The kinetics of immunological responses to microfilariae in a murine host: experimental and mathematical studies

C. J. RHODES[1]*, S. G. FOLKARD[2], A. E. BIANCO[2] *and* R. M. ANDERSON[1]

[1] *Centre for the Epidemiology of Infectious Disease, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK*
[2] *Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK*

(*Received 24 June 1996; revised 28 August 1996; accepted 3 September 1996*)

SUMMARY

We present a mathematical model which is used to interpret the dynamics of the immunological response of a mouse host to infection with the filarial worm *Onchocerca lienalis*. The model mimics changes in worm burden over time post-infection and after reinfection and its behaviour provides a good description of experimental results. Measured production of T-cells and eosinophils is also compared with the predictions of the model. Our results show that the immune response mechanism proposed on the basis of experimental results, involving $CD4^+$ T-cells and eosinophil destruction of the parasite, is supported by the insights gained from the mathematical model. Also, using the parameters estimated to describe the primary infection dynamics, the degree of acquired immunity to secondary infection is also well described by the model. Our analysis highlights the importance of obtaining quantitative measures of the many rate parameters involved in even the simplest interpretations of immunological responses to parasitic infection.

Key words: interleukin-5, eosinophil, *Onchocerca lienalis*, dynamics.

INTRODUCTION

The specific structure of the immunological response mounted by a host is often dependent upon the nature of the invading infectious agent. Such response mechanisms are the object of much detailed experimental study and often they reveal a complex interconnecting network of various cells of the immune system, together with chemical messengers (= cytokines), acting in a concerted fashion to facilitate the elimination of the invading pathogen.

Typically, the greater the organizational and developmental complexity of the infections agent, the more complex we might expect the immune response raised against it to be. Following infection with a mobile tissue-dwelling filarial macroparasite, such as *Onchocerca lienalis*, the host calls upon a broad range of the diverse cell and chemical constituents of its immune system to fight the infection. Mathematical models of the hypothesized immune response can be used to provide a detailed statement of the dominant dynamical relationships and allow an alternative way of testing the conclusions and insights generated by experiment. This has been done with some degree of success in modelling the immunological response of mice to *Trichuris muris* which is known to produce a protective Th2 response in certain mouse strains (Else & Grencis, 1991; Brass *et al*. 1992*a*, *b*). Also, the within-host dynamics during malaria infection have been discussed within a mathematical framework and compared with experiment (Hetzel & Anderson, 1996). More generally, the $CD4^+$ T-cell response to helminth infection has been discussed (Schweitzer & Anderson, 1992*a*, *b*) showing that even the simplest reduced models of the immune system exhibit a rich dynamical behaviour.

Here we specifically concentrate on the immunological mechanism that is generated after infection of murine hosts with the microfilariae of *O. lienalis*. A set of experiments is described which measure the immune response of the host to the infection and allow various hypotheses concerning the response to be tested. A mathematical model is introduced which, it is believed, captures the essential dynamics of the immune response and allows us to make good qualitative comparison with the experimental results. It reinforces the hypothesis that eosinophil destruction of the microfilariae is the dominant pathogenic mechanism acting to reduce the microfilarial burden (Folkard *et al*. 1996) and makes a good prediction of the degree of acquired immunity to secondary infection.

MATERIALS AND METHODS

A series of experiments suggest that both $CD4^+$ T-cells and eosinophils are of central importance in the development and persistence of sustained immunity

* Corresponding author: Centre for the Epidemiology of Infectious Disease, Department of Zoology, South Parks Road, Oxford OX1 3PS, UK. Tel: 01865 271264. Fax: 01865 281245. E-mail: chris.rhodes@zoology.oxford.ac.uk

https://doi.org/10.1017/S0031182096008463 Published online by Cambridge University Press

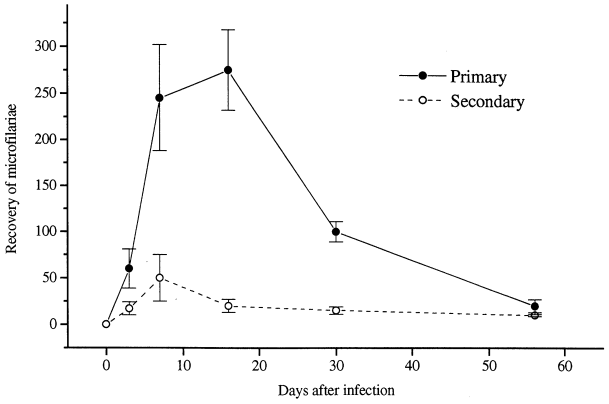

Fig. 1. Primary and secondary infection of mice with *Onchocerca lienalis*. Worm recovery (±S.D.) from the primary infection peaks around Day 20 whereas on the secondary infection the peak occurs around 7–10 days after reinfection.

to microfilariae (Folkard & Bianco, 1995; Folkard *et al*. 1996). In the experiments described below, mice received a standard dose of 5000 microfilariae by subcutaneous inoculation at the nape of the neck. The recovery of live microfilariae from the ear pinnae was measured as an index of parasite establishment and survival, shown by Townson & Bianco (1982) to be a reliable indicator of overall parasite survival.

Fig. 1 shows the microfilarial recovery (Townson *et al*. 1984) from CBA mice inoculated on Day 0 and again, with an identical dose on Day 100. The second dose is cleared faster due to acquired immunity to infection built up during the primary infection.

The importance of $CD4^+$ cells in co-ordinating immunological responses is shown in Fig. 2. CBA mice injected with an anti-$CD4^+$ antibody have, relative to the control group, an enhanced microfilarial burden over a long time-scale ($\chi^2 = 366{\cdot}4$, $P < 0{\cdot}001$) (Folkard & Bianco, 1985). The host is unable to clear infection as fast as an immunologically intact mouse (Fig. 2A). Furthermore, mice reinfected with microfilariae and treated with the anti-$CD4^+$ antibody show a reduction in their acquired immunity and therefore an increased parasite burden relative to the reinfected control group (Fig. 2B).

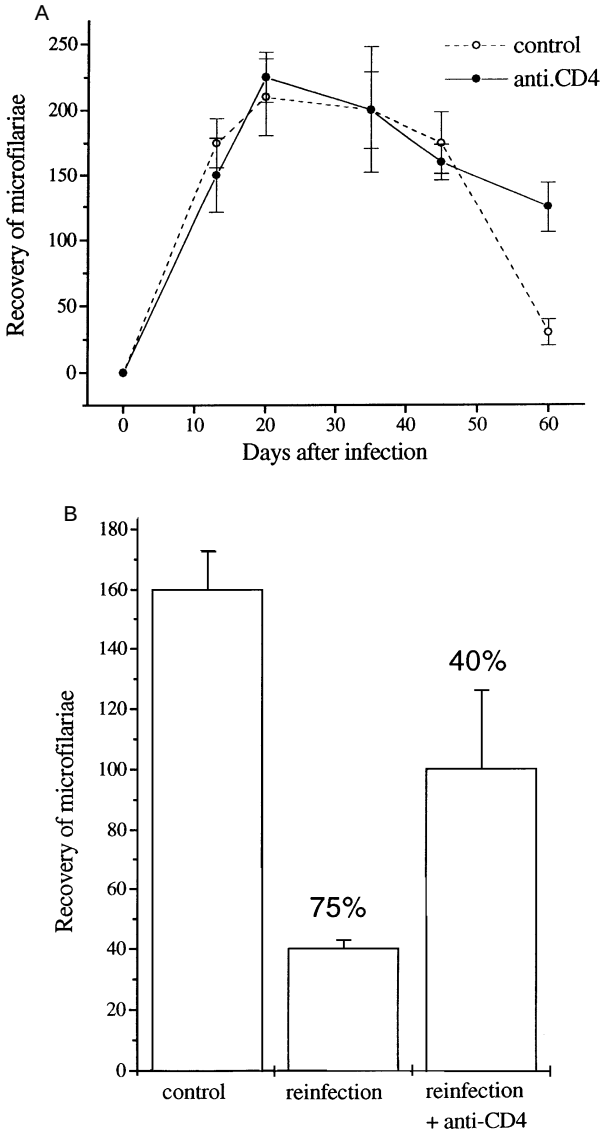

Fig. 2. (A) Worm recovery (±S.E.) from mice lacking a $CD4^+$ T-cell response compared with a control group. (B) The acquired immunity on reinfection compared with a control group. Reinfection occurred on Day 100. Thirteen days after reinfection the control group have a 75% reduction of microfilariae present compared with 13 days after the primary infection. However, $CD4^+$-deficient mice have a 40% reduction in the secondary infection showing that they are less able to clear the parasite.

Experiments were also performed to investigate the effect of eosinophils on parasite depletion (Folkard *et al*. 1996). CBA mice were injected with an anti-interleukin-5 (IL5) neutralizing antibody which inhibits eosinophil production (from approximately 14% to 1% during primary infection, and from approximately 40% to 3% during secondary infection (Folkard *et al*. 1996)), and the parasite burden measured over time. Fig. 3A shows a small but significant ($\chi^2 = 137{\cdot}1$, $P < 0{\cdot}001$) delay in parasite clearance in the eosinophil-deficient mice compared with a control group, clearly indicating that without eosinophils mice are less able to clear the infection. Reinfection took place on Day 100 and the microfilarial recovery recorded on Day 114. Relative to the control group Fig. 3B shows that the eosinophil-deficient mice are less able to clear their secondary infection, and therefore show an increased parasite burden.

The experiments also yielded interesting results concerning the rate of emergence of the eosinophil population relative to the parasite burden. Fig. 4 shows how the peak of eosinophils, as measured in the peripheral blood circulation, occurs somewhat later than that in the parasite burden.

https://doi.org/10.1017/S0031182096008463 Published online by Cambridge University Press

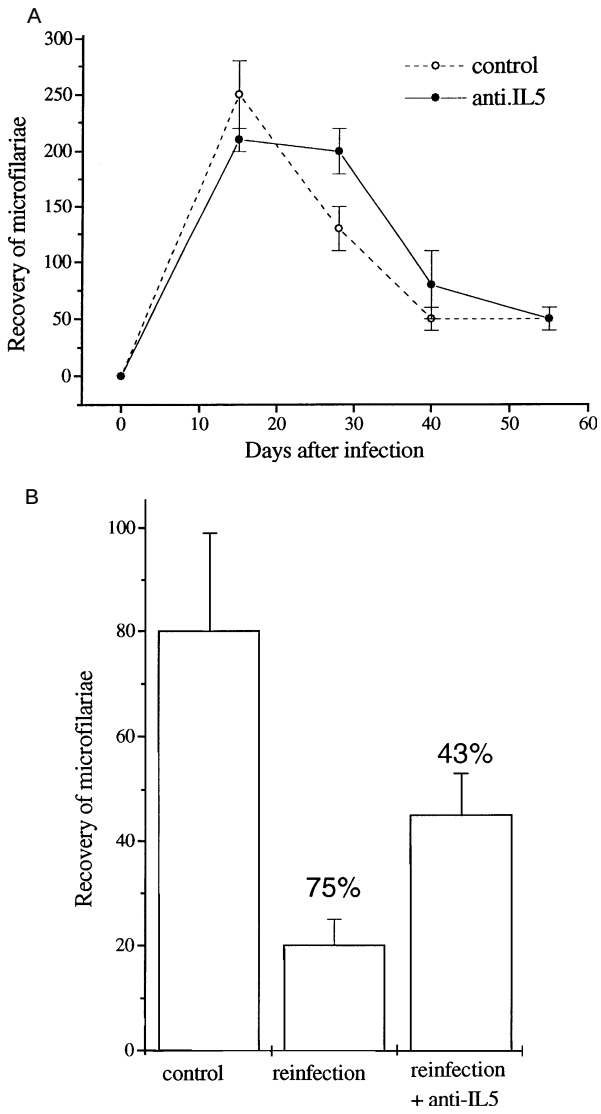

Fig. 3. (A) Worm recovery (±S.E.) from mice treated with an anti-IL5 antibody. This effectively disables the eosinophil response. There is enhanced survival of the parasite in the eosinophil-deficient mice compared to the control group showing that eosinophils are involved in parasite killing. (B) The acquired immunity on reinfection compared with a control group. Reinfection occurred on Day 100. Fourteen days after reinfection the control group have a 75% reduction of microfilariae present compared with 14 days after the primary infection. Eosinophil-deficient mice have a 43% reduction in the secondary parasite burden showing that they are less able to clear the infection.

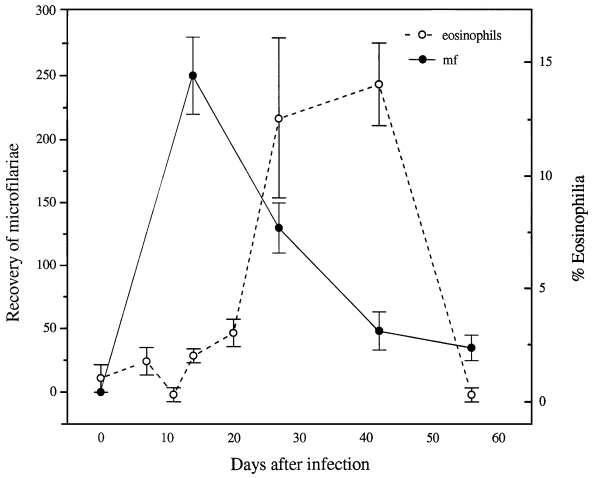

Fig. 4. Recovery of microfilariae (±S.E.) from a primary infection of a control group compared with the dynamics of the eosinophil population measured in the peripheral blood circulation.

DYNAMICAL MODEL

The experimental work described above provides quantitative insight into the dynamics of the immunological response mechanism of the mouse to microfilarial infection. A small challenge infection is depleted by the action of the immune system and the resulting temporal behaviour of the worm burden is of central interest.

Below, we set out a number of coupled differential equations that model the rate of change of the populations of filariae (P), $CD4^+$ T-cells (T) and eosinophils (E). The solutions of these equations that are based on very simple assumptions encouragingly give qualitatively similar dynamics to the experimental results.

Filarial dynamics

We expect that due to the action of the host immune system and natural parasite mortality the overall worm burden, P, will decline as time passes, so we write

$$dP/dt = -\mu P - \mu' P - \beta PE, \quad (1)$$

where μ is the natural *per capita* mortality of the worm. We assume that the main immune response mode is through $CD4^+$ T-cell directed eosinophilic killing of the parasite, represented by the term βPE, though there also appears to be a weaker secondary mortality due to the effect of $CD8^+$ cells represented by the term $\mu' P$.

We assume that worms arrive at the ear pinnae at a rate proportional to the total population of worms in the body. Once there, they remain in place and are subject to attrition from the effects of the immune system. Defining p to be the subset of the worm population found in the ears

$$dp/dt = aP - \mu p - \mu' p - \beta pE, \quad (2)$$

where a is a measure of the rate of arrival of worms to the ears. As we have no data on the distribution of eosinophils throughout the host we have to assume that parasite mortality is proportional to the total eosinophil number rather than proportional to some subset in the ear.

Experiments with severe combined immunodeficient mice (Folkard, unpublished data) indicate that worms are capable of surviving for long periods of time in the mouse tissue without appreciable

https://doi.org/10.1017/S0031182096008463 Published online by Cambridge University Press

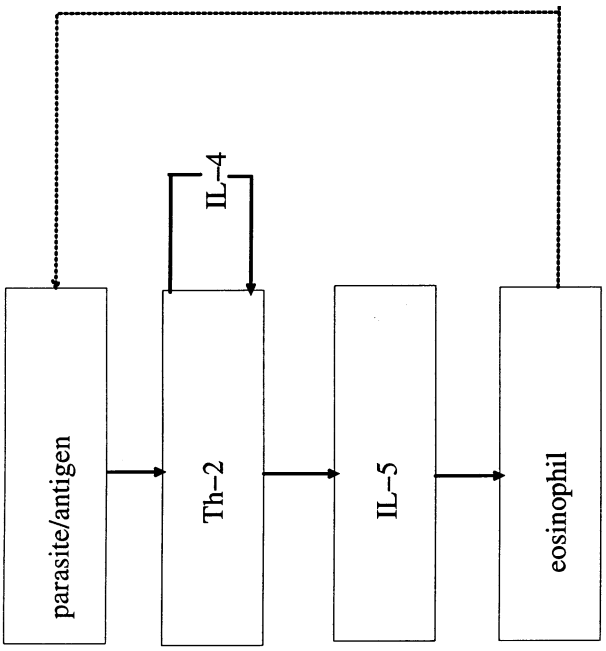

Fig. 5. Comparison of microfilarial recovery from CD4+ T-cell depleted mice with the solution of equation 4. This allows an estimate of the rate of filarial uptake to the ears and the effect of non-CD4+ T-cell related mortality.

reduction in numbers due to natural mortality, so it is a reasonable approximation to neglect the natural parasite mortality, μ, entirely, with respect to the time-scale of the experimental results used in our parameter estimation.

It is possible to estimate the constants a and μ' by selectively disabling parts of the mouse immune response i.e. the equivalent of setting $\beta = 0$ in equation 2. Most of the anti-filarial immune response arises from the actions of CD4+ T-cells. By selectively knocking out the CD4+ response the mouse can only respond with the less efficient non-CD4+ cells such as CD8+ T-cells and this greatly enhances parasite survival. Fig. 2A shows the microfilarial burden recovered from CD4+ depleted mice. Clearly, over the long term, we see a reduction in the worm burden, possibly due to the action of CD8+ T-cells. This overall effect is sub-summed into the 'effective' mortality parameter μ', which, in effect, represents any non-CD4+ related mortality. Thus, Fig. 2A is represented by the equation

$$dp/dt = aP - \mu' p. \tag{3}$$

From equation 1, with $\mu = 0$ and $\beta = 0$, the total parasite number as a function of time $P = P(0)\, e^{-\mu' t}$. Substituting this in equation 3, the resulting exact solution for the parasite number in the ears of CD4+ deficient mice is

$$p = P(0)\, a t e^{-\mu' t}. \tag{4}$$

Fig. 5 shows the solution of this equation with $a = 5{\cdot}3 \times 10^{-3}$/day and $\mu' = 0{\cdot}043$/day. The data of Fig. 2A are shown for comparison. Hence, we are able to account for the dynamics of the microfilarial recovery in the absence of any CD4+ T-cell mediated response.

CD4+ immune response

Following the initial microfilarial challenge infection a CD4+ T-cell dependent immunity emerges as the parasites are destroyed. The T-cells are activated at a rate proportional to the amount of parasite (or antigen) and then proliferate in a non-linear way. It is this T-cell proliferation that is central to the dynamics of the model. As the CD4+ Th2 population increases, the cytokines IL4 and IL5 are produced at a rate ϕ_4T and ϕ_5T and are removed at a rate μ_4 and μ_5. The IL4 stimulates the proliferation of Th2 cells at a rate which saturates for high level of this cytokine. Therefore, the net T-cell proliferation is of the form $\propto IT/1+bIT$. Such saturation effects in the T-cell response to cytokines have been observed during *in vitro* studies (Toribio *et al*. 1989). In turn, the IL5 cytokine stimulates eosinophil production at a rate proportional to the amount of IL5 present. The eosinophil is removed at a rate γ and through interaction with the parasite. We neglect any Th1/Th2 cross-regulation effect in this study. The diagram in Fig. 6 shows the relationship between the principal components of the immune mechanism believed to be operating against *O. lienalis* infection in the murine host.

A set of equations which represent this hypothesized immune response is as follows.

The total parasite number

$$dP/dt = -\mu' P - \beta PE. \tag{5}$$

The number of activated Th2 cells

$$dT/dt = \xi P + \frac{\sigma I_4}{1 + bI_4\, T} T - \alpha T. \tag{6}$$

The concentration of IL4

$$dI_4/dt = \phi_4\, T - \mu_4\, I_4. \tag{7}$$

The concentration of IL5

$$dI_5/dt = \phi_5\, T - \mu_5\, I_5. \tag{8}$$

The number of activated eosinophils

$$dE/dt = \epsilon I_5 - \gamma E - \beta_e\, PE. \tag{9}$$

The number of parasites in the ears

$$dp/dt = ap - \mu' p - \beta pE. \tag{10}$$

This set of equations can be simplified by noting that much evidence suggests that the cytokine dynamics is faster than the other time-scales of relevance in the model, i.e. $\frac{1}{\mu_4}, \frac{1}{\mu_5} \ll \frac{1}{\mu}, \frac{1}{\mu'}, \frac{1}{\gamma}, \frac{1}{\alpha}$. Assuming that the cytokines reach equilibrium very quickly, i.e. $I_4 = \frac{\phi_4}{\mu_4} T$ and $I_5 = \frac{\phi_5}{\mu_5} T$, the reduced set of equations for the

https://doi.org/10.1017/S0031182096008463 Published online by Cambridge University Press

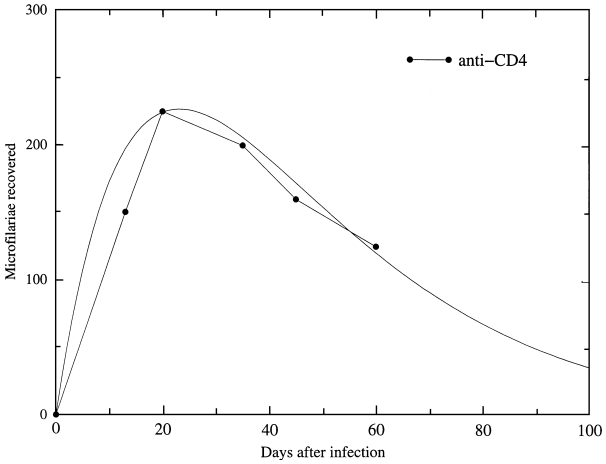

Fig. 6. A flow diagram indicating the principal components of the immunological response mechanism which are used to construct the mathematical model.

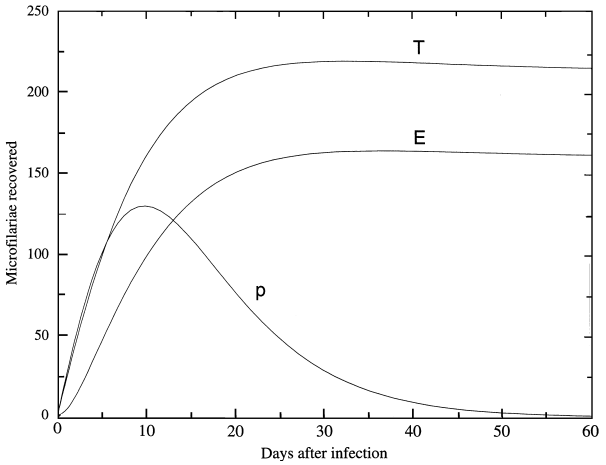

Fig. 7. The number of microfilariae recovered from the ears, p, the T-cell population, T, and number of eosinophils, E, after primary infection. T-cell and eosinophil numbers are scaled to fit on the same scale as the graph of worm recovery. The T-cell populations peak at around Day 30 and the eosinophil peaks at around Day 40. The parameters used are $\beta = 1{\cdot}0 \times 10^{-4}$ day^{-2}, $\xi = 0{\cdot}4$/day, $\sigma = 0{\cdot}4$/day, $b' = 2{\cdot}5 \times 10^{-4}$/day, $\epsilon' = 0{\cdot}015$/day and $\beta_e = 1{\cdot}0 \times 10^{-5}$ day^{-2}.

total parasite numbers, P, the parasite numbers recovered from the ears, p, the Th2 cell population, T, and the eosinophilia, E.

$$dP/dt = -\mu' P - \beta PE, \tag{11}$$

$$dT/dt = \xi P + \frac{\sigma T}{1 + b' T^2} T - \alpha T, \tag{12}$$

$$dE/dt = \epsilon' T - \gamma E - \beta_e PE, \tag{13}$$

$$dp/dt = ap - \mu' p - \beta pE. \tag{14}$$

We take this to be the simplest model which captures the dynamics of the main components of the immunological response to infection with *O. linealis* microfilariae.

PRIMARY INFECTION

At the start of each experiment 5000 microfilariae are injected into the mouse host, thus $P(0) = 5000$. A full solution of equations 11–14 can be performed, using an adaptive step-size Runge-Kutta routine (Press *et al.* 1991), to see how the population recovered from the ears, p, changes over time. However, we need to estimate appropriate parameters to do this. So far we have only been able to determine the parameters a and μ'. We set the life-time of a T-cell to be approximately 20 days ($= 1/\alpha$) and the life-time of the eosinophils to be approximately 2 days ($= 1/\gamma$). In the absence of quantitative experimental data the remaining parameters are chosen in order to place the solution in the appropriate dynamical regime.

Fig. 7 shows the time dependence of the major components involved in our model. The T-cell and eosinophil populations have been scaled in order to fit on the graph. The microfilarial population recovered from the ears increases initially, peaks at around Day 10 and then declines to zero by around Day 70. This can be compared with Fig. 1, primary infection. The Th2 cell population begins to rise immediately upon injection with the microfilariae and eventually saturates to a high level and this is reflected in the IL5 measurements made experimentally. Elevated Th2 cell populations are required to form the 'immunological memory' which facilitates faster worm killing after reinfection. Similarly, the eosinophil population also begins to rise following infection, peaking at around Day 40. Unlike the experimental results, the calculated eosinophil numbers, though declining slowly, remain elevated throughout primary infection. This disparity is believed to reflect the difference between eosinophils measured in the periphery (Fig. 4) and eosinophils in the tissue at the site of infection. Following infection, eosinophils are released into the peripheral circulation from the bone-marrow and are sequestered from the circulation to sites of infection. This results in the drop in eosinophil numbers observed in the experiment. Our eosinophil data are from the periphery, and not from the tissues. We, as yet, have little data concerning the eosinophil number in the ears of infected mice. This question will be addressed by future experimental studies.

SECONDARY INFECTION

A second dose infection (also of 5000 microfilariae) can be administered to the murine host in order to ascertain the degree of acquired immunity to reinfection. The rationale is that the host, having been exposed to the parasite, should clear the infection much quicker and destroy the parasite more efficiently on a subsequent exposure. This is

https://doi.org/10.1017/S0031182096008463 Published online by Cambridge University Press

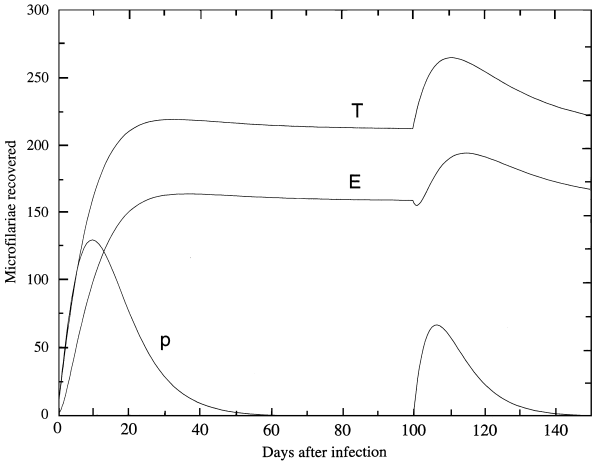

Fig. 8. The number of microfilariae recovered from the ears, p, the T-cell population, T, and number of eosinophils, E, after primary infection and followed by secondary infection with 5000 microfilariae on Day 100. T-cell and eosinophil numbers are scaled to fit on the same scale as the graph of worm recovery.

due to immunological memory of the $CD4^+$ Th2 population which is available to co-ordinate a rapid eosinophil response when reinfection occurs. As a measure of the acquired immunity, we compare the peak worm burden in the primary infection with the peak worm burden in the secondary. Typically, secondary infections lead to a 75 % drop (see Figs 2B and 3B) in the peak worm numbers. Also, the peak in worm numbers occurs sooner after the infection event in the secondary infection than in the primary, as is visible in Fig. 1, indicating that worms are eliminated from within the tissues much faster.

Fig. 8 shows the resulting parasite burden after a reinfection with 5000 microfilariae on Day 100. The same parameters are used in the secondary infection calculation as are used in the primary. There is a 50 % reduction in the peak parasite burden and the secondary peak occurs 7 days after the secondary infection event. This result is comparable with the dynamics observed in the secondary infection of mice with *O. lienalis* (Fig. 1). Also the model shows enhanced T-cell population and eosinophil population after secondary infection, which is also observed in the experimental results. However, the calculations suggest that a further source of microfilarial mortality arising from some additional action of the immune system might be taking place in order to boost the acquired immunity further.

DISCUSSION

In the murine host, experiments point towards eosinophilic destruction as the main mode of parasite killing. This expression of the immune response is clearly a very complex mechanism with many different cell types and chemical messengers involved in the processes of activation, proliferation and signalling. We have presented a simple mathematical model representing the $CD4^+$ and $CD8^+$ T-cell mediated immunological response of a murine host to infection with the microfilarial stage of *O. lienalis*. The overall structure of our model is similar to that used in other studies of the interaction of helminth infection and the immune system (Schweitzer & Anderson, 1992*a*, *b*). The model captures the essential dynamics of the system and reinforces the view that killing mediated by eosinophils is the primary cause of parasite mortality in the host. The predicted populations of parasite, Th2 T-cells and eosinophils, in both primary and secondary infection, are in accord with those observed experimentally. In particular we are able to show that the degree of acquired immunity is approximately the same as that seen in the host and that the rate of parasite removal, as measured by the time to reach peak parasite numbers in the ears, is increased after reinfection.

The model we present is only able to capture the more important and better understood relationships between the various cell and cytokine populations and does not claim to be a complete description of their dynamical inter-relationships. Indeed, our model suggests that there might be further causes of parasite mortality other than the ones we have discussed. However, we show that it can provide useful insight into this branch of the immune response as it gives a qualitative picture concordant with general immunological understanding.

Studies of this kind indicate the utility of applying a mathematical framework to the complexities of immunological response both to facilitate parameter estimation and to provide insight into the complex dynamics of host–parasite relationships. Experimental investigation of the immune response of the murine host to infection with *O. lienalis* is on-going and it remains to be seen how new insights can be incorporated into the model framework. A key component of future experimental studies is the need to generate quantitative estimates of the many parameters that are involved in the kinetics of immunological responses to infectious agents.

Research support for the authors is kindly provided by the Wellcome Trust. C.J.R. thanks D. Austin of Oxford University for useful discussions.

REFERENCES

BRASS, A., BANCROFT, A. J., CLAMP, M. E., GRENCIS, R. K. & ELSE, K. J. (1994*a*). Dynamical and critical behaviour of a simple discrete model of the cellular immune system. *Physical Review* **E50**, 1589–1593.

BRASS, A., GRENCIS, R. K. & ELSE, K. J. (1994*b*). A cellular automata model for helper T-cell subset polarisation in chronic and acute infection. *Journal of Theoretical Biology* **166**, 189–200.

ELSE, K. J. & GRENCIS, R. K. (1991). Cellular immune

https://doi.org/10.1017/S0031182096008463 Published online by Cambridge University Press

responses to the murine nematode parasite *Trichuris muris*. I. Differential cytokine production during acute or chronic infection. *Immunology* **72**, 508–513.

FOLKARD, S. G. & BIANCO, A. E. (1995). Roles for both $CD4^+$ and $CD8^+$ T-cells in protective immunity against *Onchocerca lienalis* microfilariae in the mouse. *Parasite Immunology* **17**, 541–553.

FOLKARD, S. G., HOGARTH, P. J., TAYLOR, M. J. & BIANCO, A. E. (1996). Eosinophils are the major effector cells of immunity to microfilariae in a mouse model of onchocerciasis. *Parasitology* **112**, 323–329.

HETZEL, C. & ANDERSON, R. M. (1996). The within-host cellular dynamics of bloodstage malaria: theoretical and experimental studies. *Parasitology* **113**, 25–38.

PRESS, W. H., FLANNERY, B. P., TEUKOLSKY, S. A. & VETTERLING, W. T. (1991). *Numerical Recipes in Fortran*. Cambridge University Press, Cambridge.

SCHWEITZER, A. N. & ANDERSON, R. M. (1992*a*). Dynamic interaction between $CD4^+$ T cells and parasitic helminths: mathematical models of heterogeneity in outcome. *Parasitology* **105**, 513–522.

SCHWEITZER, A. N. & ANDERSON, R. M. (1992*b*). The regulation of immunological responses to parasitic infections and the development of tolerance. *Proceedings of the Royal Society*, *B* **247**, 107–112.

TORIBIO, M. L., GUTIERREZ-RAMOS, J. C., PEZZI, I., MARCOS, M. A. R. & MARTINEZ, A. (1989). Interleukin-2 dependent autocrine proliferation in T-cell development. *Nature*, *London* **342**, 82–85.

TOWNSON, S. & BIANCO, A. E. (1982). Experimental infection of mice with the microfilariae of *Onchocerca lienalis*. *Parasitology* **85**, 283–293.

TOWNSON, S., BIANCO, A. E., DOENHOFF, M. J. & MULLER, R. (1984). Immunity to *Onchocerca lienalis* microfilariae in mice. I. Resistance induced by the homologous parasite. *Tropenmedizin und Parasitologie* **35**, 202–208.