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SMR.940 - 37

THIRD AUTUMN WORKSHOP ON MATHEMATICAL ECOLOGY

(14 October - 1 November 1996)

"Immunodominance, competition and evolution in immunological responses to helminth parasite antigens"

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Immunodominance, competition and evolution in immunological responses to helminth parasite antigens

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(*Received 25 September 1995; revised 26 February 1996; accepted 26 February 1996*)

SUMMARY

The paper describes the development and analysis of a mathematical framework for the study of the within-host population dynamics of the interaction between macroparasites and the human immune system. Simple models of this interaction based on the proliferation of T cell clones specific to parasite antigen, and the impact of clonal expansion on parasite survival, capture the basic features of age-related changes in worm loads within human communities. The model is generalized to multiple epitopes on a single antigen, and reveals competitive exclusion amongst T cells, with a single clone becoming immunodominant in the absence of cross-reactive responses and genetic variation. The introduction of genetic heterogeneity and concomitant variability in the immunogenicity of specific epitopes induces additional complexity into the dynamical interaction. Most importantly, multiple epitope models with antigenic variation suggest that the immunodominant response may not necessarily be targeted at the epitope at which some strains show the greatest immunogenicity. High immunogenicity at a particular epitope can be masked by genetic variability even though many of the variants are more immunogenic at this epitope by comparison with the epitope to which the immunodominant immunological response is directed.

Key words: antigenic variation, helminth antigens, immunological responses, immunodominance, mathematical models.

INTRODUCTION

Advances in our basic understanding of parasite immunology and in the molecular plus biochemical techniques useful in the study of helminth antigens, have raised hopes that progress in vaccine development will accelerate in the coming years. Of particular interest for schistosomes and other helminth parasites in recent years has been the identification of candidate vaccine antigens (secreted, excreted, surface or membrane) and the mappin the major B and T cell epitopes on the molec using synthetic peptides and sequence informat (Reynolds, Shoemaker & Harn, 1992). Progres the production of synthetic peptide-based vacci has been advanced by the development of mult peptides from a branching lysine core (MAP multiple antigen peptides) (Posnett, McGrath Tam, 1988).

These approaches to the construction of anti presenting multiple epitopes to the immune sys appear to hold much promise for helminth vac development in general. However, these techn and conceptual advances can only be evaluated experiments in which laboratory animal models immunized with a candidate vaccine and expose infection (or repeated infection). In these circu stances, interpreting the efficacy of a synthetic antigen will depend in part on an understanding of the dynamics of the host immunological responses to antigens that present multiple epitopes to the immune system.

Applying population biological principles to the study of the immune system has provided a new tool to probe possible dynamical phenomena within immunological response mechanisms (for a recent review see Anderson (1994*a*) and references therein).

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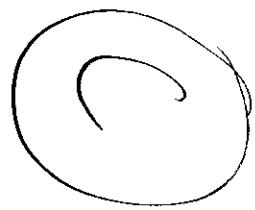

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the immunogenicity of the different epitopes (as a result of antigenic variation induced by mutation or recombination).

In this paper we examine the population dynamics of immunological responses to helminth parasite antigens that present multiple epitopes to the immune system. Our aim is to understand the basic population biology of this interaction with various assumptions concerning the evolution of the immune response under repeated exposure to infection by a genetically heterogeneous parasite population. Simple mathematical models are used to study the evolution of the immune system in individual hosts, where genetic variations within the parasite population is reflected in heterogeneity in the immunogenicities of particular epitopes on key antigens.

BASIC MODEL OF A CELLULAR IMMUNOLOGICAL RESPONSE TO HELMINTH ANTIGENS

How specific immunological resistance to helminth parasite infection operates is still unclear and current understanding rests heavily upon animal model systems and *in vitro* studies (Maizels *et al.* 1993). Individual mechanisms rarely operate in isolation and resistance is effected by a multicomponent response. The prime candidates are the humoral and cellular components most evident during infection such as elevated levels of IgE and eosinophils, but the topic is still one of much controversy (Mitchell, 1979; Sher & Coffman, 1992; Butterworth *et al.* 1992). Two subsets of differentiated helper T cells (Th1 and Th2), characterized by contrasting profiles of secreted cytokines, are believed to be cross-inhibitory so that one cell type will gain at the expense of the other (Wynn *et al.* 1995). In chronic helminth infections the Th1/Th2 ratio is typically profoundly imbalanced (Mossman & Coffman, 1989).

In our basic model of an immune response in hosts repeatedly exposed to infection (as in most natural settings of human exposure to helminth infection, or as in 'trickle' infection experimental design) we build on past work in this area (Schweitzer & Anderson, 1992*a*; Nowak *et al.* 1995). Our aim is to capture the essence of the parasite–host immunological defences: (i) the antigens of helminths commonly exposed to the immune system are excreted, secreted and surface molecules; (ii) each antigen has a number of surface epitopes; (iii) T cells proliferate in response to parasite antigens; (iv) these epitopes are subject to some genetic variation.

For simplicity we do not discriminate between the humoral and cellular components, nor do we differentiate between T cell subsets. Our knowledge of T cell subsets is based primarily on studies in mouse models. Studies of human, sheep and cattle T cell subsets (the real targets of vaccination) are still developing. Not making a distinction between subsets, though naïve, is valid given that the Th1 and Th2 subsets may be 2 manifestations of a continuous spectrum of T cells differentiated by their cytokine profiles (Kelso, 1995).

For simplicity in our basic framework we define a population of T cells that are stimulated to undergo clonal expansion (= proliferation) on contact with parasite antigens (or via the presentation of such antigens to the T cells by antigen-presenting cells). The abundance of these cells is therefore related to the abundance of the parasite, and in turn T cell abundance is assumed to be proportional to the effector arm of the immune response that is responsible for the destruction of the parasite within the host. Again for simplicity, we assume that the immune response acts to reduce parasite life-expectancy as opposed to acting on mortality and parasite establishment.

We begin by considering the *simplest* possible system consisting of a single host exposed to a parasite where the immune response that is most effective in reducing parasite life-expectancy is directed against 1 epitope on a single antigen (Schweitzer & Anderson, 1992*b*). We define the parasite abundance at time t as $p(t)$ and the abundance of T cells specific to the epitope on the major parasite antigen as $x(t)$. Assuming that the host is repeatedly exposed to infection at a constant rate A, that parasites die at a *per capita* mortality rate μ and that their net rate of death due to the immune response is $hx(t)p(t)$, then a single pair of equations to mirror the interaction between the parasite and the host's immune response is as follows:

$$dp(t)/dt = A - \mu p(t) - hx(t)p(t), \quad (1)$$

$$dx(t)/dt = cp(t)x(t) - bx(t). \quad (2)$$

Here the net rate of T cell proliferation is assumed to be proportional to parasite (= antigen) density with *per capita* rate c (its magnitude can be taken as a measure of the *immunogenicity* of the epitope on the major parasite antigen, i.e. its ability to stimulate T cell proliferation). Activated T cells are assumed to have a life-expectancy of $1/b$. In equation (1) the magnitude of the parameter h defines the degree to which the activated T cell can lead to the death of the parasite via immune responses directed at the single epitope on the major parasite antigen.

Different individuals may have different parasite exposure rates A depending on many factors including age (and hence time), sex and location. To model these more carefully we can replace A by some function $A(t)$ to mirror an age dependency in exposure or by a stochastic term to mirror variability (Anderson & May, 1991). Very briefly, the model predicts that if an immune response is activated then exposure has minimal effect on the system provided that the peak exposure rates are comparable in the two models. However, if no response is present, then

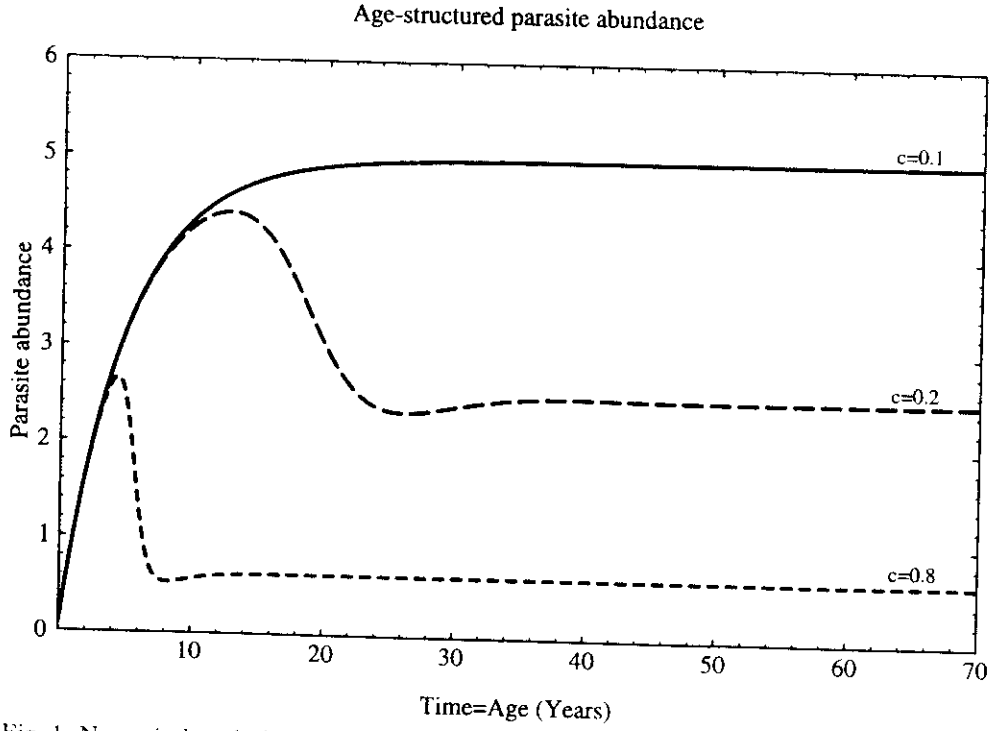

Fig. 1. Numerical evolution of equations (1) and (2). Parameters used are $\Lambda = 1$, $\mu = 0{\cdot}2$, $h = 0{\cdot}1$ and $b = 0{\cdot}5$, with initial conditions $p(0) = 0{\cdot}1$ and $x(0) = 0{\cdot}05$. In the absence of an immune response parasite abundance increases until $p^* = \Lambda/\mu = 5$. Using these parameters $\beta = 10\,c$. Increasing the immunogenicity c above the value 0·1 (ensuring $\beta > 1$) activates the immune system.

$p(t)$ roughly follows exposure which typically gives a convex worm burden with a peak at $t = 10$–15 years depending on parameters and the function $\Lambda(t)$. For simplicity we will ignore age dependency in exposure and heterogeneity - these types require further explanation in a future paper.

In the absence of an immune response the parasite abundance within the host grows over time (= age) to a stable equilibrium p^* via the solution

$$p(t) = p^*[1 - \exp(-\mu t)], \tag{3}$$

$$p^* = Q = \Lambda/\mu, \tag{4}$$

at which point new arrivals balance net losses due to mortality.

The system of equations (1) and (2) can be further simplified by rescaling such that $P(t) = p(t)/Q$, $X(t) = hx(t)/\mu$ and $t' = \mu t$. $P(t)$ is simply the ratio of parasite abundance divided by the level it would attain in the absence of an immunological response (hence it must be less than unity in value). Dropping the prime gives

$$dP(t)/dt = 1 - P(t)[1 + X(t)], \tag{5}$$

$$dX(t)/dt = \alpha X(t)[\beta P(t) - 1], \tag{6}$$

where $\alpha = b/\mu$ and $\beta = cQ/b$. Typically for most helminth parasites, their life-expectancy $(1/\mu)$ will be 1 year or more, whilst that of activated T cells is likely to be much shorter. Hence the value of α will be much greater than unity.

The system has 2 possible stable (= stationary) points. The first of these is the state in which the constant exposure of the parasite fails to activate the immune system such that $X^* = 0$ and $P^* = 1$. This arises when $\beta \leqslant 1$, and in biological terms it arises when the antigen's epitope is not sufficiently immunogenic

$$c \leqslant b/Q. \tag{7}$$

We term this state the *unactivated* state (it is a state of tolerance).

The second equilibrium arises when $B > 1$ and here the immune system is activated by the antigen's epitope and parasite abundance suppressed below the value $Q = \Lambda/\mu$. The worm burden rises rapidly to a peak at approximately

$$t \sim 1/\beta \log 1/X(0) \tag{8}$$

and then exhibits damped oscillations to the stable point $P^* = 1/\beta$, $X^* = \beta - 1$ with period τ such that

$$\tau = 4\pi/[\sqrt{\ } - \Delta], \tag{9}$$

$$\Delta = [X^* + 1]^2 - 4\alpha X^* \tag{10}$$

provided Δ is negative. Fig. 1 records 3 numerical evaluations of equations (1) and (2) for 3 different values of β, one in which the immune system is unactivated ($\beta < 1$) and the remaining cases with sufficiently high degrees of immunogenicity to elicit an immunological response. The changes in parasite abundance over time can be viewed as changes with age in parasite burden. The convex patterns recorded for the two cases in which $\beta > 1$ are similar in qualitative form to many observed age-parasite intensity curves for intestinal or other helminth

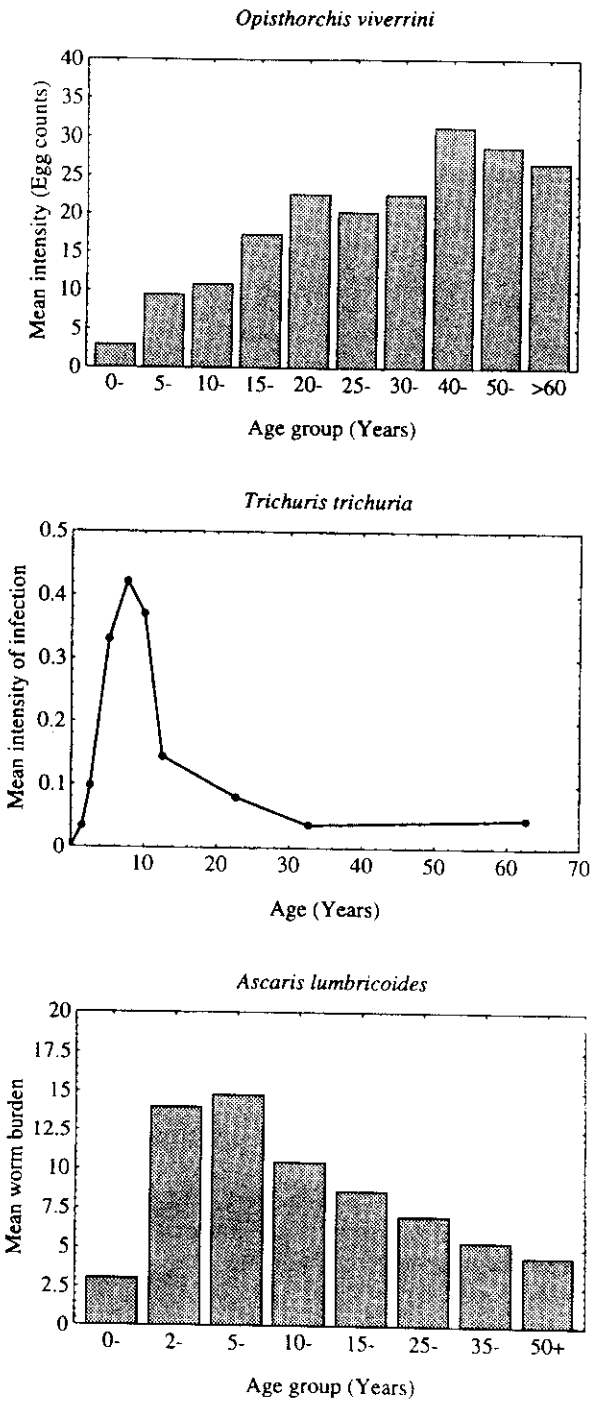

Fig. 2. Age-related intensity profiles of helminth infections for *Opisthorchis viverrini* (Upatham *et al.* 1992), *Trichuris trichuria* (Bundy, 1990) and *Ascaris lumbricoides* (Elkins, Haswell-Elkins & Anderson, 1986). All three profiles show similar qualitative behaviour to those of Fig. 1 generated by equations (1) and (2).

infections in human communities (Fig. 2) (Elkins, Haswell-Elkins & Anderson, 1986; Bundy, 1990; Upatham *et al.* 1992). Observed patterns of convexity in worm load with age are thought to be the result of a slow build up of immunity with an increased duration of exposure to infection plus age-related changes in the rate of exposure to infection (Anderson & May, 1991).

In evolutionary terms, this very simple model helps to focus attention on the selective pressures acting on both host and parasite. Note that the parasite is never cleared by the immune response, due to repeated exposure to infection and, with short-lived T cells, the failure to generate lasting and complete immunity to reinfection. However, parasite abundance can be suppressed to very low levels (Fig. 1) as observed in natural systems in the older age classes (Fig. 2). It is in the host's best interests to suppress the parasite abundance to as low a level as possible. This can be achieved either by raising the rate, c, at which T cells proliferate or increasing their life-expectancy ($1/b$). The T cell proliferation constant consists of 2 components, the rate of cell division and the sensitivity to unit quantities of parasite antigen (i.e. immunogenicity).

As shown in equation (8) the peak worm burden occurs at an age dependent on both c and the number of activated T cells present prior to infection. If T cells are present in large numbers then infection can be countered much earlier and the overall worm burden minimized. Hence it is in the host's interest to mount an effective immune response as quickly as possible via the production of activated T cells early in a child's development.

From the parasite's point of view, the objective is to try and avoid activating the immune system (thereby maximizing parasite burden) which is best achieved by reducing the immunogenicity of its antigens.

THE RATE OF T CELL PROLIFERATION

In our basic model the net rate of T cell proliferation was assumed to be directly proportional to the density of the parasite within the host (i.e. the major parasite antigen). Experimental studies suggest that the rate of T cell proliferation as a function of antigen concentration can adopt a variety of forms ranging from saturation to a constant at high antigen concentrations to more complex patterns where the rate rises to some maximum value at intermediate antigen concentrations and then declines as antigen concentration rises further (Fig. 3). Such a response suggests 'high-zone' tolerance where T cells become anergic (= unresponsive) at high antigen concentrations. These responses are commonly observed in host–parasite interactions (Jones *et al.* 1990; Maizels *et al.* 1993).

To mimic these more complex relationships between T cell population and parasite abundance we modify the basic model to

$$dp(t)/dt = A - \mu p(t) - hx(t)\,p(t), \tag{11}$$

$$dx(t)/dt = dp(t)\,R(p)\,x(t) - bx(t), \tag{12}$$

where the function $R(p)$ denotes the density-dependent proliferation rate and d is the maximum *per capita* (per cell) rate of proliferation. We consider

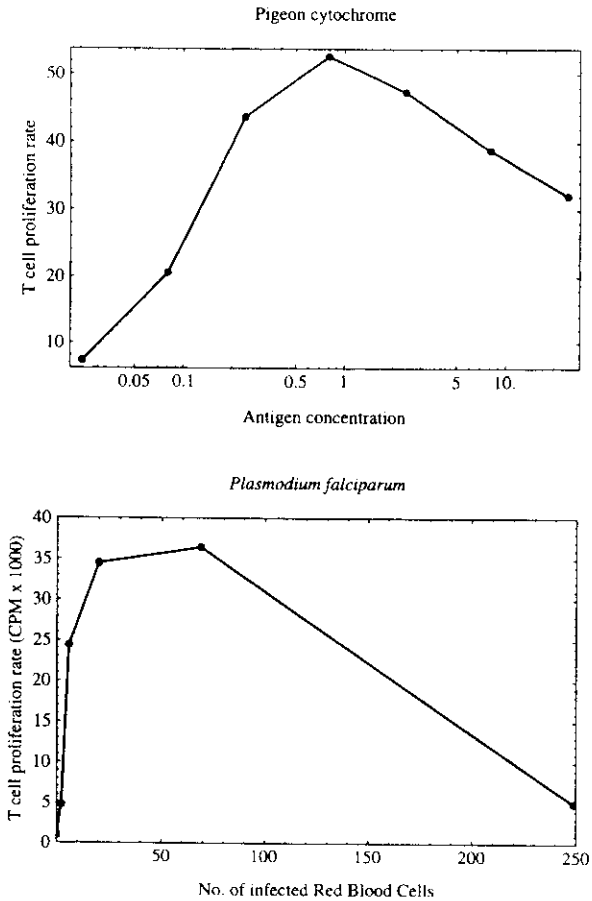

Fig. 3. Dose-response curves showing the influence of antigen (pigeon cytochrome *c*, Matis *et al.* (1982) number of *Plasmodium falciparum*-infected red blood cells, Jones *et al.* (1990)) on T cell proliferation rates. In each case a characteristic peak proliferation rate with high-zone tolerance (unresponsiveness) at high antigen concentrations is seen. These profiles are modelled using the function $P(t)\,R_1(P)$.

3 forms of the function which saturate to the constant value d at high antigen concentrations (R_1–R_3) and 1 convex function (R_4) where

$$\left.\begin{aligned} p(t)\,R_1(p) &= \frac{p(t)}{\xi+p(t)},\\ p(t)\,R_2(p) &= \frac{p^2(t)}{\xi^2+p^2(t)},\\ p(t)\,R_3(p) &= 1-e^{-p(t)/\xi}. \end{aligned}\right\} \tag{13}$$

Here the scaling parameter ξ defines the degree of density dependence in T cell proliferation (for R_1 and R_2), ξ is the antigen level which gives a proliferation rate 50% of the maximum value. For the convex function R_4 we define

$$p(t)\,R_4(t) = \frac{2\xi p(t)}{\xi^2+p^2(t)}. \tag{14}$$

All 4 functions are plotted in Fig. 4. For the 3 saturating functions (R_1–R_3) the immune system remains unactivated provided $\gamma = d/b \leqslant 1$. In other words, for the immune system to be activated (and parasite burden suppressed below the level Λ/μ), the maximum T cell proliferation rate (d), must exceed the death rate of the cells (b). Once activated, however, the system shows a similar behaviour to the earlier model, with the worm burden reaching a peak at respectively

$$\left.\begin{aligned} t_1 &\sim \delta/[\gamma-1],\\ t_2 &\sim \delta/\sqrt{\gamma-1},\\ t_3 &\sim \delta\log\gamma/[\gamma-1], \end{aligned}\right\} \tag{15}$$

and an increased tendency (over and above the basic model) to undergo damped oscillations to the stable suppressed state (see May (1973) for derivations to this and more general results).

Of greater interest is the convex proliferation function since this type of pattern is often observed (Fig. 3). As before, the immune system remains unactivated provided $\gamma \leqslant 1$. However, when $\gamma > 1$, 3 stationary states are possible. For the rescaled equations (see equations (5) and (6)),

$$dP(t)/dt = 1-P(t)\,[1+X(t)], \tag{16}$$

$$dX(t)/dt = \alpha X(t)\,[\gamma P(t)\,R(P)-1], \tag{17}$$

where $\gamma = d/b$ and the proliferation function $R(P)$ has a new scale $\zeta = \xi/Q$ ($Q = \Lambda/\mu$). The 3 equilibria are given by

$$\left.\begin{aligned} P_1^* &= \zeta[\gamma+\sqrt{\gamma^2-1}],\\ P_2^* &= \zeta[\gamma-\sqrt{\gamma^2-1}],\\ P_3^* &= 1. \end{aligned}\right\} \tag{18}$$

Assuming that $2\zeta\gamma \leqslant 1$ (i.e. that all 3 points are positive), stability analysis shows that only P_2^* and P_3^* are stable. This means that the steady state attained depends on the initial conditions of the system. Fig. 5 shows a phase portrait of the model's behaviour with the 3 stationary (= equilibrium) states plotted with corresponding isoclines ($dP(t)/dt = dX(t)/dt = 0$). Four numerical solutions of equations (16) and (17) are presented with the following initial conditions at time $t = 0$,

$$(X(0), P(0)) = (0{\cdot}2, 0),\ (2, 0),\ (1, 1) \text{ and } (2, 1). \tag{19}$$

The boundary that divides the phase plane into a region whose initial values of $P(0)$ and $X(0)$ are allocated to the state of an unactivated immune system (= high parasite burden and unactivated T cells) and a region where they are allocated to a partial immunity state (= low parasite burden and elevated T cell activity), and passes via the unstable state (X_1^*, P_1^*), is also plotted in Fig. 5. Without activation the worm burden $P(t)$ follows the solution in equation (3) which gives saturation when $P(t) = P_3^*$. With activation the worm burden again increases until

$$t_4 \sim \delta/\gamma \tag{20}$$

and then oscillates to the stable equilibrium P_2^*.

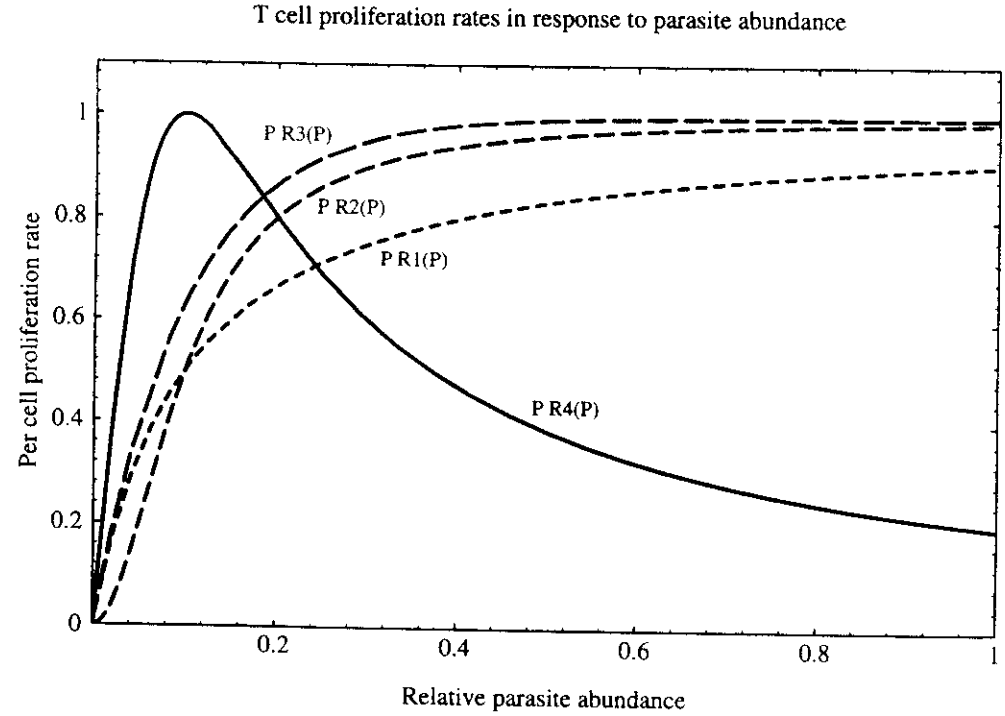

Fig. 4. Normalized per-cell T cell proliferation rates $P(t)R_i(P)$. We have chosen a scale $\xi = Q/4$ ensuring high-zone tolerance. Saturation will always induce oscillations in the immune response towards a stable equilibrium.

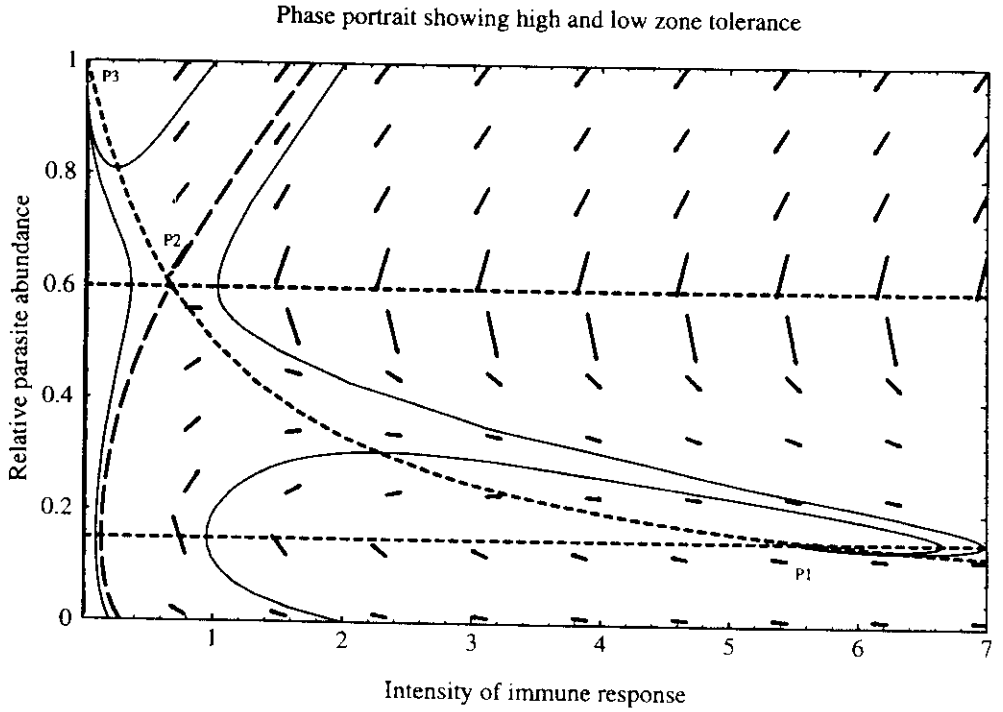

Fig. 5. *Phase portrait* of twin-zone tolerance T cell proliferation. Isoclines ($dP/dt = dX/dt = 0$) are shown as short dashed lines and the three fixed points are labelled at the intersections of these lines. Parameters used are $\alpha = 10$, $\gamma = 1{\cdot}25$ and $\delta = 0{\cdot}30$. The two critical trajectories are shown as long dashed lines. Initial background T cell levels to the left of these lines lead to tolerance and the equilibrium P_3. Increasing initial activation switches on the immune response and the system oscillaes to the stable equilibrium P_1.

Hence the unactivated state (= tolerance of high parasite burden) may arise because of high initial exposure to the parasite (say soon after birth of a child in an area of endemic infection) or because the initial density of T cells specific to the parasite's major antigen is too low (or the antigen is of low immunogenicity).

Multiple equilibria arising from simple models of the interaction between macro-parasites and the vertebrate immune system have been reported in a

number of recent publications (Anderson, 1991, 1994*b*; Schweitzer & Anderson, 1992*b*).

PARASITE ANTIGENS WITH MULTIPLE EPITOPES

Surface, secreted or excreted parasite carbohydrate or protein antigens typically bear a number of epitopes that are recognized by the T and B cells of the host's immune system. However, in a viral infection, cytotoxic T lymphocytes (CTL) recognize only a small number of the potential epitopes on the parasite's antigens. In some cases only a single epitope is recognized and this phenomenon is known as immunodominance (Townsend & Bodmer, 1989; Hill, Mullbacher & Blanden, 1993). Several explanations have been put forward to explain immunodominance and these include high affinity binding of a particular epitope to the major histocompatibility complex (MHC) molecule (an MHC-epitope complex binds T cell antigen receptor with high affinity), abundance of T cells with receptors specific to the epitope, abundance of the epitope, the concentration of the source protein, its intrinsic stability and localization plus the presence of appropriate proteolytic cleavage sites that lead to efficient antigen processing (Sercarz *et al.* 1994). Recently Nowak *et al.* have suggested an explanation based on the population dynamic principle of competitive exclusion, where one T cell specific to a particular epitope wins in competition with other T cell clones specific to other epitopes on the same antigen (Nowak *et al.* 1995).

Much less is known about immunodominance within immunological responses by humans to epitopes of macroparasites. However, work on candidate helminth vaccines with antigens that present multiple epitopes in rodent models suggests that 1 epitope may be recognized much more strongly than others in a given strain of the rodent host. With the same antigen, the pattern of recognition may vary from host to host, or more commonly between different mouse strains (Reynolds, Dahl & Harn, 1994).

In order to study the dynamics of T cell responses to multiple epitopes on a single helminth antigen we extend the basic model to mirror N epitopes on the antigen, each with its own immunogenicity, c_i. $p(t)$ still denotes parasite abundance (assumed to be directly proportional to antigen concentration) but $x_i(t)$ now denotes T cells specific to epitope i. For simplicity we use the basic assumption for T cell proliferation with a *per capita* rate of $c_i p(t)$. Rescaling the equations as before gives a coupled system of $N+1$ equations,

$$dP(t)/dt = 1 - p(t)\left[1 + \sum_{i=1}^{N} X_i(t)\right], \tag{21}$$

$$dX_i(t)/dt = \alpha X_i(t)\,[\beta_i P(t) - 1], \quad i = 1, \ldots, N. \tag{22}$$

the parameter α remains unchanged from the basic model although β_i and $X_i(t)$ will be epitope-specific ($\beta_i = c_i Q/b$ and $X_i(t) = h_i x_i(t)/\mu$). Analysis of these equations shows that there are only 2 possible steady states; (1) no activation of the immune system and (2) immunodominance of 1 epitope.

No activation implies that no activated T cells are present at equilibrium

$$P^* = 1, \quad X_i^* = 0, \quad i = 1, \ldots, N. \tag{23}$$

This is stable provided all the β_i are less than unity (i.e. when all epitopes are of weak immunogenicity relative to specific T cell expectancy).

Immunodominance of 1 epitope

$$P^* = 1/\beta_j^{-1}, \quad X_j^* = \beta_j - 1, \quad X_i^* = 0, \quad i \neq j. \tag{24}$$

When activation of the immune system occurs ($\beta_k > 1$), then it is possible that more than 1 epitope may satisfy the constraint $\beta_k > 1$. In those circumstances, it can be shown that complete exclusion occurs such that the response to only 1 epitope is elevated and all others are zero. In other words, the action of the immune system minimizes parasite abundance by expanding only those T cells stimulated by the most immunogenic epitope (i.e. maximum β_i). Hence maximum effect is produced by a minimum effort (only 1 T cell clone expanded). Because the immune system is focussed on only 1 epitope, the responses to the other $N-1$ epitopes are ineffectual and they decay to zero. The result derived from the basic model (equations (1) and (2)) therefore holds with β replaced with $\mathrm{Max}\,\beta_i$.

It is not clear at present whether immune responses to helminth multi-epitope antigens are indeed characterized by such total immunodominance of the response to a single epitope (as is sometimes the case for viral antigens). One might expect partial dominance of 1 epitope with other epitope-specific T cells present in lesser quantities. This could be induced by some form of cross-reactivity between epitopes (i.e. 1 T cell clone responds, with different efficacies, to more than 1 epitope) or as a result of genetically determined variability between parasites in the immunogenicities of a given epitope.

PARASITE ANTIGENS WITH GENETIC VARIATION AT A SINGLE CROSS-REACTIVE EPITOPE

Genetic changes in the gene that encodes for a specific epitope may arise via mutation events in asexual reproduction and as a result of recombination in sexual reproduction (the norm amongst helminth parasites). We start by considering a single epitope which may have n variants, $i = 1, \ldots, n$. We now require a subscript on the parasite abundance variable $p_i(t)$ to denote antigenic variant i. With the

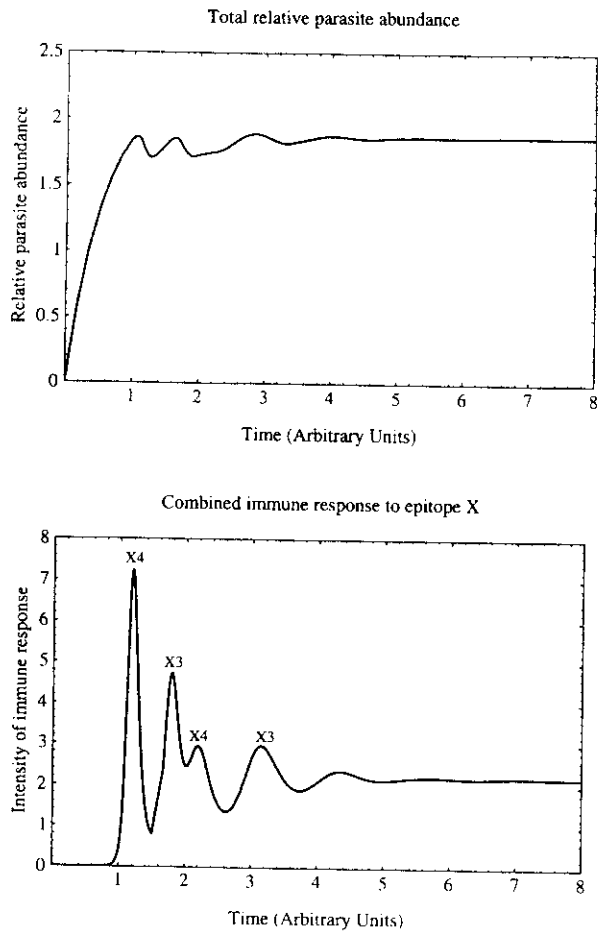

Fig. 6. *Partial coexistence* of a single cross-reacting epitope with $n = 4$ variants. Parameters used are $\alpha = 30$, $\delta = 0{\cdot}2$, with initial conditions $P_i(0) = 0$ and $x_i(0) = 0{\cdot}01$. The immunogenicities β_i are randomly chosen to lie in the range $0 < \beta_i \leqslant 4$. Only T cells x_3 and x_4 are activated and these oscillate with different periods giving the combined T cell response shown.

assumption that each variant is distinct and elicits a variant-specific T cell response, the appropriate set of scaled equations is

$$dP_i(t)/dt = 1 - P_i(t)[1 + X_i(t)], \quad i = 1, \ldots, n, \tag{25}$$

$$dX_i(t)/dt = \alpha X_i(t)[\beta_i P_i(t) - 1]. \tag{26}$$

For simplicity we have assumed that all parasite variants have the same immigration and death rates such that in the absence of an immune response $P_i^* = \Lambda/\mu = Q$.

Inspection of these equations reveals that $P_i(t)$ is only coupled with a specific T cell, $X_i(t)$, with no other T cell equation involved. As such the model behaves as n independent single-epitope basic models as detailed in the first section of the paper.

Different genetic variants will often have an underlying structural similarity and it may be the case that the gene that encodes for a particular epitope differs by only 1 base-pair substitution between 2 variants. Such similarity may induce cross-reactivity between variants where 1 T cell clone is stimulated by and responds to more than 1 variant. To mimic this biological situation we can add an extra cross-coupling term to our expression for $dP_i(t)/dt$ of the form $\delta \sum_{j=1}^{n} P_j(t)$. This is a very simple term since the degree of cross-reactivity is assumed to be the same between all variants. We do, however, assume that the value of δ is very small. We could instead have made a more complicated (and perhaps more realistic) assumption with a term such as $\delta P_i(t) \sum_{j=1, j \neq i}^{n} X_j(t)$. Unfortunately, the introduction of such a term into the more complex multiple-epitope models means we lose much of the analytical predictability of the model (such a treatment will be given elsewhere). The simple assumption, however, serves to illustrate the impact of cross-reactivity and can be viewed as a form of background immunity to antigens or epitopes that are conserved across all variants. As such its impact on parasite mortality is proportional to the sum over all strains.

The modified model is

$$dP_i(t)/dt = 1 - P_i(t)[1 + X_i(t)] - \delta \sum_{j=1}^{n} P_j(t), \quad i = 1, \ldots, n \tag{27}$$

$$dX_i(t)/dt = \alpha X_i(t)[\beta_i P_i(t) - 1]. \tag{28}$$

Analysis of this system reveals 3 possible equilibrium scenarios, namely; (1) no activation of T cells, (2) coexistence of T cells specific to all genetic variants and (3) partial coexistence of only T cells specific to some variants.

No activation, as illustrated in the basic model, implies no activated T cells present at equilibrium. With cross-reactivity created by immune responses to conserved regions of parasite antigens (conserved across variants), there is still some degree of extra mortality imposed on the total parasite population within the host, even when specific T cell abundances are zero. In the unactivated state the parasite variant abundances are

$$P_i^* = 1/[1 + \delta n], \quad i = 1, \ldots, n. \tag{29}$$

It can be shown that all genetic variants will fail to activate specific responses provided the parameters β_i remain below some critical value β_c for all variants

$$\beta_i \leqslant 1 + \delta n, \quad i = 1, \ldots, n. \tag{30}$$

In the basic model the condition was simply $\beta \leqslant 1$, hence the evolution of genetic diversity increases the likelihood that the epitope in question will not activate specific immunological responses. Here is a simple but powerful reason for sex or mutation events generating diversity in parasite antigen epitopes!

Coexistence of all the different T cell clones specific to particular variants is now possible, in contrast to the multiple-epitope model. With all epitope parasite variants present and their respective T cell populations present, the equilibrium states are

$$P_i^* = 1/\beta_i, \quad X_i^* = \beta[1 - \delta P_i^*] - 1, \quad i = 1, \ldots, n \tag{31}$$

where P_*^* is given by

$$P_*^* = \sum_{i=1}^{n} 1/\beta_i. \tag{32}$$

Coexistence of all variants is stable provided that *all* β_i are greater than $1+\delta n$. Damped oscillations to the steady state may occur with periods

$$\tau_i = 4\pi/[\sqrt{} - \Delta_i], \quad i = 1, \ldots, n, \tag{33}$$

$$\Delta_i = b_i^2[1-\delta P_*^*]^2 - \alpha[\beta_i[1-\delta P_*^*]-1], \tag{34}$$

provided that α is large enough to ensure that all Δ_i are negative.

The fact that each variant will oscillate with a different period τ_i means that the combined T cell response ($X_*(t)$) will consist of many peaks and troughs as each variant reaches a maxima and minima at different time-intervals.

Partial coexistence can occur if only m of the total n variants satisfy the constraint $\beta_i > 1+\delta n$. Fig. 6 records a numerical realization of partial coexistence with parameter values $n = 4$, $\delta = 0{\cdot}2$, $\alpha = 30$, $\beta_c = 1{\cdot}8$ and

$$\beta_i = (0{\cdot}2420,\ 0{\cdot}9575,\ 3{\cdot}7873,\ 2{\cdot}9031). \tag{35}$$

Only β_3 and β_4 are above the critical value of 1·8 and hence only these variants stimulate specific T cell responses. The stability of the partial coexistence state is important. Helminth parasite antigen epitopes may have many closely related genetic variants, some of which may not be sufficiently immunogenic to stimulate specific T cell or B cell responses. The model shows that those variants will coexist with other more immunogenic variants.

PARASITE ANTIGENS WITH GENETIC VARIATION AT MULTIPLE CROSS-REACTIVE EPITOPES

The previous section revealed that the introduction of antigenic variation reduced the likelihood of an unactivated immune system provided cross-reactivity between variants occurred. To mimic reality more closely we need to extend this analysis to encompass multiple epitopes with genetic variability possible at each epitope on the parasite antigen.

For simplicity we shall assume that the parasite antigen of interest has only 3 epitopes although a generalization to an arbitrary number will be given elsewhere. Each of these 3 epitopes is subject to genetic variation. Combining the ideas of the last two sections we now require a total of 3 subscripts on $p(t)$, 1 for each epitope. Each epitope will activate its own set of T cells which we shall denote by $x_i(t)$, $y_j(t)$ and $z_k(t)$. Hence the antigen variant p_{ijk} is acted on by T cells x_i at epitope 1, y_j at epitope 2 and z_k at epitope 3. If there are N epitopes, each with e_p different variants, there will of course be a total

$$\mathcal{N} = \prod_{p=1}^{N} e_p = e_1 e_2 e_3, \ldots, e_N \tag{36}$$

different antigenic variants, where e_1, e_2 and e_3 are the number of variants for epitopes 1, 2 and 3 respectively. Each of these parasites has many epitopes which means there is an additional form of cross-reactivity. All variants p_{i**} are acted on by T cells x_i independent of the other 2 epitope variants. This cross-reactivity will have important consequences when we come to look at the dynamics of the system. Of course introducing this cross-coupling increases the number of equations such that the total number of equations required to model the system is

$$1 + \sum_{p=1}^{N} e_p = e_1 e_2 e_3, \ldots, e_N + e_1 + e_2 + e_3 + \cdots + e_N, \tag{37}$$

all of which are coupled through the multiple T cell actions.

For simplicity we assume that all T cells again have the same lifetime $1/b$ and all antigenic parasite variants have the same immigration and death rates such that $p_{ijk}^* = Q$, in the absence of an immunological response, independent of i, j and k. With these assumptions the simplest rescaled equations governing the immune system and antigenic variants are

$$dP_{ijk}(t)/dt = 1 - P_{ijk}(t)[X_i(t) + Y_j(t) + Z_k(t)] - \delta P_{***}(t), \tag{38}$$

$$dX_i(t)/dt = \alpha X_i(t)[\beta_i^1 P_{i**}(t) - 1] \quad i = 1, \ldots, e_1, \tag{39}$$

$$dY_j(t)/dt = \alpha Y_j(t)[\beta_j^2 P_{*j*}(t) - 1], \quad j = 1, \ldots, e_2, \tag{40}$$

$$dZ_k(t)/dt = \alpha Z_k(t)[\beta_k^3 P_{**k}(t) - 1], \quad k = 1, \ldots, e_3. \tag{41}$$

As in the previous section, a subscript $*$ denotes a summation over all possible variants and hence the cross-reactivity term involving P_{***} should be written as

$$P_{***}(t) \equiv \sum_{i=1}^{e_1} \sum_{j}^{e_2} \sum_{k=1}^{e_3} P_{ijk}(t). \tag{42}$$

The rescaled immunogenicities β_q^p are both epitope- and variant-specific which means β_i^1 refers to the immunogenicity of the i'the variant of epitope 1 etc, and therefore

$$\beta_q^p = c_q^p Q/b, \tag{43}$$

implying that each antigenic variant has its own immunogenicity c_q^p. Keeping so many independent parameters in the model clearly makes the analysis much harder, although we can again divide the behaviour of the model into tolerance and immuno-activated states.

Immune unresponsiveness (anergy)

Looking at the full equations we can identify an unactivated immune system as a possible equilibrium. If we set all T cell levels to zero and sum

over all antigenic variants the equation for $dP_{ijk}(t)/dt = 0$ gives

$$P^*_{ijk} = 1/[1+\delta.\mathcal{N}], \quad X^*_i = Y^*_j = Z^*_k = 0, \tag{44}$$

independent of i, j and k. This is exactly analogous to the case of a single epitope with n antigenic variants. Instead of n, there are now $\mathcal{N}$ variants which means that cross-reactivity responses will have an even greater combined effect since we known that

$$\mathcal{N} \geqslant n. \tag{45}$$

The unactivated state will be stable if no epitopes activate an immune response. This requires that for each epitope p and variant q

$$\beta^p_q \leqslant e_p[1+\delta.\mathcal{N}]/.\mathcal{N}, \quad p = 1, \ldots, \mathcal{N}, \quad q = 1, \ldots, e_p. \tag{46}$$

Comparing this with equation (30), this stability condition has an extra factor $e_p/.\mathcal{N}$ which will make the unactivated state much less likely when many epitopes are present.

If the total number of variants is very large then the total antigen level tends to the limit

$$\lim_{\mathcal{N}\to\infty} P_{***} = 1/\delta, \tag{47}$$

independent of $\mathcal{N}$. This will only be possible provided that the stability conditions

$$\beta^p_q \leqslant e_p\delta \tag{48}$$

can be satisfied. If all epitopes have large e_p values then the unactivated state might be stable; however, if $\mathcal{N}$ is dominated by just one or two epitopes then the inequality becomes very restrictive and a failure to activate the immune system is unlikely. Of course if no cross-reactivity is present then $\delta = 0$ which means that in the large $\mathcal{N}$ limit the unactivated state will not arise irrespective of the variability distribution.

From these results we can see that adding antigenic diversity at many epitopes produces quantitatively the same level of inactivation as that predicted at one epitope, although the unactivated state is much less likely to be stable. If evolutionary pressures on the helminth are trying to avoid recognition by the immune system we might expect the generation of new antigenic variants to be unfavourable. In fact we will see that there is a mechanism whereby generating new variants enables the helminth to avoid activating the full immune response.

Immunodominance and partial coexistence

Increasing the number of epitopes and the number of variants at each reduces the likelihood that the parasite avoids recognition by the host's immune system. What happens when the system is activated? From the simple models with no antigenic variation, only T cells specific to the most immunogenic epitope will proliferate. Furthermore T cells specific to variants of the same epitope can coexist. These two observations suggest that some form of selection by the immune system (and the parasite) will take place once many genetically variable epitopes are present on the parasite antigens.

Analysis of the model reveals that selection does take place such that some T cell clones will proliferate and some parasites with particular sets of epitope variants will predominate (despite equal recruitment for all types). However, despite the presence of immunodominance reflected by the high abundances of specific T cell clones, coexistence can occur.

At equilibrium, for all T cell clones (e.g. epitope 1), the equation $dX_i(t)/dt = 0$ implies that either

$$P^*_{i**} = 1/\beta^1_i, \quad \text{or} \quad X^*_i = 0 \tag{49}$$

(and similarly for epitopes 2 and 3). Ideally we need an expression for total parasite abundance (summing across the variant types at each epitope) in the closed form

$$P^*_{***} = \sum_{i=1}^{e_1} 1/\beta^1_i. \tag{50}$$

This requires that all T cell clones (x_i) be present at equilibrium, which in turn requires

$$\beta^1_i > e_1[1+\delta.\mathcal{N}]/.\mathcal{N}, \quad i = 1, \ldots, e_1. \tag{51}$$

If this holds then equation (50) is correct.

More generally, the immune system is trying to minimize the total worm burden given the specifications of the immunogenicities of the variants at each epitope and the cell proliferation and death rates. This implies competition between T cell clones and hence some form of immunodominance of clones to a particular epitope.

The model suggests that the immunodominant epitope will be that which has a full set of activated T cells to each of the genetic variants and the lowest reciprocal β sum where

$$P^*_{***} = \text{Min} \sum_{q=1}^{e_p} 1/\beta^p_q. \tag{52}$$

In the multiple epitope model without genetic variation no coexistence between T cell clones specific to the different epitopes is possible. Once variation arises coexistence is possible with a number of distinct epitopes activating reasonable immune responses. More formally this can be seen from the fact that the sums P^*_{i**}, P^*_{*j*} and P^*_{**k} may each contain a different number of elements. For example, if epitope 1 is immunodominant (as assumed in equation (51)), then to a good approximation the summations over parasite variants at the other two epitopes are:

$$P^*_{*j*} \simeq P^*_{***}/e_2 \quad \text{and} \quad P^*_{**k} \simeq P^*_{***}/e_3. \tag{53}$$

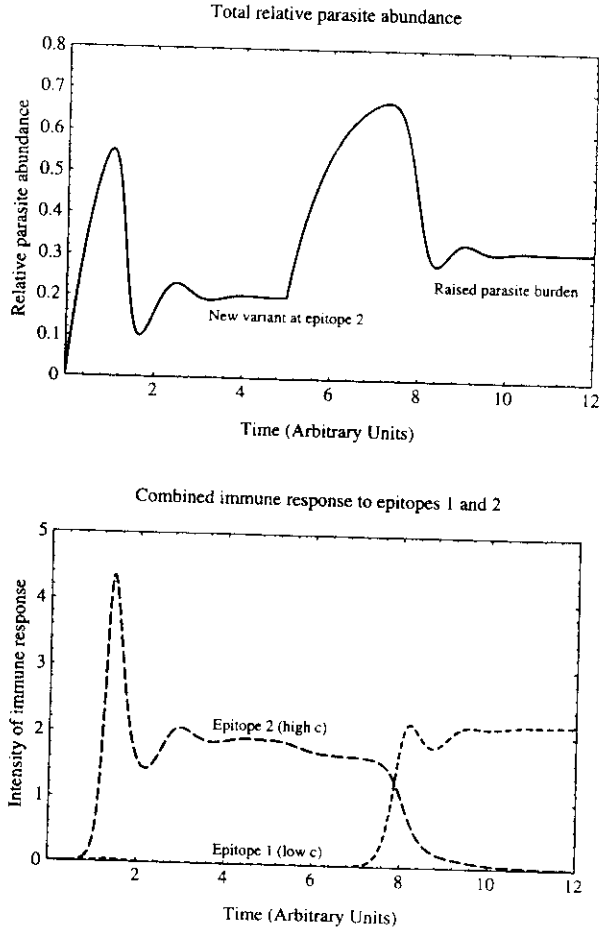

Fig. 7. *Switching in immunodominance* from epitope 2 (most immunogenic) to epitope 1 (less immunogenic). Initially there is a single parasite variant P_{11} present which elicits an immune response from both epitope 1 and 2-specific T cells. Epitope 2 has the highest immunogenicity and is therefore immunodominant. At time $t = 5$, a second genetic variant is generated at this epitope (introducing P_{12} into the parasite population). This causes a switch in immunodominance and an increase in parasite abundance. Parameters used are $\alpha = 5$ and $\delta = 0{\cdot}2$. The immunogenicities are $\beta^1 = (3{\cdot}7180)$ and $\beta^2 = (5{\cdot}0, 3{\cdot}7811)$. Initial conditions are those of Fig. 6.

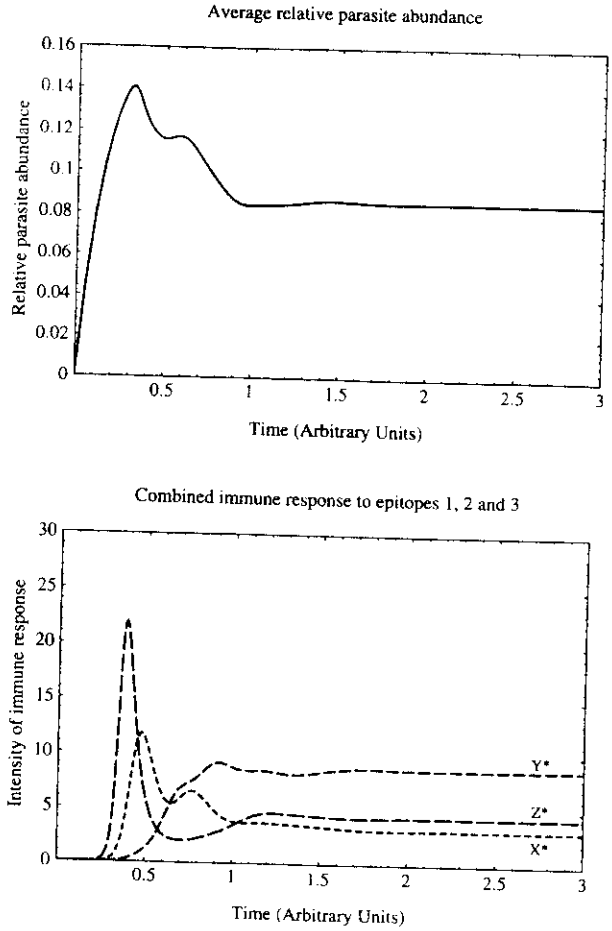

Fig. 8. Coexistence of three T cell responses to an antigen with $A = 16$ variants. Parameters used are $\alpha = 30$ and $\delta = 0{\cdot}2$ with initial conditions $P_{ijk}(0) = 0$, $X_i(0) = Y_j(0) = Z_k(0) = 0{\cdot}01$. The immunogenicities β_q^p for epitope p-variant q are randomly chosen to lie in the range $0 < \beta_q^p \leqslant 3$. Epitope 2 ($y_j$) is immunodominant although T cells responding to the other two epitopes are also present at equilibrium.

T cell clones y_j and z_k will also be present at equilibrium provided

$$\beta_q^p \geqslant \beta_a^p = e_p/P^*_{***}. \quad P \neq 1, \tag{54}$$

where a subscript a denotes an approximation. (This approximation holds good except in the case when β_q^p is only slightly smaller than the critical value e_p/P^*_{***}, in which case the other T cell clones persist even though they are ruled out by the approximation.)

A series of important biological insights are generated from this analysis of a rather complex biological model (i.e. multiple epitopes and multiple genetic variants deriving from variations at each epitope). First, coexistence between all variants in the genetically diverse parasite population is possible although T cell clones specific to every one of the variants may not be activated and hence detectable. Secondly, the epitope with the most immunogenic parasite variant may not stimulate the strongest and most effective (in terms of reducing parasite burden) immune response. At first sight these results appear counter-intuitive. The reason lies in equation (52) and the surrounding biological interpretation of this result. The immune system is acting to try and minimize the total worm burden and in doing so selects the epitope which elicits a T cell response to all variants and in which the sum of the individual immunogenicities to each variant is greatest (given equal parasite strain life-expectancies, rates of recruitment and T cell clone life-expectancies). Hence, a lowly immunogenic epitope may be targeted instead of a highly immunogenic one if the former has few variants and the latter many. In other words, the parasite may evade immunological responses to a highly immunogenic antigen epitope by evolving high mutation rates in the gene that encodes for that part of the antigen (or other genetic mechanisms to

Table 1. Immunogenicities used in simulation of coexistence

Epitope	Variants e_p	β_c^p	β_q^p	$\sum_{q=1}^{e_p} 1/\beta_q^p$
1 (x_i)	3	0·725	(1·3430, 1·9070, 0·2963)	4·6434
2 (y_j)	2	0·483	(0·9076, 1·0511)	2·0532
3 (z_k)	4	0·967	(0·2615, 3·0, 1·1116, 1·4057)	5·7681

generate variability in the epitope). Conversely, the parasite can direct the immune response to a low immunogenic epitope via conservation of the genetic material coding for it. We refer to this phenomenon as the process of *masking* potentially immunodominant epitopes by antigenic variation. Third, and related to the second point, a single non-varying epitope (a conserved epitope) can dominate the immune response even when more immunogenic variants exist at other epitopes.

An illustration of the last two points is presented in Fig. 7 where a numerical simulation of the model presented in equations (38–41) is depicted (for simplicity we have restricted the model to only 2 epitopes). The graph records the parasite population growth in a single host where at time $t = 0$ the parasite population is genetically homogeneous and the host's response is directed at 2 epitopes on a single antigen. Epitope 2 is immunodominant (i.e. has greater immunogenicity, $c_2 > c_1$) and the T cell clones specific to this epitope outcompete those targeted at epitope 1 (competitive exclusion). At time $t = 5$, a new genetic variant emerges with variation at epitope 2 (now 2 strains in the population). The new variation at epitope 2 is still more immunogenic than the conserved epitope 1, but the condition defined in equation (52) now produces a shift in immunodominance to epitope 1 (the less-immunogenic epitope) with a consequent elevation in the total parasite burden. Thus in this example we see antigenic variation acting to 'mask' the most immunogenic epitope to the benefit of the parasite's overall abundance within the host. Note, however, that this will not always occur. The quantitative details of the relative immunogenicities of the variants and the number present in the population for each epitope at the time of emergence influence which epitope is targeted by the immune system (equation (51)). We consider each of the 3 biological phenomena outlined here in greater detail in the following subsections.

Coexisting T cell responses to more than 1 epitope. In the example presented in Fig. 7, the parameter values chosen resulted in competitive exclusion between the T cells specific to each of the two epitopes. Coexistence can occur and a specific example serves to illustrate this phenomenon. Suppose epitope 1 (in a 3-epitope antigen) has a lowest β sum (equation (52)) and all its variants stimulate measurable T cell responses. Then equation (54) implies that if the other two epitopes have a least 1 variant with $\beta_q^p > e_p P_{***}^*$, then they will have at least 1 set of T cell clones activated at equilibrium.

Fig. 8 records an example of coexisting T cell responses to 3 epitopes on a single antigen (summed responses to all variants at each epitope). We randomly selected the number of variants at each epitope and the individual β_q^p (= immunogenicities) values which lie in the interval 0–3, such that the highest immunogenicity is always exactly 3·0. We set the cross-reactivity parameter δ (extra death rate imposed by immune response to conserved antigens/epitopes) to a low value of 0·2 and α to 30. There are a total of $A = 24$ parasite antigenic variants which means that T cell activation is possible provided equation (46) is satisfied, i.e.

$$\beta_q^p > \beta_c^p \equiv e_p[1 + 0{\cdot}2 \times 24]/24 = 0{\cdot}2417 e_p. \tag{55}$$

The parameter values chosen for the simulation are shown in Table 1. T cell clones to all variants can be activated and proliferate except to variants x_3 and z_1. Epitope 2 has the lowest reciprocal β sum and T cell clones proliferate to all variants (i.e. equation (51) is satisfied). The average (scaled) worm burden is therefore

$$P_{***}^*/A = 2{\cdot}0532/24 = 0{\cdot}0836. \tag{56}$$

Having determined the value of P_{***}^*, and demonstrated that epitope 2 will be immunodominant, we can predict the likelihood of coexistence between T cell clones to the 3 epitopes by estimating critical β_a^p values (equation (54)).

$$\beta_a^1 \simeq 1{\cdot}4511 \quad \text{and} \quad \beta_a^3 \simeq 1{\cdot}9482. \tag{57}$$

Considering Table 1 and equation (57) we can see that $\beta_2^1 > \beta_a^1$ and $\beta_2^3 > \beta_a^3$, which means that T cell clones to x_2 and z_2 will be present at equilibrium. also β_1^1 is very close to its critical value, and in the numerical results x_1 T cells are also present. This specific example clearly illustrates how immunodominance and coexistence of a subset of T cell clones can occur concomitantly.

Masking of immunodominant epitopes. For the coexistence of T cell clones specific to all the epitopes present on an antigen we require that at least 1 variant on each epitope has a degree of immunogenicity greater than a specified value (equation (54)). Now suppose that the most immunogenic epitope is subject to a great deal of genetic diversity. In the absence of variation we would expect this

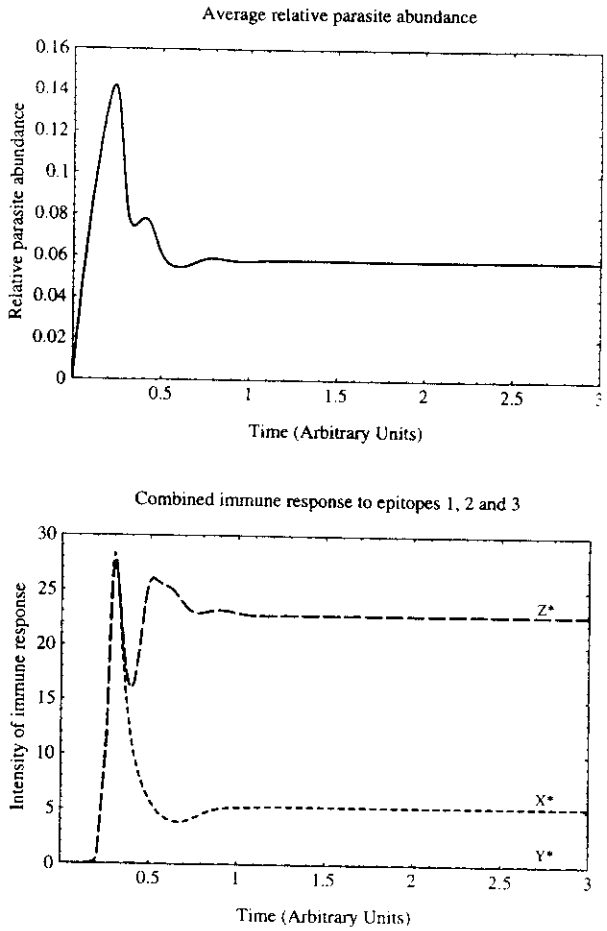

Fig. 9. *Masking* of a highly immunogenic epitope on the surface of an antigen with $A = 24$ variants. Parameters used are $\alpha = 30$ and $\delta = 0{\cdot}2$ with initial conditions $P_{ijk}(0) = 0$, $X_i(0) = Y_j(0) = Z_k(0) = 0{\cdot}01$. The immunogenicities β_q^p are randomly chosen to lie in the range $0 < \beta_q^p \leqslant 3$. Epitope 2 ($y_j$) is the most immunogenic although no epitope 2-specific T cells are present at equilibrium.

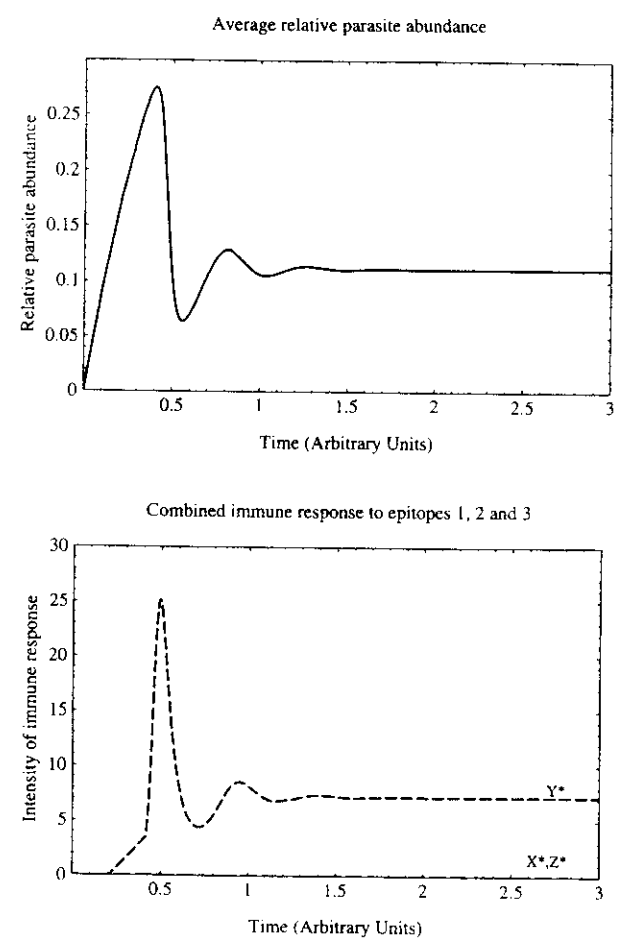

Fig. 10. *Single-epitope immunodominance* by an epitope on the surface of an antigen with $A = 4$ variants. Parameters used are $\alpha = 30$ and $\delta = 0{\cdot}2$ with initial conditions $P_{ijk}(0) = 0$, $X_i(0) = Y_j(0) = Z_k(0) = 0{\cdot}01$. The immunogenicities β_q^p are randomly chosen to lie in the range $0 < \beta_q^p \leqslant 3$. Epitope 2 ($y$) is immunodominant and no other T cells are present, even though epitope 3 (z_k) is the most immunogenic.

epitope to be immunodominant. However, as noted earlier, high diversity can act to mask immunodominance.

Again a simple numerical example well illustrates this phenomenon (Fig. 9). Retaining the parameter values of the previous example (Fig. 8), but choosing a new set of β values and numbers of variants, we arrive at a set of $A = 16$ antigenic variants where T cell activation requires

$$\beta_q^p > \beta_c^p \equiv e_p[1 + 0{\cdot}2 \times 16]/16 = 0{\cdot}2625e_p. \tag{58}$$

The specific β_q^p values and their reciprocal sums are shown in Table 2. All the T cell clones except y_4 can proliferate and epitope 3 is the immunodominant epitope (lowest reciprocal β sum and all its T cells can proliferate). Estimating the total worm burden and approximate critical β values for the persistence of each T cell clone at equilibrium gives

$$P^*_{***}/A = 0{\cdot}0579 \tag{59}$$

with approximate critical β_a^p values

$$\beta_a^1 \simeq 2{\cdot}1584 \quad \text{and} \quad \beta_a^2 \simeq 4{\cdot}3169. \tag{60}$$

For epitope 1, only T cell clones to variant x_1 will be present and, more importantly, none of the T cell

Table 2. Immunogenicities used in simulation of masking of immunodominance

Epitope	Variants e_p	β_c^p	β_q^p	$\Sigma_{q=1}^{e_p} 1/\beta_q^p$
1 (x_i)	2	0·525	(2·6501, 0·8596)	1·5407
2 (y_j)	4	1·050	(2·0803, 2·6911, 3·0, 0·9867)	2·1991
3 (z_k)	2	0·525	(2·6311, 1·8298)	0·9266

Table 3. Immunogenicities used in simulation of single epitope immunodominance

Epitope	Variants e_p	β_r^p	β_q^p	$\Sigma_{q=1}^{e_p} 1/\beta_q^p$
1 (x_i)	2	0·525	(2·311, 2·073)	0·9114
2 (y_j)	1	0·2625	(2·2458)	0·4453
3 (z_k)	2	0·525	(2·2313, 3·0)	0·7815

clones to the 4 variants of epitope 2 will be present at equilibrium (i.e. $\beta_a^2 > 3{\cdot}0$). In other words, some of the immunogenic variants at the epitope 2 site will not elicit the expansion of specific T cell clones. The potentially immunodominant epitope is masked by epitope 3 which has a limited number of variants by comparison with epitope 2.

Single variant immunodominance. The role of masking a potentially immunodominant but variable epitope is made even easier if only 1 variant is present at one of the other epitopes. In the example detailed above, suppose that epitope 2 is conserved (only 1 variant). In this situation there will be only 1 immunogenicity β^2, and if its reciprocal is smaller than the sums of the immunogenicity reciprocals at the other 2 epitopes then epitope 2 will be immunodominant. If the immunogenicity of the conserved epitope is exactly one half of the highest immunogenicity of the variants at all other epitopes, and if all other variants have at least 2 variants, this implies that no other T cell clones will be activated (i.e. immunodominance of epitope 2 with complex exclusion of T cell clones to all variants at other epitopes).

An example of this phenomenon is plotted in Fig. 10 with numerical values similar to the two previous examples (Figs 8 and 9) but with new randomly generated e_p and β_q^p values shown in Table 3.

All epitopes other than 2 fail to activate an immune response and we are left with a single epitope which is immunodominant, despite the fact that variants at other epitopes have greater immunogenicities. This dynamical outcome which induces masking is an obvious advantage to a helminth parasite. For highly immunogenic epitopes, high genetic variability in the gene that codes for this site which induces distinctive variants that can potentially elicit T cell proliferation acts to shift the focus of the immune system to less immunogenic sites.

DISCUSSION

We started with a very simple model of the interaction between a parasite expressing 1 major antigen with a single epitope and the human immune system. The insights gained from the simple model facilitated the analysis and interpretation of much more complex models representing multiple antigens and genetic variation at each epitope. A number of important biological insights emerge from these analyses. First, in the absence of genetic variation or significant degrees of cross-reactivity of immune responses across different epitopes, competitive exclusion results in the interaction between different T cell clones specific to each epitope with the clone that induces the maximum depression of the parasite population 'winning' and becoming immunodominant. Once a degree of cross-reactivity occurs then coexistence of different T cell clones may occur but again the immunodominant clone will be the one which most effectively controls parasite abundance. Although the focus of our models has been on a single antigen with multiple epitopes, similar conclusions emerge if we consider multiple antigens each with a single major epitope. The response to 1 antigen (whether humoral or cellular) is likely to dominate in accord with its success in limiting parasite abundance.

However, helminth parasites are characterized, in part, by their ability to produce very large numbers of transmission stages by sexual reproduction. In the make up of the organism a great deal is invested in sex and reproduction. The adult sexually reproducing parasite lives in a very hostile environment and is confronted with a wide range of immunological responses targeted at key antigens that are central to parasite survival and transmission. One consequence of sex is the generation of variation in these antigens and undoubtedly this is of great importance to the long-term persistence of parasite populations within their human host. In areas of endemic infection with filarial worms, intestinal helminths and schistosome flukes the majority of people harbour worms for the major part of their lives.

The simple conclusions concerning the dynamical interaction between the parasite and the human host immune system change radically once genetic variation is taken into account. This greatly enhanced dynamical complexity very much parallels that discovered recently for antigenic variation in viruses and immunological responses to this variation (Nowak *et al.* 1995*a*). In many ways, however, the complexity is somewhat easier to unravel for helminths due to the basic immigration (= infection) – death nature of the processes that influence population growth in the host. For multiple epitope antigens, the immunodominant epitope, in terms of the abundance of activated T or B cells to that epitope, may not necessarily be the epitope with the genetic variant of the greatest immunogenicity. The immune system is still striving to minimize parasite abundance, but genetic variation at the most immunogenic epitope can result in a shift in immunodominance to a less immunogenic site. This shift

may be of advantage to the parasite since the targeting of the immune response to a less immunogenic (but more conserved) site will result in greater parasite abundance than would be the case if the response stayed focussed on the site with more immunogenic variants (but greater variability). The strategy for the parasite is clear—generate variability at immunogenic sites on antigens of key importance to survival and transmission. Similar conclusions apply if we consider multiple antigens with genetic variability at a single epitope on each. The phenomenon of 'masking' immunogenic site by a conserved epitope or antigen is an interesting one. One test of this prediction would be the detection of immunodominant responses to conserved antigen epitopes in humans in areas of endemic helminth infection. Another would be (in the same setting) the identification of highly immunogenic but variable epitopes that do not elicit immunodominant responses.

In conclusion, the aim of this paper has been to construct a theoretical framework with which to begin to explore the population dynamics of the within-host interaction between helminth parasites and vertebrate hosts. The framework generated is capable of addressing problems involving multiple antigens, multiple epitopes on each antigen and antigenic variation generated by recombination (or mutation). Although a simple beginning, the current framework reveals much dynamical complexity and, in particular, some counter intuitive notions concerning immunodominance and immunogenicity. In a further paper we will examine the implications of these results for the choice of antigens and epitopes for candidate helminth vaccines.

D.J.A. and R.M.A. thank the Wellcome Trust for fellowship and research grant support, respectively. We thank our colleagues, M. Nowak, R. M. May and G. Barker for stimulating discussions.

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