenic responses to S. mansoni antigens were not detected until relatively late in the study period, despite continued exposure to the parasite. Similarly, human studies conducted in areas of low endemicity have demonstrated that longer periods of time are required before positive delayed hypersensitivity reactions can be detected (McKay et al., 1973; Colley et al., 1977). McKay et al. (1973) suggest that lighter infections which expose the host to smaller amounts of antigen, may require a longer time to stimulate the same degree of hypersensitivity that results in a shorter time from heavier infections. Thus, the intensity and duration of infection will both effect the levels of cell-mediated immunity (CMI) elicited. This would also seem to apply to the present study where statistically significant blastogenic responses to larval and egg antigens were detected much earlier following higher intensities of exposure by comparison with lower levels. That this earlier induction of cellular reactivity is associated with the eventual acquisition of resistance cannot be concluded from this study. However, Phillips et al. (1977) have reported that the onset

of resistance in the rat model is dependent upon the sensitization of T-lymphocytes.

The exposure of mice to 3 or 10 cercariae/mouse/week elicited a rapid and strong cellular response to the schistosomula antigen. However, immediately following the peak response, delta CPM values were observed to decline to stable plateaus. This decline in blastogenic activity was significant at the highest intensity of exposure and by 15 weeks, proliferative responses of the two experimental groups were similar. This occurred despite the presence of higher worm burdens in the most heavily exposed animals. Similarly, an increase in hyporesponsiveness to the T-cell mitogen ConA with increasing intensity of infection was observed in the present study. The decreased ability of lymphocytes from heavily infected human subjects to respond to mitogens and S. mansoni antigens, has been previously observed (Ellner et al., 1981; Feldmeier et al., 1985b). As granulomas formed around S. mansoni eggs in the tissues are primarily a manifestation of delayed hypersensitivity (Warren et al., 1967), modulation of cell-mediated immunity may reduce parasite-induced pathology (Nash et al., 1982).

Overall, lymphoproliferative responses to schistosomula antigen were higher than those to SEA and the adult membrane preparation, with the latter antigen being the least effective stimulus. Furthermore, T-cell reactivity towards the schistosomula preparation was observed prior to anti-SEA activity. The extremely high blastogenic responses to the larval antigen may be due to factors associated with the preparation of the antigen. Alternatively, repeated exposure of the host to the larval forms of the parasite may have induced elevated responses to this antigen preparation. Indeed, Katz and Colley (1976) have shown that multiple exposures produce optimal blastogenic activity to cercarial antigens. Whether or not these anti-

larval responses (in the absence of anti-SEA reactivity) contribute to the acquisition of resistance is uncertain. However, the presence of host resistance to S. mansoni infection, in the absence of patent infections under field conditions, has been considered to result from effective immune responses elicited by exposure to larval forms (Colley, 1981; Wilkins et al., 1987). Furthermore, it is clear from studies involving the use of irradiated cercariae that extended exposure to schistosomula , in the absence of patent infections, can induce significant levels of resistance.

Lymphoproliferative responses to SEA were lower than those to the schistosomula antigen and at the moderate dose of infection, were not consistently elevated above control values. Low blastogenic responses to the egg antigen may be due to the SEA-sensitized lymphocytes having less access to the circulation, as they may be trapped in granulomas (El-Bassiouny et al., 1987). Surprisingly, anti-SEA responses of heavily exposed mice did not decline from peak levels. This has been observed in human situations and may be due to repeated stimulation by large quantities of egg antigen (Reiner et al., 1979).

The inability of adult membrane antigens to stimulate lymphocytes to blastogenesis has been observed previously using human cell cultures (Gazzinelli et al., 1983). Furthermore, the same authors also observed schistosomula tegument preparations to be strong stimulants of blastogenic activity. Together, these observations would appear to support the view that adult worms are somewhat impervious to immune attack while larval forms are the main focus of immune reactivities (Smithers and Terry, 1969).

In contrast to the pattern of CMI, IgG responses to S. mansoni antigens increased gradually to peak levels at the two highest intensities of exposure, and remained unchanged for the remainder of the study irrespective of the antigen considered. The concentration of IgG antibody in

the sera of mice exposed to 1 cercaria/mouse/week increased linearly throughout the study and did not appear to stabilize. In contrast, irrespective of dose and antigen, IgM antibody levels were observed to peak between 12 and 20 weeks post-initial exposure and remained unchanged thereafter.

In general, the concentration of IgG antibody measured to each antigen appeared to correlate with exposure and worm burden. A similar relationship between levels of IgG antibody and intensity of infection in humans has been described (Butterworth et al., 1985; Roberts et al., 1987)

A role for humoral mechanisms in the acquisition of resistance to S. mansoni infection has been demonstrated by studies involving the passive transfer of immune sera (Sher et al., 1975; 1977). It is generally believed that IgG antibodies are responsible for the mediation of this immunity (Butterworth et al., 1977). In contrast, IgM antibodies are not cytotoxic to schistosomula in vitro but rather, appear to inhibit eosinophil-dependent killing mediated by IgG antibodies (Khalife et al., 1986; Yi et al., 1986).

In the present study, the role of each antibody class in the acquisition of resistance to S. mansoni cannot be determined. It may not be appropriate to compare responses between the different antigen preparations, as the number and affinity of epitopes in each assay is unknown (Dunne et al., 1988).

However, important points are raised when considering the pattern of humoral responses observed in the present study. First , it has been suggested that the quantity of protective antibody in the sera of the host may be crucial for the expression of resistance to schistosome infection (Butterworth, 1984; Vignali et al., 1989c). Thus, it was observed that concentrations of IgG antibody in the sera of mice exposed to 1 cercaria/mouse/week were lower than in groups of mice which did develop levels of acquired resistance. Furthermore, IgG activity in mice exposed to 1

cercaria/mouse/week did not appear to attain maximum levels.

The dichotomy of decreased T-cell reactivity and increasing antibody levels has been previously observed in both murine and human situations (Boros et al., 1975; Feldmeier et al., 1985a). This has led to the suggestion that the early stimulation of cell-mediated immunity is sufficient to promote resistance to S. mansoni infection, before antibodies become elevated and begin to assume a protective role (McLaren and Smithers, 1987). It is also possible that serum-derived factors contribute to the eventual suppression of cell-mediated activity and, consequently, granuloma formation in the tissues of the host (Boros et al., 1975).

In conclusion, mice exposed to repeated low level doses of S. mansoni cercariae generate age-intensity profiles and patterns of immune reactivity, which are dependent upon the intensity and duration of cercarial exposure. Although these trends are similar to those observed in endemic situations, the factors which are responsible for their generation in the murine host may not be the same as those operating in human subjects. Nevertheless, results derived from trickle infection studies in the laboratory, may help to unravel the complex and dynamic relationship between epidemiological and immunological parameters, and resistance to S. mansoni infection.

Chapter 4

Dynamics of Residual Resistance and Immune Responsiveness Following the Interruption of a Trickle Infection with Praziquantel

4.1 Introduction

One approach to studying resistance to schistosome infection in experimental models has been to follow the pattern of parasite acquisition following chemotherapeutic treatment (Dean, 1983). In this way, worm burdens derived from a primary exposure are eliminated, allowing for the evaluation of host protection in the absence of adult worms (i.e. the effects of concomitant immunity). Control programmes in endemic communities frequently include the treatment of infected individuals (Warren, 1982). Thus, experimental studies of this nature may have particular relevance to the human situation where concomitant immunity may also play a role in host protection (Bradley and McCullough, 1973). If concomitant immunity is eliminated, it is possible that future infections may be of greater intensity and induce greater levels of morbidity (Andrade and de Brito, 1982).

Recent studies in Kenya (Butterworth et al., 1984; 1985) and Brazil (Dessein et al., 1988) have reported resistance to S. mansoni infection following chemotherapeutic cure. In both situations resistance appeared to be dependent upon the duration of primary infection prior to treatment, with older individuals being more resistant than younger subjects. Similarly, using the mouse model, Tawfik and Colley (1986) have shown that mice infected for 20 weeks demonstrate higher levels of resistance following drug treatment than 10-week infected animals. As susceptibility to reinfection in

the human studies could not be attributed to differences in water contact patterns, a role for acquired immunity was suggested. Consequently, attempts have been made to correlate immune responses with resistance, with a view towards identifying potential vaccinating antigens. Indeed, results from the Brazilian study suggest a relationship between levels of resistance and IgG responses to a 37-kDa surface antigen. Alternatively, a role for cell-mediated immunity has been suggested by Colley et al. (1986) who observed that Egyptian subjects with low peripheral blood mononuclear cell (PBMN) proliferative responses were more susceptible to reinfection with S. mansoni than high responding individuals.

It was demonstrated in the previous chapter that mice exposed to a trickle infection of 3 cercariae/mouse/week exhibit an age-intensity profile similar to that observed in human communities. The pattern of cellular and humoral responses to schistosome preparations was also characteristic of that frequently encountered in human communities with endemic infection.

The aim of the present study was to investigate the relationship between the duration of a primary trickle infection and resistance to reinfection with S. mansoni following chemotherapeutic treatment. Mice were exposed to either a 10 or 18 week trickle exposure prior to the administration of praziquantel. These time periods were previously observed to represent acute and chronic phases of infection, respectively.

The pattern of cellular and humoral responses to S. mansoni antigens post-treatment was also monitored in an attempt to relate levels of immune responsiveness with host protection.

4.2 Problems in the maintenance of the mouse host.

During the course of this study, two problems were encountered which have complicated the interpretation of the results.

The first problem became apparent by 4-6 weeks post-infection, when a number of experimental and treated control mice developed a bacterial eye infection. A small number of severely affected animals were immediately destroyed while the remaining animals were successfully treated within 10 days with Neomycin^{R} antibiotic ointment. As age/sex matched controls remained unaffected by the bacterial infection, it is possible that S. mansoni-induced immunosuppressive effects contributed to the expression of the eye infection. Alternatively, as infection tanks were not regularly sterilized, it is possible that the bacteria were transmitted during the infection procedure thereby exposing experimental and treated control animals only. Furthermore, age/sex matched control animals were housed separately from S. mansoni exposed mice and could therefore avoid contact with animals harbouring the bacterial infection.

A second problem became apparent at the time of blood collection. It was observed that mice purchased from two external sources were susceptible to death during the tail bleeding procedure. This occurred irrespective of infection status and could not be attributed to any latent viral, bacterial or fungal infections as determined by microbiological screening of the animals. Alternatively, it is suggested that unusually warm conditions encountered in the Animal Unit during the course of the experiment, may have rendered some mice susceptible to stress related mortality.

The combined effect of the two problems along with natural and S. mansoni induced host mortality reduced sample sizes in mouse groups. As a result, insufficient numbers of treated control mice infected for 18 weeks prior to chemotherapy were available for blood collection. While significant

effects on epidemiological or immunological parameters were not detected, reduced sample sizes may have contributed to elevated variances and the masking of potentially significant results.

4.3 Methods

The experimental design is illustrated in Fig. 4.1.

Three hundred and thirty, 3 week old male CBA/Ca mice were divided into three groups of 120, 120 and 90 animals. These groups comprised the experimental (E), treated control (T) and age/sex matched control (C) groups respectively. Experimental mice received either a 10 or 18-week primary trickle infection before praziquantel was administered. At 1, 5 or 10 weeks post-treatment, groups of 20 mice were re-exposed to a 6 week trickle infection. Challenged animals were than killed (at 7, 11 or 16 weeks after treatment) and either perfused or examined for their in vitro cellular reactivity to egg, larval and adult worm antigens. Blood samples were collected from all animals. The length of time between treatment and challenge was varied to help assess the duration of residual resistance following chemotherapy.

Treated control mice received the primary trickle infection and praziquantel treatment but no challenge dose. The purpose of this group was to monitor residual worm burdens surviving chemotherapeutic treatment and to assess the effect of post-treatment challenge on immune reactivities.

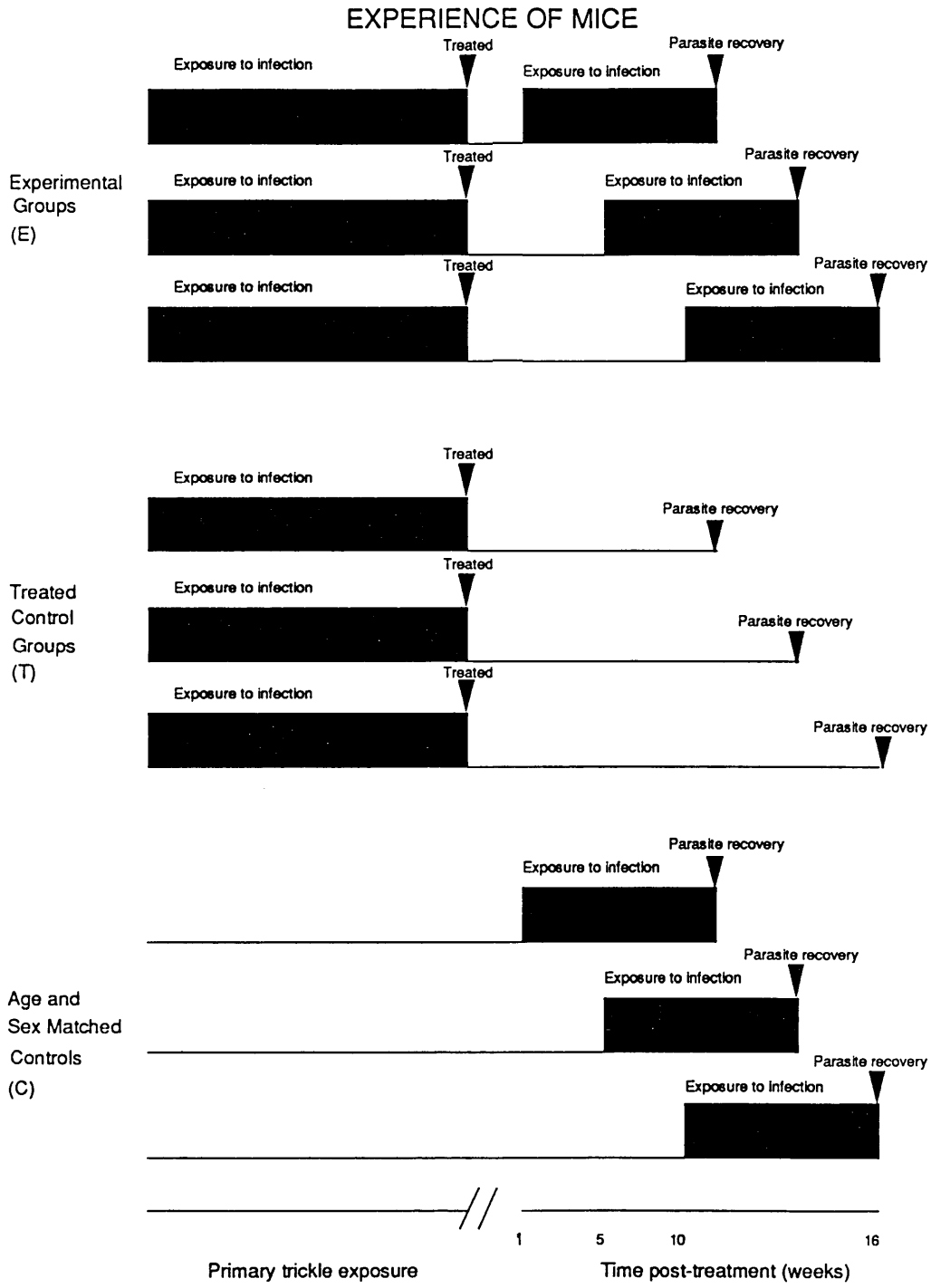
The age/sex matched control group did not receive the primary trickle infection or praziquantel treatment but were exposed to the 6-week challenge infection at the same time as the corresponding group of experimental mice. As these mice had no previous experience of infection, they could be used to evaluate levels of resistance acquired by experimental animals. Resistance was also expressed as percent reduction in parasite establishment as

Figure 4.1

Experimental protocol used for assessing residual resistance after chemotherapy*

Levels of residual resistance were compared between groups of mice receiving primary trickle exposures of either 10 or 18 weeks.

*Adopted from Sithithaworn (1987)


EXPERIENCE OF MICE
Experimental Groups (E)
Treated
Parasite recovery
Exposure to infection
Exposure to infection
Treated
Parasite recovery
Exposure to infection
Exposure to infection
Treated
Parasite recovery
Exposure to infection
Exposure to infection
Treated Control Groups (T)
Treated
Exposure to infection
Parasite recovery
Treated
Exposure to infection
Parasite recovery
Treated
Exposure to infection
Parasite recovery
Age and Sex Matched Controls (C)
Parasite recovery
Exposure to infection
Parasite recovery
Exposure to infection
Parasite recovery
Exposure to infection
1
5
10
16
Primary trickle exposure
Time post-treatment (weeks)

calculated from the formula: C-(E-T)/C x100.

4.4 Results

4.4.1 Worm Burdens:

Parasite recoveries from experimental animals and their respective treated controls at all times post-treatment are shown in Figs. 4.2a & b. Since the distribution of the parasites in the samples of mice conformed with the Poisson probability distribution, square root transformed data is presented. Experimental worm burdens remained unchanged during the post-treatment period for both the 10 and 18-week infected mice (F-test, $p>0.05$). Treated control values from the 10-week infected animals showed a slight decline by 16 weeks post-treatment, although no significant change was detected (F-test, $p>0.05$). Similarly, these worm burdens remained stable post-chemotherapy for the 18-week infected group (F-test, $p>0.05$). Worm burdens derived from treated control animals did not differ significantly between 10 and 18-week infected mice at corresponding times post-treatment (t-test, $p>0.05$).

In order to determine the net parasite burden derived from reinfection, the difference between the mean burden of the experimental and corresponding treated control group was calculated. This corrected mean was determined by subtracting the mean treated control value from the mean of each experimental group of mice.

The corrected mean parasite burdens and the age/sex matched controls are compared in Figs. 4.3a & b. For the 10-week infected group, the corrected mean was significantly less than that of the age/sex matched controls at 7 weeks post-treatment (t-test, $p<0.05$). No significant difference was detected at 11 and 16 weeks. Interestingly, no significant differences

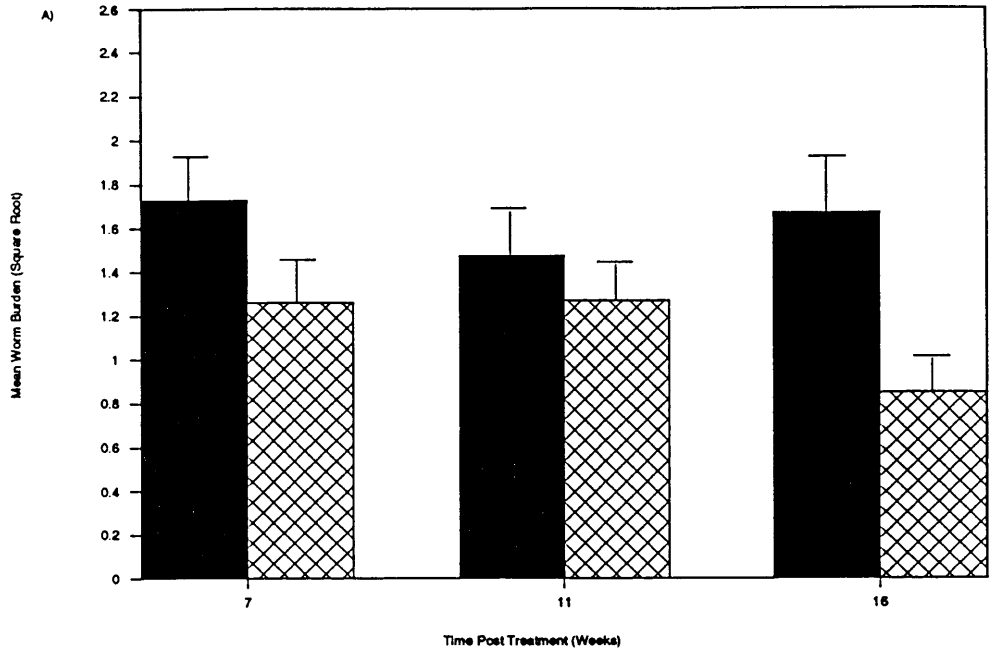
Figure 4.2

Parasite recovery following treatment with praziquantel

A) Histogram bars represent mean parasite burden (square roots) and standard error of the experimental groups (solid) and the treated control groups (hatched) at pre-determined intervals post-treatment.

Mice were exposed to 3 cercariae/mouse/week for 10 weeks prior to treatment. At 1, 5 and 10 weeks post-treatment experimental mice were re-exposed to the previous level of infection for 6 weeks whereas treated control animals received no challenge dose.

B) As for A) except that mice received primary trickle infection for 18 weeks prior to treatment.


A)
Mean Worm Burden (Square Root)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
7
11
16
Time Post Treatment (Weeks)

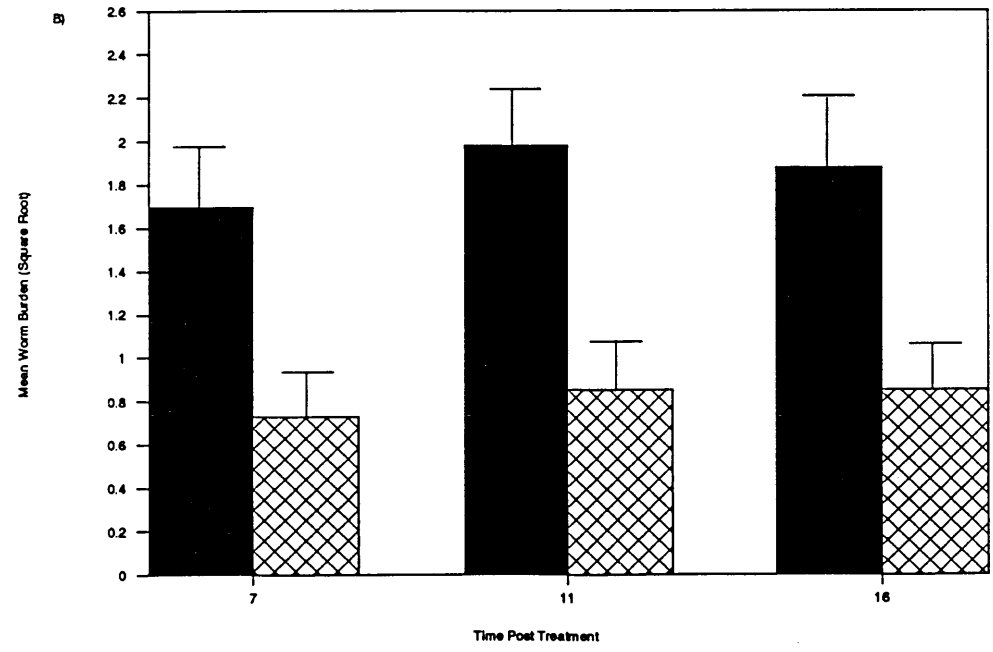

B)
Mean Worm Burden (Square Root)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
7
11
16
Time Post Treatment

Figure 4.3

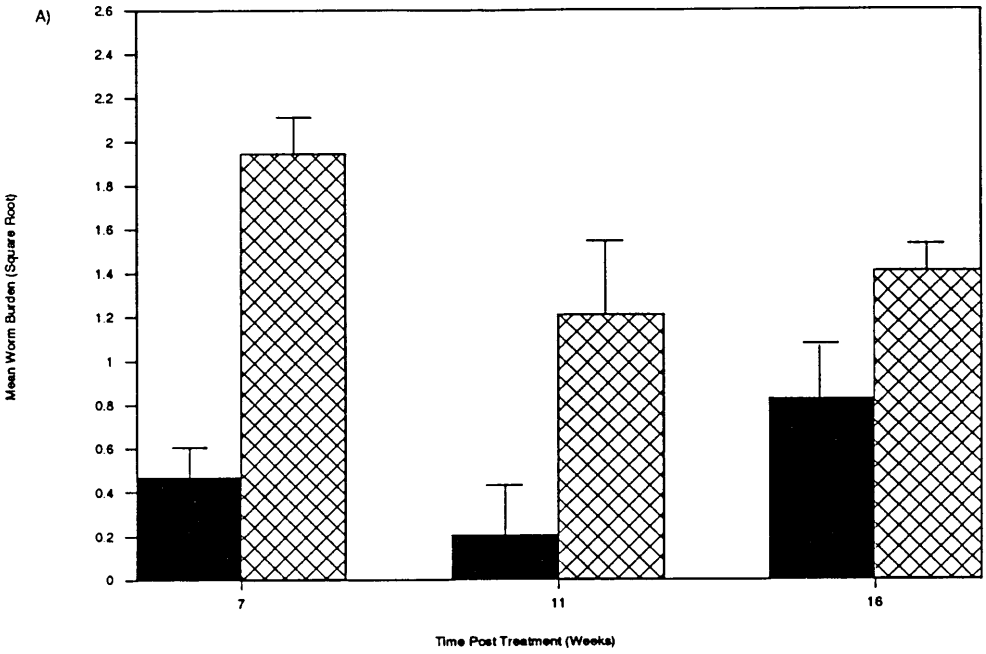
Persistence of residual resistance following chemotherapy

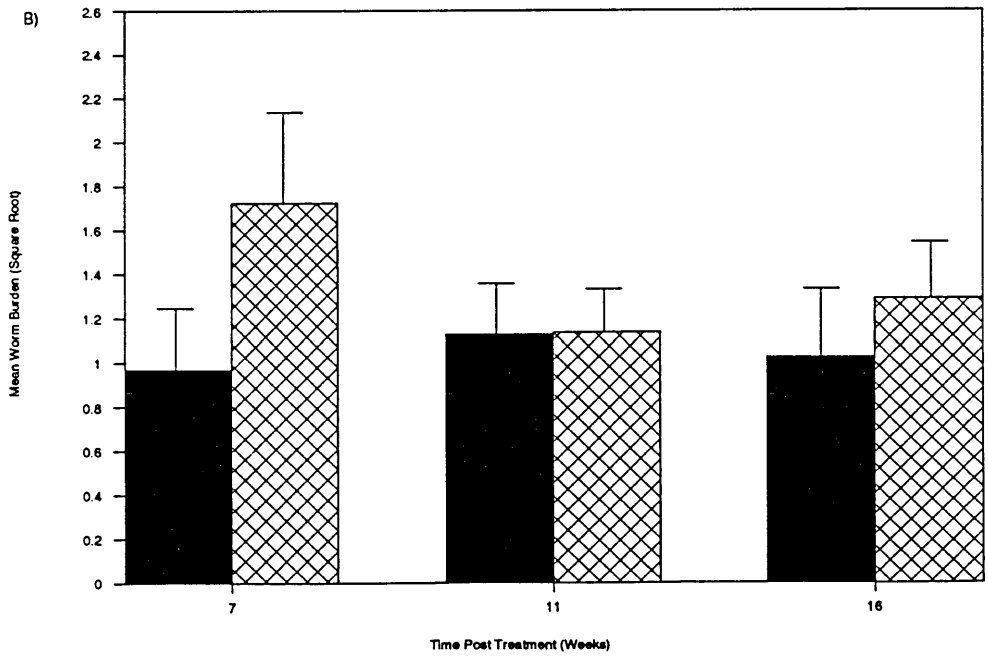
Histogram bars represent the mean parasite burden (square roots) and standard error of the corrected groups (solid) and age/sex matched control groups (hatched) at pre-determined intervals post-treatment.

The duration of primary exposure was:

A) 3 cercariae/mouse/week for 10 weeks

B) 3 cercariae/mouse/week for 18 weeks


A)
Mean Worm Burden (Square Root)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
7
11
16
Time Post Treatment (Weeks)


B)
Mean Worm Burden (Square Root)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
7
11
16
Time Post Treatment (Weeks)

were observed between corrected and age/matched control groups at any time post-chemotherapy for mice treated at 18 weeks. The general pattern of resistance over time post-treatment is illustrated by the observed levels of percent partial protection (Fig. 4.4). For the 10-week infected group, resistance levels of 76%, 87% and 41% were observed at 7, 11 and 16 weeks post-treatment respectively. This compared with corresponding levels 44%, 1% and 20% for mice exposed to an 18-week trickle infection prior to treatment. In support of these observations, the number of challenge juveniles recovered from experimental animals was found to be slightly higher from mice receiving the 18-week primary exposure at all times following chemotherapeutic treatment (Fig. 4.5).

These results suggest that resistance to challenge is more stable if mice are exposed to S. mansoni infection for 10 weeks rather than 18 weeks prior to chemotherapy. Thus, treatment of mice following an 18-week trickle infection leads to a rapid decrease in resistance to reinfection. In contrast, mice receiving a 10-week trickle exposure display high levels of resistance to challenge immediately post-treatment with a slow decline thereafter.

4.4.2 In vitro Proliferative Responses to S. mansoni Antigens:

a) Responses following a 10 week primary exposure:

Cells from mice infected for 10 weeks prior to praziquantel treatment proliferated more than cells from naive mice when cultured with schistosomula antigen and SEA at 7 and 11 weeks post-chemotherapy. At 11 weeks post-treatment these responses were significantly higher than treated control values, but similar at other times post-treatment. Fig. 4.6a illustrates the pattern of cellular responses of experimental mice to S. mansoni antigens post-treatment. Statistical analyses are provided in Table A4.2.

Figure 4.4

Persistence of residual resistance as percent partial protection

The histogram bars represent levels of percent partial protection as described in section 4.3. Mice received a primary trickle infection for either 10 weeks (///) or 18 weeks (\\\).

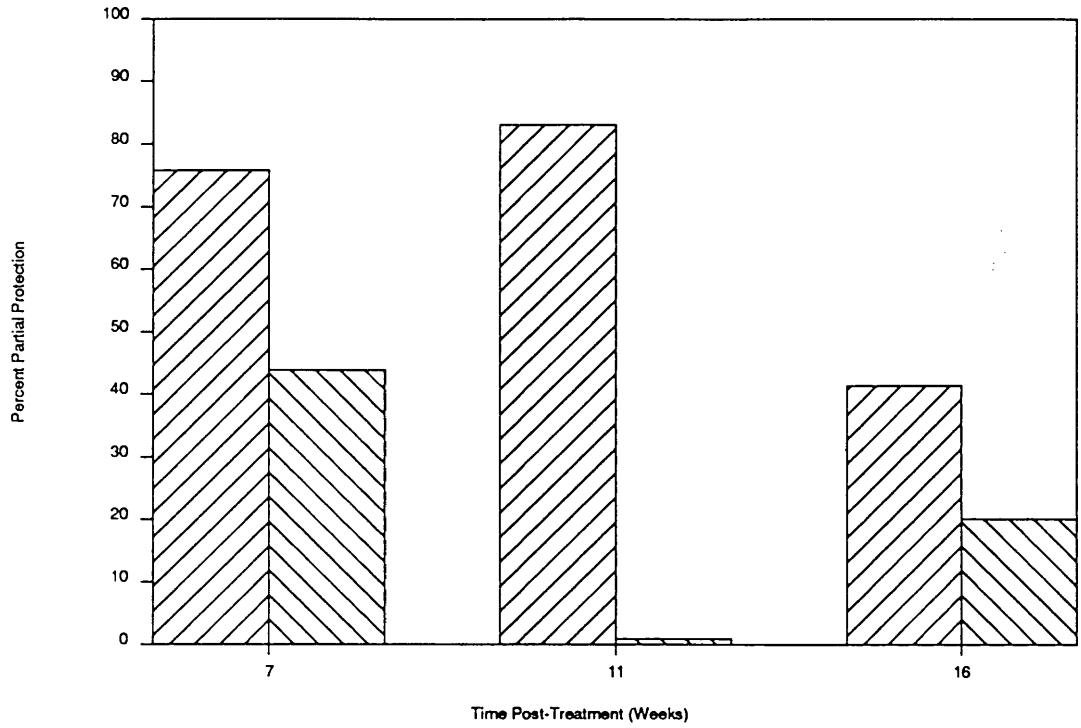
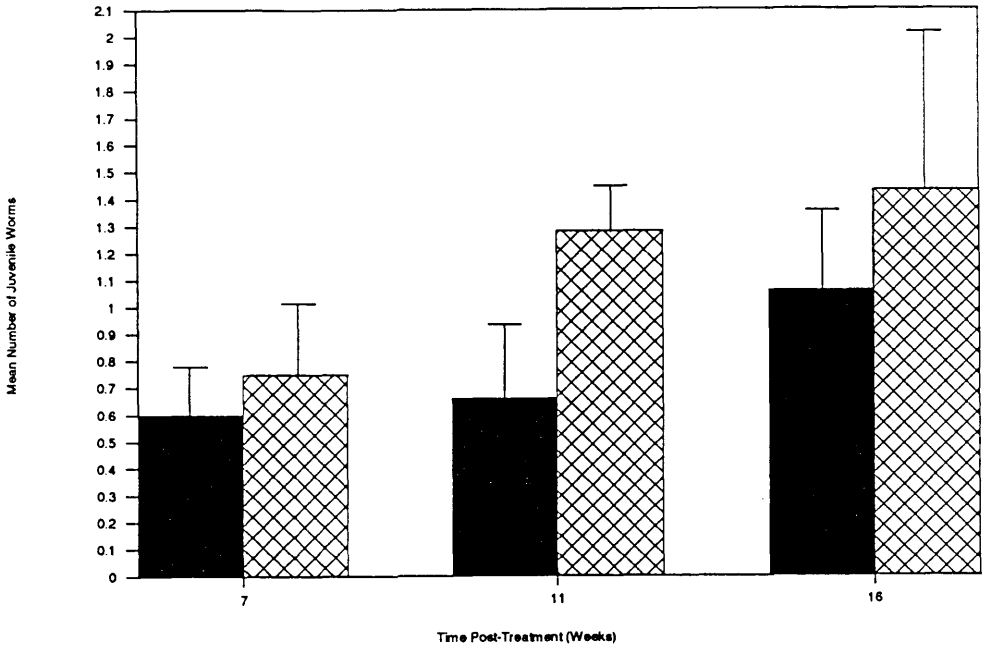

100
90
80
70
60
50
40
30
20
10
0
Percent Partial Protection
7
11
16
Time Post-Treatment (Weeks)

Figure 4.5

Recovery of juvenile worms following chemotherapeutic treatment.

Histogram bars represent the mean number of juvenile worms and standard error of mice receiving a 10 (solid) or 18 (hatched) week primary trickle infection prior to chemotherapeutic treatment.


Mean Number of Juvenile Worms
2.1
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
7
11
16
Time Post-Treatment (Weeks)

Schistosomula-antigen stimulated responses were equally elevated at 7 and 11 weeks post-treatment but declined significantly by 16 weeks post-chemotherapy. Responses to SEA peaked by 11 weeks post-treatment and declined to insignificant levels 16 weeks after treatment. Proliferation in response to the adult membrane antigen remained low throughout the post-treatment period. These responses were significantly lower than those observed in response to the schistosomula antigen at all times post-treatment, but did not differ significantly from SEA-stimulated values. Responses to the larval antigen consistently exceeded responses to SEA, this difference being significant at 7 weeks post-treatment.

b) Responses following an 18 week primary exposure:

Lymphocytes derived from mice receiving an 18-week trickle infection exhibited insignificant proliferative responses to schistosomula and adult antigens at 7 weeks post-treatment. Blastogenic responses to the schistosomula antigen became significantly higher than those recorded for naive mice at 11 and 16 weeks post-treatment. SEA-elicited cellular reactions were significantly elevated at 7 and 16 weeks post-chemotherapy but not at 11 weeks post-treatment. Statistical analyses are provided in Table A4.3. At no time did antigen-stimulated cells from experimental mice proliferate more than similarly cultured cells from corresponding treated controls.

The pattern of blastogenic activity of cells derived from experimental mice is shown in Fig. 4.6b. Schistosomula-elicited responses are similar to that observed for the 10-week infected mice at 7 weeks post-treatment. This observation must be interpreted cautiously however, as the response of cells derived from the 18-week infected animals was not significantly higher than that observed for the age/sex matched control group. Thus, immunogenic

Figure 4.6

Lymphoproliferative responses following chemotherapeutic treatment

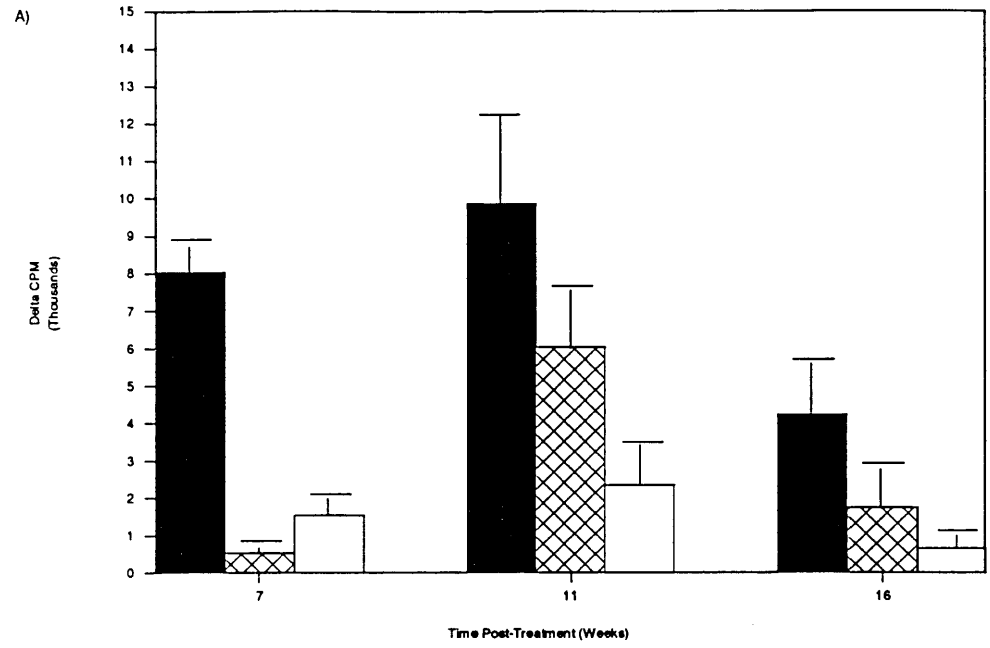
Histogram bars represent mean levels proliferation and standard error of spleen cells derived from experimental mice, to schistosomula antigen (solid), SEA (hatched) and adult antigen (clear) at pre-determined intervals post-treatment.

Duration of primary trickle infection was:

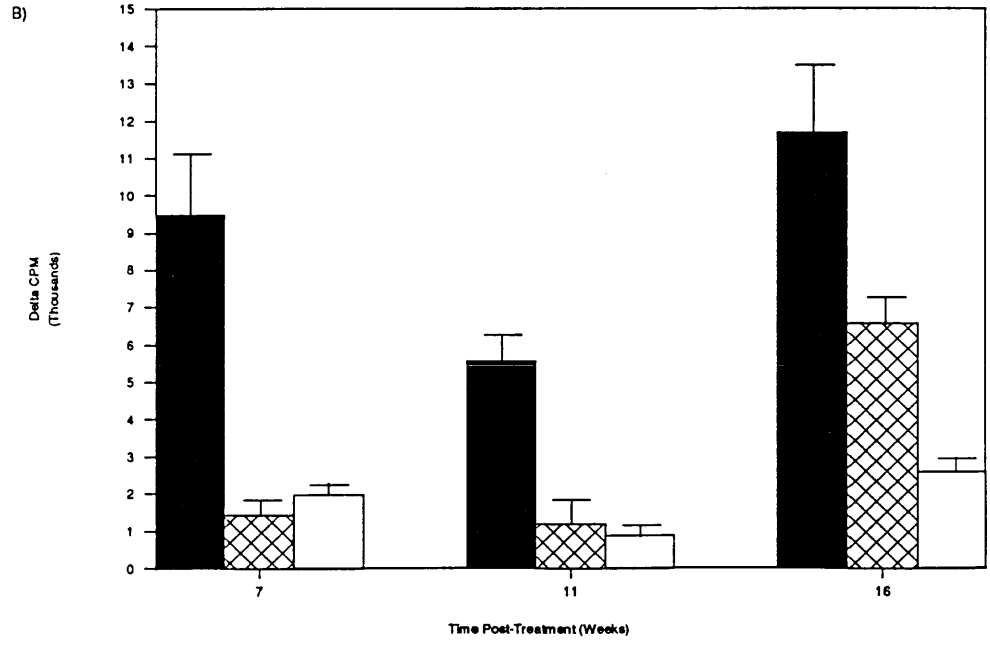
A) 3 cercariae/mouse/week for 10 weeks

B) 3 cercariae/mouse/week for 18 weeks

A)


Delta CPM (Thousands)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
7
11
16
Time Post-Treatment (Weeks)

B)


Delta CPM (Thousands)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
7
11
16
Time Post-Treatment (Weeks)

responses to all three antigenic preparations would appear to be quite low at this time post-treatment. Cellular responsiveness to all three antigens declined even further by 11 weeks post-treatment. At this time, responses to S. mansoni antigens were lower than those generated by mice receiving the 10 week primary infection, with SEA elicited responses being significantly lower. At 16 weeks post-treatment there was a dramatic increase in schistosomula and SEA-elicited responses relative to the observations made 5 weeks previously. At this time, responses to all antigens were significantly higher than those generated by the 10-week infected mice.

4.4.3 Immunoglobulin Levels:

a) Responses following a 10 week primary exposure: (Fig. 4.7a, 4.8a).

IgG and IgM responses did not differ from respective treated control values at any time post-treatment. With few exceptions, mean IgG and IgM responses to all schistosome antigens remained at a constant level throughout the post-treatment period. Statistical analyses are provided in Table A4.5. IgG response to adult antigen was consistently higher than that to SEA which was in turn, higher than that to schistosomula antigen. IgM titres tended to be highest in response to SEA and lowest in response to the adult membrane antigen.

b) Responses following a 18 week primary exposure: (Fig. 4.7b, 4.8b.)

The pattern of antibody responsiveness in this group was similar to that observed for mice receiving the 10-week primary exposure. IgG responses tended to be higher in mice receiving the 18-week rather than the 10-week infection, particularly at 7 and 11 weeks post-treatment. Statistical analyses are provided in Table A4.6. At 7 weeks post-treatment, anti-

Figure 4.7

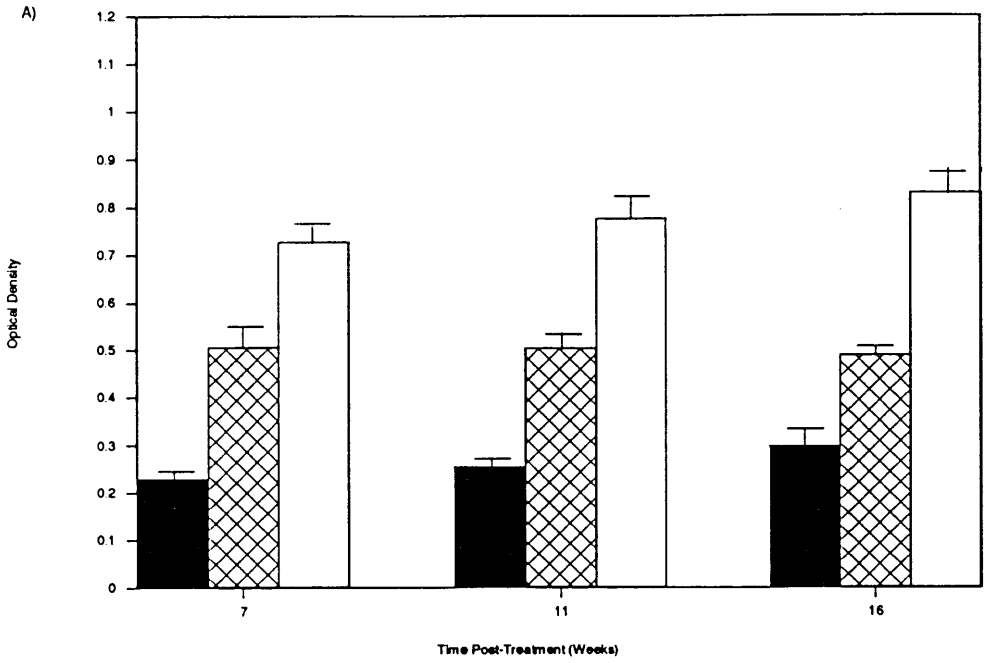
Immunoglobulin levels (IgG) following chemotherapeutic treatment.

Histogram bars represent the mean IgG antibody responses and standard error to schistosomula antigen (solid), SEA (hatched), and adult antigen (clear) at pre-determined intervals post-treatment.

Duration of primary trickle infection was:

A) 3 cercariae/mouse/week for 10 weeks

B) 3 cercariae/mouse/week for 18 weeks


A)
Optical Density
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
7
11
16
Time Post-Treatment (Weeks)

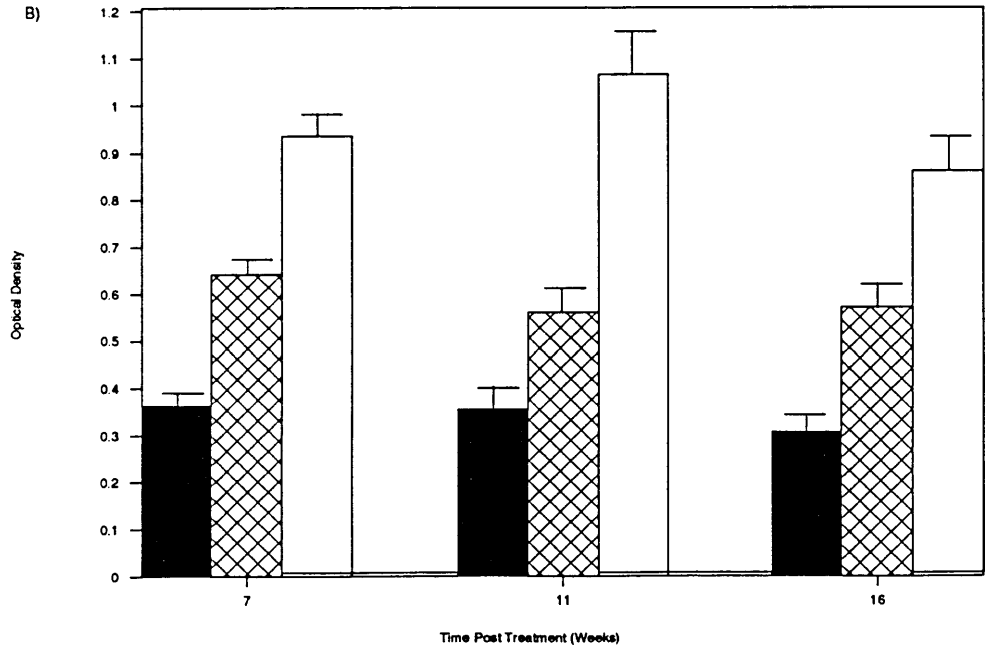

B)
Optical Density
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
7
11
16
Time Post Treatment (Weeks)

Figure 4.8

Immunoglobulin levels (IgM) following chemotherapeutic treatment.

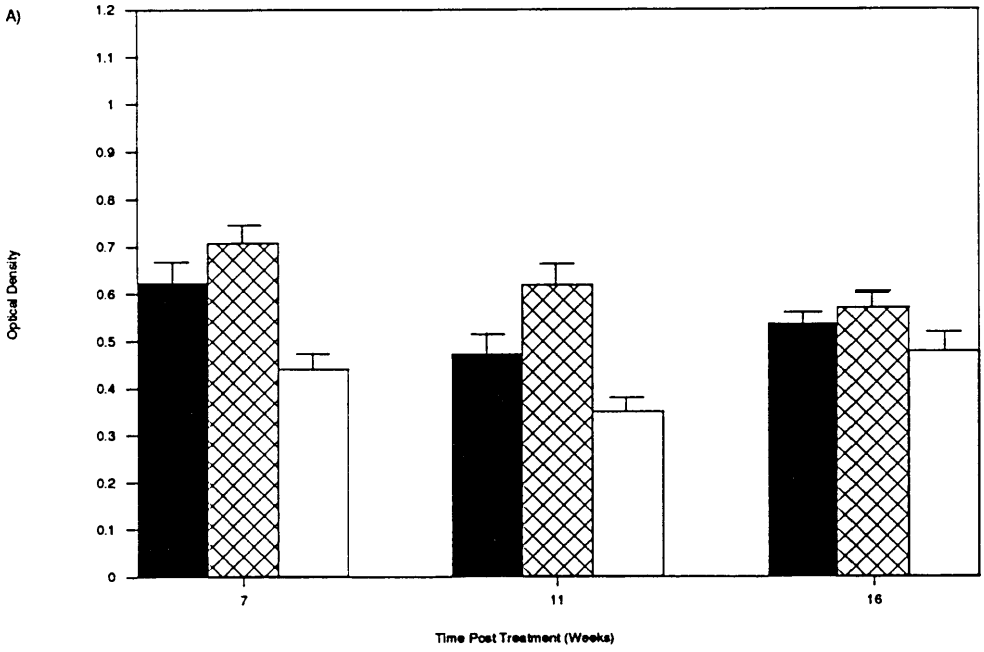
Histogram bars represent the mean IgM antibody responses and standard error to schistosomula antigen (solid), SEA (hatched), and adult antigen (clear) at varying intervals post-treatment.

Duration of primary trickle infection was:

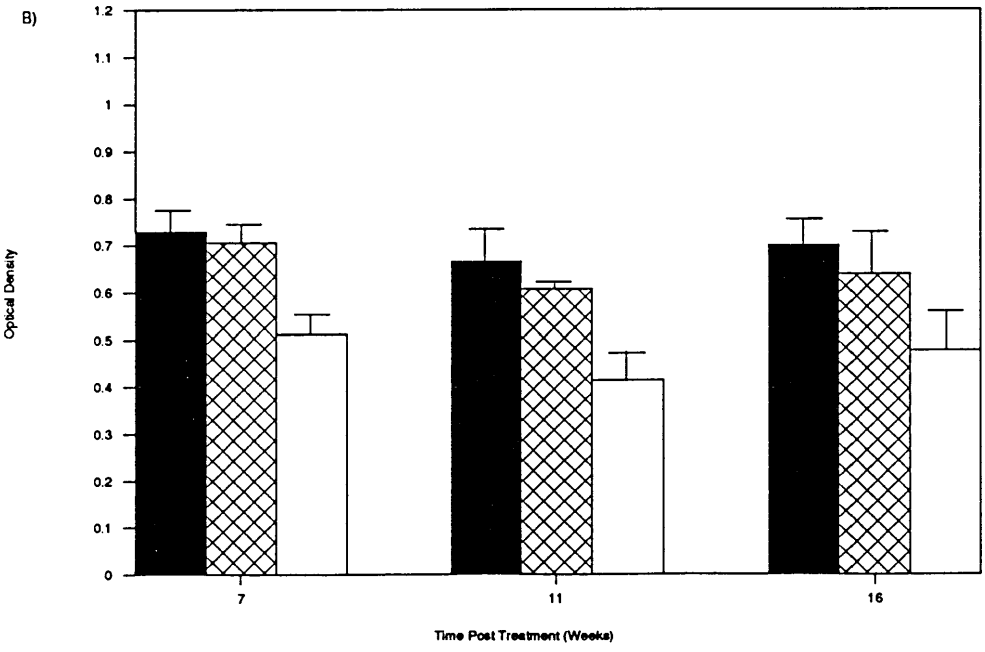
A) 3 cercariae/mouse/week for 10 weeks

B) 3 cercariae/mouse/week for 18 weeks

A)


1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Optical Density
7
11
16
Time Post Treatment (Weeks)

B)


1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Optical Density
7
11
16
Time Post Treatment (Weeks)

schistosomula IgM responses were significantly higher than levels obtained from sera of 10-week infected animals.

4.5 Discussion

Interpretation of the results has been complicated by the inability of drug treatment to eliminate all worms derived from the primary trickle exposure. Following a trickle infection, the host is likely to harbour parasite burdens composed of mixed developmental stages. Juvenile stages are known to be capable of avoiding the effects of praziquantel treatment using the dose regime employed in this study and can subsequently mature to the adult stage (Sabah et al., 1986). A similar situation is thought to occur under field conditions where children in particular are likely to harbour prepatent infections at the time of chemotherapeutic treatment (Sturrock et al., 1987). Therefore, infection of experimental animals with repeated low level doses of cercariae, rather than single primary infections, may provide a better understanding of the consequences of drug treatment in endemic communities.

The results of the present study have shown that mice receiving a trickle infection for 10 weeks prior to treatment, exhibit more stable levels of resistance immediately post-chemotherapy than 18-week infected animals. This difference is not likely to be due to the effects of worm burdens surviving chemotherapeutic treatment, as parasite loads did not differ between treated control groups of 10 and 18-week infected animals at corresponding times post-treatment. Nevertheless, the potential impact of the residual worm burdens on resistance cannot be ignored.

These results would appear to differ from those derived from previous experimental (Tawfik and Colley, 1986) and field (Butterworth et al., 1984; 1985; Wilkins et al., 1987; Dessein et al., 1988) studies where the duration of exposure prior to treatment was observed to be directly related to levels of residual resistance. Although Tawfik and Colley (1986)

employed the mouse model and used similar durations of primary exposure, their primary infection was derived from a single dose rather than a trickle infection. Previous studies using baboons have demonstrated that the pattern of immune reactivity expressed following a single primary exposure, differs from that resulting from a trickle infection (Damian et al., 1981). Consequently, trickle infections may also induce patterns of residual resistance which differ from those induced by a single primary exposure. To extrapolate from mice to humans may also be unwarranted as it is impossible to know what time scale of infection in the mouse model is comparable with that in the human situation (Colley et al., 1977).

The pattern of cell-mediated immunity (CMI) post-treatment appeared to be related to the duration of the primary trickle exposure. Thus, treatment of 10-week, acutely infected mice, led to the gradual diminution of cellular activation, with initially high levels of T-cell reactivity towards schistosomula antigen correlating with observed levels of residual resistance. In contrast, treatment of chronically infected animals led to an almost immediate abrogation of resistance to S. mansoni infection parallelled by very low levels of T-cell responsiveness. However, by the end of the post-treatment period, blastogenic responses to larval and egg antigens increased dramatically, although levels of residual resistance remained low. Therefore, the expression of anti-schistosome blastogenic activity did not necessarily correlate with levels of host protection.

Similarly, studies derived from human communities have demonstrated that treatment of acutely infected patients leads to the diminution of cellular responsiveness to schistosome preparations while treatment of chronically infected patients leads to an eventual increase in cellular responses to worm antigens (Ottesen et al., 1979; Gazzinelli et al., 1985; El-Bassouiny et al., 1987). This pronounced elevation of

lymphoproliferative responses to schistosome preparations by chronic individuals, has been attributed to either the massive release of cross-reactive antigens into the circulation or the reversal of parasite-induced modulatory effects by anti-schistosome chemotherapy (El-Bassouiny et al., 1987). Both explanations may be relevant to the present study. However, the absence of a chronically-infected, untreated control group, makes any critical assessment concerning the direct impact of chemotherapy on immune function impossible. Unfortunately, these human studies did not attempt to correlate patterns of CMI post-treatment with levels of residual resistance.

Recently, Colley et al. (1986) have observed that individuals with low blastogenic responses to schistosome preparations post-treatment were more susceptible to reinfection than high responding donors. This conclusion cannot be drawn from the present study. Alternatively, it is suggested that the pattern of blastogenic activity post-treatment is a reflection of the host's immune status at the time of chemotherapeutic cure and not necessarily a correlate of residual resistance.

A similar conclusion may be drawn regarding the pattern of humoral responses post-treatment. In particular, IgG responses of 18-week infected animals were generally higher than those generated by 10-week infected mice at 7 and 11 weeks post-treatment. Although these responses did not correlate with observed levels of resistance, they did correspond with pre-treatment levels described in the Chapter 3. As expected, IgM levels were similar between the two groups at corresponding times post-treatment, as unlike IgG titres, IgM levels plateau by 10 weeks post-infection. A similar relationship between pre- and post-treatment antibody levels was observed upon serological analysis of S. mansoni-treated Kenyan patients (Roberts et al., 1987).

The results of the present study have demonstrated an inverse relationship between the duration of primary exposure and residual resistance to reinfection with S. mansoni. An immune basis for this observation could not be detected although it is suggested that the use of more sophisticated immunological techniques may help to expose the immune mechanisms underlying this resistance. A role for anatomical changes to the parasite migration route should also be considered. Of particular interest was the relationship between the duration of primary exposure and the pattern of immune reactivity post-treatment. As these patterns appeared to parallel those observed following the treatment of human subjects, it is suggested that this model may provide some insight into potentially protective immune mechanisms operating under conditions of repeated exposure.

Chapter 5

General Discussion

The present study describes the relationship between epidemiological and immunological parameters associated with chronic S. mansoni infection in inbred mice. More specifically, the factors involved in the acquisition and persistence of resistance to the helminth were investigated following weekly cercarial exposures over extended periods of time. This method of examining the dynamics of acquisition and loss of resistance to S. mansoni was selected over the more conventional single dose/challenge method, in an attempt to mimic patterns of exposure experienced in human communities in which the infection is endemic.

Prior to embarking on a final analysis of the results presented in this study, the limitations of using animal models to mimic what is essentially a disease of humans, must be re-emphasized. This is particularly true of the S. mansoni/mouse model, where the relatively small size of the rodent, combined with its susceptibility to disease, frequently leads to the premature death of the host. This point is highlighted by the paradigm that a single worm pair in a 20g mouse is the equivalent of 3500 worm pairs in a 70kg man (Cheever, 1969).

To chronologically extrapolate from mice to humans is also unwarranted. Thus, it is impossible to know whether a mouse infected for 8 weeks is equivalent to an early, 1 year or 2 year infected human (Colley et al., 1977). Additionally, it is impossible to determine the duration of

infection in the human host.

Despite these problems, it is known that mice harbouring a single worm pair can survive indefinite periods of time (Warren, 1963) and that such infections are accompanied by portal inflammation and fibrosis which is similar to that observed in human patients (Andrade and Warren, 1964). Of great significance is that, like humans, mice are permissive to schistosome infection and develop a partial resistance to reinfection (Dean, 1983). Although such levels of resistance may be partially attributed to the non-specific effects of portal disruption (Wilson et al., 1983), it is likely that at least some of the resistance generated is immunologically based (Pons et al., 1989). Furthermore, the mouse host is able to elicit humoral and cellular responses to S. mansoni antigens. Overall, the S. mansoni/mouse model may still provide a useful system for enhancing the understanding of the epidemiology and immunology of schistosome infection in humans. However, the interpretation of results derived from experimental models, must include a clear understanding of the consequences of overestimating their relevance to what is observed in human communities.

In the first set of experiments, described in Chapter 3, mice were exposed to repeated low levels doses of S. mansoni cercariae in an attempt to generate age-intensity profiles, similar to those found in human communities. It was considered that an understanding of the dynamic changes in immune responsiveness during the course of such chronic infections might provide some information on the role of immunity in the acquisition of resistance to S. mansoni.

It was observed that the age-intensity profiles generated by mice were dependent upon the intensity of cercarial exposure. Thus, at the lowest dose of infection, average helminth burdens rose monotonically throughout the 35-week study period without attaining a stable plateau. In contrast, under

the same experimental conditions, Crombie and Anderson (1985) observed that worm burdens began to stabilize between 20 and 30 weeks post-initial exposure. The authors proposed that this infection pattern is characteristic of conditions where there is a constant exposure to infection, constant worm mortality and no acquired resistance. It is possible that such an equilibrium between parasite immigration and mortality would have been established in the present study had the experimental period been extended beyond 35 weeks. Nevertheless, the absence of any decline in infection intensity with time, suggests the absence of any acquired resistance to infection by mice of this experimental group.

As the intensity of cercarial exposure was increased, worm burdens rose more rapidly and to higher peak levels. At the moderate dose of infection, average helminth burdens began to decline as the mice entered older age groups. A reduction in parasite load with increasing experience of infection is known to occur in certain endemic situations (Kloetzel and da Silva, 1967; Jordan, 1985). However, the infection patterns generated in human communities likely result from the combined effects of acquired resistance and decreased exposure to transmission sites with increasing age (Wilkins, 1987). The experimental protocol employed in this study precludes any impact of age-related changes in water contact on the infection patterns observed. This tentatively suggests that acquired resistance may also play an important role in the generation of infection patterns in endemic communities.

Mice exposed to the highest density of cercariae exhibited an age-intensity profile which peaked by 12 weeks post-initial exposure and then remained unchanged for the duration of the study. That the profile did not become convex in form was surprising, as it has been hypothesized that the degree to which the intensity of S. mansoni infection is likely to decline

relative to the maximum level attained, is often directly related to the intensity of transmission (Anderson and May, 1985). It is suggested that the stable plateau achieved at the highest intensity of exposure may reflect in part the partial effectiveness of the immune response to S. mansoni. Thus, under conditions of intense exposure the immune system is unable to reduce the rate of parasite establishment below that of helminth loss via mortality. The sharp decline in the number of juveniles recovered by perfusion suggests that the host had acquired a degree of resistance to infection. A parallel situation may exist in highly endemic communities where egg output may fail to decline in older age classes, but instead remains stable or increases throughout adult life (Smith et al., 1979). It has been argued that under such conditions of exposure the immune system serves to maintain parasite burdens at a stable and tolerable level (Wilkins, 1987).

Alternatively, it is possible that the profile may have become convex in form had the experimental period been extended. Unfortunately, such intensities of exposure often reduce the life expectancy of the mouse host, limiting the duration of the study period.

In the present study, liver egg burdens appeared to increase to a stable plateau at all three infection densities. Of potentially greater significance was the observation that similar numbers of eggs per worm pair were recovered from mice at each time period, irrespective of infection intensity. This suggests that parasite fecundity is not restricted by the density of worms being harboured by the host, in contrast to what has been described by Medley and Anderson (1985).

The impact of immune regulation on egg production with increasing dose of infection must also be considered. Thus, the suppression of parasite fecundity has been observed to be one manifestation of host resistance in both the S. mansoni/mouse model (Doenhoff et al., 1978) and the S.

bovis/bovine model (Bushara et al., 1983a).

The pattern of immune responsiveness was also observed to be dependent upon the intensity of cercarial exposure. Of particular interest was the inability of cells derived from mice exposed to the lowest density of cercariae to consistently respond to antigenic stimulation despite continued exposure to infection. In contrast, responses of cells from the more heavily exposed animals, particularly to the schistosomula preparation, were both immediate and long-lasting. As mice exposed to 1 cercaria/mouse/week did not appear to express any resistance to infection, it is possible that a consistent and strong cellular response to the larva is required before the host can mount an effective defence against the parasite. This is supported by observations which have demonstrated the necessary activation of cellular mechanisms before protective immunity can be stimulated in both rats (Phillips et al., 1977) and rhesus monkeys (Maddison et al., 1979b).

Another interesting aspect of this study was the observed relationship between S. mansoni infection and the suppression of the blastogenic response to the T-cell mitogen, ConA. The apparently transient nature of this suppression has been previously noted in S. mansoni infected mice (Trizio et al., 1980) and rhesus monkeys (Maddison et al., 1979b). This schistosome-induced suppression of lymphocyte responsiveness to non-specific mitogen has been documented previously in both the murine (Pelley et al., 1976; Diab et al., 1989) and human systems (Reiner et al., 1979; Lewert et al., 1979; Cottrell et al., 1982). However, it should be noted that in many instances, lymphocytes derived from human schistosomal patients have not been found to be hyporesponsive to T-cell mitogens (Colley et al., 1977; Ottesen et al., 1978; Abdel-Salam et al., 1981; El-Bassiouny et al., 1987).

Generalized cellular immunosuppression has been observed to occur as a result of a number of parasitic infections other than schistosomiasis. Indeed, lymphocytes derived from human patients infected with Plasmodium falciparum (Troye-Blomberg et al., 1983) and leishmaniasis (Castes et al., 1989) express depressed proliferative responses to ConA.

Whether or not the immunosuppressive effects of parasitic infections can enhance host susceptibility to infection is uncertain. However, results obtained from a study employing the Taenia taeniaeformis/mouse model have indicated that suppressed responses to ConA, correlate with host susceptibility to the parasite (Letonja et al., 1987).

Results from the current investigation have suggested that cellular responses of infected mice to S. mansoni antigens decline with increasing chronicity of infection. This was particularly true of responses to the schistosomula antigen at the two higher intensities of exposure, although responses of moderately exposed mice to SEA did decline to insignificant levels by the end of the study.

Previous studies using the murine model have also demonstrated a decline in delayed hypersensitivity reactions to schistosome antigens during the chronic phase of infection (Colley, 1972; Boros et al., 1975; Olds and Ellner, 1984). As the pathology associated with the host response to eggs in the tissues is considered to be a cell-mediated event (Warren et al., 1967), it is considered that a decline in cell-mediated immunity may reduce granuloma formation and allow animals to survive the chronic stage of infection (Boros et al., 1975). However, there is some evidence to suggest that the decline in cell-mediated immunity may also enhance the susceptibility of the host to superinfection (Olds and Ellner, 1984).

The examination of cellular and humoral responsiveness of mice infected with S. mansoni was conducted following the exposure of the host

to repeated low level doses of cercariae. A number of experimental studies have also considered the effects of a chronic S. mansoni infection on the immune status of the host. However, with only a few exceptions, such studies have monitored changes in immune reactivity following a single primary dose rather than a trickle infection. The question arises as to whether or not these different infection regimes induce different patterns of immune responsiveness.

In a study using the S. mansoni/baboon model, Damian et al. (1981) monitored the pattern of humoral reactivity following a trickle infection, and compared the results with that of a parallel study where baboons were exposed to only a single exposure (Suzuki et al., 1981). Of particular interest was the observation that average levels of IgM antibodies were maintained for longer periods of time following a trickle infection. This difference was attributed to the repeated exposures of cercariae. (Damian et al., 1981).

Dunne et al., (1984) have reported that anti-SEA IgM antibody responses of mice exposed to a single S. mansoni infection peak between 8 and 10 weeks post-infection and slowly decline thereafter. This differs from the observations made in the present study where anti-SEA IgM reactivity did not decline from peak levels. This tentatively suggests that repeated stimulation of the mouse host may also serve to maintain strong levels of anti-schistosome IgM reactivity. However, as a direct comparison between singly and repeatedly infected hosts was not made in the present study, no formal conclusion can be drawn.

The possibility that the pattern of exposure to S. mansoni cercariae can effect the pattern of cellular reactivity has been suggested by the observations of Katz and Colley (1976). These authors demonstrated the necessity of repeated exposures to the cercarial stage to induce dependable

levels of cellular reactivity against a larval preparation.

It would appear therefore that repeated stimulation of the host can induce patterns of immune reactivity which differ from those elicited by a single primary exposure. Although the total worm burden of individuals living in endemic regions is thought to result from repeated exposure to infective cercariae, it cannot be said with any certainty as to whether responses generated by trickle infections in the laboratory are more or less likely to represent patterns of immune reactivity generated in the field. Nevertheless, the use of multiple cercarial exposures to investigate the dynamic nature of the immune response to S. mansoni infection may still provide useful information when considering the concurrent role of immunological and ecological factors that influence the generation of age-intensity profiles.

The experiment described in Chapter 4, was designed to determine the relationship between the duration of a primary trickle exposure and residual resistance to S. mansoni infection following the application of praziquantel.

It was observed that mice treated following a 10-week infection were more resistant than 18-week infected animals to reinfection with the cercariae of S. mansoni. This apparently inverse relationship between the persistence of resistance following chemotherapeutic treatment and the duration of a primary infection would appear to be in direct conflict with the results of previous studies derived from experimental (Tawfik and Colley, 1986) and field studies (Butterworth et al., 1984; 1985; Wilkins et al., 1987; Dessein et al., 1988).

The results of the present study have been slightly complicated by the inability of drug treatment to eliminate all the parasites derived from the primary exposure. The use of trickle exposures increases the likelihood that

certain juvenile stages, which are not susceptible to praziquantel treatment (Sabah et al., 1986) are likely to be present when the drug is applied. Hence, it is not surprising that complete elimination of the primary worm burden was not achieved in the present study. Although this inability to clear the host of infection has complicated the interpretation of the results, it may serve to better mimic the situation in endemic communities, where young children in particular may harbour very recent infections at the time of chemotherapeutic treatment (Sturrock et al., 1987).

The impact of these residual worm burdens on the persistence of resistance is unclear. However, as no significant difference between worm burdens of 10 and 18-week treated control animals was observed, the impact of such residual worm burdens is not likely to explain the enhanced susceptibility of 18-week exposed mice. Nevertheless, any conclusions regarding the persistence of resistance in the absence of concomitant immunity cannot be derived from this study.

As levels of parasite-induced host pathology are known to become less severe with increasing chronicity of infection (Warren, 1977), it is possible that non-specific mechanisms of resistance were of greater consequence in the 10-week infected animals at the time of reinfection.

Of particular interest was the observed relationship between the duration of primary exposure and the pattern of immune reactivity following chemotherapeutic treatment. As described previously, the patterns of immune reactivity observed may have reflected the epidemiological and immunological status of the host prior to chemotherapy rather than the ability of the host to resist reinfection.

Interestingly, mean anti-schistosome IgG activity was inversely related to the mean levels of resistance generated by the mice. In a Kenyan study, a similar relationship between residual resistance and humoral

responsiveness was observed (Butterworth et al., 1985). The authors attributed this to the high levels of reinfection and the continued stimulation of antibody responses by schistosome-related antigens

Although there was no apparent correlation between the patterns of immune reactivity and residual resistance, it may be that the techniques employed in this study were not sufficiently sensitive to identify potentially protective cellular or humoral responses. Thus, future studies may include specific antigenic preparations which are considered to have protective properties. Indeed, a recent study using serum samples from Brazilian patients has described a specific antigen on the surface of the schistosomula which is recognized by IgG antibodies of protected subjects (Dessein et al., 1988).

Overall, the present study has described an experimental model which may be useful in helping to understand the complex and dynamic relationship between epidemiological and immunological parameters associated with schistosome infection. Central to the theme of this study was the use of trickle infections. It was thought that such an infection regimen might reflect the pattern of exposure experienced in endemic situations. Indeed, the infection and immune patterns generated in this study are somewhat similar in broad terms to those observed in human communities.

Future studies of this nature may be expanded to include ecological considerations, which may affect the acquisition of resistance to schistosome infection. Thus, it may be interesting to consider the effects of malnutrition on the immune capabilities of the host. This may have particular relevance to the human situation, where the immunosuppressive effects of the parasite may be enhanced by high levels of malnourishment (Lewert et al., 1979).

It has been suggested in this thesis that repeated exposure to live schistosomes may induce immune responses, similar to those generated

following exposure to irradiated cercariae. This is not an unreasonable assumption, as both models involve the exposure of the host to large amounts of schistosomula antigen. If this is in fact the case, it may be that trickle exposures enhance the protective response to the larva in the absence of potentially harmful anti-egg responses. Studies which consider the role of T-cell subsets and their products in relation to the generation of immunity and disease, would be of great importance in examining such a problem. The results of a recent study clearly indicate that the generation of disease and/or immunity may well depend upon the specific T-cell subset stimulated (Scott et al., 1989). This would appear to be a profitable area for future research.

Acknowledgements

I would like to thank all the members of PERG for their part in making my stay in London so enjoyable.

Many thanks to my supervisor, Professor R.M. Anderson FRS, for his support and for allowing Jennie Wilner to sign all those blank cheques. I am indebted to Christopher Langford, Paiboon Sithithaworn, Graham Medley, James Nokes and Jane Lillywhite for their invaluable assistance. I could not thank Helen Guyatt Baby enough, for her life saving manoeuvres during my final days of panic. I also wish to express my appreciation to Alison Agnew and Dr. A.J.G Simpson for their time and constructive advice. I would also like to recognize the generally unappreciated efforts of the Animal Unit staff, whose expertise and care are necessary for the successful completion of all animal based studies in the Department of Biology.

A heartfelt thank you to Edwin Michael and Marie-Claude Boily for their friendship and ability to make me laugh when it wasn't funny. I would like to apologize to Martin Cox, a great person and friend, for never ever managing that third pint. Thank you to Helen Udy and Neil Slade who had to bear with me all day, everyday. Special thanks to Gary Childs for introducing me to Second Division Football and to Phil Edmunds who made me appreciate the skills of Gary Lineker.

This work could not have been accomplished without the constant love and support of my family who always seem to understand.

Most of all, thank you Lindsay, for making me a better person and for making each day more special than the last.

Bibliography

Abdel-Salam, E., Higashi, G.I., Kamal, K.A. and Ishaac, S. (1981). Cell-mediated immune assay in children with Schistosoma haematobium and the effect of praziquantel therapy. Transactions of the Royal Society of Tropical Medicine and Hygiene. **75**: 207-214.

Abraham, K.M. and Teale, J.M. (1987). The contribution of parasite-specific T-cells to isotype restriction in Mesocestoides corti-infected mice. Journal of Immunology. **139**: 2530-2537.

Anderson, R.M. (1978). Population dynamics of snail infection by miracidia. Parasitology. **77**: 201-224.

Anderson, R.M. (1987). Determinants of infection in human schistosomiasis. In "Baillieres Clinical Tropical Medicine and Communicable Diseases, Vol. 2. No. 2" (ed. A.A.F. Mahmoud),

Anderson, R.M. and May, R.M. (1985). Vaccination and herd immunity to infectious diseases. Nature. **318**: 323-329.

Anderson, R.M. and Schad, G.A. (1985). Hookworm burdens and faecal egg counts: an analysis of the biological basis of variation. Transactions of the Royal Society of Tropical Medicine and Hygiene. **79**: 812-825.

Andrade, Z.A. and de Brito, P.A. (1982). Curative chemotherapy and resistance to reinfection in murine schistosomiasis. American Journal of Tropical Medicine and Hygiene. **31**: 116-121.

Andrade, Z.A. and Warren, K.S. (1964). Mild prolonged schistosomiasis in mice: alterations in host response with time and the development of portal fibrosis. Transactions of the Royal Society of Tropical Medicine and Hygiene. **58**: 53-57.

Asch, H.L. (1972). Rhythmic emergence of Schistosoma mansoni cercariae from Biomphalaria glabrata: control by illumination. Experimental Parasitology. **53**: 189-198.

Barral-Netto, M., Hofstetter, M., Cheever, A.W. and Ottesen, E.A. (1983). Specificity of antibody and cellular immune responses in human schistosomiasis. American Journal of Tropical Medicine and Hygiene. **32**: 106-113.

Barreto, M.L., Silva, J.T.F., Mott, K.E. and Lehman Jr., J.F. (1978). Stability of faecal egg excretion in Schistosoma mansoni infection. Transactions of the Royal Society of Tropical Medicine and Hygiene. **72**: 181-187.

Bickle, Q., Bain, J., McGregor, A. and Doenhoff, M. (1979a). Factors affecting the acquisition of resistance against Schistosoma mansoni in the mouse: III. The failure of primary infections with cercariae of one sex to induce resistance to reinfection. Transactions of the Royal Society of Tropical Medicine and Hygiene. **73**: 37-41.

Bickle, Q., Long, E. and Doenhoff, M. (1980). Schistosoma mansoni: Influence of the mouse host's sex, age, and strain on resistance to reinfection. Experimental Parasitology. **50**: 222-232.

Bickle, Q.D., Taylor, M.G., Doenhoff, M.J. and Nelson, G.S. (1979b). Immunization of mice with gamma-irradiated intramucularly infected schistosomula of Schistosoma mansoni. Parasitology. **79**: 209-222.

Boros, D.L., Pelley, R.P. and Warren, K.S. (1975). Spontaneous modulation of granulomatous hypersensitivity in schistosomiasis mansoni. Journal of Immunology. **114**: 1437-1441.

Boros, D.L. and Warren, K.S. (1970). Delayed hypersensitivity-type granuloma formation and dermal reaction induced and elicited by a

soluble factor isolated from Schistosoma mansoni eggs. The Journal of Experimental Medicine. **132**: 488-507.

Bout, D.T., Joseph, M., David, J.R. and Capron, A.R. (1981). In vitro killing of S. mansoni schistosomula by lymphokine-activated mouse macrophages. Journal of Immunology. **127**: 1-5.

Bout, D., Rousseaux, R., Carlier, Y. and Capron, A. (1980). Kinetics of classes and subclasses of total immunoglobulins and specific antibodies to Schistosoma mansoni during murine infection. Parasitology. **80**: 247-256.

Bradley, D.J. and McCullough, F.S. (1973). Egg output stability and the epidemiology of Schistosoma haematobium Part II. An analysis of the epidemiology of endemic S. haematobium. Transactions of the Royal Society of Tropical Medicine and Hygiene. **67**: 491-500.

Bushara, H.O., Hussein, M.F., Majid, M.A., Musa, B.E.H. and Taylor, M.G. (1983a). Observations on cattle schistosomiasis in the Sudan, a study in comparative medicine. IV. Preliminary observations on the mechanism of naturally-acquired resistance. American Journal of Tropical Medicine and Hygiene. **32**: 1065-1070.

Bushara, H.O., Majid, B.Y.A., Majid, A.A., Khitma, I., Gameel, A.A., Karib, E.A., Hussein, M.F. and Taylor, M.G. (1983b). Observations on cattle schistosomiasis in the Sudan, a study in comparative medicine. V. The effect of praziquantel therapy on naturally acquired resistance to Schistosoma bovis. American Journal of Tropical Medicine and Hygiene. **32**: 1370-1374.

Butterworth, A.E. (1984). Cell-mediated damage to helminths. Advances in Parasitology. **23**: 143-235.

Butterworth, A.E. (1987). Immunity in human schistosomiasis. In "Acta Tropica: Prospects for immunological intervention in human schistosomiasis". Vol. 44. Supp. 12. 31-40.

Butterworth, A.E., Capron, M., Cordingley, J.S., Dalton, P.R., Dunne, D.W., Kariuki, H.C., Kimani, G., Koech, D., Mugambi, M., Ouma, J.H., Prentice, M.A., Richardson, B.A., Arap Siongok, T.K., Sturrock, R.F. and Taylor, D.W. (1985). Immunity after treatment of human schistosomiasis mansoni II. Identification of resistant individuals, and analysis of their immune responses. Transactions of the Royal Society of Tropical Medicine and Hygiene. **79**: 393-408.

Butterworth, A.E., Dalton, P.R., Dunne, D.W., Mugambi, M., Ouma, J.H., Richardson, B.A., Arap Siongok, T.K. and Sturrock, R.F. (1984). Immunity after treatment of human schistosomiasis mansoni. I. Study design, pretreatment observations and the results of treatment. Transactions of the Royal Society of Tropical Medicine and Hygiene. **78**: 108-123.

Butterworth, A.E., Remold, G.H., Houba V., David, J.R., Franks, D., David, P.H. and Sturrock, R.F. (1977). Antibody-dependent eosinophil-mediated damage to 51Cr-labeled schistosomula of Schistosoma mansoni. Mediation by IgG and inhibition by antigen-antibody complexes. Journal of Immunology. **118**: 2230-2236.

Butterworth, A.E., Wassom, D.L., Gleich, G.J., Loegering, D.A. and David, J.R. (1979). Damage to schistosomula of Schistosoma mansoni induced directly by eosinophil major basic protein. Journal of Immunology. **122**: 221-229.

Castes, M., Moros, Z., Martinez, A., Trujillo, D., Castellanos, P.L., Rondon, A.J. and Convit, J. (1989). Cell-mediated immunity in localized cutaneous leishmaniasis patients before and after treatment with immunotherapy or chemotherapy. Parasite Immunology. **11**: 211-222.

Chapman, C.B., Knopf, P.M., Hicks, J.D. and Mitchell, G.F. (1979). IgG1 hypergammaglobulinaemia in chronic parasitic infections in mice: magnitude of the response in mice infected with various parasites.

Australian Journal of Experimental Biology and Medical Science. **57**: 369-387.

Cheever, A.W. (1968). A quantitative post-mortem study of schistosomiasis mansoni in man. American Journal of Tropical Medicine and Hygiene. **17**: 38-64.

Cheever, A.W. (1969). Quantitative comparison of the intensity of Schistosoma mansoni infections in man and experimental animals. Transactions of the Royal Society of Tropical Medicine and Hygiene. **63**: 781-795.

Cheever, A.W., DeWitt, W.B. and Warren, K.S. (1965). Repeated infection and treatment of mice with Schistosoma mansoni: functional, anatomic and immunologic observations. American journal of Tropical Medicine and Hygiene. **14**: 239-253.

Cheever, A.W. and Duvall, R.H. (1974). Single and repeated infections of grivet monkeys with Schistosoma mansoni: parasitological and pathological observations over a 31-month period. American Journal of Tropical Medicine and Hygiene. **23**: 884-894.

Cheever, A.W., Duvall, R.H., Hallack Jr., T.A., Minker, R.G., Malley, J.D. and Malley, K.G. (1987). Variation of hepatic fibrosis and granuloma size among mouse strains infected with Schistosoma mansoni. **37**: 85-97.

Cheever, A.W., Kamel, I.A., Elwi, A.M., Mosimann, J.E. and Danner, R. (1977). Schistosoma mansoni and S. haematobium infections in Egypt. II. Quantitative parasitological findings at necropsy. American Journal of Tropical Medicine and Hygiene. **26**: 702-716.

Cioli, D. (1983). Metazoan parasites: The schistosomes. An introductory guide to the literature. In "Molecular biology of parasites" (eds. J. Guardiola, L. Luzzatto and W. Trager), Raven Press, New York.

Civil, R.H., Warren, K.S. and Mahmoud, A.A.F. (1978). Conditions of bacille Calmette-Guerin induced resistance to Schistosoma mansoni in mice. Journal of Infectious Diseases. **137**: 550-558.

Colley, D.G. (1972). Schistosoma mansoni: eosinophilia and the development of lymphocyte blastogenesis in response to soluble egg antigen in inbred mice. Experimental Parasitology. **32**: 520-526.

Colley, D.G. (1975). Immune responses to a soluble Schistosoma egg antigen preparation during chronic primary infection with Schistosoma mansoni. Journal of Immunology. **115**: 150-156.

Colley, D.G. (1981). Immune responses and imminoregulation in experimental and clinical schistosomiasis. In "Parasitic diseases Vol. I. The Immunology" (ed. J.M. Mansfield), Marcel Decker, Inc., New York.

Colley, D.G. (1983). Schistosome antigen laboratory workshop: summary of a meeting sponsored by the Edna McConnell Clark Foundation. Journal of Parasitology. **69**: 45-48.

Colley, D.G., Barsoum, I.S., Dahawi, H.S.S., Gamil, F., Habib, M. and El Alamy, M.A. (1986). Immune responses and immunoregulation in relation to human schistosomiasis in Egypt. III. Immunity and longitudinal studies of in vitro responsiveness after treatment. Transactions of the Royal Society of Tropical Medicine and Hygiene. **80**: 952-957.

Colley, D.G. and Colley, M.D. (1989). Protective immunity and vaccines to schistosomiasis. Parasitology Today. **5**: 350-354.

Colley, D.G., Cook, J.A., Freeman, G.L., Bartholomew, R.K. and Jordan, P. (1977). Immune responses during human schistosomiasis mansoni. I. In vitro lymphocyte blastogenic responses to heterogeneous antigenic preparations from schistosome eggs, worms and cercariae.

International Archives of Allergy and Applied Immunology. **53**: 420-433.

Colley, D.G. and Freeman, G.L. (1980). Schistosoma mansoni: worm burden requirements for the establishment of resistance to reinfection in inbred mice. I. CBA/J and C57BL/6 mice. American Journal of Tropical Medicine and Hygiene. **29**: 1279-1285.

Colley, D.G. and Freeman, G.L. (1983). Differences in adult Schistosoma mansoni worm burden requirements for the establishment of resistance to reinfection in inbred mice. American Journal of Tropical Medicine and Hygiene. **32**: 543-549.

Colley, D.G. and Wikel, S.K. (1974). Schistosoma mansoni: simplified method for the production of schistosomules. Experimental Parasitology: **35**: 44-51.

Corradin, G., Etlinger, H.M., Chiller, J.M. (1977). Lymphocyte specificity to protein antigens. I. Characterization of the antigen-induced in vitro T-cell dependent proliferative response with lymph node cells from primed mice. Journal of Immunology. **119**: 1048-1053.

Cottrell, B.J., Humber, B., Sturrock, R.F., Seitz, H. and Rees, P. (1982). Non specific cell mediated immunity in patients infected with Schistosoma mansoni in Kenya. Transactions of the Royal Society of Tropical Medicine and Hygiene. **76**: 234-237.

Coulie, P.G., Coutelier, J.P., Uytenhove, C., Lambotte, P. and Van Snick, J. (1985). In vivo suppression of T-dependent antibody responses by treatment with a monoclonal anti-L3T4 antibody. European Journal of Immunology. **15**: 638-640.

Croll, N.A., Anderson, R.M., Gyorkos, T.W. and Ghadrian, E. (1982). The population biology and control of Ascaris lumbricoides in a rural community in Iran. Transactions of the Royal Society of Tropical Medicine and Hygiene. **76**: 187-197.

Crombie, J.A. (1985). Infection dynamics of Schistosoma mansoni in the laboratory mouse. Ph.D. Thesis. University of London.

Crombie, J.A. and Anderson, R.M. (1985). Population dynamics of Schistosoma mansoni in mice repeatedly exposed to infection. Nature. **315**: 491-493.

Dalton, P.R. and Pole D. (1978). Water contact patterns in relation to Schistosoma haematobium infection. Bulletin of the World Health Organization. **56**: 417-426.

Damian, R.T., Greene, N.D. and Fitzgerald, K. (1974). Schistosomiasis mansoni in baboons. II. Acquisition of immunity to challenge infection after repeated small exposures to cercariae of Schistosoma mansoni. American Journal of Tropical Medicine and Hygiene. **23**: 78-80.

Damian, R.T., Greene, N.D., Suzuki, T. and Dean, D.A. (1981). Schistosomiasis mansoni in baboons. V. Antibodies and immediate hypersensitivity in multiply infected Papo cynocephalus. American Journal of Tropical Medicine and Hygiene. **30**: 836-843.

Dean, D.A. (1983). Schistosoma and related genera: acquired resistance in mice. Experimental Parasitology. **55**: 1-104.

Dean, D.A., Bukowski, M.A. and Cheever, A.W. (1981). Relationship between acquired resistance, portal hypertension, and lung granulomas in ten strains of mice infected with Schistosoma mansoni. American Journal of Tropical Medicine and Hygiene. **30**: 806-814.

Dean, D.A. and Mangold, B.L. (1984). Autoradiographic analysis of resistance to reinfection with Schistosoma mansoni in mice. Evidence that the liver is a major site of worm elimination. American Journal of Tropical Medicine and Hygiene. **33**: 97-103.

Dean, D.A., Minard, P., Murrell, K.D. and Vannier, W.E. (1978b). Resistance of mice to secondary infection with Schistosoma mansoni. II. Evidence for a correlation between egg deposition and worm elimination. American Journal of Tropical Medicine and Hygiene. **27**: 957-965.

Dean, D.A., Minard, P., Stirewalt, M.A., Vannier, W.E. and Murrell, K.D. (1978a). Resistance of mice to secondary infection with Schistosoma mansoni. I. Comparison of bisexual and unisexual initial infections. American Journal of Tropical Medicine and Hygiene. **27**: 951-956.

Delgado, V.S. and McLaren, D.J. (1989). Variable generation of specific acquired resistance in CBA/Ca mice chronically infected with schistosomiasis mansoni. Parasitology. **99**: 357-364.

Delgado, V.S. and McLaren, D.J. (1990). Evidence for enhancememt of IgG1 subclass expression in mice polyvaccinated with radiation-attenuated cercariae of Schistosoma mansoni and the role of this isotype in serum transferred immunity. Parasite Immunology. **12**: 15-32.

Dessein, A.J., Begley, M., Demeure, C., Caillol, D., Fueri, J., Galvo dos reis, M., Andrade, Z.A., Prata, A. and Bina, J.C. (1988). Human resistance to Schistosoma mansoni is associated with IgG reactivity to a 37-kDa larval surface antigen. Journal of Immunology. **140**: 2727-2736.

Diab, M., Kamel, K., Cavender, D. and Higashi, G. (1989). Modulation of cell-mediated immunity in mice with chronic unisexual or bisexual Schistosoma mansoni cercarial infection. Annals of Tropical Medicine and Parasitology. **83**: 25-30.

Doenhoff, M., Bickle, Q., Bain, J., Webbe, G. and Nelson, G. (1980). Factors affecting the acquisition of resistance against Schistosoma mansoni in the mouse. V. Reduction in the degree of resistance to

reinfection after chemotherapeutic elimination of recently patent primary infections. Journal of Helminthology. **54**: 7-16.

Doenhoff, M., Bickle, Q., Long, E., Bain, J. and McGregor, A. (1978). Factors affecting the acquisition of resistance against Schistosoma mansoni in the mouse. I. Demonstration of resistance to reinfection using a model system that involves perfusion of mice within three weeks of challenge. Journal of Helminthology. **53**: 173-186.

Doenhoff, M. and Long, E. (1979). Factors affecting the acquisition of resistance against Schistosoma mansoni in the mouse. IV. The inability of T-cell deprived mice to resist reinfection, and other in vivo studies on the mechanisms of resistance. Parasitology. **78**: 171-183.

Duncan, E. and Colley, D. (1986). Effects of in vivo depletion of L3T4+ lymphocytes on immune responses in mice infected with Schistosoma mansoni. Federation Proceedings. **45**: 622 (Abstract).

Dunne, D.W., Bain, J., Lillywhite, J. and Doenhoff, M. (1984). The stage-strain and species specificity of a Schistosoma mansoni egg antigen fraction (CEF6) with serodiagnostic potential. Transactions of the Royal Society of Tropical Medicine and Hygiene. **78**: 460-470.

Dunne, D.W., Grabowska, A.M., Fulford, A.J.C., Butterworth, A.E., Sturrock, R.F., Koech, D. and Ouma, J.H. (1988). Human antibody responses to Schistosoma mansoni: the influence of epitopes shared between different life-cycle stages on the responses to the schistosomulum. European Journal of Immunology. **18**: 123-131.

El-Bassiouny, A.E.I., Shaker, Z.A., El-Kalouby, A.H. and El-Raziky, E.H. (1987). Effect of praziquantel on certain immune responses of schistosomal Egyptian patients. II. Cell-mediated lymphoproliferative response. Parasitological Research. **73**: 334-340.

Ellner, J.J., Olds, G.R., Lee, C.W., Kleinhenz, M.E. and Edmonds, K.L. (1982). Destruction of the multicellular parasite Schistosoma mansoni by T-lymphocytes. Journal of Clinical Investigations. **70**: 369-378.

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. Journal of Immunology. **126**: 309-312.

Esuruoso, G.O. (1976). The demonstration in vitro of the mitogenic effects of trypanosomal antigen on the spleen cells of normal, athymic and cyclophosphamide-treated mice. Clinical and Experimental Immunology. **23**: 314-317.

Fanning, M.M. and Kazura, J.W. (1984). Genetic-linked variation in susceptibility of mice to schistosomiasis mansoni. Parasite Immunology. **6**: 95-103.

Feldmeier, H., Gastl, G.A., Pogensee, U., Kortmann, C., Daffalla, A.A. and Peter, H.H. (1985a). Relationship between intensity of infection and immunomodulation in human schistosomiasis. I. Lymphocyte subpopulations and specific antibody responses. Clinical and Experimental Immunology. **60**: 225-233.

Feldmeier, H., Gastl, G.A., Pogensee, U., Kortmann, C., Daffalla, A.A. and Peter, H.H. (1985b). Relationship between intensity of infection and immunomodulation in human schistosomiasis. II. NK cell activity and in vitro lymphocyte proliferation. Clinical and Experimental Immunology. **60**: 234-240.

Foster, R. and Broomfield, K.E. (1971). Preliminary studies on the development of Schistosoma mansoni in rhesus monkeys following different regimes of infection. Annals of Tropical Medicine and Parasitology. **65**: 159-168.

Frandsden, F. (1987). Introduction. In "Danish bilharziasis laboratory biennial report 1985 & 1986" (eds. N.O. Christensen, F. Frandsden

and E. Svenningsen). Danish Bilharziasis Laboratory, Charlottenlund, Denmark.

Gajewski, T.F., Schell, S.R., Nau, G. and Fitch, F.W. (1989). Regulation of T-cell activation: differences among T-cell subsets. Immunological Reviews. **111**: 79-108.

Gazzinelli, G., Katz, N., Rocha, R.S. and Colley, D.G. (1983). Immune responses during human schistosomiasis mansoni. VIII. Differential in vitro cellular responsiveness to adult worm and schistosomular tegumental preparations. American Journal of Tropical Medicine and Hygiene. **32**: 326-333.

Gazzinelli, G., Lambertucci, J.R., Katz, N., Rocha, R.S., Lima, M.S. and Colley, D.G. (1985). Immune responses during human schistosomiasis mansoni. XI. Immunologic status of patients with acute infections and after treatment. Journal of Immunology. **135**: 2121-2127.

Georgi, J.R., Wade, S.E. and Dean, D.A. (1986). Attrition and temporal distribution of Schistosoma mansoni and S. haematobium schistosomula in laboratory mice. Parasitology. **93**: 55-70.

Gold, D. and Lengy, J. (1975). Failure to immunise mice against Schistosoma mansoni by therapeutic eradication of the adult worm burden. Annals of Tropical Medicine and Parasitology. **69**: 265-266.

Golenser, J., Marva, E., Spira, D.T., Gabrielsen, A.A. and Jensen, J.B. (1985). Mitogenic and antigenic activity of Plasmodium falciparum in primate and rodent lymphocytes. International Journal for Parasitology. **15**: 435-440.

Gonnert, R. and Andrews P. 1977. Praziquantel, a new broad-spectrum antischistosomal agent. Zeitschrift fur Parasitenkunde. **52**: 129-150.

Hagan, P., Blumenthal, U.J., Chaudri, M., Greenwood, B.M., Hayes, R.J., Hodgson, J., Kelly, C., Knight, M., Simpson, A.J.G., Smithers, S.R.

and Wilkins, H.A. (1987). Resistance to reinfection with Schistosoma haematobium in Gambian children: analysis of their immune responses. Transactions of the Royal Society of Tropical Medicine and Hygiene. **81**: 938-946.

Hagan, P., Wilkins, H.A., Blumenthal, A.J., Hayes, R.J., and Greenwood, B.M. (1985). Eosinophilia and resistance to Schistosoma haematobium in man. Parasite Immunology. **7**: 625-632.

Harrison, R.A., Bickle, Q. and Doenhoff, M.J. (1982). Factors affecting the acquisition of resistance against Schistosoma mansoni in the mouse. Evidence that the mechanisms which mediate resistance during early patent infections may lack immunological specificity. Parasitology. **84**: 93-110.

Hillyer, G.V. (1982). Schistosomiasis. In "Parasitic Zoonoses. Vol. III." (eds. G.V. Hillyer and C.E. Hopla), CRC Press Inc., Florida, USA.

Hillyer, G.V., Tiben, E.R., Knight, W.B., Gomez de Rios, I. and Pelley, R.P. (1979). Immunodiagnosis of infection with Schistosoma mansoni: Comparison of ELISA, radioimmunoassay and precipitation tests performed with antigens from eggs. American Journal of Tropical Medicine and Hygiene: **28**: 661-669.

Hsu, L.S.Y., Hsu, H.F. and Barmeister, L.F. (1981). Schistosoma mansoni: vaccination of mice with highly x-irradiated cercariae. Experimental Parasitology. **52**: 91-104.

Incani, R.N. and McLaren, D.J. (1981). Neutrophil-mediated cytotoxicity to schistosomula of Schistosoma mansoni in vitro: studies on the kinetics of complement and/or antibody-dependent adherence and killing. Parasite Immunology. **3**: 107-126.

Incani, R.N. and McLaren, D.J. (1984). Histopathological and ultrastructural studies of cutaneous reactions elicited in naive and chronically

infected mice by invading schistosomula of Schistosoma mansoni. International Journal for Parasitology. **14**: 259-276.

James, E.R. and Taylor, M.G. (1976). Transformation of cercariae to schistosomula: a quantitative comparison of transformation techniques and of infectivity by different injection routes of the organisms produced. Journal of Helminthology. **50**: 223-233.

James, S.L. (1981). In vitro proliferative response to living schistosomula by T lymphocytes from mice infected with Schistosoma mansoni. Parasitology. **83**: 147-162.

James, S.L. (1985). Induction of protective immunity against Schistosoma mansoni by a nonliving vaccine is dependent on the method of antigen presentation. Journal of Immunology. **134**: 1956-1960.

James S.L. and Cheever, A.W. (1985). Comparison of immune responses between high and low responder strains of mice in the concomitant immunity and vaccine models of resistance to Schistosoma mansoni. Parasitology. **91**: 301-315.

James, S.L. and Glaven, J. (1987). Effects of inhibitors of tumoricidal activity upon schistosomulum killing by activated macrophages. Infection and Immunity. **55**: 3174-3178.

James, S.L., Glaven, J., Goldenberg, S., Meltzer, M.S. and Pearce, E. (1990). Tumor necrosis factor (TNF) as a mediator of macrophage helminthtoxic activity. Parasite Immunology. **12**: 1-13.

James, S.L., Labine, M. and Sher, A. (1981). Mechanisms of protective immunity against Schistosoma mansoni infection in mice vaccinated with irradiated cercariae. I. Analysis of antibody and T lymphocyte responses in mouse strains developing differing levels of immunity. Cellular Immunology. **65**: 75-83.

James, S.L., Lazdins, J.K., Hieny, S. and Natovitz, P. (1983b). Macrophages as effector cells of protective immunity in murine schistosomiasis. VI. T cell-dependent, lymphokine-mediated, activation of macrophages in response to Schistosoma mansoni antigens. Journal of Immunology. **131**: 1481-1486.

James, S.L., Lazdins, J.K., Meltzer, M.S. and Sher, A. (1982). Macrophages as effector cells of protective immunity in murine schistosomiasis. I. Activation of peritoneal macrophages during natural infection. Cellular Immunology. **67**: 255-266.

James, S.L. and Sher, A. (1983). Mechanisms of protective immunity against Schistosoma mansoni in mice vaccinated with irradiated cercariae. III. Identification of a mouse strain, P/N, that fails to respond to vaccination. Parasite Immunology. **5**: 567-575.

James, S.L., Skamene, E. and Meltzer, M.S. (1983a). Macrophages as effector cells of protective immunity in murine schistosomiasis. V. Variation in macrophage schistosomulacidal and tumoricidal activities among mouse strains and correlation with resistance to reinfection. Journal of Immunology. **131**: 948-953.

Jordan, P. (1985). Schistosomiasis. Cambridge University Press, Cambridge.

Jourdane, J. and Theron, A. (1987). Larval development: eggs to cercariae. In "The biology of schistosomes, from genes to latrines" (eds. D. Rollinson and A.J.G. Simpson), Academic Press, London.

Kamel, I.A., Cheever, A.W., Elwi, A.M., Mosimann, J.E. and Danner, R. (1977). Schistosoma mansoni and Schistosoma haematobium infections in Egypt. I. Evaluation of techniques for recovery of worms and eggs at necropsy. American Journal of Tropical Medicine and Hygiene. **26**: 696-701.

Kamiya, H., Smithers, S.R. and McLaren, D.J. (1987). Schistosoma mansoni: autoradiographic tracking studies of isotopically labelled

challenge parasites in naive and vaccinated CBA/Ca mice. Parasite Immunology. **9**: 515-529.

Katz, S.P. and Colley, D.G. (1976). Induction of cellular and humoral immunological responsiveness to a soluble cercarial antigen preparation from Schistosoma mansoni. Infection and Immunity. **14**: 502-508.

Kayes, S.G. and Colley, D.G. (1984). Immunoregulation in experimental schistosomiasis. III. Role of macrophages in soluble egg antigen-induced chronic spleen cell augmentation of baseline lymphocyte reactivity. Cellular Immunology. **83**: 161-169.

Kelly, E.A.B. and Colley, D.G. (1988). In vivo effects of monoclonal anti-L3T4 antibody on immune responsiveness of mice infected with Schistosoma mansoni. Reduction of irradiated cercariae-induced resistance. Journal of Immunology. **140**: 2737-2745.

Keymer, A. E. (1982). Density dependent mechansisms in the regulation of intestinal helminth populations. Parasitology. **84**: 573-587.

Khalife, J., Capron, M., Capron, A., Grzych, J., Butterworth, A.E., Dunne, D.W. and Ouma, J.H. (1986). Immunity in human schistosomiasis mansoni. Regulation of protective immune mechanisms by IgM blocking antibodies. Journal of Experimental Medicine. **164**: 1626-1640.

Kloetzel, K. and da Silva, J.R. (1967). Schistosomiasis mansoni acquired in adulthood: behavior of egg counts and the intradermal test. American Journal of Tropical Medicine and Hygiene. **16**: 167-169.

Knight, S.C. (1987). Lymphocyte proliferation assays. In " Lymphocytes, a practical approach" (ed. G.G.B. Klaus), IRL Press, Oxford.

Letonja, T., Hammerberg, C. and Schurig, G. (1987). Evaluation of spleen lymphocyte responsiveness to a T-cell mitogen during early infection

with larval Taenia taeniaeformis. Parasitological Research. **73**: 265-270.

Lewert, R.M., Yogore Jr., M.G. and Blas, B.L. (1979). Lymphocyte responsiveness to phytohemagglutinin and to worm and egg antigens in human schistosomiasis japonica. American Journal of Tropical Medicine and Hygiene. **28**: 92-98.

Lewis, F.A., Kennell, C.J. and James, S.L. (1987). Vaccine-induced immunity in mice against Schistosoma mansoni trickle cercarial infections. American Journal of Tropical Medicine and Hygiene. **37**: 98-105.

Lillywhite, J.E., Burgess, P.J. and Stewart, T.J. (Undated). A laboratory manual for IFAT and ELISA. London School of Hygiene and Tropical Medicine Internal Publication.

Ljungstrom, I. and Sundquist, K.G. (1979). Lymphocyte activation induced by Trichinella spiralis infection reflected as spontaneous DNA synthesis in vitro. Clinical and Experimental Immunology. **38**: 381-388.

Lloyd, S. and Soulsby, E.J.L. (1988). Immunological responses of the host. In "Parasitology in focus, facts and trends" (ed. H. Mehlhorn), Springer-Verlag, Berlin.

Long, E., Doenhoff, M.J. and Bain, J. (1978). Factors affecting the acquisition of resistance against Schistosoma mansoni in the mouse. 2. The time at which resistance to reinfection develops. Journal of Helminthology. **52**: 187-191.

Long, E., Harrison, R., Bickle, Q., Bain, J., Nelson, G. and Doenhoff, M.J. (1980). Factors affecting the acquisition of resistance against Schistosoma mansoni in the mouse. The effect of varying the route and the number of primary infections, and the correlation between the

size of the primary infection and the degree of resistance that is acquired. Parasitology. **81**: 355-371.

Maddison, S.E. and Kagan, I.G. (1979a). Adoptive transfer of protective immunity in experimental schistosomiasis in the mouse. Journal of Parasitology. **65**: 515-519.

Maddison, S.E., Kagan, I.G., Chandler, F.W., Gold, D., Hillyer, G.V., Slemenda, S.B. and Tsang, V. (1979b). Immunization against Schistosoma mansoni in rhesus monkeys and the requirement of activation of both cellular and humoral mechanisms. Infection and Immunity. **25**: 237-248.

Mahmoud, A.A.F., Peters, P.A., Civil, R.H. and Remington, J.S. (1979). In vitro killing of schistosomula of Schistosoma mansoni by BCG and C. parvum activated macrophages. Journal of Immunology. **122**: 1655-1657.

Mahmoud, A.A.F., Warren, K.S. and Peters, P.A. (1975). A role for the eosinophil in acquired resistance to Schistosoma mansoni infection as determined by antieosinophil serum. Journal of Experimental Medicine. **142**: 805-813.

Maizels, R.M. and Robertson, B.D. (1988). Molecular parasitology laboratory manual. Department of Biology, Imperial College of Science, Technology and Medicine. Internal Publication.

Maluish, A.E. and Strong, D.M. (1986). Lymphocyte proliferation. In "Manual of clinical laboratory immunology, third edition" (eds. N.R. Rose, H. Friedman and J.L. Fahey), American Society for Microbiology, Washington, D.C..

Mangold, B.L. and Dean, D.A. (1986). Passive transfer with serum and IgG antibodies of irradiated cercaria-induced resistance against Schistosoma mansoni in mice. Journal of Immunology. **136**: 2644-2648.

Mansfield, J.M., Craig, S.A. and Stelzer, G.T. (1976). Lymphocyte function in experimental African trypanosomiasis: mitogenic effects of trypanosome extracts in vivo. Infection and Immunity. **14**: 976-981.

Mastin, A.J., Bickle, Q.D. and Wilson, R.A. (1983). Schistosoma mansoni: migration and attrition of irradiated and challenge schistosomula in the mouse. Parasitology. **87**: 87-102.

McCullough, F.S. and Bradley, D.J. (1973). Egg output stability and the epidemiology of Schistosoma haematobium. I. Variation and stability of S. haematobium egg counts. Transactions of the Royal Society of Tropical Medicine and Hygiene. **67**: 475-490.

McHugh, S.M., Coulson, P.S. and Wilson, R.A. (1987). The relationship between pathology and resistance to reinfection with Schistosoma mansoni in mice: a causal mechanism of resistance in chronic infections. Parasitology. **94**: 81-91.

McKay, D.A., Warren, K.S., Cook, J.A. and Jordan, P. (1973). Immunologic diagnosis of schistosomiasis. III. The effects of nutritional status and infection intensity on intradermal test results in St. Lucian children. American Journal of Tropical Medicine and Hygiene. **22**: 205-210.

McLaren, D.J. (1989). Will the real target of immunity to schistosomiasis please stand up. Parasitology Today. **5**: 279-282.

McLaren, D.J., Clegg, J.A. and Smithers, S.R. (1975). Acquisition of host antigens by young Schistosoma mansoni in mice: correlation with failure to bind antibody in vitro. Parasitology. **70**: 67-75.

McLaren, D.J. and James, S.L. (1985). Ultrastructural studies of the killing of schistosomula of Schistosoma mansoni by activated macrophages in vitro. Parasite Immunology. **7**: 315-331.

McLaren, D.J., McKean, J.R., Olsson, I., Venge, P. and Kay, A.B. (1981). Morphological studies on the killing of schistosomula of Schistosoma mansoni by human eosinophil and neutrophil cationic proteins in vitro. Parasite Immunology. **3**: 359-373.

McLaren, D.J. and Smithers, S.R. (1987). The immune response to schistosomes in experimental hosts. In "The biology of schistosomes, from genes to latrines" (eds. D. Rollinson and A.J.G. Simpson), Academic Press, London.

McLaren, D.J., Strath, M. and Smithers, S.R. (1987). Schistosoma mansoni: evidence that immunity in vaccinated and chronically infected CBA/Ca mice is sensitive to treatment with a monoclonal antibody that depletes cutaneous effector cells. Parasite Immunology. **9**: 667-682.

Medley, G.F. and Anderson, R.M. (1985). Density-dependent fecundity in Schistosoma mansoni infections in man. Transactions of the Royal Society of Tropical Medicine and Hygiene. **79**: 532-539.

Mendlovic, F., Tarab-Hazdai, R. and Arnon, R. (1987). Role of cellular and humoral immunity and helper cell involvement in permissiveness to infection by Schistosoma mansoni. European Journal of Immunology. **17**: 1151-1157.

Miller, K.L. and Smithers, S.R. (1980). Schistosoma mansoni: the attrition of a challenge infection in mice immunized with highly irradiated live cercariae. Experimental Parasitology. **50**: 212-221.

Miller, P. and Wilson, R.A. (1978). Migration of the schistosomula of Schistosoma mansoni from skin to lungs. Parasitology. **77**: 281-302.

Miller, P. and Wilson, R.A. (1980). Migration of the schistosomula of Schistosoma mansoni from the lungs to the hepatic portal system. Parasitology. **80**: 267-288.

Minard, P., Dean, D.A., Jacobson, R.H., Vannier, W.E. and Murrell, K.D. (1978). Immunization of mice with Cobalt-60 irradiated Schistosoma mansoni cercariae. American Journal of Tropical Medicine and Hygiene. **27**: 76-87.

Mishell, B.B. and Shiigi, S.M. (1980). Selected methods in cellular immunology. W.H. Freeman and Company, San Francisco.

Moloney, N.A., Hinchcliffe, P. and Webbe, G. (1986). The ability of single sex infections of Schistosoma japonicum to induce resistance to reinfection in mice. Journal of Helminthology. **60**: 250-254.

Moloney, N.A., Hinchcliffe, P. and Webbe, G. (1987b). Loss of resistance to reinfection with Schistosoma japonicum in mice after treatment with praziquantel. Transactions of the Royal Society of Tropical Medicine and Hygiene. **81**: 247-254.

Moloney, N.A., Luty, A.J.F., Hinchcliffe, P. and Webbe, G. (1987a). The involvement of eggs and soluble egg antigens in resistance to Schistosoma japonicum. Journal of Helminthology. **61**: 9-17.

Moloney, N.A. and Webbe, G. and Luty, A.J.F. (1984). Factors affecting the acquisition of resistance to Schistosoma japonicum in the mouse. I. The correlation between egg deposition and worm elimination. Parasitology. **89**: 345-360.

Mott, K.E. (1987). Epidemiological considerations for development of a schistosome vaccine. In "Acta Tropica: Prospects for immunological intervention in human schistosomiasis." Vol. 44. Supp. 12: 13-20.

Murare, H.M. and Doenhoff, M.J. (1987). Parasitological observations of Schistosoma bovis in normal and T-cell deprived mice. Parasitology. **95**: 507-516.

Murrell, K.D., Clark, S., Dean, D.A. and Vannier, W.E. (1979). Influence of mouse strain on induction of resistance with irradiated Schistosoma mansoni cercariae. Journal of Parasitology. **65**: 829-830.

Nash, T.E., Cheever, A.W., Ottesen, E.A. and Cook, J.A. (1982). Schistosome infections in humans: perspectives and recent findings. Annals of Internal Medicine. **97**: 740-754.

Nash, T.E., Ottesen, E.A. and Cheever, A.W. (1978). Antibody response to a polysaccharide antigen present in the schistosome gut. II. Modulation of the antibody response. American Journal of Tropical Medicine and Hygiene. **24**: 944-950.

Olds, G.R. and Ellner, J.J. (1984). Modulation of macrophage activation and resistance by suppressor T lymphocytes in chronic murine Schistosoma mansoni infection. Journal of Immunology. **133**: 2720-2724.

Olivotto, M., Boddi, V., Sbarba, P. and Arcangeli, A. (1982). A comparative kinetic analysis of proliferation in vitro of Con A-treated splenocytes and syngeneic leukaemia cells. Cellular and Tissue Kinetics. **15**: 623-636.

Omer-Ali. P., Smithers, S.R., Bickle, Q., Phillips, S.M., Harn, D. and Simpson, A.J.G. (1988). Analysis of the anti-Schistosoma mansoni surface antibody response during murine infection and its potential contribution to protective immunity. Journal of Immunology. **140**: 258-264.

Oppenheim, J.J. and Schecter, B. (1980). Lymphocyte transformation . In " Manual of clinical immunology, second edition", (eds. N.R. Rose and H. Friedman), American Society for Microbiology, Washington, D.C..

Ottesen, E.A. (1979). Modulation of the host response in human schistosomiasis. I. Adherent suppressor cells that inhibit lymphocyte

proliferative responses to parasite antigens. Journal of Immunology. **123**: 1639-1644.

Ottesen, E.A., Hiatt, R.A., Cheever, A.W., Sottomayor, Z.R. and Neva, F.A. (1978). The acquisition and loss of antigen-specific cellular immune responsiveness in acute and chronic schistosomiasis in man. Clinical and Experimental Immunology. **33**: 38-47.

Pearce, E.J., Basch, P. and Sher, A. (1986). Evidence that the reduced antigenicity of developing Schistosoma mansoni schistosomula is due to antigen shedding rather than host molecule acquisition. Parasite Immunology. **8**: 79-84.

Pearce, E.J. and James, S.L. (1986). Post-lung stage schistosomula of Schistosoma mansoni exhibit transient susceptibility to macrophage-mediated cytotoxicity in vitro that may relate to late phase killing in vivo. Parasite Immunology. **8**: 513-527.

Pearce, E.J. and McLaren, D.J. (1983). Reappraisal of the guinea-pig model as an experimental host for studies of schistosomiasis mansoni. Parasitology. **87**: 455-464.

Pelley, R.P., Ruffier, J.J. and Warren, K.S. (1976). Suppressive effect of a chronic helminth infection, schistosomiasis mansoni, on the in vitro responses of spleen and lymph node cells to the T-cell mitogens, phytohemagglutin and concanavalin A. Infection and Immunity. **13**: 1176-1183.

Phillips, S.M., Reid, W.A., Khoury, P.B. and Doughty, B.L. (1977). The cellular and humoral immune response to Schistosoma mansoni infections in inbred rats. IV. A posteriori interpretations. American Journal of Tropical Medicine and Hygiene. **6**: 48-53.

Pons, A., Morgan, J.S., Hutchinson, M.L., Rojkind, M., Groszmann, R.J. and Stadecker, M.J. (1989). Resistance to reinfection in experimental

murine schistosomiasis: role of porto-hepatic hemodynamics. American Journal of Tropical Medicine and Hygiene. **41**: 189-197.

Ramalho-Pinto, F.J., de Rossi, R. and Smithers, S.R. (1979). Murine schistosomiasis mansoni: anti-schistosomula antibodies and the IgG subclasses involved in the complement and eosinophil-mediated killing of schistosomula in vitro. Parasite Immunology. **1**: 295-308.

Reiner, N.E., Kamel, R., Higashi, G.I., Naggar, A.E., Aguib, M., Ellner, J.J. and Mahmoud, A.A.F. (1979). Concurrent responses of peripheral blood and splenic mononuclear cells to antigenic and mitogenic stimulation in human hepatosplenic schistosomiasis. Journal of Infectious Diseases. **140**: 162-168.

Roberts, S.M., Wilson, R.A., Ouma, J.H., Kariuki, H.C., Koech, D., Arap Siongok, T.K., Sturrock, R.F. and Butterworth, A.E. (1987). Immunity after treatment of human schistosomiasis mansoni: quantitative and qualitative antibody responses to tegumental membrane antigens prepared from adult worms. Transactions of the Royal Society of Tropical Medicine and Hygiene. **81**: 786-793.

Rocklin, R.E. (1986). Introduction to cellular components. In "Manual of clinical laboratory immunology, third edition" (eds. N.R. Rose, H. Friedman and J.L. Fahey), American Society for Microbiology, Washington D.C..

Rollinson, D. and Southgate, V.R. (1987). The genus Schistosoma: a taxonomic appraisal. In " The biology of schistosomes, from genes to latrines" (eds. D. Rollinson and A.J.G. Simpson), Academic Press, London.

Sabah, A.A., Fletcher, C., Webbe, G. and Doenhoff, M.J. (1986). Schistosoma mansoni: chemotherapy of infections of different ages. Experimental Parasitology. **61**: 294-303.

Sadun, E.H., von Lichtenberg, F., Cheever, A.W. and Erickson, D.G. (1970). Schistosomiasis mansoni in the chimpanzee. The natural history of chronic infections after single and multiple exposures. The American Journal of Tropical Medicine and Hygiene. **19**: 258-277.

Samuelson, J.C., Sher, A. and Caulfield, J.P. (1980). Newly transformed schistosomula spontaneously lose surface antigens and C3 acceptor sites during culture. Journal of Immunology. **124**: 2055-2063.

Scott, P., Pearce, E., Cheever, A.W., Coffman, R.L. and Sher, A. (1989). Role of cytokines and CD4+ T-cell subsets in the regulation of parasite immunity and disease. Immunological Reviews. **112**: 161-182.

Severinson, E. and Larson, E.L. (1986). Lymphocyte response to polyclonal B and T cell activators. In "Handbook of experimental immunology, Vol. 2, fourth edition" (ed. D.M. Weir), Blackwell Scientific Publications, Oxford.

Sher, A., Hieny, S. and James, S.L. (1984). Mechanisms of protective immunity against S. mansoni infection in mice vaccinated with irradiated cercariae. VI. Influence of the major histocompatibility complex. Parasite Immunology. **6**: 319-328.

Sher, A., Hieny, S., James, S.L. and Asofsky, R. (1982a). Mechanisms of protective immunity against Schistosoma mansoni infection in mice vaccinated with irradiated cercariae. II. Analysis of immunity in hosts deficient in T lymphocytes, B lymphocytes or complement. Journal of Immunology. **128**: 1880-1884.

Sher, A., James, S.L.,Simpson, A.J.G., Lazdins, J.K. and Meltzer, M.S. (1982b). Macrophages as effector cells of protective immunity in murine schistosomiasis. III. Loss of susceptibility to macrophage-mediated killing during maturation of S. mansoni schistosomula from the skin to the lung stage. Journal of Immunology. **128**: 1876-1879.

Sher, A. and Moser, G. (1981). Immunologic properties of developing schistosomula. American Journal of Pathology. **102**: 121-126.

Sher, A., Smithers, S.R. and Mackenzie, P. (1975). Passive transfer of acquired resistance to Schistosoma mansoni in laboratory mice. Parasitology. **70**: 347-357.

Sher, A., Smithers, S.R., Mackenzie, P. and Broomfield, K. (1977). Schistosoma mansoni: immunoglobulins involved in passive immunozation of laboratory mice. Experimental Parasitology. **41**: 160-166.

Simpson, A.J.G., Schryer, M.D., Cesari, I.M., Evans, W.H. and Smithers, S.R. (1981). Isolation and partial characterization of the tegumental outer membrane of adult Schistosoma mansoni. Parasitology. **83**: 163-168.

Sithithaworn, P. (1987). Population dynamics of Schistosoma mansoni in laboratory mice exposed to repeated infections. Ph.D. Thesis. University of London.

Smith, M.A. and Clegg, J.A. (1976). Different levels of acquired immunity to Schistosoma mansoni in two strains of hamster. Parasitology. **73**: 47-53.

Smithers, S.R. and Doenhoff, M.J. (1982). Schistosomiasis. In "Immunology of parasitic infections" (eds. S. Cohen and K.S. Warren), Blackwell Scientific Publications, London.

Smithers, S.R. and Gammage, K. (1980). Recovery of Schistosoma mansoni from the skin, lungs and hepatic portal system of naive mice and mice previously exposed to S. mansoni: evidence for two phases of parasite attrition in immune mice. Parasitology. **80**: 289-300.

Smithers, S.R. and Miller, K.L. (1980). Protective immunity in murine schistosomiasis mansoni: evidence for two distinct mechanisms. American Journal of Tropical Medicine and Hygiene. **29**: 832-841.

Smithers, S.R. and Terry, R.J. (1965). The infection of laboratory hosts with cercariae of Schistosoma mansoni and the recovery of adult worms. Parasitology. **55**: 695-700.

Smithers, S.R. and Terry, R.J. (1967). Resistance to experimental infection with Schistosoma mansoni in rhesus monkeys induced by the transfer of adult worms. Transactions of the Royal Society of Tropical Medicine and Hygiene. **61**: 517-533.

Smithers, S.R. and Terry, R.J. (1969). Immunity in schistosomiasis. Annals of the New York Academy of Sciences. **160**: 826-840.

Sturrock, R.F., Bensted-Smith, R., Butterworth, A.E., Dalton, P.R., Kariuki, H.C., Koech, D., Mugambi, M., Ouma, J.H. and Arap Siongok, T.K. (1987). Immunity after treatment of human schistosomiasis mansoni. III. Long-term effects of treatment and retreatment. Transactions of the Royal Society of Tropical Medicine and Hygiene. **81**: 303-314.

Sturrock, R.F., Cottrell, B.J. and Kimani, R. (1984). Observations on the ability of repeated light exposures to Schistosoma mansoni cercariae to induce resistance to reinfection in Kenyan baboons (Papio anubis). Parasitology. **88**: 505-514.

Sturrock, R.F., Kimani, R., Cottrell, B.J., Butterworth, A.E., Seitz, H.M., Siongok, T.K. and Houba, V. (1983). Observations on possible immunity to reinfection among Kenyan schoolchildren after treatment for Schistosoma mansoni. Transactions of the Royal Society of Tropical Medicine and Hygiene. **77**: 363-371.

Suzuki, T. and Damian, R.T. (1981). Schistosomiasis mansoni in baboons. IV. The development of antibodies to Schistosoma mansoni adult

worm, egg and cercarial antigens during acute and chronic infections. American Journal of Tropical Medicine and Hygiene. **30**: 825-835.

Tawfik, A.F., Carter, C.E. and Colley, D.G. (1986). Effects of anti-schistosomal chemotherapy on immune responses, protection and immunity. I. Changes in cellular and humoral responses. American Journal of Tropical Medicine and Hygiene. **35**: 100-109.

Tawfik, A.F. and Colley, D.G. (1986). Effects of anti-schistosomal chemotherapy on immune responses, protection and immunity. II. Concomitant immunity and immunization with irradiated cercariae. American Journal of Tropical Medicine and Hygiene. **35**: 110-117.

Teesdale, C.H., Fahringer, K. and Chitsolo, L. (1985). Egg count variability and sensitivity of a thin smear technique for the diagnosis of Schistosoma mansoni. Transactions of the Royal Society of Tropical Medicine and Hygiene. **69**: 369-373.

Trizio, D., Della Bruna, C. and Isetta, A.M. (1980). Modification of immune responsiveness in murine schistosomiasis mansoni. Immunology. **40**: 353-358.

Troye-Blomberg, M., Sjoholm, P.E., Perlmann, H., Pattarroyo, M.E. and Perlmann, P. (1983). Regulation of the immune response to Plasmodium falciparum malaria. I. Non-specific proliferative responses in vitro and characterization of lymphocytes. Clinical and Experimental Immunology. **53**: 335-344.

Vignali, D.A.A., Bickle, Q.D. and Taylor, M.G. (1988). Studies on immunity to Schistosoma mansoni in vivo: whole-body irradiation has no effect on vaccine-induced resistance in mice. Parasitology. **96**: 49-61.

Vignali, D.A.A., Bickle, Q.D. and Taylor, M.G. (1989c). Immunity to Schistosoma mansoni in vivo: contradiction or clarification? Immunology Today. **10**: 410-416.

Vignali, D.A.A., Crocker, P., Bickle, Q.D., Cobbold, S., Waldman, H. and Taylor, M.G. (1989b). A role for CD4+ but not CD8+ T-cells in immunity to Schistosoma mansoni induced by 20krad-irradiated and Ro 11-3128-terminated infections. Immunology. **67**: 466-472.

Vignali, D.A.A., Klaus, S.N., Bickle, Q.D. and Taylor, M.G. (1989a). Histological examination of the cellular reactions around schistosomula of Schistosoma mansoni in the lungs of sublethally irradiated and unirradiated, immune and control rats. Parasitology. **98**: 57-65.

Waithe, W.I. and Hirschorn, K. (1978). Lymphocyte response to activators. In "Handbook of experimental immunology, Vol. 3, third edition" (ed. D.M. Weir), Blackwell Scientific Publications, Oxford.

Ward, R.E.M. and McLaren, D.J. (1988). Schistosoma mansoni: evidence that eosinophils and/or macrophages contribute to skin-phase challenge attrition in vaccinated CBA/Ca mice. Parasitology. **96**: 63-84.

Warren, K.S. (1963). The contribution of worm burden and host response to the development of hepatosplenic schistosomiasis mansoni in mice. American Journal of Tropical Medicine and Hygiene. **12**: 34-39.

Warren, K.S. (1966). The pathogenesis of clay-pipe stem cirrhosis in mice with chronic schistosomiasis mansoni, with a note on the longevity of the schistosomes. American Journal of Pathology. **49**: 477-489.

Warren, K.S. (1973). Regulation of the prevalence and intensity of schistosomiasis in man: immunology or ecology. Journal of Infectious Diseases. **127**: 595-609.

Warren, K.S. (1977). Modulation of immunopathology and disease in schistosomiasis. American Journal of Tropical Medicine and Hygiene. **26**: 113-118.

Warren, K.S. (1982). Selective primary health care: strategies for control of disease in the developing world. I. Schistosomiasis. Reviews of Infectious Diseases. **4**: 715-726.

Warren, K.S., Cook, J.A., Littell, A.S., Kagan, I.G. and Jordan, P. (1973b). Immunological diagnosis of schistosomiasis. II. Further studies of the sensitivity and specificity of delayed intradermal reactions. American Journal of Tropical Medicine and Hygiene. **22**: 199-204.

Warren, K.S., Domingo, E.O. and Cowan, R.B.T. (1967). Granuloma formation around eggs as a manifestation of delayed hypersensitivity. American Journal of Pathology. **51**: 735-756.

Warren, K.S., Kellermeyer, R.W., Jordan, P., Littell, A.S., Cook, J.A. and Kagan, I.G. (1973a). Immunologic diagnosis of schistosomiasis. I. A controlled study of intradermal (immediate and delayed) and serologic tests in St. Lucians infected with Schistosoma mansoni and uninfected St. Vincentians. American Journal of Tropical Medicine and Hygiene. **22**: 189-198.

Warren, K.S., Pelley, R.P. and Mahmoud, A.A.F. (1977). Immunity and immunopathology following reinfection of mice cured of chronic schistosomiasis mansoni. American Journal of Tropical Medicine and Hygiene. **26**: 957-962.

Wertheimer, S.P., Vermund, S.H., Lumley, L.H. and Singer, B. (1987). Lack of demonstrable density-dependent fecundity of schistosomiasis mansoni: analyses of Egyptian quantitative human autopsies. American Journal of Tropical Medicine and Hygiene. **37**: 79-84.

Wilkins, H.A. (1987). The epidemiology of schistosome infections in man. In "The biology of schistosomes, from genes to latrines" (eds. D. Rollinson and A.J.G. Simpson), Academic Press, London.

Wilkins, H.A., Blumenthal, U.J., Hagan, P., Hayes, R.J. and Tulloch, S. (1987). Resistance to reinfection after treatment of urinary schistosomiasis. Transactions of the Royal Society of Tropical Medicine and Hygiene. **81**: 29-35.

Wilkins, H.A., Goll, P.H., Marshall, T.F. de C. and Moore, P.J. (1984a). Dynamics of Schistosoma haematobium infection in a Gambian community. I. The pattern of human infection in the study area. Transactions of the Royal Society of Tropical Medicine and Hygiene. **78**: 216-221.

Wilkins, H.A., Goll, P.H., Marshall, T.F. de C. and Moore, P.J. (1984b). Dynamics of Schistosoma haematobium infection in a Gambian community. III. Acquisition and loss of infection. Transactions of the Royal Society of Tropical Medicine and Hygiene. **78**: 227-232.

Wilkins, H.A. and Scott, A. (1978). Variation and stability in Schistosoma haematobium egg counts: a four year study of Gambian children. Transactions of the Royal Society of Tropical Medicine and Hygiene. **72**: 397-404.

Wilson, R.A. and Coulson, P.S. (1989). Where and how does immune elimination of Schistosoma mansoni occur. Parasitology Today. **5**: 274-278.

Wilson, R.A., Coulson, P.S. and Dixon, B. (1986). Migration of the schistosomula of Schistosoma mansoni in mice vaccinated with radiation-attenuated cercariae, and normal mice: an attempt to identify the site and timing of parasite death. Parasitology. **92**: 101-116.

Wilson, R.A., Coulson, P.S. and McHugh, S.M. (1983). A significant part of the concomitant immunity of mice to Schistosoma mansoni is the consequence of a leaky hepatic portal system, not immune killing. Parasite Immunology. **5**: 595-601.

Wofsky, D., Mayes, D.C., Woodcock, J. and Seaman, W.E. (1985). Inhibition of humoral immunity in vivo by monoclonal antibody to L3T4: studies with soluble antigens in intact mice. Journal of Immunology. **135**: 1698-1781.

Yi, X., Omer-Ali, P., Kelly, C., Simpson, A.J.G. and Smithers, S.R. (1986). IgM antibodies recognizing carbohydrate epitopes shared between schistosomula and miracidia of Schistosoma mansoni that block in vitro killing. Journal of Immunology. **137**: 3946-3954.

Appendix 1: Description of Reagents from Chapter 2

Table A2.1: List of buffers and reagents used for ELISA.

PBS. . .	..85g NaCl, 1.28g Na_2HPO_4, 0.156g $NaH_2PO_4.2H_2O$, 1L dist. H_2O.
Incubation Buffer.	..100ml PBS, 0.05ml Tween 20 (Sigma Ltd., U.K.).
Coating Buffer.	.1.59g Na_2CO_3, 8.9g $NaHCO_3$, 1L dist. H_2O, pH 9.6.
Washing Buffer.	..9g NaCl, 0.5ml Tween 20, 1L dist. H_2).
Substrate Buffer.	.7.19g Na_2HPO_4, 5.19g citric acid, 1L dist. H_2O.
Stock substrate.	..100mg orthophenylamine diamine, 10ml methanol.
Substrate solution.	..100ml substrate buffer, 1ml stock substrate, 10ul 30% H_2O_2.

Table A2.2: List of reagents used for in vitro lymphocyte proliferation assay.

Constituents of cell growth medium (100ml)

1.0ml.	.100mM sodium pyruvate
1.0ml.	.200mM L-glutamine
1.0ml.	.100x solution of non-essential amino acids
1.0ml.	.Hepes buffer
1.5ml.	.7.5% sodium bicarbonate
1.0ml.	.5000 units/ml pennicillin/streptomycin
0.2ml.	.10mg/ml gentamycin
93.3ml.. . . .	.RPMI 1640

All reagents were purchased from Gibco LtD., U.K.

Hypotonic lysis solution (1L) (Enriquez et al., 1986).

NH_4Cl.	.8.19g
$KHCO_3$.	.1g
EDTA.	.0.037g
Dist. H_2O. . . .	.1L

Appendix 2: Statistical Analysis of Results from Chapter 3

Table A3.1: Baseline lymphoproliferative responses of cells of CBA/Ca mice.

a) Comparison: Baseline lymphoproliferative responses with time post-initial exposure.

Experimental mice

	Statistic	df	Probability
ANOVA with time-Dose 1	F=3.45	7, 25	p<0.05
ANOVA with time-Dose 3	F=2.89	6, 25	p<0.05
ANOVA with time-Dose 10	F=14.40	3, 14	p<0.01

Naive control mice

ANOVA with time-Dose 1	F=2.20	7, 23	p=0.05
ANOVA with time-Dose 3	F=65.54	6, 22	p<0.01
ANOVA with time-Dose 10	F=18.57	3, 16	p<0.01

b) Comparison: Baseline proliferation of cells of experimental mice vs. naive control mice.

Test ANOVA			
Dose 1	F=4.56	1, 48	p<0.01
Dose 3	F=18.86	1, 45	p<0.01
Dose 10	F=29.22	1, 22	p<0.01

Table A3.2: Non-specific lymphoproliferative responses of cells of naive CBA/Ca mice to S. mansoni antigens.

a) Comparison: Response to each antigen and in the absence of antigenic stimulation.

Dose 1:

Test			
ANOVA with antigen	F=61.17	3, 128	p<0.01
Scheffe-Baseline vs. Schistsomula			p<0.01
Scheffe-Baseline vs. SEA			p>0.05
Scheffe-Baseline vs. Adult			p<0.01
Scheffe-Schistosomula vs. Adult			p<0.01
Scheffe-SEA vs. Adult			p<0.01

Dose 3:

Test			
ANOVA with antigen	F=44.59	3, 124	p<0.01
Scheffe-Baseline vs. Schistosomula			p<0.01
Scheffe-Baseline vs. SEA			p>0.05
Scheffe-Baseline vs. Adult			p<0.01
Scheffe-Schistosomula vs. SEA			p<0.01
Scheffe-Schistosomula vs. Adult			p>0.05

Scheffe-SEA vs. Adult			p<0.01
Dose 10:			
Test			
ANOVA with antigen	F=28.30	3,68	p<0.01
Scheffe-Baseline vs. Schistosomula			p<0.01
Scheffe-Baseline vs. SEA			p>0.05
Scheffe-Baseline vs. Adult			p<0.05
Scheffe-Schistosomula vs. SEA			p<0.01
Scheffe-Schistosomula vs. Adult			p<0.01
Scheffe-SEA vs. Adult			p>0.05

Table A3.3: Lymphoproliferative responses of cells from CBA/Ca mice to S. mansoni antigens following a trickle infection.

a) Comparison: Response of cells of experimental mice vs. naive controls.

Schistosomula antigen

Test			
ANOVA			
Dose 1	F=31.96	1,48	p<0.01
Dose 3	F=74.20	1,46	p<0.01
Dose 10	F=97.52	1,28	p<0.01

	Cercarial dose/mouse/week		
Time (Weeks)	1	3	10
6	p>0.05	p<0.05	p<0.01
9	p>0.05	p>0.05	p<0.01
10		p<0.01	
12	p<0.01	p<0.01	p<0.01
15	p>0.05	p<0.01	p<0.01
18		p<0.01	
20	p>0.05	p<0.01	
25	p>0.05		
30	p<0.05		
35	p<0.01		

SEA

Test			
ANOVA			
Dose 1	F=5.01	1,48	p<0.05
Dose 3	F=11.61	1,46	p<0.01
Dose 10	F=47.76	1,28	p<0.01

	Cercarial dose/mouse/week		
Time (Weeks)	1	3	10
6	p>0.05	p>0.05	p>0.05
9	p>0.05	p>0.05	p<0.01
10		p<0.05	
12	p>0.05	p<0.05	p<0.01
15	p>0.05	p>0.05	p<0.01
18		p<0.05	
20	p>0.05	p>0.05	
25	p>0.05		
30	p>0.05		
35	p<0.05		

Adult worm antigen

Test
ANOVA

Dose 1	F=5.54	1,48	p<0.05
Dose 3	F=0.66	1,46	p>0.05
Dose 10	F=4.74	1,28	p<0.05

	Cercarial dose/mouse/week		
Time (Weeks)	1	3	10
6	p<0.05	p>0.05	p>0.05
9	p>0.05	p<0.05*	p<0.05
10		p>0.05	
12	p<0.05	p>0.05	p>0.05
15	p>0.05	p<0.05*	p>0.05
18		p>0.05	
20	p>0.05	p>0.05	
25	p>0.05		
30	p>0.05		
35	p>0.05		

* Response of cells from naive mice exceeded response of cells from experimental animals

b) Response of one antigen vs. another.

Dose 1:

Schistosomula vs. SEA	F=43.87	1,50	p<0.01
Schistosomula vs. Adult	F=34.44	1,60	p<0.01
SEA vs. Adult	F=2.75	1,50	p>0.05

Dose 3:

Schistosomula vs. SEA	F=29.34	1,50	p<0.01
Schistosomula vs. Adult	F=99.03	1,50	p<0.01
SEA vs. Adult	F=4.15	1,50	p<0.05

Dose 10:

Schistosomula vs. SEA	F=37.76	1,28	p<0.01
Schistsomula vs. Adult	F=129.2	1,28	p<0.01
SEA vs. Adult	F=21.83	1,28	p<0.01

c) Comparison: Proliferation for each antigen considered separately.

Schistosomula antigen
ANOVA with time

Dose 1	F=2.15	7,25	p>0.05
Dose 3	F=9.73	6,25	p<0.01
Dose 10	F=5.37	3,14	p<0.05

ANOVA with dose

Dose 1 vs. Dose 3	F=0.55	1,55	p>0.05
Dose 1 vs. Dose 10	F=12.94	1,43	p<0.01
Dose 3 vs. Dose 10	F=12.82	1,42	p<0.01

SEA
ANOVA with time

Dose 1	F=1.56	7,25	p>0.05
Dose 3	F=2.18	6,25	p>0.05
Dose 10	F=7.37	3,14	p<0.01

ANOVA with dose

Dose 1 vs. Dose 3	F=0.96	1,55	p>0.05
Dose 1 vs. Dose 10	F=16.28	1,43	p<0.01
Dose 3 vs. Dose 10	F=15.75	1,42	p<0.01

Adult worm antigen
ANOVA with time

Dose 1	F=2.55	7,25	p<0.05
Dose 3	F=4.78	6,25	p<0.01
Dose 10	F=4.13	3,14	p<0.05

ANOVA with dose

Dose 1 vs. Dose 3	F=7.90	1,55	p<0.01
Dose 1 vs. dose 10	F=4.14	1,43	p<0.05
Dose 3 vs. dose 10	F=2.71	1,42	p>0.05

Table A3.4: Lymphoproliferative responses of cells from CBA/Ca mice to concanavalin A following a trickle infection.

a) Comparison: Responses within each dose of exposure.

Dose 1:

ANOVA with time	F=9.40	7,25	p<0.01
Experimental vs. naive controls	F=5.02	1,48	p<0.05

Dose 3:

ANOVA with time	F=20.08	6,25	p<0.01
Experimental vs. naive controls	F=46.90	1,46	p<0.01

Dose 10:

Anova with time	F=4.56	3,14	p<0.01
Experimental vs. naive controls	F=16.78	1,28	p<0.01

Table A3.5: Immunoglobulin levels in mice exposed to repeated low level doses of S. mansoni cercariae.

a) Comparison: IgG reactivity to each S. mansoni antigen.

Schistosomula antigen
ANOVA with time

Dose 1	F=30.35	6,50	p<0.01
Dose 3	F=39.85	6,45	p<0.01
Dose 10	F=86.33	5,34	p<0.01

ANOVA with dose

Dose 1 vs. Dose 3	F=21.64	1,80	p<0.01
Dose 1 vs. Dose 10	F=51.19	1,67	p<0.01
Dose 3 vs. Dose 10	F=11.06	1,77	p<0.01

SEA
ANOVA with time

Dose 1	F=21.57	6,50	p<0.01
Dose 3	F=32.38	6,45	p<0.01
Dose 10	F=49.60	5,34	p<0.01

ANOVA with dose

Dose 1 vs. Dose 3	F=6.59	1,80	p<0.05
Dose 1 vs. Dose 10	F=9.90	1,67	p<0.01
Dose 3 vs. Dose 10	F=2.21	1,77	p>0.05

Adult worm antigen
ANOVA with time

Dose 1	F=28.32	6,50	p<0.01
Dose 3	F=41.13	6,45	p<0.01
Dose 10	F=61.52	5,34	p<0.01

ANOVA with dose

Dose 1 vs. Dose 3	F=11.21	1,80	p<0.01
Dose 1 vs. Dose 10	F=34.16	1,67	p<0.01
Dose 3 vs. Dose 10	F=15.94	1,77	p<0.01

ANOVA with antigen

Dose 1	F=9.50	2,150	p<0.01
Scheffe-Schistosomula vs. SEA			p>0.05
Scheffe-Schistosomula vs. Adult			p<0.01
Scheffe-SEA vs. Adult			p<0.01
Dose 3	F=11.91	2,135	p<0.01
Scheffe-Schistosomula vs. SEA			p>0.05
Scheffe-Schistosomula vs. Adult			p<0.01
Scheffe-SEA vs. Adult			p<0.01
Dose 10	F=39.31	2,99	p<0.01
Scheffe-Schistosomula vs. SEA			p>0.05
Scheffe-Schistosomula vs. Adult			p<0.01
Scheffe-SEA vs. Adult			p<0.01

b) Comparison: IgM reactivity to each S. mansoni antigen.

Schistosomula antigen
ANOVA with time

Dose 1	F=13.03	6,50	p<0.01
Dose 3	F=22.54	6,45	p<0.01
Dose 10	F=38.08	5,34	p<0.01

ANOVA with dose

Dose 1 vs. Dose 3	F=16.82	1,80	p<0.01
Dose 1 vs. Dose 10	F=70.18	1,67	p<0.01
Dose 3 vs. Dose 10	F=18.80	1,77	p<0.01

SEA

ANOVA with time

Dose 1	F=13.86	6, 50	p<0.01
Dose 3	F=25.98	6, 45	p<0.01
Dose 10	F=61.77	5, 34	p<0.01

ANOVA with dose

Dose 1 vs. Dose 3	F=11.28	1, 80	p<0.01
Dose 1 vs. Dose 10	F=65.94	1, 67	p<0.01
Dose 3 vs. Dose 10	F=29.79	1, 77	p<0.01

Adult worm antigen
ANOVA with time

Dose 1	F=17.56	6,50	p<0.01
Dose 3	F=16.19	6, 45	p<0.01
Dose 10	F=22.98	5, 34	p<0.01

ANOVA with dose

Dose 1 vs. Dose 3	F=8.01	1, 80	p<0.01
Dose 1 vs. Dose 10	F=44.43	1, 67	p<0.01
Dose 3 vs. Dose 10	F=21.99	1, 77	p<0.01

ANOVA with antigen

Dose 1	F=8.87	2, 150	p<0.01
Scheffe-Schistosomula vs. SEA			p>0.05
Scheffe-Schistosomula vs. Adult			p<0.01
Scheffe-SEA vs. Adult			p<0.01
Dose 3	F=30.33	2, 135	p<0.01
Scheffe-Schistosomula vs. SEA			p>0.05
Scheffe-Schistosomula vs. Adult			p<0.01
Scheffe-SEA vs Adult			p<0.01
Dose 10	F=46.64	2, 99	p<0.01
Scheffe-Schistosomula vs. SEA			p>0.05
Scheffe-Schistosomula vs. Adult			p<0.01
Scheffe-SEA vs Adult			p<0.01

Table A4.1: Worm recovery (mean ± 95% C.L) from various groups of mice in the residual acquired resistance experiments.

A) 3 cercariae/mouse/week for 10 weeks:

Time Post-Treatment	Experimental Group	n	Treated Control Group	n	Corrected Mean	n	Age-Matched Controls	n
7	1.73± 0.421	15	1.26± 0.42	15	0.471± 0.42	15	1.95± 0.61	5
11	1.47± 0.50	15	1.27± 0.30	13	0.204± 0.49	15	1.21± 1.01	5
16	1.67± 0.59	15	0.85± 0.47	15	0.83 ± 0.59	15	1.41± 0.33	8

B) 3 cercariae/mouse/week for 18 weeks:

Time Post-Treatment	Experimental Group	n	Treated Control Group	n	Corrected Mean	n	Age-Matched Controls	n
7	1.70± 0.69	8	1.73± 0.42	15	0.97± 0.69	8	1.73± 0.96	6
11	1.98± 0.59	7	0.85± 0.96	4	1.13± 0.59	7	1.14± 0.52	7
16	1.88± 0.80	7	0.85± 0.65	8	1.03± 0.80	7	1.29± 0.58	7

Appendix 3: Statistical Analysis of Results from Chapter 4

Table A4.2: Results of lymphocyte proliferation assays from mice receiving a 10-week primary trickle infection.

a) Comparison: Proliferation of cells of experimental mice vs. naive mice.

Test	Weeks After Treatment		
ANOVA	7	11	16
Schistosomula	F=22.1,p<0.05	F=11.26,p<0.05	F=1.08,p>0.05
SEA	F=8.66,p<0.05	F=13.7,p<0.01	F=2.62,p>0.05
Adult	F=2.74,p>0.05	F=0.98,p>0.05	F=0.53,p>0.05

b) Comparison: Proliferation of cells of experimental mice vs. treated control mice.

Test	Weeks After Treatment		
ANOVA	7	11	16
Schistosomula	F=0.04,p>0.05	F=9.61,p<0.05	F=1.11,p>0.05
SEA	F=1.71,p>0.05	F=7.94,p<0.05	F=0.02,p>0.05
Adult	F=0.27,p>0.05	F=2.81,p>0.05	F=4.19,p>0.05

c) Comparison: Response of experimental mice to schistosomula antigen with time post-treatment.

Test	Probability
Scheffe-Week 7 vs. Week 11	p>0.05
Scheffe-Week 7 vs. Week 16	p<0.05
Scheffe-Week 11 vs. Week 16	p<0.01

d) Comparison: Response of experimental mice to SEA with times post-treatment.

Test	Probability
Scheffe-Week 7 vs. Week 11	p<0.01
Scheffe-Week 7 vs. Week 16	p>0.05
Scheffe-Week 11 vs. Week 16	p<0.05

e) Comparison: Response of experimental mice to adult antigen with time.

Test	Probability
Scheffe-Week 7 vs. Week 11	p>0.05
Scheffe-Week 7 vs. Week 16	p>0.05
Scheffe-Week 11 vs. Week 16	p>0.05

f) Response of experimental mice to S. mansoni antigens at 7 weeks post-treatment.

Test	Probability
Scheffe-Schistosomula vs. SEA	p<0.01
Scheffe-Schistosomula vs. Adult	p<0.01
Scheffe-SEA vs. Adult	p>0.05

g) Comparison: Response of experimental mice to S. mansoni antigens at 11 weeks

post-treatment.

Test	Probability
Scheffe-Schistosomula vs. SEA	p>0.05
Scheffe-Schistosomula vs. Adult	p<0.01
Scheffe-SEA vs. Adult	p=0.058

h) Comparison: Response of experimental mice to S. mansoni antigens at 16 weeks post-treatment.

Test	Probability
Scheffe-Schistosomula vs. SEA	p>0.05
Scheffe-Schistosomula vs. Adult	p<0.05
Scheffe-SEA vs. Adult	p>0.05

Table A4.3: Results of lymphocyte proliferation assays from mice receiving an 18-week primary trickle infection.

a)Comparison: Proliferation of cells of experimental mice vs. naive mice.

Test	Weeks After Treatment		
ANOVA	7	11	16
Schistosomula	F=2.34,p>0.05	F=23.3,p<0.01	F=19.04,p<0.01
SEA	F=18.17,p<0.01	F=2.11,p>0.05	F=33.66,p<0.01
Adult	F=0.27,p>0.05	F=7.51,p=0.052	F=0.10,p>0.05

b)Comparison: Proliferation of cells of experimental mice vs. treated control mice.

Test	Weeks After Treatment		
ANOVA	7	11	16
Schistosomula	F=0.58,p>0.05	F=0.18,p>0.05	F=0.09,p>0.05
SEA	F=0.55,p>0.05	F=0.71,p>0.05	F=4.22,p>0.05
Adult	F=0.09,p>0.05	F=0.08,p>0.05	F=0.09,p>0.05

c)Comparison: Response of experimental mice to schistosomula antigen with time post-treatment.

Test	Probability
Scheffe-Week 7 vs. Week 11	p<0.05
Scheffe-Week 7 vs. Week 16	P>0.05
Scheffe-Week 11 vs. Week 16	p<0.01

d)Comparison: Response of experimental mice to SEA with time post-treatment.

Test	Probability
Scheffe-Week 7 vs. Week 11	p>0.05
Scheffe-Week 7 vs. Week 16	p<0.01
Scheffe-Week 11 vs. Week 16	p<0.01

e)Comparison: Response of experimental mice to adult antigen with time post-treatment.

Test	Probability
Scheffe-Week 7 vs. Week 11	p>0.05
Scheffe-Week 7 vs. Week 16	p>0.05
Scheffe-Week 11 vs. Week 16	p>0.05

f)Comparison: Response of experimental mice to S. mansoni antigens at 7 weeks post-treatment.

Test	Probability
Scheffe-Schistosomula vs. SEA	p<0.01
Scheffe-Schistosomula vs. Adult	p<0.01
Scheffe-SEA vs. Adult	p>0.05

g)Comparison: Response of experimental mice to S. mansoni antigens at 11 weeks post-treatment.

Test	Probability
Scheffe-Schistosomula vs. SEA	p<0.01
Scheffe-Schistosomula vs. Adult	p<0.01
Scheffe-SEA vs. Adult	p>0.05

h)Comparison: Response of experimental mice to S. mansoni antigens at 16 weeks post-treatment.

Test	Probability
Scheffe-Schistosomula vs. SEA	p<0.01
Scheffe-Schistosomula vs. Adult	p<0.01
Scheffe-SEA vs. Adult	p<0.01

Table A4.4:Results of lymphocyte proliferation assays from experimental mice receiving a 10 or 18 week primary trickle infection.

a)Comparison: Response to schistosomula antigen at corresponding times post-treatment.

Test	Weeks After Treatment				
ANOVA					
	7		11		16
10 vs. 18 weeks	F=0.78,p>0.0	5	F=2.8,p>0.05		F=10.04,p=0.019

b)Comparison: Response to SEA at corresponding times post-treatment.

Test	Weeks After Treatment		
ANOVA			
	7	11	16
10 vs. 18 weeks	F=5.91,p<0.05	F=7.42,p<0.05	F=17.82,p<0.01

c)Comparison: Response to adult antigen at corresponding times post-treatment.

Test	Weeks After Treatment

ANOVA

	7	11	16
10 vs. 18 weeks	F=0.69,p>0.05	F=19.53,p>0.05	F=19.53,p<0.01

Table A4.5:Immunoglobulin levels in mice receiving a 10 week primary trickle infection.

a)Comparison: IgG levels of experimental mice vs. treated control mice.

Test ANOVA	Weeks After Treatment		
	7	11	16
Schistosomula	F=0.11,p>0.05	F=0.14,p>0.05	F=1.08,p>0.05
SEA	F=0.75,p>0.05	F=0.95,p>0.05	F=1.99,p>0.05
Adult	F=0.59,p>0.05	F=1.26,p>0.05	F=0.12,p>0.05

b)Comparison: IgM levels of experimental mice vs. treated control mice.

Test ANOVA	Weeks After Treatment		
	7	11	16
Schistosomula	F=1.22,p>0.05	F=0.17,p>0.05	F=1.04,p>0.05
SEA	F=0.08,p>0.05	F=0.09,p>0.05	F=2.17,p>0.05
Adult	F=0.12,p>0.05	F=0.01,p>0.05	F=0.05,p>0.05

c)Comparison: IgG responses of experimental mice to S. mansoni antigens with time post-treatment.

Test	Statistic	Probability
ANOVA with time-Schistosomula	F=1.99	p>0.05
ANOVA with time-SEA	F=0.07	p>0.05
ANOVA with time-Adult	F=2.19	p>0.05

d)Comparison: IgM response of experimental mice to S. mansoni antigens with time post-treatment.

Test	Statistic	Probability
ANOVA with time-Schistosomula	F=5.62	p<0.01
Scheffe-Week 7 vs. Week 11		p<0.01
ANOVA with time-SEA	F=3.23	p<0.05
Scheffe-Week 7 vs. Week 16		p=0.05
ANOVA with time-Adult	F=5.43	p<0.01
Scheffe-Week 11 vs. Week 16		p<0.01

e)Comparison: IgG response of experimental mice to S. mansoni antigens at 7 weeks post-treatment.

Test	
Probability	
Scheffe-Schistosomula vs. SEA	p<0.01
Scheffe-Schistosomula vs. Adult	p<0.01
Scheffe-SEA vs. Adult	p<0.01

f)Comparison: IgM response of experimental mice to S. mansoni antigens at 7 weeks post-treatment.

Test

Probability	
Scheffe-Schistosomula vs. SEA	p>0.05
Scheffe- Schistosomula vs. Adult	p<0.01
Scheffe-SEA vs. Adult	p<0.01

g)Comparison: IgG response of experimental mice to S. mansoni antigens at 11 weeks post-treatment.

Test

Probability	
Scheffe-Schistosomula vs. SEA	p<0.01
Scheffe-Schistosomula vs. Adult	p<0.01
Scheffe-SEA vs. Adult	p<0.01

h)Comparison: IgM response of experimental mice to S. mansoni antigens at 11 weeks post-treatment.

Test

Probability	
Scheffe-Schistosomula vs. SEA	p<0.05
Scheffe-Schistosomula vs. Adult	p<0.05
Scheffe-SEA vs. Adult	p<0.01

i)Comparison: IgG response of experimental mice to S. mansoni antigens at 16 weeks post-treatment.

Test

Probability	
Scheffe-Schistosomula vs. SEA	p<0.01
Scheffe-Schistosomula vs. Adult	p<0.01
Scheffe-SEA vs. Adult	p<0.01

j)Comparison: IgM response of experimental mice to S. mansoni antigens at 16 weeks post-treatment.

Test	Statistic	Probability
ANOVA with antigens	F=2.34	p>0.05

Table A4.6: Immunoglobulin levels in mice receiving an 18 week primary trickle infection.

a)Comparison: IgG response of experimental mice to S. mansoni antigens with time post-treatment.

Test	Statistic	Probability
ANOVA with time-Schistosomula	F=0.76	p>0.05
ANOVA with time-SEA	F=1.72	p>0.05
ANOVA with time-Adult	F=1.90	p>0.05

b)Comparison: IgM response of experimental mice to S. mansoni antigens with time post-treatment.

Test	Statistic	Probability

ANOVA with time-Schistosomula	F=0.42	p>0.05
ANOVA with time-SEA	F=1.30	p>0.05
ANOVA with time-Adult	F=1.12	p>0.05

c)Comparison: IgG response of experimental mice to S. mansoni antigens at 7 weeks post-treatment.

Test	Probability
Scheffe-Schistosomula vs. SEA	p<0.01
Scheffe-Schistosomula vs. Adult	p<0.01
Scheffe-SEA vs. Adult	p<0.01

d)Comparison: IgM response of experimental mice to S. mansoni antigens at 7 weeks post-treatment.

Test	Probability
Scheffe-Schistosomula vs. SEA	p>0.05
Scheffe-Schistosomula vs. Adult	p<0.01
Scheffe-SEA vs. Adult	p<0.01

e)Comparison: IgG response of experimental mice to S. mansoni antigens at 11 weeks post-treatment.

Test	Probability
Scheffe-Schistosomula vs. SEA	p<0.05
Scheffe-Schistosomula vs. Adult	p<0.01
SEA vs. Adult	p<0.01

f)Comparison: IgM response of experimental mice to S. mansoni antigens at 11 weeks post-treatment.

Test	Probability
Scheffe-Schistosomula vs. SEA	p>0.05
Scheffe-Schistosomula vs. Adult	p<0.05
Scheffe-SEA vs. Adult	p>0.05

g)Comparison: IgG response of experimental mice to S. mansoni antigens at 16 weeks post-treatment.

Test	Probability
Scheffe-Schistosomula vs. SEA	p<0.05
Scheffe-Schistosomula vs. Adult	p<0.01
Scheffe-SEA vs. Adult	p=0.05

h)Comparison: IgM response of experimental mice to S. mansoni antigens at 16 weeks post-treatment.

Test	Statistic	Probability
ANOVA with antigens	F=1.38	p>0.05

Table A4.7: Immunoglobulin levels of experimental mice receiving a 10 or 18 week primary trickle infection.

a)Comparison: Antibody response to schistosomula antigen at corresponding times post-treatment.

Test ANOVA	Weeks After Treatment		
	7	11	16
10vs.18 weeks-IgG	F=32.95,p<0.01	F=5.59,p<0.05	F=.057,p>0.05
10vs.18 weeks-IgM	F=5.77,p<0.05	F=5.34,p<0.05	F=4.31,p>0.05

b)Comparison: Antibody response to SEA at corresponding times post-treatment.

Test ANOVA	Weeks After Treatment		
	7	11	16
10vs.18 weeks-IgG	F=6.35,p<0.05	F=0.63,p>0.05	F=0.98,p>0.05
10vs.18 weeks-IgM	F=0.01,p>0.05	F=0.00,p>0.05	F=0.58,p>0.05

c)Comparison: Antibody response to adult antigen at corresponding times post-treatment.

Test ANOVA	Weeks After Treatment		
	7	11	16
10vs.18 weeks-IgG	F=14.42,p<0.01	F=21.56,p<0.01	F=0.1,p>0.05
10vs.18 weeks-IgM	F=3.08,p>0.05	F=1.15,p>0.05	F=0.01,p>0,05