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THE KINETICS OF T CELL RESPONSES

Martin John Allan

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Abstract

The proliferation of T cells in response to an antigenic stimulus is a vital component of the adaptive immune response. Recently it has been demonstrated that, following a brief encounter with antigen, T cells enter a developmental program that can produce a full response in the absence of further antigenic stimulation. The striking similarity of T cell kinetics in different hosts in response to different stimuli demonstrates that the program strictly regulates cell number. Although T cells are receptive to a diverse range of modulatory signals, this invariance of key aspects of their kinetics suggests that regulation of cell number may be produced by a single mechanism.

The first aim of this thesis is to identify the most plausible mechanism that could regulate the T cell kinetics during an acute response. Using an ODE compartmental model to keep track of the number of cells in each generation, the predictions of a number of plausible regulatory mechanisms are compared to experimental data to determine the potential of each to regulate cell number. One revealing conclusion is that all successful mechanisms progressively increase the apoptosis rate during the response. The most plausible mechanisms are then further assessed to determine which produces the least wasteful response (in terms of unnecessary T cell death). It is concluded that the most plausible mechanism is one that progressively increases death rates and decreases division rates.

The second aim of this thesis is to investigate how the programmed nature of the regulatory mechanism affects the outcome of infection. Two aspects of the outcome of infection are considered: the size of the generated memory population, and the success, or otherwise, of pathogen clearance. The previous compartmental model is

extended to incorporate the formation of memory cells, and the impact of the program parameters on the final memory size following an acute infection is established. Situations when the pathogen can persist beyond the acute phase are then considered, and a discrete-time population model is developed to predict the long-term behaviour of the response. It is found that if the developmental program always produces a net increase in cell population size then pathogen clearance is guaranteed. A further conclusion is that during this long-term infection the sensitivity of the specific memory cells to re-stimulation diminishes.

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CHAPTER 1
MOTIVATION FOR THIS STUDY

On numerous occasions the adaptive immune system has saved your life. Without the precision and power of this complex cell network, which recognises and eradicates foreign particles from your body, viruses and bacteria would have overwhelmed you. The operation of this defensive system, however, is fallible. Its subversion or evasion by pathogens leads to chronic infection. Its erroneous activation can cause self-harm in the form of allergies and auto-immunity. To correct these failures and malfunctions it is necessary to understand the mechanisms that control the response.

The action of the adaptive immune system is orchestrated by T lymphocyte cells and recent studies demonstrate that a program strictly regulates their number during a typical response. A more robust understanding of this regulatory mechanism could enable techniques of immune modulation to be developed, to boost insufficient responses and inhibit unwanted ones.

A huge amount of experimental research has been focused on this area, and, given the complexity of the immune system, experimentalists have increasing sought the advice of theoreticians. Experimental and theoretical work powerfully complement each other. Laboratory observations provide the inspiration and data for mathematical studies, which in turn motivate and help direct new experiments.

Mathematical models of T cell:pathogen systems are being increasingly utilized. Some are concerned principally with the interaction between the immune system and a particular pathogen [4, 5]. The response to HIV infection has been modelled most extensively [6]. Others concentrate on the underlying mechanisms that regulate the T cell kinetics to all infections [7, 8]. Many interesting insights have been gleaned from the work [7, 9, 8, 10, 11]. However, recent evidence suggests that a standard assumption of the majority of models is incorrect. It was believed that during an

adaptive immune response the T cell kinetics were tightly coupled to those of the pathogen. But this view has been challenged by observations demonstrating that, following antigenic stimulation, the proliferative T cell response is largely controlled by a developmental program that fully unfolds even in the absence of antigen [12, 13, 14]. The mathematical models developed in the past may therefore make false predictions or fail to reveal important relationships. Thus there is an imperative to develop a new model of the T cell response that incorporates this fundamental feature of a developmental program and consider its consequences. Some work has already been done in this direction [2, 15, 1] but much still remains concerning how T cell number is regulated by this program.

Despite a myriad of potential modulatory signals in lymphoid tissue, proliferating T cells exhibit similar kinetics in response to different stimuli in different hosts. This suggests that cell number is regulated by a single mechanism controlled by the developmental program. The aims of this thesis are to identify the most plausible regulatory mechanism, and then investigate how its programmed nature affects the outcome of infection. Two aspects of the outcome of infection are considered: the size of the generated memory population following a primary response, and the success, or otherwise, of pathogen clearance.

The results are laid out in the next seven chapters. In Chapter 2 the biology of the immune system is introduced along with a critical discussion of relevant modelling work. In Chapter 3 a number of plausible regulatory mechanisms are assessed, by comparison with experimental data, to determine the potential of each to regulate cell number (this work has been published [16]). Chapter 4 assesses the wastage (defined in terms of unnecessary T cell death) associated with each of the successful mechanisms to determine the most plausible. In Chapter 5 the most plausible mechanism is analysed to find out what determines the final size of the memory population following an acute response and how this may be modulated by changes to the mechanism. In Chapter 6, the ability of the regulatory mechanism to achieve pathogen clearance is determined. Chapter 7 addresses the hypothesis that the declining size of T cell populations observed following some infections is a consequence of the action of the regulatory mechanism during low level antigen persistence. Finally, in Chapter 8, the

main findings of the thesis are discussed along with future areas of investigation.

CHAPTER 2
BACKGROUND

T lymphocytes are an integral part of the adaptive immune system. Each T cell is capable of recognising and responding to one particular antigen (a foreign particle such as a fragment of bacteria or virus). An appropriate encounter with antigen will induce the activation of those cells with the correct specificity (i.e. those that recognise it). Once activated, the cells proliferate, their numbers expanding enormously. They then migrate to the site of infection where they orchestrate the destruction of the pathogen. Once the infection is cleared the antigen-specific T cells undergo apoptosis (cell death) that dramatically reduces their number. A small fraction survives and persists as a stable memory population that is capable of responding to subsequent encounters with its specific antigen with greater effectiveness than could the naive population.

In this chapter the three main phases of the T cell response are first discussed: the encounter with antigen and activation of a T cell population, the proliferative response, and the formation and maintenance of a long-lived memory population. Past mathematical investigations of T cell responses are then critically examined.

2.1 The T cell proliferative response

2.1.1 Encounter and Activation

After developing in the primary lymphoid organs (the bone marrow and the thymus), mature T cells enter the circulation. Initially they are known as naive T cells since they have not encountered their specific antigen. These cells continuously circulate around the body, passing between the blood stream and the secondary lymphoid organs, such as the lymph nodes and the spleen [17].

The naive pool comprises two main subsets: T helper cells (CD4) and cytotoxic T lymphocyte cells (CD8). In an adaptive response, activated CD8 T cells act against intracellular pathogens by inducing apoptosis in their host cells. CD4 T cells help orchestrate the immune response through the release of signalling proteins known as cytokines and by activating other components of the adaptive system such as macrophages and B cells. Macrophages are cells capable of ingesting and destroying pathogens through a process known as phagocytosis. B cells are able to secrete antibodies which facilitate the phagocytosis of antigen.

In the absence of antigen naive cell numbers are homeostatically balanced: slow proliferation and entry of new cells from the thymus balance a gradual death. It is believed that this homeostasis is maintained by a combination of T cell receptor (TCR) ligation and contact with cytokines, in particular IL7 (reviewed in [18]).

To participate in an adaptive response a T cell must be activated by an encounter with its specific antigen. For naive T cells this always occurs in the lymphoid tissues, through which large numbers of naive T cells continuously migrate. Several processes direct antigen toward these tissues, where reside antigen-presenting cells (APCs) that specialise in capturing and presenting antigen to the T cells. An encounter between a naive T cell and its specific antigen will only produce full activation if two conditions are met: the antigen must be bound to a self-MHC molecule, and a co-stimulatory signal must be delivered. These stipulations endow APCs with a critical role in the response since they are the only cells capable of fulfilling both these requirements. They present antigen complexed with MHC I and II, and express co-stimulatory cell surface molecules, the most important being CD28.

Dendritic cells (DCs), macrophages and B cells are the three main types of APC. DCs are the most important and specialised, acting as the scouts of the adaptive immune system. Residing in most peripheral tissues in the body, they sample the environment, capturing antigens [19, 20]. Once internalised, these antigens are processed into peptides, and then loaded onto surface MHC class I and II molecules, a process known as antigen presentation. In peripheral tissues DCs exist in an 'immature' state which is ineffective at presentation. Appropriate stimulation by the antigen however, initiates a developmental program that matures DCs into potent

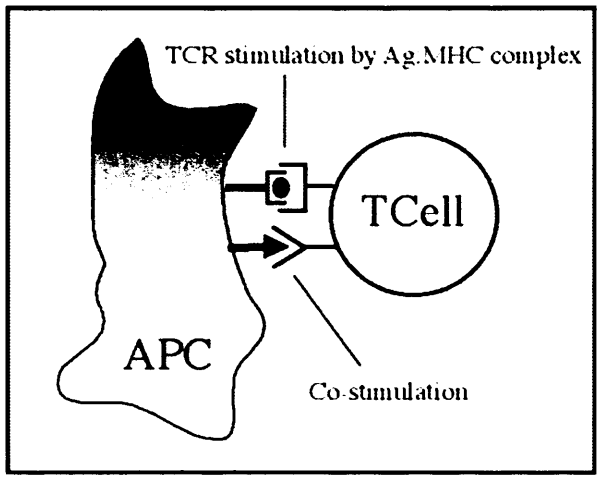


Figure 2.1: Activation of a naive T cell

presenters. They migrate to secondary lymphoid organs, transporting their antigenic cargo for presentation to naive T cells.

As the T cells migrate through the lymphoid tissues they make frequent contacts with APCs. If in one of these contacts a T cell recognises its specific antigen, it will cease migration [21] and initiate a tight, highly organized interaction, known as an immunological synapse [22], with the APC. The T cell will begin to undergo a number of biochemical and morphological changes that are known as activation. Among these changes the T cell cytoskeleton polarises toward the APC and the TCR and other molecules move towards the cell-cell interface [23]. After several days of this intimate interaction the T cell disengages and embarks on a period of proliferation, followed by migration to the site of infection and destruction of pathogen.

2.1.2 The Proliferative Response

Once activated, T cells divide extensively in the lymphoid tissues. They continue to divide 2-4 times every 24 hours for 4-6 days [24, 25, 26, 27]. Each cell therefore has the potential to produce thousands of daughter cells all with the same specificity. Alongside this proliferative response phenotypical changes occur. Over 4-5 days the

activated cells gradually differentiate into armed effector cells that possess the functions necessary to participate in the extirpation of the pathogen. CD8 T cells develop cytolytic (cell rupturing) functions and secrete the cytokine IFN-γ to remove intracellular pathogens from host cells. CD4 T cells can differentiate along two distinct paths to form Th1 or Th2 cells. Th1 cells specialise in activating macrophages and promoting inflammation. Th2 cells initiate the antibody response (humoral response) by activating B cells and producing anti-inflammatory cytokines. The T cells down-regulate lymph node homing receptors such as CCR7 and CD62L, allowing them to migrate to the site of infection.

The number of proliferating T cells peaks around $7-8$ days after activation. By this time the enormous clonal pool of armed effectors should have achieved pathogen clearance. Over the next few weeks the population steadily declines though apoptosis to approximately 5-10 % of the peak cell number [24, 28]. This small fraction then persists as a stable memory population for many years [27].

It was originally believed that the unfolding of the primary response was tightly coupled to the antigen kinetics. The presence of antigen, via repeated stimulation, was believed to drive T cell proliferation and clonal expansion, while its absence resulted in apoptosis and clonal contraction. This was backed by observations that in general the onset of contraction coincided with the time of antigen clearance [29, 30, 31, 32, 33, 34]. Recent studies, however, have strongly disputed this idea [35, 25, 36, 12, 36, 13, 14]. They suggest that T cells, once activated by antigen, enter an antigen-independent developmental program that can produce a complete primary response. In the past few years observations have shown that the kinetics of the response are, to a large degree, uncoupled from the infection kinetics [36]. After a brief encounter with antigen, both CD8 [12, 36, 13, 14] and CD4 [37, 38, 39] T cell populations have been observed to undergo substantial expansion. Then, in the continued absence of antigen, the effector population can contract to a stable memory population [12]. The onset time of contraction and its subsequent kinetics are observed to be independent of current antigen levels [35, 25]. The proliferation of CD8 T cells in response to an acute infection is observed to peak several days after the pathogen appears to be cleared [35]. Following contraction the size of the memory pool is proportional to

the size of the peak response [25, 40, 27, 41], seemingly unaffected by the previous antigen kinetics. These observations strongly suggest that after initiation by antigen, the subsequent kinetics of the T cell response are regulated by an antigen-independent mechanism.

It also appears that the continued presence of antigen is not essential for T cells to differentiate into effectors. CD8 T cells have been observed to acquire full cytolytic function, including IFN-γ production, after brief antigen stimulus [38, 12, 36]. CD4 T cells are also able to complete their effector differentiation in the absence of antigen [38], committing to a particular differentiation pathway after 24-48hrs of stimulation [42, 37, 43]. This suggests that the differentiation of activated T cells may be largely controlled by an antigen-independent mechanism.

Many antigen-independent regulatory mechanisms have been proposed. One possible mechanism involves each T cell regulating itself. Each cell may alter the expression of the genes that influence its proliferation. It is observed that the expression of survival genes Bcl-2 and Bcl-X_L decreases during the course of the response [44, 45, 46, 47]. This may explain the increasing apoptosis rates of cells over the same period [48, 27, 49, 50]. A similar downregulation of division promoting genes, such as c-myc and shc, may similarly explain the declining division rates of cells [27, 51]. This modulation may occur at the intracellular level or via autocrine cytokine signalling. A potential cytokine is the growth factor IL2. It is produced by cycling T cells and can act autocrinally to promote both division and survival [52]. By modulating the production of this cytokine, cells could regulate their proliferation. It is observed that its rate of secretion and receptor expression decrease during the response [53, 54] in line with the decrease in proliferation. However, although believed to be essential for *in vitro* proliferation [55, 56, 57] its role *in vivo* is less clear. A recent study has shown that the effect of IL2 *in vivo* depends on the differentiation status of the T cell [58]. During the expansion phase IL2 has a detrimental effect on survival, whilst during the contraction phase it has a beneficial effect. Other studies have shown that IL2 can potentiate greater apoptosis [59]. This enhanced death may, however, result from the pro-proliferative action of the cytokine producing more high generation cells which suffer greater rates of death [49].

Another mechanism could operate by each T cell being regulated by the action of other T cells. This could occur via cytokine signalling. The cytokine IL2 is observed to act paracrinally and is a potential candidate for reasons previously discussed. Alternatively regulation may be achieved via contact interactions between the cells. One candidate is the process known as activation-induced cell death (AICD) in which activated T cells induce death in their neighbours via direct cell-cell interactions. This contact killing of like-cells, known as fractricide, would result in density dependent apoptosis. This mechanism is putatively mediated by the receptor and ligand pair Fas and FasL [60, 61, 62]. These surface molecules are upregulated during the response [63] so the level of cell death would increase during the response. Surface bound TGF-β may mediate a similar mechanism that inhibits division rather than survival [64].

Regulation could also be mediated by external cells such as APCs or stromal cells [65]. Such cells could exert their effect via cytokines or contact interactions. Potential cytokines include IL7 and IL15. They are secreted by many cells in the body, including DCs, and are known to play important roles in the homeostasis of naive and memory T cells [66, 67, 68] and to augment the clonal expansion of activated cells [69, 14]. IL7 and IL15 are known to reduce apoptosis, possibly by the upregulation of the survival gene Bcl-2 [70, 71, 72, 73]. The main effect of IL15 however, is thought to be the promotion of division. Contact interactions would most likely be mediated by APCs. Dendritic cells express a number of surface molecules that can affect T cell proliferation. The B7 family, constitutively expressed by DCs, can induce both pro- and anti-proliferative effects on cycling T cells. Ligation of the T cell receptor CD28 by B7 molecules together with TCR stimulation by specific antigen induces IL2 production [74, 75, 76], while ligation of CTLA-4 inhibits both division and survival [77, 48], possibly by suppressing the production of IL2 [78, 79]. Ligation of the T cell surface molecule OX40 by the DC surface molecule OX40L enhances the survival of T cells [80].

Cytokines are believed to be vital modulators of effector differentiation. They are known to be instrumental in determining the specialisation of CD4 effector cells [43]. The flavour of the cytokine mix along with the level of co-stimulation determines

whether CD4 T cells go down the Th1 or Th2 pathway. Cytokines IL12 and IL18 are known to promote expression of Th1 function while IL4 and IL10 promote Th2. Appropriate cytokines may be released by APCs or the T cells themselves. They may not be however, the main driving force of the process since gene changes associated with effector cells are observed in their absence [81, 82]. The differentiation may be driven by contact interactions with APCs [83] or controlled by an internal program that operates via internal signals.

It is likely that a developmental program would link its regulatory effects to cell division. The cell cycle provides a perfect opportunity to modulate gene expression to create new cellular phenotypes [53, 84]. A tight coupling has been demonstrated between the generation number of a dividing cell and its levels of receptor expression and rates of cytokine secretion, including those known to affect division and apoptosis rates [85, 53, 86, 87, 54]. By altering these rates, the response kinetics can be regulated. Since the cell cycle, once initiated, can proceed independently of external stimuli (such as antigen stimulation) it would be a mechanism for uncoupling the regulatory program from the infection kinetics. Alternatively, the program may link its steps to a time-dependent process such as the duration of cytokine stimulus.

2.1.3 Memory

Not all the effector cells generated in a T cell response succumb to apoptosis. A small fraction survives as a long-lived memory population capable of responding to a second challenge by the same antigen. The response of this memory clone is much greater than the naive precursor clone [31, 88, 89, 90]. Although this enhanced function is believed to be mainly due to the far higher numbers of cells in the memory clone [89], changes in the phenotype of the cell also clearly play a role. Memory cells require lower levels of antigen and co-stimulation to activate than do naive cells [91], and, once activated, initiate proliferation sooner and secrete larger quantities of cytokines more rapidly than naive cells [92, 93]. They also express a range of surface proteins that enable them to enter non-lymphoid tissues and mucosal sites (reviewed in [94]).

Originally it was believed that the persistence of antigen was required for the

formation and maintenance of T cell memory [90]. Several studies showed that to maintain a long-lived memory clone the component cells required continued antigenic stimulation [95, 96]. It was also argued that the long-term storage of antigen complexes on the surface of follicular dendritic cells [97] demonstrated the requirement for antigen persistence [98]. Recent work however, showing that memory T cells can form [99, 100, 12] and survive [101, 40, 102, 103, 104, 105, 93] in the complete absence of antigen after the initial exposure, suggests that antigen is not required.

The precise manner of mémory formation has generated much debate. Until recently a common view was that the memory population represented a cohort that received inadequate stimulation and as a result failed to differentiate into effector cells (this is known as the decreasing potential model; see figure 2.2a) [106].

The general consensus now however, is that a naive cell must pass through the effector phenotype in order to become a memory cell [100, 107, 89]; a model known as linear differentiation and shown in figure 2.2b. This idea is strongly backed by the observations that memory precursors undergo extensive proliferation during the primary response [108, 107, 91] and express effector functions [109, 107, 110]. It has been suggested that a particular subset of naive cells are predestined to become memory cells. This destiny may be programmed during activation, or present in the cell since its creation. Recent studies, however, strongly suggest that memory selection is stochastic [99, 54]. Even if memory is formed by a stochastic linear differentiation the details concerning the timing of memory formation are presently unclear. Are cells gradually 'peeled-off' the effector pool during the response and placed in memory where they remain while the entire effector pool is removed by apoptosis? Or can memory only form at the end of the response (when the fully differentiated effector pool has contracted down to a stable size)? One study showed that it took around 3-4 weeks for effector CD4 T cells to acquire the memory phenotype [101]. It is now believed that differentiation to memory is the natural culmination of the developmental program initiated by antigenic stimulation.

The journey of a cell from naive to memory may be locked into the cell cycle where each division triggers changes that take the cell one more step towards the memory phenotype; several studies have reported that memory formation only begins after a

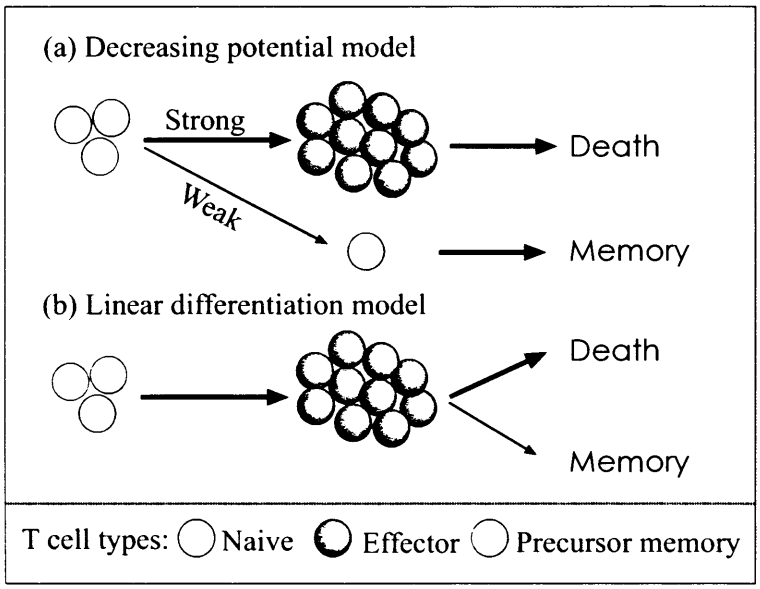


Figure 2.2: The two main T cell differentiation models.

minimum number of divisions [107, 87]. Alternatively the differentiation may follow a smoother path where exposure to cytokines gradually elicits cellular changes. IL15 and IL7 are believed to be instrumental in promoting the formation of memory. It has been demonstrated that over-expression of IL15 generates a larger memory pool [73] while addition of anti-IL15 results in a smaller one [111]. Its effect may stem from its pro-proliferative effect [112] or its maintenance of the survival factor Bcl-2 [72, 73]. IL7 has been found to be essential for the generation of memory [68], possibly through its positive effect on Bcl-2 [70, 71] or specific memory molecules [113]. The differentiation most likely involves a combination of division-induced stepwise changes and smoother cytokine-mediated ones.

The nature of homeostatic control is one of the central mysteries of immunology [114]. How is the memory pool able to exactly balance the division and apoptosis rates of its component cells to achieve long-term stability? A number of antigen-independent mechanisms have been proposed. A popular idea is that competition for pro-proliferative signals controls the pool size. Large pools would tend to contract because each individual clone would receive an inadequate frequency of signals, whereas

smaller pools would tend to expand due to the high level of signals received by each cell. It was originally postulated that contact with self-MHC molecules provided these signals. This was disputed however, by studies showing that memory homeostasis existed in MHC deficient mice [115, 116]. The general view now is that the regulation of homeostasis is mediated by a combination of cytokines, in particular IL7 and IL15 [66, 67, 68] and self-MHC. As previously discussed they affect the proliferation and survival of T cells. They are produced by many cells in the body and may therefore provide an even pool of resource for which the T cells compete. IL2 has been shown to affect memory cells but its action is unclear. In a chronically infected mouse it was observed to increase proliferation [58], whereas in another system it lead to a decrease [66]. Recently Yates and Callard [117] proposed that homeostasis could be mediated by fratricide where contact interactions between T cells produced a density-dependent death rate. Their theoretical investigations revealed the effectiveness of this mechanism and its predictions showed close parallels with experimental observations (for further discussion of this idea see ref. [118]).

Although it is believed that the formation and maintenance of memory does not require the continued presence of antigen, memory cells are exquisitely sensitive to antigenic re-challenge. On re-encounter with antigen a memory clone undergoes a rapid proliferative response. This secondary response is of far greater magnitude than the primary [119, 120, 121, 122]. Contraction then results in a stable memory pool of greater size than the original. Persistence of antigen can have a variety of effects on the memory pool. It can cause the continuous reactivation of the memory cells [114] thus resulting in a larger memory pool. One study showed the steady accumulation of CD8 T cells specific for the persisting mouse cytopathic γ-herpesvirus (MCMV) following the typical expansion and contraction of the acute response [123]. HIV is infamous for the declining CD4 population it produces. Persistent infections can also result in intermittent fevers [124].

The experimental elucidation of the T cell regulatory mechanisms has been impeded by the challenge of accurately tracking through time several distinct cell populations. Therefore the aid of theoreticians has increasingly been sought and mathematical studies of the system have become common.

2.2 Mathematical Approaches

The kinetics of the primary T cell response are determined by two opposing processes: division, which causes a population to expand, and death, which produces contraction. The majority of primary response mathematical models have been based on the following simple ODE:

$$\dot{x} = (\delta - \alpha)\, x, \tag{2.1}$$

where x is the number of proliferating cells and δ and α are the division and apoptosis rates respectively. This equation produces exponential expansion if $\delta > \alpha$ and exponential contraction if $\alpha > \delta$. Since the primary response exhibits expansion early in the response and contraction at late stages it is obvious that the division and death terms cannot both be constant. Various forms to represent these processes have therefore been proposed. The idea that continuous T cell proliferation required constant re-stimulation persuaded many studies to assume that the division rate was proportional to the viral or bacterial concentration in the host (pathogen load) [7]. This led to equations of the form

$$\dot{x} = (\delta\, p - \alpha)\, x, \tag{2.2}$$

where p is the pathogen load. This model is able to produce the characteristic expansion followed by contraction observed in a normal primary response. In the early stages of a response the high level of pathogen will produce a dominating division term leading to expansion, whereas late in the response the falling concentration of pathogen allows the death term to dominate and produce contraction. An interesting prediction of this simple model, as noted by De Boer and Perelson [7], is that expansion only occurs when the pathogen load rises above the threshold value, α/δ. This supports an experimental hypothesis put forward by Brunner *et al* [125] that the number of T cells clustered around an APC must exceed some critical value to initiate proliferation.

De Boer and Perelson believed that equation 2.2 was too simple because it did not provide a maximum proliferation rate for T cells [126]. They viewed proliferation of

T cells in response to pathogen encounter as analogous to an ecological system where T cells (predators) competed for a pathogen (prey). They first extended the equation to the following form

$$\dot{x} = \left(\frac{\delta p}{(1/\eta) + p} - \alpha \right) x,$$

where $1/\eta$ is the pathogen load that produces a half-maximum division rate, to force the proliferation rate to saturate at high pathogen loads. They realised that this was still unrealistic since the rate of proliferation did not saturate for large numbers of T cells. Competition for access to sites on the APCs is known to occur in such circumstances [127]. Therefore taking an idea from another study [128] they derived a new function that saturated at high T cell numbers. It took a similar form to the following

$$\dot{x} = \left(\frac{\delta p}{(1/\eta) + x + p} - \alpha \right) x. \tag{2.3}$$

They investigated the T cell response to two types of pathogen behaviour: growing and non-growing. They assumed that a growing pathogen would increase in size exponentially until its level saturated at very high concentrations. They further assumed that the rate of antigen clearance would be proportional to the frequency of T cell:pathogen interactions. These assumptions were represented by the following equation

$$\dot{p} = \psi p \left(1 - \frac{p}{p_M} \right) - \kappa x p, \tag{2.4}$$

where p_M is the maximum possible pathogen load and ψ its replication rate. Non-growing pathogens could be modelled by setting $\psi = 0$. The model produced responses that exhibited many of the characteristics observed experimentally. The study's key assumption that competition is an essential element of the T cell response was backed by a later study that compared a variety of T cell proliferation functions [8]. They found that functions that included competition produced superior fits to *in vitro* experimental data.

Many of the subsequent theoretical studies of the primary T cell response used models based on the predator-prey scenario. Bocharov [129] constructed a model with a similar basis to predict the T cell response to varying levels of virus (represented

with a similar equation to that used by De Boer and Perelsons [7]). He extended the model by incoporating a variety of further observations. He assumed that the population of activated T cells comprised two subsets: proliferating precursor effectors, and non-proliferating effectors that were able to kill pathogens. Although De Boer and Perelsons models produced fairly realistic dynamics for medium levels of pathogen they were not able to accurately predict the behaviour at high levels. It is observed in experiment that excessive levels of pathogen can actually result in a greatly diminished response. Bocharov incorporated two potential mechanisms for this effect into a general proliferation model. The first represented AICD, where excessive TCR stimulation potentiates high levels of activated T cell death. He included a death rate that was proportional to the square of the pathogen concentrations. The mechanism would therefore have a far greater effect for high doses of pathogen than for low doses. The second mechanism represented the phenomenon of clonal anergy, where excessive stimulation inhibited the cell cycle. This was modelled by setting the division rate inversely proportional to the viral load. This model was tested by comparing its predictions to *in vivo* data. Fairly good fits were obtained for a wide range of pathogen concentrations, but not all the parameters fell into realistic values. Also, with a greater number of parameters than data points it is not surprising that good fits were obtained. Overall the paper was commendable for its deep appreciation of the underlying biology, but in attempting to explain multiple immune observations the inclusion of a large number of assumptions weakened belief in the model's predictions.

A number of studies have investigated the long term response to antigen. A study by Fraser *et al* [3] investigated the response to continuous antigenic stimulation of a T cell pool that was divided into resting and cycling effectors. When stimulated by antigen, resting cells entered the cycling population and then divided once before dropping back to the resting pool. They assumed a density dependent death rate during the time in the cycling compartment. They explicitly modelled the antigen kinetics in a similar manner to De Boer and Perelson [7]. The main result of the study was the potential resolution of a paradox in the field of HIV that high levels of viral replication can occur when only a small fraction of cells are infected. McLean

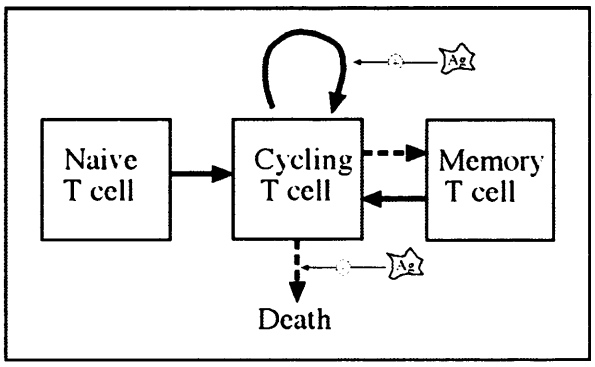


Figure 2.3: Model used by De Boer *et al* 2001[2]

[130] developed a three compartmental model (naive, activated and memory T cells) to investigate long term infection when proliferation was largely driven by the cytokine IL2. The model was able to mimic a range of experimental observations and predicted the persistence of slow growing pathogens. Another group investigated how the avidity distribution of a T cell pool evolved in response to a persistent infection [131]. Avidity describes the strength of attraction between the receptor and ligand of two cells. They found that the domination of the most avid cells during the primary and secondary responses diminished for long-term responses when there was a limit to the number of divisions that a T cell could undergo before dying. This result however, seemed an intuitive consequence of their model structure. They linked the division rates of cell populations to the level of antigen such that more avid clones divided faster and therefore reached their maximum number of divisions sooner. Late in the response the more avid clones consequently died at a much faster rate. One group studied the requirements for the coexistence of a T cell pool and a low level of virus using numerical bifurcation analysis [132]. They found that coexistence was only possible if the pathogen significantly reduced its replication rate after the acute response.

The main criticism of these models is that they fail to incorporate a period of programmed proliferation, which, as discussed previously, is a key aspect of the T cell response. Recently theoretical studies have adopted this idea, De Boer *et al* [2] were among the first to do so. Besides obtaining estimates for the the kinetic parameters

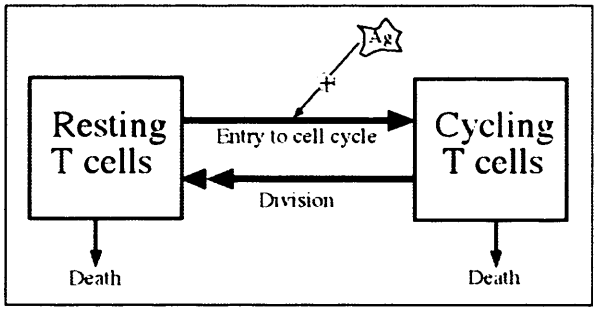


Figure 2.4: the two compartment proliferative model of Fraser et al [3]

of T cell proliferation, they found that small differences between initial pathogen loads could manifest as large differences in the magnitude of expansion. The model however, was perhaps over-simplistic: the T cells expanded at a fixed rate for a fixed number of days and then contraction commenced. The rounded peak characteristic of a normal response was therefore not obtained. Another study compared the predator-prey style models to a programmed response and demonstrated the superiority of the latter in matching experimental observations [15]. De Boer *et al* [1] used an extension of their past model to compare the full responses of CD4 and CD8 T cells kinetics, focussing on the stage of memory formation and found that CD4 and CD8 T cells exhibit fundamentally different dynamics.

Despite the inclusion of programmed proliferation in recent theoretical studies, much still remains to be understood about its nature and effects on the overall response. Does the program modulate both division and apoptosis rates, and are changes coupled to time after activation or number of divisions? Also how does the program affect the outcome of an immune response?

CHAPTER 3
COMPARING POTENTIAL T CELL REGULATORY MECHANISMS

What goes up must come down...
Anonymous

The activation and proliferation of T lymphocytes in response to antigen is a key feature of adaptive immunity. After contact with antigen-presenting cells (APCs), activation through the T cell receptor, costimulation and a delay of around a day, antigen-specific T cells divide extensively in lymphoid tissue, peaking in number after approximately seven days *in vivo* [24, 133, 26, 25]. This population then declines through cell death (apoptosis) to approximately 5-10% of its peak value over the next few days [24, 28]. The typical kinetics are shown in figure 3.1. The insensitivity of these kinetics to variations in the nature of the stimulus is surprising considering the many factors believed to influence proliferation, such as antigen dose, costimulation cytokines [134]. This suggests that an unknown mechanism tightly regulates cell number.

A prevailing view was that the proliferation of T cells is driven by the presence of antigen, while death results from its absence. This has led many previous theoretical investigations of the T cell response to link the rates of division [3, 2, 8, 129, 7] and/or apoptosis [2, 131] to the level of antigen. However, recent experiments have cast doubt on this enticingly simple idea. Several groups have reported that T cells undergo multiple rounds of division after a brief initial stimulus [39, 12, 13, 14] and that the time and kinetics of contraction are antigen-independent [35, 12]. These results suggest that an antigen-independent mechanism may govern regulation and various candidates have been proposed. It has been suggested that the T cells are

regulated by an internal program [12]. This idea is supported by a number of experiments in which T cells are able to undergo a normal proliferative response after the withdrawal of antigen [135, 36, 13]. This program may regulate the response by gradually decreasing the expression of genes promoting proliferation; either directly or by reducing production of pro-proliferative cytokines such as IL2 [136, 52], IL4 [137, 138], or IL15 [72]. Another potential means of regulation by AICD [139] (for further details see Chapter 2), in which activated T cells induce apoptosis in their neighbours via direct cell-cell interactions. External agents, such as dendritic cells, may also be critical for the control of T cell number though contact interactions or secretion of cytokines. The various forms of regulation are discussed in more detail in the Chapter 2.

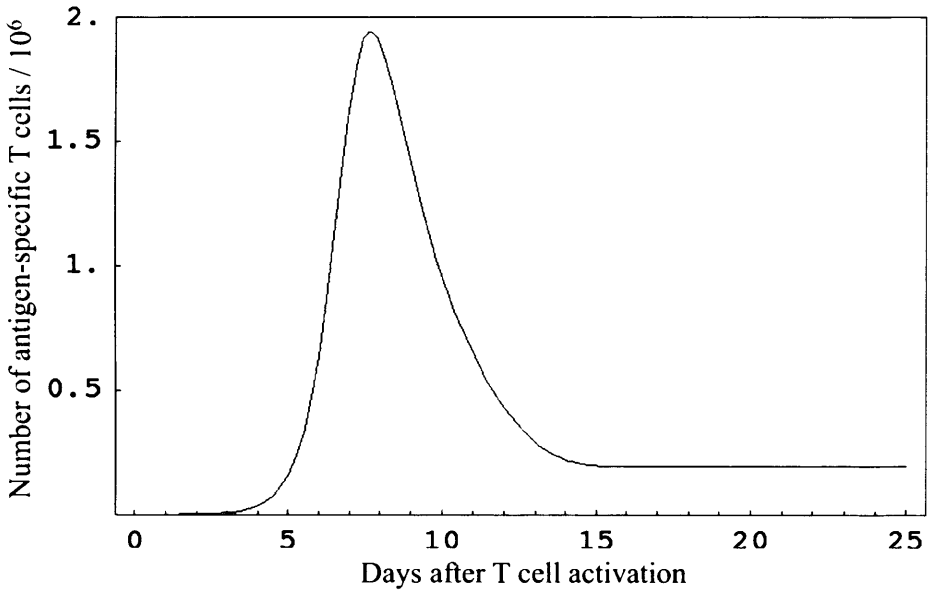


Figure 3.1: The typical kinetics of a T cell population following activation.

Although regulation of the T cell response may require the interplay of a number of different mechanisms it is possible that one mechanism may be capable of regulating by itself. Discovering such a sole regulator would greatly enhance our ability to manipulate T cell responses. This chapter details the theoretical analysis of a variety of potential regulatory mechanisms to assess the ability of each to regulate

in the absence of other mechanisms. For each mechanism a mathematical model is constructed to represent its behaviour. The model characteristics are then compared to *in vitro* experimental data. This type of experimental data is suitable since the mechanisms described here involve only the elements of *in vitro* proliferation assays. From this comparison can be deduced the ability of each mechanism to act as a sole regulator.

3.1 Modelling the Regulation of T cell Proliferation

A general mathematical model representing T cell proliferation is constructed. This is used as the basis for creating a range of specific models, each representing a particular regulatory mechanism. Note that when comparing these models to data, the terms 'model' and 'mechanism' are used interchangeably, although strictly one can only make statements regarding the former.

The general model represents a pool of cells that over time can either divide or die. If the dividing T cell population was phenotypically homogeneous then the pool could be treated as a single entity with a growth and death rate. However, as cells are observed to change their behaviour with division [54, 140], a multi-compartment model is used to keep track of the number of cells in each generation. Thus in the i-th compartment there are x_i cells that have divided i times. The last compartment, $i = I$, contains all the cells that have divided I times or more. Cells continue to proliferate in this compartment but information about division number is no longer required as I is chosen to be sufficiently high that the relevant cell properties are no longer division-dependent. Cells leave a compartment by undergoing division or apoptosis, and cells enter by dividing in the previous compartment. The fluxes of cells between compartments are determined simply by the division rate, δ_i and the apoptosis rate, α_i (Figure 3.2).

One can envisage two distinct, general mechanisms controlling T cell proliferation - one in which proliferation and death rates are functions of time, or one in which they vary strictly as a function of division number. In this chapter situations are investigated in which δ_i and α_i vary with *either* time *or* division, but not both. The

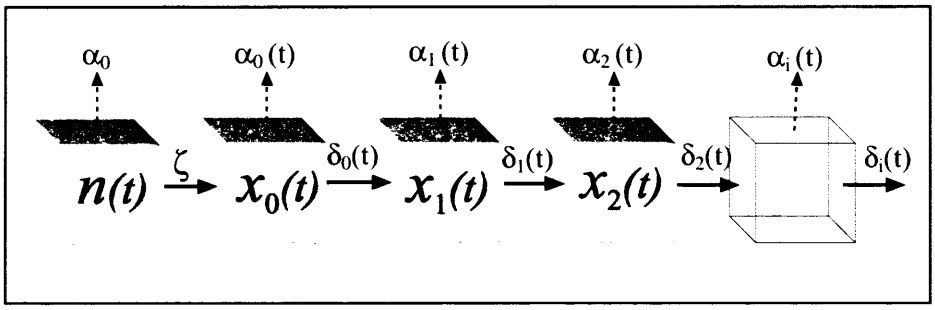


Figure 3.2: Partition model of T cell proliferation. The passage of cells through the compartments (depicted as numbered boxes) is controlled by the division rate, $\delta_i(t)$, and the apoptosis rate, $\alpha_i(t)$.

general model has similarities with that used in a recent paper [15] to investigate the level and nature of antigen-dependence of the T cell primary response. The model presented here, however, allows for the separate control of division and apoptosis rates.

The activation step

A population of N naive T cells is considered to comprise two subpopulations: cells that are responsive to a particular level of antigenic stimulation, $n(t)$; and cells that are unresponsive to this stimulation, $u(t)$. Both *in vivo* and *in vitro* the fraction, γ, of naive cells that respond to an APC stimulus by activating and undergoing proliferation is determined by a variety of factors such as antigen dose [12]. γ is made a parameter in the model, and $n(0) = \gamma N$. Activation induces changes to the cells' structure and initiates a period of growth leading to a division after a lag time in the range τ to a time $\tau + \Delta\tau$. Assuming that the naive cells also undergo apoptosis at a constant rate α_0,

$$\frac{\mathrm{d}n}{\mathrm{d}t} = -(\zeta(t) + \alpha_0)n(t). \tag{3.1}$$

where t is time and $\zeta(t)$ is the rate of entry into the first division compartment and is defined as

$$\zeta(t) = \begin{cases} \zeta_0 & \tau \leq t \leq (\tau + \Delta\tau), \\ 0 & \text{otherwise.} \end{cases} \tag{3.2}$$

The normalisation constant, ζ_0, is calculated by assuming that 99% of activatable cells enter the first compartment between the times τ and $\tau + \Delta\tau$ and integrating Equation (3.1) over this period of entry. This gives

$$\zeta_0 = \frac{\ln(100)}{\Delta\tau} - \alpha_0. \tag{3.3}$$

The unresponsive naive cells will gradually die during the response according to the equation

$$\frac{\mathrm{du}}{\mathrm{dt}} = -\alpha_0 u(t). \tag{3.4}$$

where $u(0) = N(1 - \gamma)$; therefore $u(t) = N(1 - \gamma)e^{-\alpha_0 t}$. Keeping track of the total number of naive cells is essential for comparisons between the model predictions and experimental data since experimental cell counting techniques do not distinguish between those that can be activated and those that cannot.

The cycling step

The rate equations governing the other compartments are as follows:

$$\frac{\mathrm{d}x_i}{\mathrm{d}t} = \begin{cases} 2\zeta(t)n(t) - (\delta_1(t) + \alpha_1(t))\, x_1(t) & i = 1, \\ 2\delta_{i-1}(t)\, x_{i-1}(t) - (\delta_i(t) + \alpha_i(t))\, x_i(t) & i = 2 \ldots I - 1, \\ 2\delta_{I-1}(t)\, x_{I-1}(t) + (\delta_I(t) - \alpha_I(t))\, x_I(t) & i = I. \end{cases} \tag{3.5}$$

The division rate $\delta_i(t)$ and apoptotic rate $\alpha_i(t)$ determine the characteristics of pool expansion and contraction. These functions are defined according to the mechanism being investigated. These mechanisms are grouped into four categories (Figure 3.3):

1. *Auto-regulation*, in which each T cell regulates its own proliferation;

2. *Para-regulation - by cytokine*, in which the T cells regulate each other via cytokine signalling;

3. *Para-regulation - by contact*, in which the T cells regulate each other via contact interactions;

4. *APC regulation - by contact*, in which T cells are regulated by contact interactions with APC surface molecules.

Each of the above can be further classified into mechanisms that affect only survival, only division, or both. In addition, the influence of each can be time- or division-dependent. Furthermore, the two main subsets of T cells, T helper and cytotoxic T cells, commonly referred to as CD4 and CD8 T cells respectively, are investigated.

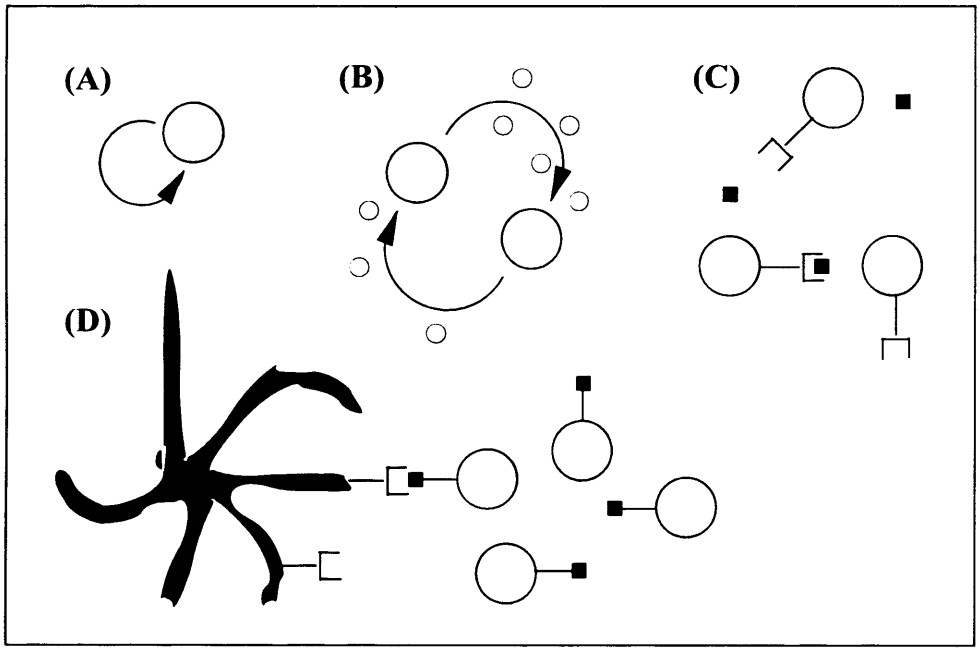


Figure 3.3: Types of T cell regulatory mechanism. (A) Auto-regulation. (B) Para-regulation - By cytokines. (C) Para-regulation - By contact interactions. (D) Regulation by APC - By contact interactions (represented by a dendritic cell).

3.1.1 Auto-regulation

Here it is assumed that T cell proliferation is regulated by an internal program that operates independently of external signals. It is further assumed that this program operates by downregulating division and/or survival genes during the course of a response (both have been observed to decrease in experiment [141, 142]) and that this causes the division rate to fall from its maximum value, δ_M, to zero and/or the apoptosis rate to rise from a low level, α_L, to a high level, α_H. The rates can change slowly or rapidly but the change occurs most rapidly at a particular time or division number. This behaviour is modelled with weighting functions f and r. These functions change between zero and one as a particular time or division number is passed and the maximum rate of change is determined by the slope factor, ω. A weighting function, f, is used to model a fall in a rate over time or division,

$$f(t) = \frac{1}{1 + e^{\omega(t-t_f)}}, \qquad \text{or } f_i = \frac{1}{1 + e^{\omega(i-i_f)}}. \tag{3.6}$$

The values t_f and i_f are respectively the time or division number at which the function is at half maximum: the greater these values, the later in the response the downregulation of division genes occurs. Similarly, a weighting function r is used to model a rise in a rate, where

$$r(t) = \frac{e^{\omega(t-t_r)}}{1 + e^{\omega(t-t_r)}}, \qquad \text{or } r_i = \frac{e^{\omega(i-i_r)}}{1 + e^{\omega(i-i_r)}}. \tag{3.7}$$

The values t_r and i_r denote the time or division number at which the function is at half maximum; the greater these values, the later into the response the downregulation of survival genes occurs. The time-dependent form of these functions for different slope factors is shown in Figure 3.4.

The case in which division rate is decreased with time is represented with $\delta_i(t) = \delta_M f(t)$, and with division as $\delta_i = \delta_M f_i$. If survival genes are decreased with time then apoptotic rates will rise as

$$\alpha_i(t) = \alpha_L + (\alpha_H - \alpha_L) r(t), \tag{3.8}$$

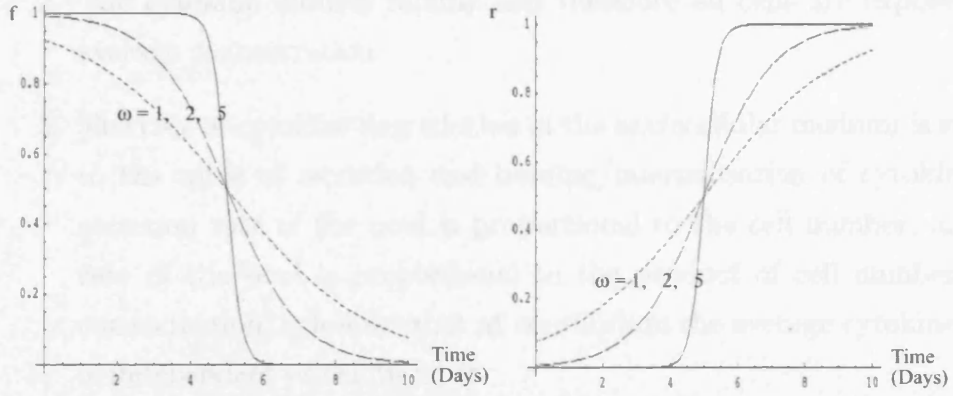


Figure 3.4: Weighting functions f and r for different slope factors.

while if the decrease occurs with division number

$$\alpha_i(t) = \alpha_L + (\alpha_H - \alpha_L) r_i. \tag{3.9}$$

The instances in which survival or division are unaffected are represented by the following:

$$\delta_i(t) = \delta_M, \tag{3.10}$$

$$\alpha_i(t) = \alpha_L. \tag{3.11}$$

3.1.2 Para-regulation – by cytokines

A variety of cytokines are produced by proliferating T cells. Many have been observed to influence proliferation and survival, such as IL2 or IL4 [52, 143]. In addition, rates of cytokine production per cell have been observed to change during the first few rounds of proliferation [54]. Here a model is developed to represent a regulatory mechanism in which a cytokine promoting division and/or survival is produced in decreasing amounts by activated T cells.

The following assumptions are made:

1. All cells produce a cytokine with a rate of production that falls with time or division according to the function f.

2. The cytokine diffuses rapidly and therefore all cells are exposed to the same average concentration.

3. The rate of cytokine degradation in the extracellular medium is small compared to the rates of secretion and binding/internalisation of cytokines. Since the secretion rate of the pool is proportional to the cell number, and the binding rate of the pool is proportional to the product of cell number and cytokine concentration, it follows that at equilibrium the average cytokine concentration is independent of cell number.

4. If a cytokine is required to maintain division then the division rate is proportional to the average cytokine concentration (itself proportional to the average rate of production). Therefore

$$\delta_i(t) = \begin{cases} \delta_M \frac{\sum_{i=1}^{I} f_i x_i(t)}{\sum_{i=1}^{I} x_i(t)} & \text{(decreases with division number).} \\ \\ \delta_M f(t) & \text{(decreases with time).} \end{cases} \tag{3.12}$$

5. If the cytokine is required to maintain survival gene expression then as its concentration falls the apoptotic rate rises according to the equation

$$\alpha_i(t) = \begin{cases} \alpha_L + (\alpha_H - \alpha_L) \frac{\sum_{i=1}^{I} r_i x_i(t)}{\sum_{i=1}^{I} x_i t} & \text{(decreases with division number).} \\ \alpha_L + (\alpha_H - \alpha_L) r(t) & \text{(decreases with time).} \end{cases} \tag{3.13}$$

In the instances when either division or survival are unaffected by the cytokine

$$\delta_i(t) = \delta_M. \tag{3.14}$$

$$\alpha_i(t) = \alpha_L. \tag{3.15}$$

Note that when cytokine production decreases with time, the equations are identical to those of time-dependent auto-regulation and therefore will produce the same results.

3.1.3 Para-regulation – by cell contact

Here a mechanism is modelled in which contact interactions between T cells can regulate pool expansion either by inducing apoptosis or inhibiting division. The *per capita* rates of these processes will increase as clonal expansion increases the cell density.

Triggering a death pathway

It is assumed that a cell is induced to die when its death receptor is bound by another cell's death ligand and that the expression levels of both receptor and ligand rise in unison with time or division number according to the weighting function r. It is further assumed that the probability of apoptosis of a given cell is proportional to both its level of death receptor expression and the total amount of death ligand expression in the T cell pool: therefore the apoptotic rate is given by

$$\alpha_i(t) = \alpha_L + \phi_a r \sum_{i=1}^{I} r x_i(t). \tag{3.16}$$

where ϕ_a is a constant of proportionality. The division rate is unaffected by this mechanism and therefore obeys the equation

$$\delta_i(t) = \delta_M. \tag{3.17}$$

Inhibiting Division

It is also possible that a similar cell-cell contact mechanism exists that leads to inhibition of division; a candidate mediator is membrane-bound TGF-β [64]. It is assumed that the level of inhibition of a cell's division rate is determined by its level of inhibitory receptor expression and the total amount of inhibitory ligand present in the pool. Both increase with r. This gives

$$\delta_i(t) = \frac{\delta_M}{1 + \phi_d r \sum_{i=1}^{I} r x_i(t)}. \tag{3.18}$$

where ϕ_d is a constant of proportionality. The rate of apoptosis is constant,

$$\alpha_i(t) = \alpha_L. \tag{3.19}$$

APC regulation – by contact

Here a mechanism is modelled where simple interactions between APCs and T cells, via surface molecules such as B7 and CTLA-4, regulate proliferation. The particular case is considered where the number of APC surface molecules are non-limiting and the following conditions hold:

1. The interaction decreases T cell division rate and/or increases their apoptosis rate.

2. The magnitude of the decrease induced by each interaction is proportional to the expression level of the relevant surface molecules on the T cell.

3. The expression level of these T cell surface molecules increases during the response according to the function r.

4. APC surface molecules are expressed at a constant level.

Therefore if division is affected, the division rate will fall as the expression of the T cell surface molecules rises

$$\delta_i(t) = \delta_M f. \tag{3.20}$$

and if survival is affected then the apoptotic rate will rise with surface molecule expression

$$\alpha_i(t) = \alpha_L + (\alpha_H - \alpha_L)r. \tag{3.21}$$

When neither survival nor division are affected,

$$\delta_i(t) = \delta_M, \tag{3.22}$$

$$\alpha_i(t) = \alpha_L. \tag{3.23}$$

These equations are identical to those of auto-regulation and will consequently produce the same results.

3.2 Testing the mechanisms

It is desirable to know if a mechanism can produce a good fit to experimental data for biologically reasonable parameter values. To do this, the following approach is used.

In a similar manner to ref.[129], one obtains, from published experimental data, a maximum observed range of values for each parameter. These ranges define a hyper-cuboid in parameter space that is denoted R_0.

For each of the n parameters q_i, $i = 1 \ldots n$, a calculation is made of the standard deviation σ_i of the experimental estimates. Then R_0 is searched for a good fit to experimental data. If no good fit exists a new region is defined $R_1 \supset R_0$ by extending the search range of each parameter q_i by σ_i in each direction (note that this choice of increment is arbitrary), while ensuring $q_i > 0$ for all i. R_1 is then searched. This process is repeated, extending the search region by two standard deviations, until a good fit is found. This procedure ensures that the good-fit parameter set $\{q_i\}$ is found that is closest to the original region R_0. Goodness of fit is assessed by visual inspection, and in each case one decides whether the good fit corresponds to biologically reasonable parameter values. If it does not, it is argued that the mechanism is unlikely to be able to regulate T cell proliferation.

Table 3.1 summarises the parameter definitions and the ranges of parameter values that define R_0. Below is described how these ranges were obtained. Note that for each parameter the values estimated for CD4 T cells are distinct from those of CD8 T cells. Table 3.2 displays the standard deviations of each parameter range.

1. It is assumed that any fraction of naive cells (γ) could potentially be activated [12].

2. The division rate δ is obtained from experimental calculations of the average cell cycle time; for CD4 T cells, see [144, 38, 145, 51]; for CD8 T cells, see

[93, 12, 86, 146, 13].

3. The maximal apoptotic rate α_H is obtained from the percentage of cells that die during the first 24 hrs following *in vitro* activation *without* costimulation. Costimulation via the T cell surface molecule CD28 induces an upregulation of survival factors bcl-X_L and bcl-2 [147, 148, 149, 150], which then drop to low levels after several days into the response [47, 46, 44, 45]. Activation without costimulation produces a normal division rate but high apoptotic rate [151, 146] resulting from the low survival gene expression [44]. Values were obtained for both CD4 [152, 153, 63, 154, 155, 156, 157] and CD8 T cells [39, 151, 158, 155].

4. The baseline apoptotic rate α_L is obtained from the percentage of fully activated cells (those that had received costimulation) that die in 24hrs at an early stage in their proliferation. Ranges are found for CD4 [99, 159, 48, 49, 160, 161, 152, 50] and CD8 T cells [151, 50, 159, 162].

5. After activation, cells enter the first division compartment after a time delay in the range $[\tau, \tau + \Delta\tau]$. An experimental technique known as CFSE staining (for an example see ref. [145]) is able to detect the number of divisions undergone by each cell. Using results for CD4 [163, 145, 38, 54] and CD8 T cells [163, 14, 12, 13, 93] obtained from this technique τ is calcuated, from the time at which first division cells are first observed, and an upper bound on $\tau + \Delta\tau$ from the time after which no new cells enter the first division compartment.

6. The slope factor ω is calculated from the rate of change in a parameter at half-maximum. This is obtained by differentiating the functions f or r with respect to time or division number at this half-maximum. For f, this gives

$$\omega = -4 \left. \frac{\mathrm{d}f}{\mathrm{d}t} \right|_{t=t_f} . \tag{3.24}$$

The range of possible gradients are obtained from experimental observations of the rates at which apoptosis and division rates of T cells, and the receptor and

cytokine levels associated with these processes, change during T cell responses [49, 48, 50, 164, 54, 165, 166, 167, 65, 168, 149].

7. Limits are placed on ϕ_a, the proportionality constant for the rate of contact-dependent apoptosis, by making assumptions about the effect of the mechanism at the peak response (the moment when rate of cell contact events is greatest). A simple equation for T cell kinetics is considered in which compartmental effects are ignored, division occurs at the maximum rate and the death rate is a simplified version of Equation (3.16); this gives

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \delta_M x - \phi_a x^2. \tag{3.25}$$

It is suggested that the lower bound on ϕ_a, $\phi_{a,\min}$, would give a steady state response ($\mathrm{d}x/\mathrm{d}t = 0$) at $x = x_m$, the peak cell number, giving $\phi_{a,\min} = \delta_M/x_m$. An upper bound $\phi_{a,\max}$ is estimated from the situation when 99% of cells die in one division cycle ($t \sim 1/3$ day). This ensures that the biological realistic value falls within these limits. Solving Equation (3.25) and setting $p = 0.01x_m$ gives

$$\phi_{a,\max} = \frac{\delta_M}{x_m} \frac{100\exp(\delta_M/3) - 1}{\exp(\delta_M/3) - 1}. \tag{3.26}$$

Typical values of x_m are chosen for CD4 and CD8 cells from *in vitro* experimental data [169, 170, 165], although clearly the peak number of cells in an *in vitro* response is likely to depend on the initial cell number. Using an estimate of the maximum division rate δ_M ranges for ϕ_a can be calculated for CD4 and CD8 T cells.

8. Limits are placed on ϕ_d, the proportionality constant for inhibition of division through cell contact, in a similar way. An upper bound $\phi_{d,\max}$ is calculated for a situation where the division rate is reduced to 1% of its maximal value at the peak of the response. This gives $\phi_{d,\max} \sim 100/x_m$. Likewise the minimum value $\phi_{d,\min}$, is taken when the division rate is reduced to 99% of the maximum value, which gives $\phi_{d,\min} \sim 1/x_m$. As above, *in vitro* estimates of x_m are used.

Parameter		Experimentally observed or biologically plausible range	
		CD4	**CD8**
γ	Fraction of responsive naive T cells	$0.1 - 1$	$0.1 - 1$
τ	Delay between activation and 1^{st} division	$1 - 3$ days	$1 - 2$ days
$\Delta\tau$	Spread of τ from activation to 1^{st} division	$1 - 2.5$ days	$1 - 3$ days
δ_M	Maximum division rate	$1.2 - 1.7$ day^{-1}	$2.1 - 2.8$ day^{-1}
α_H	High apoptosis rate	$0.1 - 0.8$ day^{-1}	$0.2 - 0.9$ day^{-1}
α_L	Low apoptosis rate	$0 - 0.1$ day^{-1}	$0 - 0.1$ day^{-1}
ϕ_a	Proportionality constant - contact death	$10^{-4} - 10^{-2}$	$10^{-5} - 10^{-3}$
ϕ_d	Proportionality constant - contact inhibition	$10^{-6} - 10^{-4}$	$10^{-7} - 10^{-5}$
ω	Slope factor	$1 - 6$	$1 - 5.5$
t_f (t_r)	Time at which $f(t)$ or $r(t)$ is half-maximum	$1 - 10$ days	$1 - 10$ days
i_f (i_r)	Division no. at which f_i or r_i is half-maximum	$1 - 10$ divisions	$1 - 10$ divisions

Table 3.1: Maximum ranges of parameter values observed in experiment or biologically plausible for the two main subsets of T cell

When searching for a good fit a comparison is made between the model's predictions and experimental *in vitro* data taken directly from published papers. Although these *in vitro* responses are of a smaller magnitude than a typical *in vivo* response they exhibit all the qualities, such as a proliferative delay followed by rapid expansion and then sudden contraction, of a normal primary response. For CD4 T cells the source is ref. [169] (in Figure 5 - black circles). For CD8 T cells, data is taken from four similar assays using the same type of cell - ref. [170] (in Figure 1C - black circles and black squares; Figure 2A - white circles) and ref. [165] (in Figure 3F - circles). A simple line search algorithm is used to find the least-squares fit between model predictions and experimental data of the total number of T cells. The algorithm operates by fixing all but one parameter and then searching the free parameter for the value that minimises the following

$$\chi^2 = \sum_{j=1}^{J} \frac{\left(e_j - \left(u(t_j) + n(t_j) + \sum_{i=1}^{I} x_i(t_j)\right)\right)^2}{\epsilon_j^2}. \tag{3.27}$$

Parameter	Standard deviations of parameter ranges	
	CD4	**CD8**
τ	0.9 days	0.4 days
$\Delta\tau$	0.6 days	0.8 days
δ_M	0.2 day^{-1}	0.4 day^{-1}
α_H	0.2 day^{-1}	0.3 day^{-1}
α_L	0.06 day^{-1}	0.05 day^{-1}
ω	2.1	1.6

Table 3.2: Standard deviations of the parameter ranges obtained from experimental data for the two main subsets of T cell. Note that some parameter ranges are derived from the logarithm of the experimental data and therefore the standard deviation about the mean is not symmetrical. This table displays the larger values in these cases.

The value e_j, with error on the mean ϵ_j, is the experimentally determined number of T cells at time t_j, where j denotes the j-th experimental data out of J points. The free parameter is then fixed at the value that minimises χ^2 and a previously fixed parameter is freed. This process is then repeated until a value for χ^2 is obtained that does not decrease for any parameter change. The parameter values that give this χ^2 value produce the best fit of the model's prediction to experimental data.

3.3 Results

The results are summarised in Table 3.3.

Autoregulation

Apoptosis rate increasing with time or division

This mechanism is found able to fit the experimental data for biologically reasonable parameter values for CD4 T cells when apoptosis is increased with time but not when it increases with division number. CD8 T cells cannot be regulated by either form of this mechanism – for parameters within R_0, the mechanism fails to halt the expansion of the pool, and merely slows it. For CD4 T cells, when the increase occurs with time

(Figure 3.6a), the good fit closest to R_0 has a low maximum division rate of 0.8/day representing slow division (once every 20 hours). Although this rate is low it is not sufficiently low to rule out the mechanism. When the apoptosis rate increases with division number, again no good fit is found within R_0 (Figure 3.5a). The good fit closest to R_0 exhibits very fast division (once every 3-4 hours) and extremely rapid death (95% die in around 14 hours). These values are considered to be biologically unreasonable. Similarly, for CD8 T cells when the apoptosis rate increases with division number no good fit exists within R_0 (Figure 3.5b). No good fit exists even for very wide parameter ranges representing division times from less than one hour to more than a day, and levels of apoptosis from 95% dead in a few hours to less than 5% dead in one week. When the apoptosis rate increases with time no good fit is found within the reasonable parameter ranges (Figure 3.6b), and the good fit closest to R_0 has a very high apoptosis rate of 6.9/day which represents 95% of cells dying in around 10 hrs. The model therefore suggests that for CD8 T cells, since no good fit exists for biologically reasonable parameter values, autoregulation of apoptosis rate alone cannot regulate by itself. For CD4 T cells this mechanism can only regulate if apoptosis increases with time.

Division rate decreasing with time or division

This mechanism is also unable to fit the experimental data for biologically reasonable parameter values. For CD4 T cells when the division rate decreases with division number no good fit exists within R_0 (Figure 3.7a). The good fit closest to R_0 has a division rate of 3.4/day, representing one division every 5 hours. This is much faster than is observed for CD4 T cells. When the division rate decreases with time, again no good fit exists within the reasonable ranges, and the good fit lying closest to R_0 again has an unreasonably high division rate representing one division every 5 hours. For CD8 T cells when the division rate decreases with division number (Figure 3.7b),

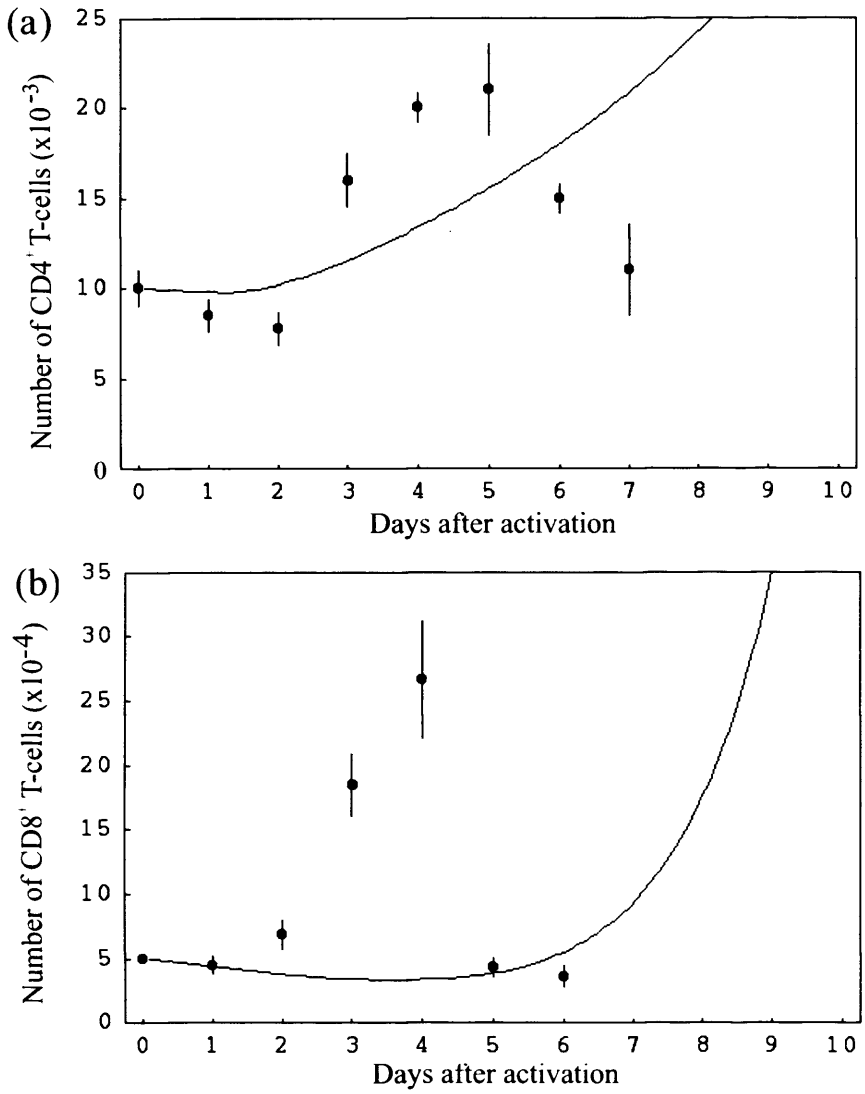


Figure 3.5: Total number of T cells vs. time when auto-regulated by internal program (or regulated by contact with APC) which increases the apoptosis rate with division number. (a) The model's best fit (χ^2 = 118.9) response for CD4 T cells (line) to experimental data (circles) sourced from [169] (within this ref. see figure 5-black circles) has the parameter values: f = 0.1, τ = 1 day, $\Delta\tau$ = 1 day, δ_M = 1.1/day, α_L = 0.01/day, α_H = 0.69/day, i_r = 4 divisions, ω=1.9. (b) The model's best fit (χ^2 = 78.7) response for CD8 T cells (line) to experimental data (circles) sourced from [170] (within this ref. see figure 1C-black circles and black squares; Figure 2A-white circles) and [165] (within this ref. see figure 3F-circles) has the parameter values: f = 0.1, τ = 1.9 days, $\Delta\tau$ = 3 days, δ_M = 2.0/day, α_L = 0.1/day, α_H = 1.3/day, i_r = 1 division, ω=3.4. Plots are of the best fits for biological reasonable parameter values

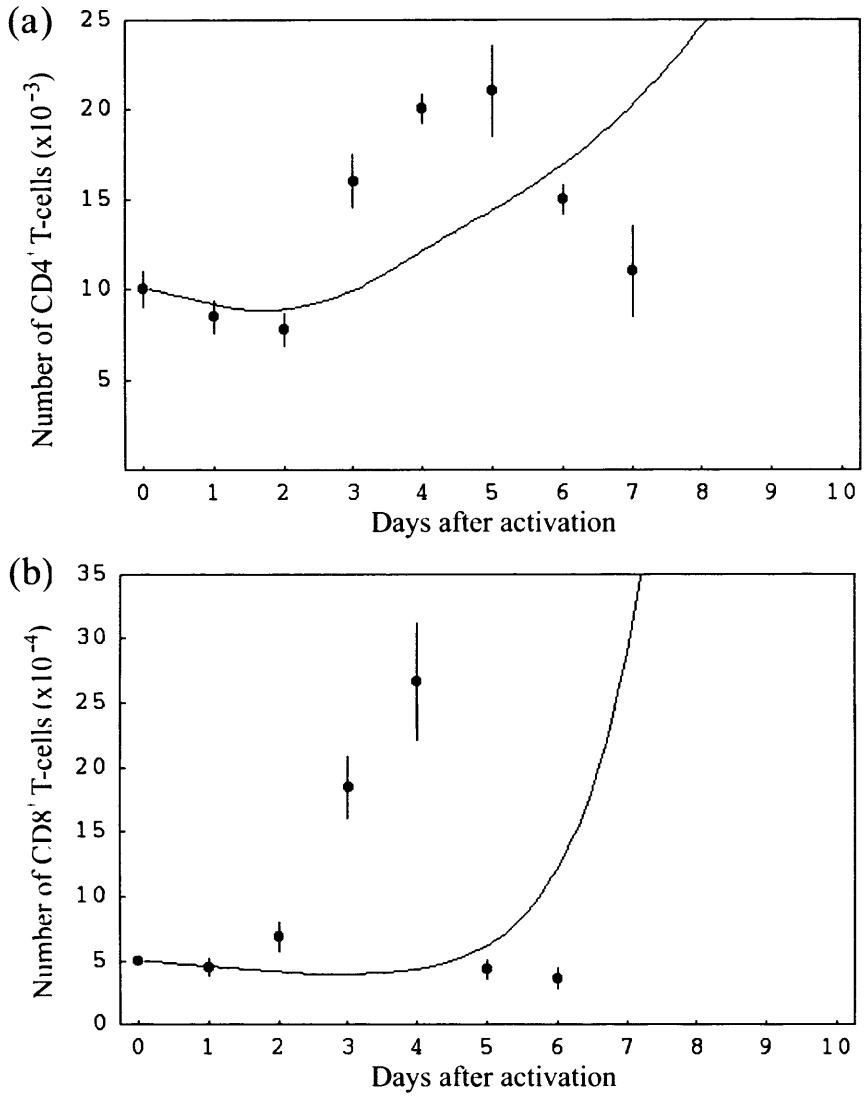


Figure 3.6: Total number of T cells vs. time when auto-regulated by internal program (or regulated by contact with APC) which increases the apoptosis rate with increasing time. (a) The model's best fit (χ^2 = 136.4) response for CD4 T cells (line) to experimental data (circles) sourced from [169] (within this ref. see figure 5-black circles) has the parameter values: f = 0.1, τ = 1 day, $\Delta\tau$ = 3 day, δ_M = 1.1/day, α_L = 0.09/day, α_H = 0.84/day, i_r = 3.7 divisions, ω=4.3 (b) The model's best fit (χ^2 = 94.0) response for CD8 T cells (line) to experimental data (circles) sourced from [170] (within this ref. see figure 1C-black circles and black squares; Figure 2A-white circles) and [165] (within this ref. see figure 3F-circles) has the parameter values: f = 0.1, τ = 1.9 days, $\Delta\tau$ = 4.0 days, δ_M = 1.9/day, α_L = 0.1/day, α_H = 0.9/day, i_r = 1 division, ω=3.4. Plots are of the best fits for biological reasonable parameter values.

or time, no good fit exists within the reasonable ranges. Furthermore, no good fit exists even for very wide parameter ranges representing times of division from less than one hour to more than a day, and levels of apoptosis from 95% dead in a few hours to less than 5% dead in one week. Since no good fits exist for reasonable parameter values autoregulation of division rate alone is discounted as a mechanism that can regulate by itself.

Apoptosis rate increasing, division rate decreasing with time or division

For CD4 T cells the mechanism predicts good fits to experimental data for biologically reasonable parameter values for time- (Figure 3.9a; χ^2=8.5) and division-dependent (Figure 3.8a) versions of the mechanism. For CD8 T cells a time-dependent mechanism produces a reasonable fit within the biological reasonable range (Figure 3.9b) and a good fit within R_0 (χ^2=4.5). For a division number-dependent mechanism, however, no good fit exists for the reasonable range (χ^2 = 18.2; Figure 3.8b) or even within very wide ranges representing times of division from less than one hour to more than a day, and levels of apoptosis from 95% dead in a few hours to less than 5% dead in one week. Therefore the results suggest that an internal program can self-regulate the response of CD4 and CD8 T cells if it affects both survival and division with time. CD4 T cells can also be regulated by a similar mechanism that changes these properties with division number.

Para-Regulation – by cytokine

Cell properties changing with time

A cytokine mechanism where the production of a cytokine decreases with time is represented by a model identical to that of time-dependent auto-regulation (see above) and consequently will produce the same results. Therefore the results suggest that

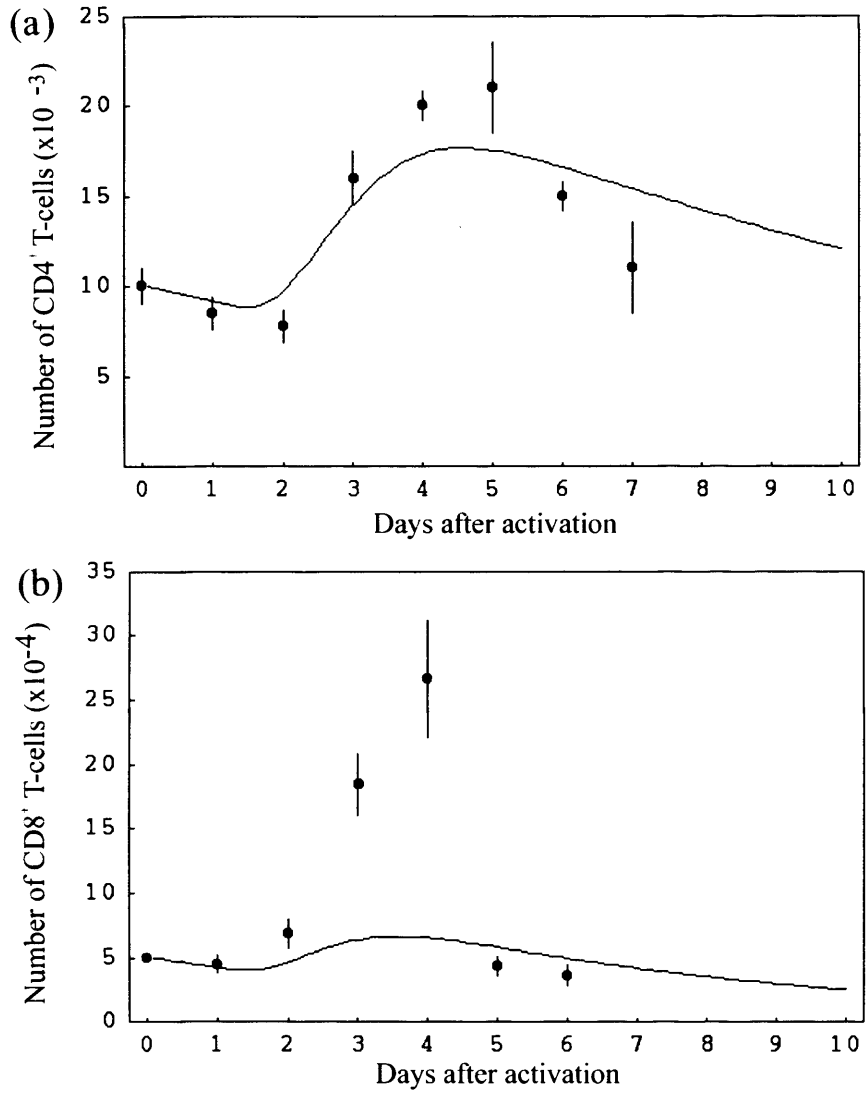


Figure 3.7: Total number of T cells vs. time when auto-regulated by internal program (or regulation by contact with APC) which decreases the division rate with division number. (a) The model's best fit (χ^2 = 26.0) response for CD4 T cells (line) to experimental data (circles) sourced from [169] (within this ref. see figure 5-black circles) has the parameter values: $f = 0.12$, $\tau = 1.3$ day, $\Delta\tau = 1$ day, $\delta_M = 1.9$/day, $\alpha_L = 0.1$/day, $\alpha_H = 0.65$/ day, $i_f = 4$ divisions, ω=5.4. (b) The model's best fit (χ^2 = 52.6) response for CD8 T cells (line) to experimental data (circles) sourced from [170] (in ref. Figure 1C-black circles+black squares; Figure 2A-white circles) and [165] (within this ref. see figure 3F-circles) has the parameter values: $f = 0.12$, $\tau = 1$ day, $\Delta\tau = 1$ day, $\delta_M = 2.9$/day, $\alpha_L = 0.1$/day, $\alpha_H = 1.3$/day, $i_f = 4$ divisions, ω=4.1. Plots are of the best fits within the biologically reasonable parameter ranges.

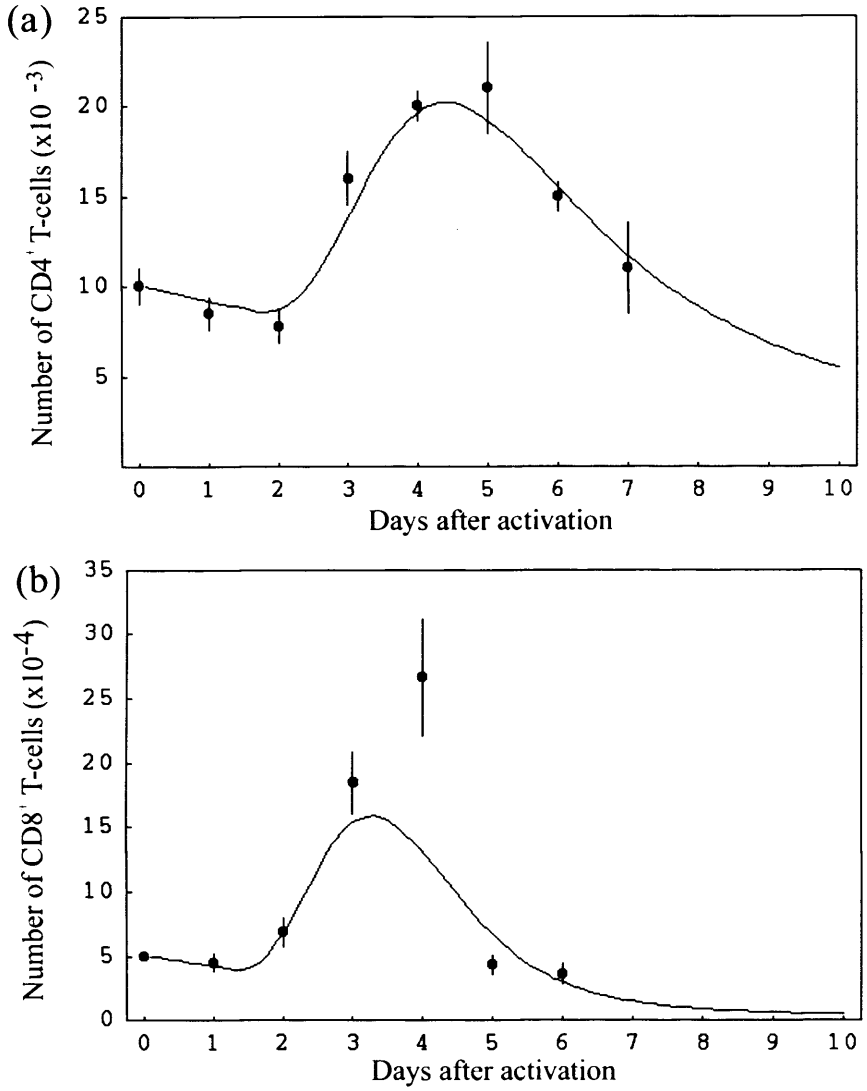


Figure 3.8: Total number of T cells vs. time when auto-regulated by internal program (or regulated by contact with APC) which decreases division rates *and* increases apoptosis rates with division number. (a) The model's best fit ($\chi^2 = 3.7$) response for CD4 T cells (line) to experimental data (circles) sourced from [169] (within this ref. see figure 5-black circles+black squares) has the parameter values: $f = 0.12$, $\tau = 1.6$ days, $\Delta\tau = 1$ days, $\delta_M = 2.6$ day, $\alpha_L = 0.1$/day, $\alpha_H = 0.47$/day, $i_f = 5$ divisions, $i_r = 5$ divisions, ω=3.7. (b) The model's best fit ($\chi^2 = 18.2$) response for CD8 T cells (line) to experimental data (circles) sourced from [170] (within this ref. see figure 1C-black circles+black squares; Figure 2A-white circles) and [165] (within this ref. see figure 3F-circles) has the parameter values: $f = 0.4$,$\tau = 1.1$ days, $\Delta\tau = 1.0$ days, $\delta_M = 2.9$ /day, $\alpha_L = 0.18$/day, $\alpha_H = 1.3$/day, $i_f = 5$ divisions, $i_r = 5$ divisions, ω=4.4. Plots are of the best fits for biological reasonable parameter values.

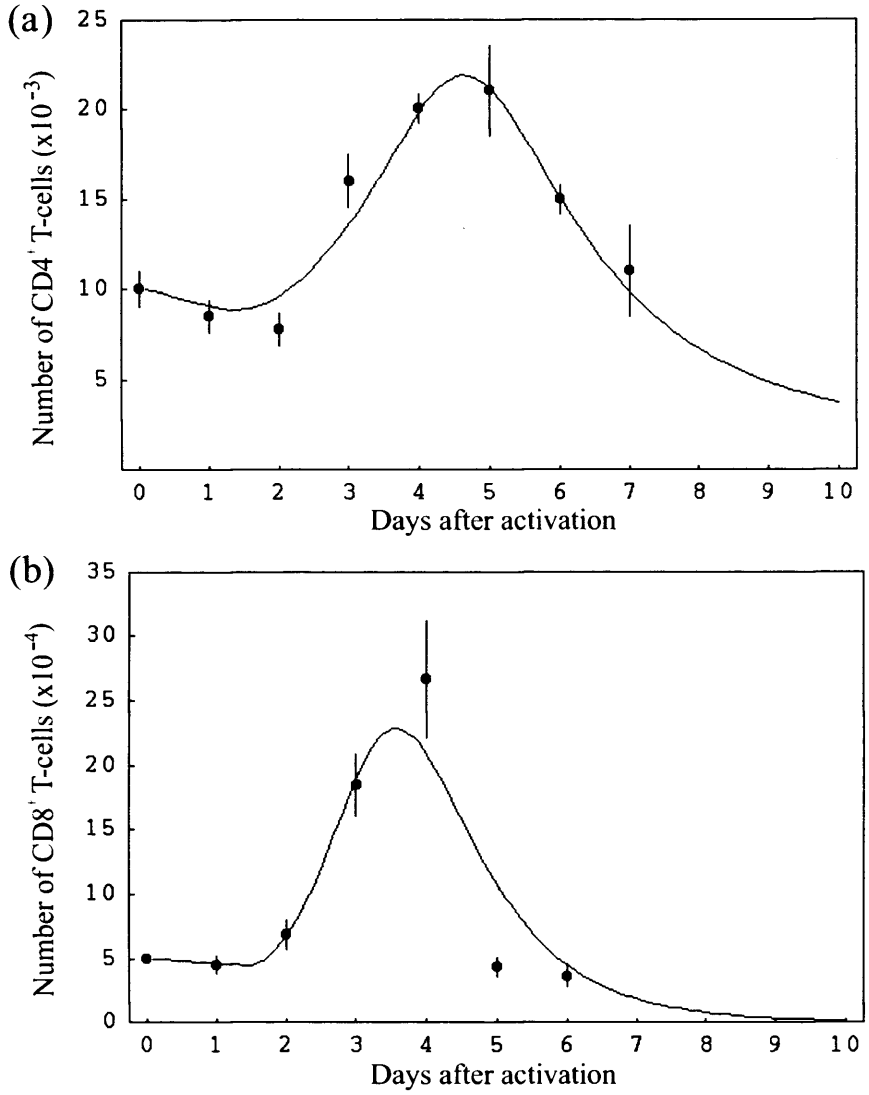


Figure 3.9: Total number of T cells vs. time when auto-regulated by internal program (or regulated by contact with APC) which decreases division rates *and* increases apoptosis rates with time. (a) The model's best fit ($\chi^2 = 8.5$) response for CD4 T cells (line) to experimental data (circles) sourced from [169] (within this ref. see figure 5-black circles+black squares) has the parameter values: $f = 0.28$, $\tau = 1.0$ days, $\Delta\tau = 3.4$ days, $\delta_M = 1.4$ day, $\alpha_L = 0.09$/day, $\alpha_H = 0.6$/day, $i_f = 4.3$ days, $i_r = 2.1$ days, ω=5.3 (b) The model's best fit ($\chi^2 = 21.2$) response for CD8 T cells (line) to experimental data (circles) sourced from [170] (within this ref. see figure 1C-black circles+black squares; Figure 2A-white circles) and [165] (within this ref. see figure 3F-circles) has the parameter values: $f = 1, \tau = 1.3$ days, $\Delta\tau = 3.3$ days, $\delta_M = 1.9$ /day, $\alpha_L = 0.08$/day, $\alpha_H = 0.9$/day, $i_f = 3$ divisions, $i_r = 3.1$ divisions, ω=4.7. Plots are of the best fits for biological reasonable parameter values.

a time-dependent cytokine mechanism that affects both survival and division can regulate both CD4 and CD8 T cell responses. CD4 T cells can also be regulated by a similar mechanism affecting survival alone.

Apoptosis rate increasing with division

It is found that this mechanism is unable to produce a good fit to experimental data for either CD4 T cells (χ^2=127.5; similar to Figure 3.5a) or CD8 T cells (χ^2 = 59.9; similar to Figure 3.5b). For CD4 T cells the good fit closest to R_0 occurs for a high division rate of 2.9, which represents a division time of less than 6 hours and a very high apoptosis rate of 3.1/day which represents 95% of cells dying in 24 hours. These values are not biologically reasonable for CD4 T cells. For CD8 T cells the good fit closest to R_0 occurs for a very high apoptosis rate of 6.9 which represents 95% of the cells dying in about 10 hours. This is a much faster death than is observed in experiment and the results therefore suggest that this is not a feasible mechanism.

Division rate decreasing with division

For this mechanism no good fit exists within R_0 for CD4 (Figure 3.10a; χ^2=71.6) or CD8 T cells (Figure 3.10b; χ^2=58.0). Furthermore, no good fit exists even for very wide ranges representing times of division from less than one hour to more than a day, and levels of apoptosis from 95% dead in a few hours to less than 5% dead in one week. Therefore these results suggest that this mechanism cannot regulate either type of T cell.

Apoptosis rate increasing, division rate decreasing with division

This mechanism produces a good fit within R_0 for CD4 T cells (Figure 3.11a; χ^2=2.5). For CD8 T cells, however, no good fit is found even for very large parameter ranges representing times of division from less than one hour to more than a day, and levels

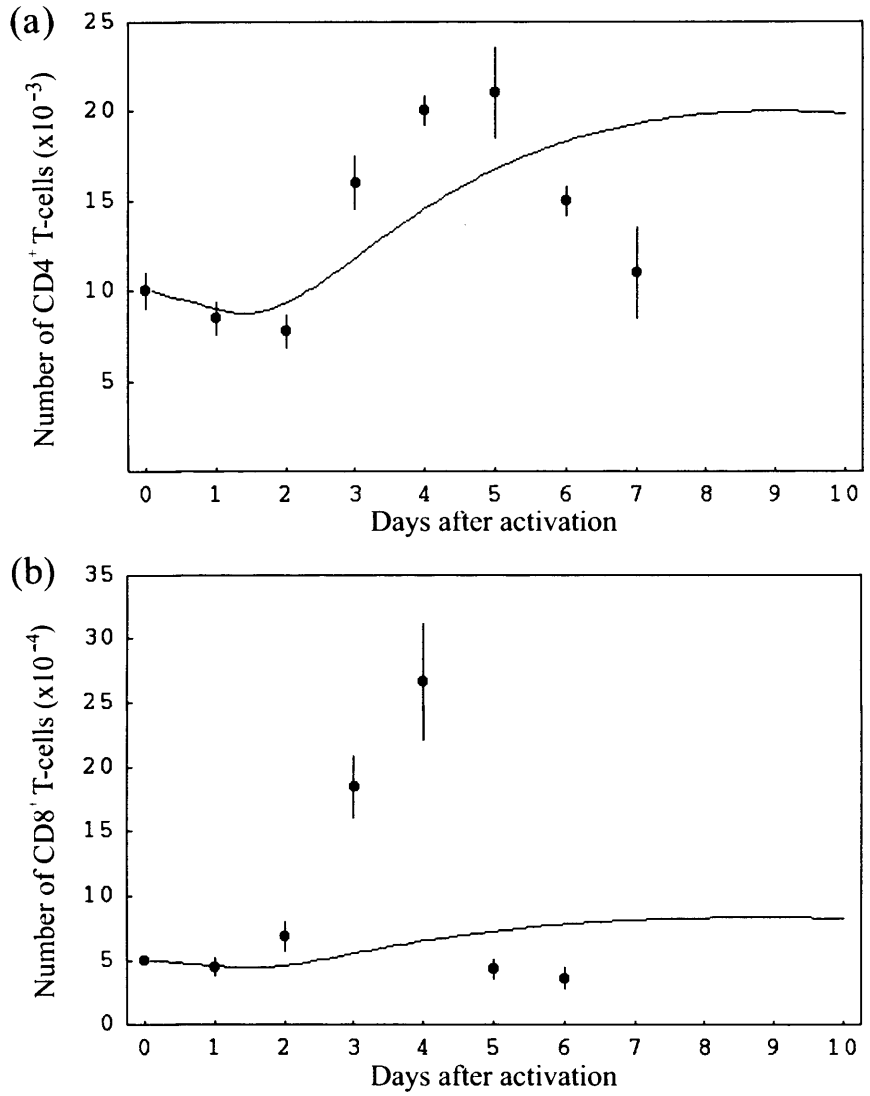


Figure 3.10: Total number of T cells vs. time when regulated by a cytokine that decreases division rates with increasing division number. (a) The model's best fit (χ^2 = 86.8) response for CD4 T cells (line) to experimental data (circles) sourced from [169] (within this ref. see figure 5-black circles+black squares) has the parameter values: f = 0.2, τ = 1 days, $\Delta\tau$ = 2.8 days, δ_M = 1.3 day, α_L = 0.1/day, α_H = 0.8/day, i_f = 4 divisions, ω=6.1 (b) The model's best fit (χ^2 = 67.0) response for CD8 T cells (line) to experimental data (circles) sourced from [170] (within this ref. see figure 1C-black circles+black squares; Figure 2A-white circles) and [165] (within this ref. see figure 3F-circles) has the parameter values: f = 0.1,τ = 1.1 days, $\Delta\tau$ = 2.1 days, δ_M = 1.9 /day, α_L = 0.1/day, α_H = 0.9/day, i_f = 4 divisions, ω=3.9 Plots are of the best fits for biological reasonable parameter values.

of apoptosis from 95% dead in a few hours to less than 5% dead in one week (Figure 3.11b). Therefore the results suggest that a cytokine mechanism can regulate the CD4 and CD8 T cell response provided that it affects both survival and division with time, and the CD4 response can also be regulated if the properties change with division number.

Para-Regulation - by contact interactions

The contact death mechanism produces a good best fit for CD4 T cells when the death receptors and ligands are increased with division number (Figure 3.12a; χ^2=4.7) or time (not shown). For CD8 T cells, division-dependent increases are unable to produce a good fit within R_0 (Figure 3.12b; χ^2 =14.8) or even for a very large ranges representing times of division from less than one hour to more than a day, and levels of apoptosis from 95% dead in a few hours to less than 5% dead in one week. Time-dependent increases, however, do produce a good best fit for reasonable parameter values (Figure 3.12c; χ^2 =3.8). It is concluded that contact-dependent death is a potential mechanism for regulating the responses of CD4 T cells. In addition, if receptor and ligand expression increases with time, this mechanism can also regulate CD8 T cells. If the mechanism also leads to inhibition of division then very similar results are produced (not shown) and the same conclusions are drawn. If the mechanism affects *only* division then the model predicts poor best fit responses for reasonable parameter values. For CD4 T cells the good fit closest to R_0 predicts a division rate of 3.6, or one division every 4.5 hours, and an apoptosis rate of 3.5/day which represents 95% cell death in less than one day. For changes with time the closest fit occurs for a division rate of 2.8 which represents a division in less than 6 hours. These parameter values are not reasonable for CD4 T-cells. For CD8 T cells, for changes with division number or time, no good fits exist over division from less than one hour to more than a day, and levels of apoptosis from 95% dead in a few hours to less than 5% dead in one week. Since the model fails to predict good fits for either type of T cell for biologically reasonable parameter values, it therefore suggests that contact inhibition

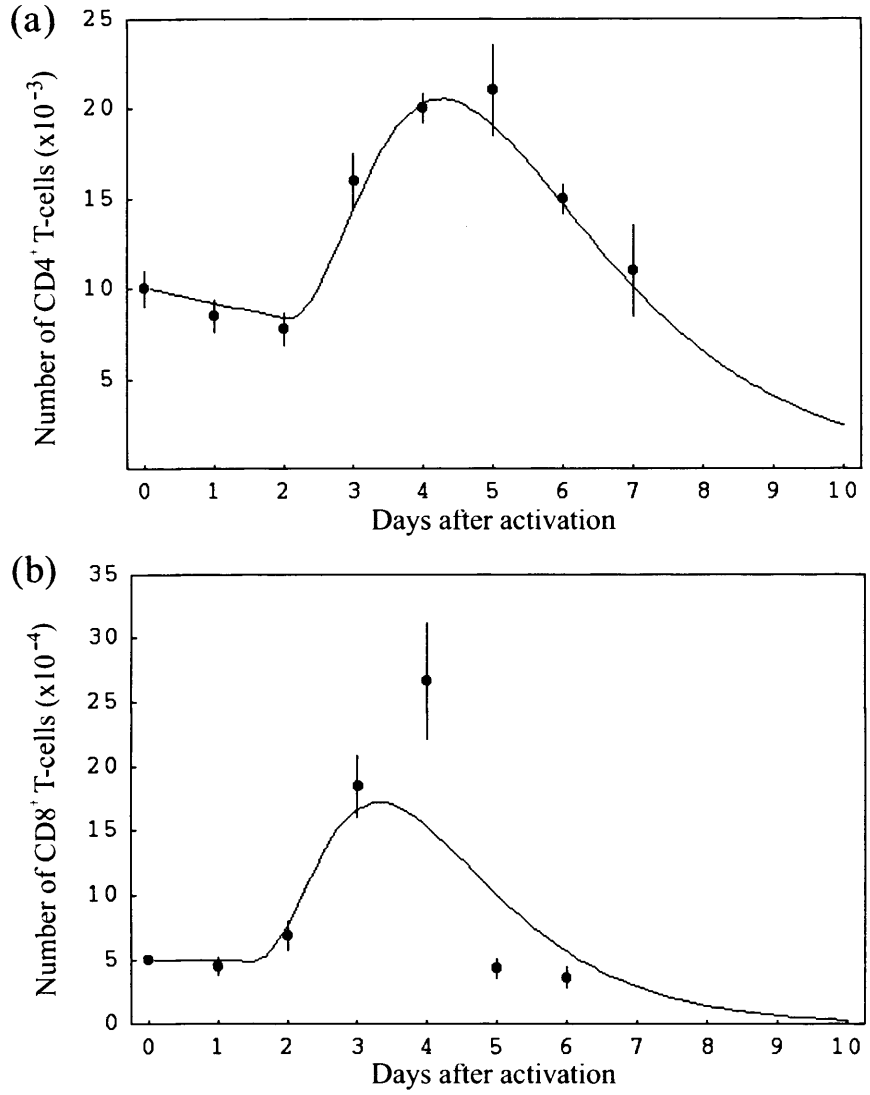


Figure 3.11: Total number of T cells vs. time when regulated by a cytokine that decreases division rates *and* increases apoptosis rates with increasing division number. (a) The model's best fit ($\chi^2 = 3.5$) response for CD4 T cells (line) to experimental data (circles) sourced from [169] (within this ref. see figure 5-black circles+black squares) has the parameter values: $f = 0.7$, $\tau = 1.9$ days, $\Delta\tau = 2.5$ days, $\delta_M = 1.5$ day, $\alpha_L = 0.1$/day, $\alpha_H = 0.7$/day, $i_f = 4$ divisions, $i_r = 3$ divisions, ω=5.3. (b) The model's best fit ($\chi^2 = 33.7$) response for CD8 T cells (line) to experimental data (circles) sourced from [170] (within this ref. see figure 1C-black circles+black squares; Figure 2A-white circles) and [165] (within this ref. see figure 3F-circles) has the parameter values: $f = 1$,$\tau = 1.4$ days, $\Delta\tau = 3.0$ days, $\delta_M = 2.3$ /day, $\alpha_L = 0.02$/day, $\alpha_H = 0.9$/day, $i_f = 4$ divisions, $i_r = 3$ divisions, ω=4.4. Plots are of the best fits for biological reasonable parameter values.

of division alone is not able to regulate T cell responses by itself.

It is concluded that a cell contact mechanism that induces apoptosis, and either inhibits or has no effect on division, is able to regulate the CD4 T cell response. If the increase in receptor/ligand expression is time-dependent then this mechanism can also regulate CD8 T-cells.

APC regulation – by contact interactions

As discussed in the methods, the model for this mechanism produces the same predictions as auto-regulation. For an APC contact-dependent mechanism, for reasonable parameter values, that affects only survival see Figure 3.5, for one that affects only division see Figure 3.7, and for one that affects both see Figure 3.8. Therefore from the auto-regulation results it is predicted that T cell interactions with APC surface molecules that affect both survival *and* division with time is a potential mechanism for regulating the responses of CD4 and CD8 T cells, while CD4 T cells can also be regulated when these properties change with division number. The model also suggests that CD4 T cells can be regulated by a similar mechanism that affects only apoptosis, and exerts its changes with time.

3.4 Discussion

Within the framework of a simple discrete structured population model of T cell proliferation, four regulatory mechanisms which influenced T cell division and survival were investigated to establish which could singlehandedly regulate the T cell response. It was found that mechanisms that modulated both apoptosis and division rates between two levels could regulate both the CD4 and the CD8 T cell responses. CD4 T cells, however, could also be regulated by a time-dependent mechanism that affected only apoptosis rates. It is further predicted that a T cell fratricidal mechanism can regulate both types of cell. Of greatest interest was the observation that rates of

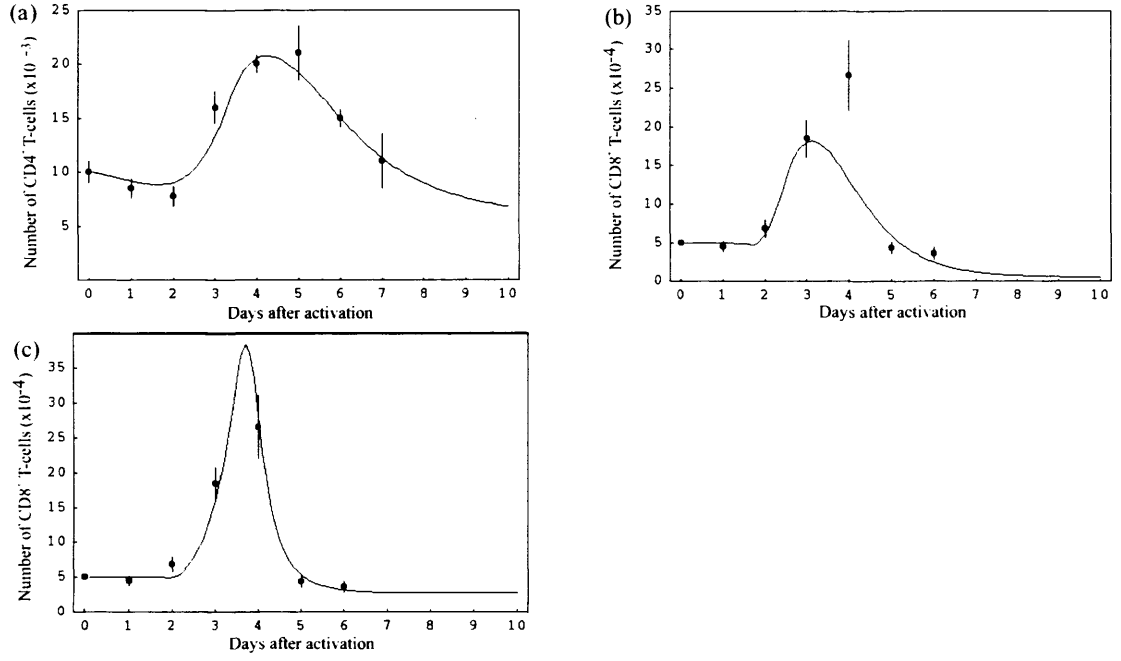


Figure 3.12: Total number of T cells vs. time when T cells pararegulated via contact death signals when receptors and ligands are upregulated during the course of the response. (a) The model's best fit ($\chi^2 = 4.7$) response for CD4 T cells (line), compared to experimental data (circles) sourced from [169] (in ref. Figure 5-black circles) when upregulation of receptors/ligands is division-dependent. The parameter values are: $f = 0.6$, $\tau = 1.5$ days, $\Delta\tau = 1.5$ days, $\delta_M = 0.9$/day, $\alpha_L = 0.09$/day, $i_r = 4$ divisions, $\phi_a = 0.00017$,ω=1.94. (b) The model's best fit ($\chi^2 = 14.8$) response for CD8 T cells (line) to experimental data (circles) when receptors and ligands are upregulated with division. The parameter values are: $f = 0.97$, $\tau = 1.5$, $\Delta\tau = 1$, $\delta_M = 2.1$/day, $\alpha_L = 0.02$/day, $i_r = 5$ days, $\omega = 5.4$, $\phi_a = 0.00059$. (c) The model's best fit ($\chi^2 = 3.8$) response for CD8 T cells (line) to experimental data (circles) when upregulation of receptors/ligands is time-dependent. The parameter values are: $f = 0.80$, $\tau = 1.7$, $\Delta\tau = 1.8$, $\delta_M = 2.0$/day, $\alpha_L = 0.1$/day, $t_r = 3.8$ days,$\omega = 4.1$, $\phi_a = 0.0002$. For (b) and (c) the experimental data is sourced from [170] (within this ref. see figure 1C-black circles+black squares; Figure 2A-white circles) and [165] (within this ref. see figure 3F-circles). Plots are of the best fits for biological reasonable parameter values.

Type of Mechanism		Potential sole-regulator?	
		CD4	**CD8**
Autoregulation			
↓ Survival	with time	**Yes**	No
	with division	No	No
↓ Division	with time	No	No
	with division	No	No
↓ (Survival + Division)	with time	**Yes**	**Yes**
	with division	**Yes**	No
Pararegulation By cytokine			
↓ Survival	with time	**Yes**	No
	with division	No	No
↓ Division	with time	No	No
	with division	No	No
↓ (Survival + Division)	with time	**Yes**	**Yes**
	with division	**Yes**	No
Pararegulation By contact			
↓ Survival	with time	**Yes**	**Yes**
	with division	**Yes**	No
↓ Division	with time	No	No
	with division	No	No
↓ (Survival + Division)	with time	**Yes**	**Yes**
	with division	**Yes**	No
APC Regulation			
↓ Survival	with time	**Yes**	No
	with division	No	No
↓ Division	with time	No	No
	with division	No	No
↓ (Survival + Division)	with time	**Yes**	**Yes**
	with division	**Yes**	No

Table 3.3: The ability of each mechanism to regulate the T cell response by itself as predicted by the model.

death must be progressively increased during a response to regulate the the T cell number.

Auto-regulation involves each T cell following an internal program that modulates its own division and/or apoptosis rates during proliferation. This program could operate entirely internally or by modulating the production of a cytokine acting in a autocrine manner. The on-off model of De Boer *et al* [2] is similar to the time-dependent version of this model.

Pararegulation by cytokine involves each T cell following an internal program that produces declining quantities of a cytokine that promotes survival and division in itself *and* other T cells. The cytokine IL2 is a candidate – it is secreted by activated T cells and is believed to act autocrinally or paracrinally to promote both survival and division [52]. IL2 production declines late in a response [54, 53, 170], as does IL2 receptor expression [171].

APC regulation involves a receptor-mediated decrease of T cell survival and division rates, mediated by APC surface molecules. It is assumed that the ligands expressed by the APCs are non-limiting and the corresponding receptor on T cells is simply upregulated during the response. The B7 - CTLA-4 interaction is a strong candidate. Surface expression of CTLA-4 is gradually increased on proliferating T cells [48, 170] and its ligation by the dendritic cell surface molecules B7-1 or B7-2 (also known as CD80/CD86) inhibits proliferation. Absence of CTLA-4 ligation results in a far greater amount of T cell proliferation [172, 173]. This inhibition induced by ligation of the receptor may affect both survival and division genes [48, 77], possibly by inhibiting IL2 production [174, 78]. It is noted that the type of APC used in the experimental assays determines to what surface molecules the results apply. The CD4 T cells are stimulated with dendritic cells and therefore the results potentially apply to any surface molecule expressed by dendritic cells, of which the B7 family are examples. The CD8 T cells are stimulated by anti-TCR and B7 molecules attached to beads, and therefore the results only apply to these molecules.

An important observation common to the three mechanisms above is that modulation of *either* apoptosis *or* division rates alone with increasing division number is unable to successfully regulate either CD4 or CD8 T cell responses. Inhibiting

survival alone fails to halt the expansion. Inhibiting division alone halts expansion but the subsequent contraction is far slower than observed in experiment. This shows that an internal program coupled to the division cycle, if the regulatory mechanism, must affect both survival genes (such as bcl-X_L and bcl-2) and division genes (such as c-myc and shc); this effect can either be internal or via an autocrine cytokine interaction. Likewise, signalling by APC surface molecules must induce changes in both pathways to successfully regulate. This suggests that receptor-ligand interactions that appear to change only survival, such as OX40 and CD134 [80], or only division, such as TGF-β [64], could not be sole regulators if their effects increase with division number.

In the fourth category of mechanism, contact between T cells triggers death and/or inhibits division. This interaction is mediated by a receptor-ligand pair, both upregulated during a response. The death part of this mechanism is analogous to that of fratricidal AICD in which the ligation of Fas by FasL on another cell triggers apoptosis. Contact-mediated cell death is a potential regulator, with or without the additional effect of division inhibition. In order to fit *in vitro* proliferation assays, the model requires that AICD begins 3-4 days after stimulation. Experiments indicate that T cells become susceptible to AICD 2-4 days after activation [63, 175], and so it is predicted that AICD is a potential regulator. Some authors have argued that AICD is a cell-autonomous (suicidal) process which gives a linear death term. At high cell densities, the form $\phi_a x^2$ for the rate of death among a cell population of size p is likely not to apply as the mixing of a population becomes restricted. In the limit of very large x, a contact-dependent death rate will saturate and become linear in the population size. If indeed AICD is a suicidal process, or cell populations in lymph nodes exceed the level at which the rate of fratricidal AICD saturates, the model essentially takes the same form as for auto-regulation of survival with time or division and the same parameter estimations and conclusions drawn there apply.

If AICD is fratricidal and non-saturating at physiological cell densities, the model predicts that the CD8 T cell response can only be regulated if upregulation of receptors/ligands is time- (rather than division-) dependent. This suggests a decoupling of the $CD8^+$ proliferative program from the apoptotic one, perhaps requiring a threshold

level of accumulated signalling from antigen or cytokines before AICD can be initiated. This is supported by the observation that effective AICD requires the presence of both antigen [176, 177] and IL2 [59].

The work could be extended in a number of ways. Further mechanisms could be investigated, such as the action of suppressor cells. The proliferation could be modelled with greater accuracy by including a stochastic phase in the cell cycle based on the Smith-Martin model [178].

Our results raise a number of questions. Firstly, why is it that irrespective of the details of the mechanism, apoptosis is always required to control the response? This would provide a much more active form of control than merely switching off proliferation, and waiting for natural wastage to bring T-cell numbers down. Given that T-cells can be quite long lived, the latter method could leave large numbers of potentially harmful T-cells circulating much longer than necessary. Secondly, why does contact dependent apoptosis appear to be more effective, to the extent that it can control the $CD8^+$ response on its own, unlike all other signalling mechanisms which also require control of proliferation? One could speculate that this is because juxtacrine apoptosis is the only mechanism consistent with known biological facts that can directly sense T-cell numbers and becomes increasingly effective at higher cell densities. By comparison, control of cell death via cytokines works by reducing survival signals either due to reduced cytokine production (due to an innate program) or to dropping cell numbers. It is thus much less effective at reducing numbers rapidly after the peak of the response. Similar arguments hold for APC-regulation and auto-regulation.

In the next chapter a number of the mechanisms presented here are further assessed by their efficiency. More precisely the amount of T cell death associated with each mechanism is calculated and these levels of death are compared. Based on the idea that aspects of immune system appear to have evolved according to an optimisation strategy, it is proposed that the most plausible regulatory mechanism is the one that minimises the levels of T cell death.

CHAPTER 4
DETERMINING THE LEAST WASTEFUL REGULATORY MECHANISM

Efficiency is intelligent laziness.
David Dunham

Many characteristics of the mammalian immune system are thought to have evolved to produce an optimal response to infection [179, 180, 181]. A number of modelling studies have investigated the T cell response in this context. It has been hypothesised that aspects of the T cell kinetics evolved to minimise the total damage experienced by the host during an infection [182]. This damage is inflicted not only by the pathogens, but also by the responding T cells as they release a cocktail of toxic chemicals to clear the infection [183, 184]. Reducing one form of damage will increase the other and a compromise must therefore be struck to minimise total damage. Two modelling studies appear to demonstrate that the T cell kinetics observed during an acute response are a solution to this optimisation strategy [182]. This conclusion seems incompatible, however, with the programmed nature of the response. Dissimilar infections, pathogens with different replication rates and pathogenicities for instance, produce similar T cell kinetics. The invariant features are discussed in Chapter 3. Given that key features of T cell kinetics appear insensitive to the pathogen properties, the mechanism that regulates the cell number may arise from an alternative optimal strategy.

The regulatory mechanism may have evolved to minimise the energy expenditure of the immune response. Immune responses are known to be metabolically costly (for

a review see [185]) and may divert energy and nutrients away from other physiological processes [186, 187, 188]. For example two days of immune activation in one species of bird expended energy equivalent to that of producing an egg [189].

It is therefore likely that the regulatory mechanism would have evolved to minimise wastage, in particular reducing the number of newly generated T cells that undergo apoptosis before serving their purpose. In this chapter it is proposed that of the plausible regulatory mechanisms from the previous chapter, the most plausible is the one that minimises T cell death during the expansion and contraction of an acute response. Simple models are developed to represent each mechanism, and the total death associated with each is calculated. A comparison of these values reveals that the least wasteful mechanism is one that increases the death rate *and* decreases the division rate. It is further shown that there is a strong selective pressure towards suppressing apoptosis until late in the expansion phase.

4.1 Method

In this chapter calculations are made of the total cell death associated with each of four different mechanisms that may regulate T cell number during a proliferative response. As in Chapter 3, each mechanism regulates by altering cell division and/or apoptosis rates. Four possible mechanisms are considered:

1. Constant division rate. Increasing suceptibility to apoptosis.

2. Constant susceptibility to apoptosis. Decreasing division rate.

3. Increasing suceptibility to apoptosis and decreasing division rate.

4. Constant division rate. Increasing suceptibility to density-dependent death.

A representation is shown in Figure 4.1.

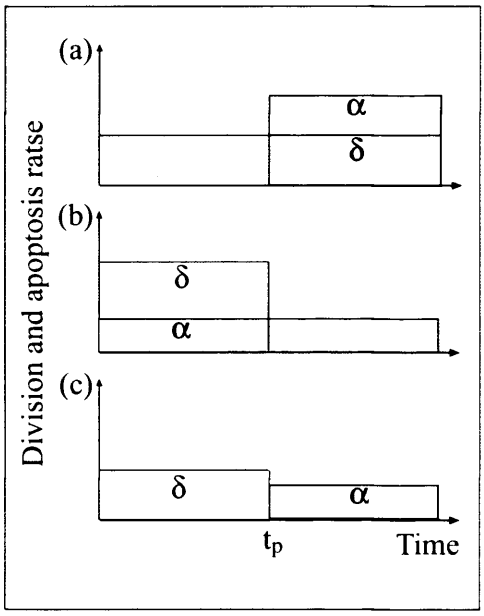


Figure 4.1: The three categories of regulatory mechanism. (a) Constant division rate and increasing suceptibility to density- dependent or independent apoptosis. (b) Constant susceptibility to apoptosis and decreasing division rate. (c) Increasing suceptibility to apoptosis and decreasing division rate.

For simplicity the changes are modelled to occur instantaneously at the peak of the response, time t_p. This assumption has been used previously [2], and is appropriate because the fine detail of the kinetics is not considered, only two macro-properties:

1. At the peak of the response, time t_p, the intial number of T cells, N, must have expanded by a factor R_E, where

$$R_E = x(t_p)/N. \tag{4.1}$$

2. The rapid cell death following the peak terminates at time T and reduces peak

size by a factor R_C, where

$$R_C = x(T)/x(t_p).$$

The quantities R_E and R_C are used because their values are easy to extract from experimental T cell kinetics data.

4.1.1 Delayed death and a constant division rate

Here the total death is calculated for a regulatory mechanism that 'turns on' apoptosis at time t_p but leaves the division rate constant. Given this delay before death, only the death after the peak needs to be calculated when the rate of change in cell number is

$$\dot{x} = (\delta - \alpha)x.$$

This equation is solved by integrating between $x(t_p)$ and $x(t)$, where $t > t_p$, to obtain

$$x(t) = x(t_p)\mathrm{e}^{(\delta-\alpha)(t-t_p)}. \tag{4.2}$$

The total death, D_α, caused by this mechanism will be

$$D_\alpha = \alpha \int_{t_p}^{t} x \, \mathrm{d}t,$$

which, taking $x(t)$ and $x(t_p)$ from Equations 4.2 and 4.1 respectively, solves to give

$$D_\alpha = \frac{N R_E \alpha}{\alpha - \delta}\left(1 - \mathrm{e}^{-(\alpha-\delta)(t-t_p)}\right). \tag{4.3}$$

The next step is to replace α and δ with other quantities such R_E and R_C that are easier to extract from experimental data. It is known that before the peak the cell population will expand at rate δ until time t_p after which the increased apoptosis rate will force contraction. Prior to the peak the T cell number therefore increases according to

$$\dot{x} = \delta x.$$

Integrating this equation between the start of the response, $t = 0$, and the peak, $t = t_p$, and letting $R_E = x(t_p)/x(0)$ produces,

$$\delta = \frac{\ln R_E}{t_p}. \tag{4.4}$$

During the contraction phase the number of T cells decreases from $x(t_p)$ to $x(t_p)\mathrm{e}^{(\delta-\alpha)(T-t_p)}$ by time T, where $T > t_p$. Since R_C is defined as the final number of T cells divided by the peak number,

$$R_C = \mathrm{e}^{(\delta-\alpha)(T-t_p)}.$$

Rearranging and substituting in the expression for δ (equation 4.4), gives

$$\alpha = \frac{\ln R_C}{t_p - T} + \frac{\ln R_E}{t_p}. \tag{4.5}$$

Finally, substituting the expressions for the the division and apoptosis rates (Equations (4.4) and (4.5) respectively) into the equation for the total death (4.3), gives

$$D_\alpha = (1 - R_C)NR_E\left(\frac{(t_p - t)\ln R_E}{t_p \ln R_C} + 1\right).$$

4.1.2 Termination of division and a constant death rate

Here the total death is calculated for a mechanism that decreases the division rate of cells while maintaining a constant susceptibility to apoptosis. The equations governing this system are

$$\dot{x} = \begin{cases} (\delta - \alpha)\, x & t < t_p, \\ -\alpha\, x & t \geq t_p. \end{cases} \tag{4.6}$$

To obtain the number of T cells before the peak, this equation is integrated between $x(0)$ and $x(t)$ where $t < t_p$ to obtain

$$x(t) = N\mathrm{e}^{(\delta-\alpha)\,t}. \tag{4.7}$$

If D_δ^b is the total death before the peak then

$$D_\delta^b = \alpha \int_0^{t_p} x \, dt.$$

Substituting Equation 4.7 into this expression and solving gives

$$D_\delta^b = \frac{\alpha x_0}{\delta - \alpha}(e^{(\delta-\alpha)t_p} - 1). \tag{4.8}$$

The next step is to find expressions for δ and α in terms of R_E and R_C. Integrating Equation (4.6), the kinetics after the peak, from $t = t_p$ to $t = T > t_p$, and rearranging gives

$$\alpha = \frac{\ln R_C}{t_p - T}.$$

From equation (4.7) at $t = t_p$,

$$R_E = e^{(\delta-\alpha)t_p}, \tag{4.9}$$

which, after substitution of the expression for α and rearranging, gives

$$\delta = \frac{\ln R_E}{t_p} + \frac{\ln R_C}{t_p - T}.$$

Substituting these expressions for δ and α into equation (4.8) gives the death that occurs before the peak with this mechanism.

$$D_\delta^b = N \frac{t_p \ln R_C(R_E - 1)}{(T - t_p)\ln R_E}.$$

The number of dead cells after the peak, D_δ^a, is simply the peak number $N R_E$ minus the final number $N R_E R_C$, which gives

$$D_\delta^a = N R_E(1 - R_C).$$

The total death, D_δ, is the death before the peak plus the death after,

$$D_\delta = N \left(\frac{t_p \ln R_C(R_E - 1)}{(T - t_p)\ln R_E} + R_E(1 - R_C) \right).$$

Next, the mechanism that decreases the division rate and increases the death rate is considered.

4.1.3 Delayed death and the termination of division

Here the total death is calculated for a mechanism where before the peak the cells divide but do not die, whereas after they die but do not divide. The resulting kinetics of the response are therefore governed by the equations

$$\dot{x} = \begin{cases} \delta\, x & t < t_p, \\ -\alpha\, x & t \geq t_p. \end{cases} \tag{4.10}$$

With this mechanism there is no death before the peak, and after the number of dead cells is simply the peak number $N\,R_E$ minus the final number $N\,R_E\,R_C$. Therefore the total death, $D_{\alpha\delta}$, is given by

$$D_{\alpha\delta} = N\,R_E\,(1 - R_C).$$

4.1.4 Delayed contact-dependent death and a constant division rate

Here the total death is calculated for a mechanism where the death of T cells is induced by contact with other T cells. It is assumed that this death commences at the peak. If the death occurred from $t = 0$ then the characteristic peak would not be produced, the population size would move directly to a fixed point (as is observed with the standard logistic equation). If the death starts at the peak then only the kinetics after this point need be considered. These will be governed by the equation

$$\dot{x}(t) = \delta\, x - \psi\, x^2 \quad t \geq t_p, \tag{4.11}$$

which solves to give

$$x(t > t_p) = \frac{N\,R_E\,\delta \mathrm{e}^{\delta\, t}}{N\,R_E\,\psi \mathrm{e}^{\delta t} + (\delta - N\,R_E \psi)\mathrm{e}^{\delta t_p}}. \tag{4.12}$$

For this mechanism the total death would be

$$D_{\psi} = \psi \int_{t_p}^{T} x^2 \mathrm{d}t.$$

Substituting in equation (4.12) and solving gives

$$\begin{aligned} D_{\psi} &= N R_E \left(1 - \frac{\delta \, \mathrm{e}^{\delta T}}{\mathrm{e}^{\delta T} N R_E \psi + \mathrm{e}^{\delta t_p}(\delta - N R_E \psi)}\right) \\ &\quad + \frac{\delta}{\psi}\left(\ln\left(N R_E \psi \, \mathrm{e}^{T\delta} + (\delta - N R_E \psi)\mathrm{e}^{\delta t_p}\right) - \ln\left(\mathrm{e}^{\delta t_p}\delta\right)\right). \end{aligned} \tag{4.13}$$

Next expressions are calculated for δ and ψ in terms of R_E and R_C. Since there is no death before the peak we can take the δ from equation (4.4). Then ψ is calculated by assuming that the size of the memory population represents a fixed point of Equation 4.11. At this fixed point $\dot{x} = 0$ and therefore $\psi = \delta / x(T)$. Since it is known that $x(T) = R_C x(t_p)$ and $x(t_p) = N R_E$ we find that

$$\psi = \frac{\delta}{R_C N R_E}.$$

Substituting this expression for ψ, and that for δ (Equation 4.4), into equation (4.13) gives an expression for the total death

$$\begin{aligned} D_{\psi x} &= N R_E \left(1 - R_C + \frac{(R_C - 1) R_C R_E}{(R_C - 1) R_E + {R_E}^{T/t_p}} - R_C \ln\left(\frac{R_E \ln R_E}{t_p}\right)\right. \\ &\quad \left. + R_C \ln \frac{\left((R_C - 1) R_E + {R_E}^{T/t_p}\right) \ln R_E}{R_C t_p}\right). \end{aligned} \tag{4.14}$$

The mechanism where at the peak the cells stop dividing and start dying by contact death would result in an identical amount of death to the similar mechanism where the death is not contact dependent.

4.2 Comparing the mechanisms

Armed with expressions for the death associated with each mechanism, a comparison can now be made for both CD8 and CD4 T cells using experimental data (Figure 4.2). CD8 T cells generally exhibit a very large expansion and rapid contraction. They typically increase in number by around 1000-fold, peak after about 7 days, and then undergo rapid contraction decreasing to about 10% in a few days [27, 35]. For CD8 T cells it is observed that a mechanism that changes both division and death rates can produce up to 200,000 fewer dead cells than mechanisms that vary only one rate (Figure 4.2a). A mechanism in which the death rate is density-dependent produces almost 100,000 extra dead cells. This suggests that a mechanism that changes both rates is substantially less wasteful when regulating the CD8 T cell response. Combined with the results of the previous chapter this suggests that this is the most plausible regulatory mechanism for CD8 T cells.

The CD4 T cell proliferative response is of a smaller magnitude and exhibits slower kinetics. Expansion is about 100-fold, peaking after about 8 days and contracting down to 10% of the peak size after several weeks [35, 142]. It is observed that for this response, increasing apoptosis alone produces far greater levels of death than the other mechanisms (Figure 4.2b), up to 4–5 times higher. The two mechanisms that decrease the division rate produce the lowest amount of death; both produce comparable levels. A contact death mechanism produces over twice the amount of death produced by these division rate decreasing mechanisms. The previous chapter demonstrated that the regulatory mechanism must increase the apoptosis rate. Therefore the most plausible regulatory mechanism for CD4 T cells, using the criterion of minimum wastage, is one that turns on the apoptosis rate *and* and turns off the division rate.

4.3 The effect of early death on the response wastage

It has been shown that modulating both death and division rates during the response is less wasteful than modulating a single rate. From the results of Chapter 3 it appears that the apoptosis rate rises late in the expansion phase. The reason for this late increase is not known but one possibility is that it further reduces cell wastage.

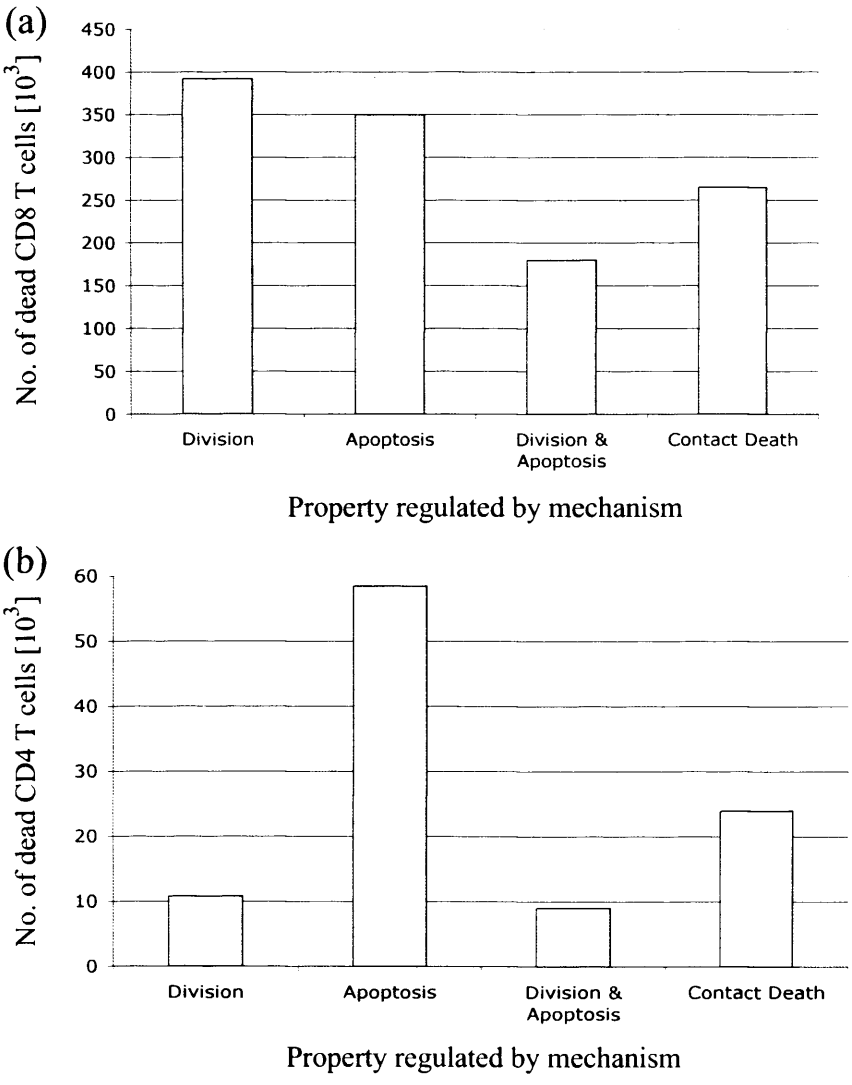


Figure 4.2: The level of cell death associated with each regulatory mechanism for (a) CD8 T cells; parameter values: $R_E = 1000, R_C = 0.1, N = 100, t_p = 7, T = 10$, (b) CD4 T cells; parameter values: $R_E = 100, R_C = 0.1, N = 100, t_p = 8, T = 30$

Here, the impact on total cell death of an earlier rate increase is considered.

A simple model based on the autoregulation mechanism proposed in Chapter 3 is constructed; a cartoon representation is shown in Figure 4.3. It was not determined in that chapter whether the effects of this regulation are linked to division-number or time. However, the majority of recent experimental studies suggest that regulation is largely linked to the numbers of divisions; many properties such as susceptibility to apoptosis, division rate and memory formation are shown to change with each division for both CD4 and CD8 T cells [50, 53, 54, 87]. Therefore, here, and in subsequent chapters, the autoregulatory mechanism is modelled with the T cell kinetic parameters linked to the number of divisions undergone by the cells.

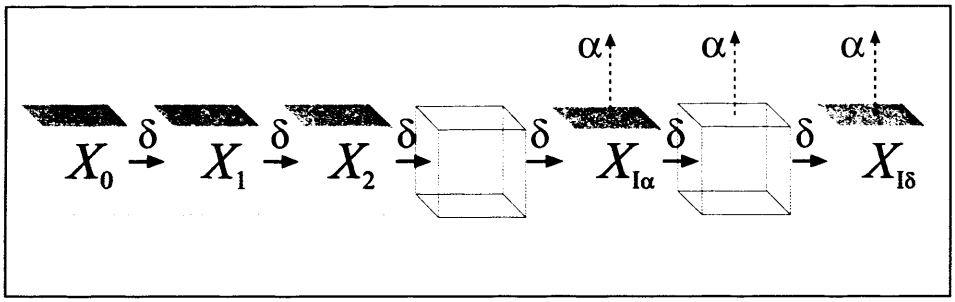


Figure 4.3: A cartoon representation of the model, where X_i are number of effectors cells that enter division i, and δ and α are the division and apoptosis rates respectively.

For simplicity, the division and apoptosis rates are modelled to change abruptly between their two extreme values as the cells reach a particular division number. Cells will divide a maximum number of times, I_δ, at the rate δ. No death will occur until the cells have divided I_α times, whereupon they will die at rate α. It is not necessary to consider the response after the cells have reached the final division because the subsequent wastage of all the model variants will be same (all will have zero division rate and α death rate).

The total amount of death during the expansion phase is calculated as a function of the difference in divisions, ΔI, between the division number of apoptosis rate increase, I_α, and the maximum number of divisions, I_δ. Therefore if X_{I_α} is the number of cells

that enter division I_α

$$X_{I_\alpha} = N\,2^{I_\alpha}. \tag{4.15}$$

These cells will, on average, remain in this division compartment for $1/\delta$ days. During this time they will die at rate α. Therefore if A_{I_α} is the number of dead cells of this division number then

$$A_{I_\alpha} = f\,X_{I_\alpha},$$

where $f = \alpha/\delta$. This relationship will hold for any division number, I, where $I \geq I_\alpha$, so the above equation can be generalised,

$$A_I = f\,X_I. \tag{4.16}$$

The number of cells that enter the next division, $X_{I_\alpha+1}$ will be twice the number of division I_α survivors,

$$X_{I_\alpha+1} = 2(X_{I_\alpha} - f\,X_{I_\alpha}).$$

or, after substituting in Equation 4.15,

$$X_{I_\alpha+1} = N\,2^{I_\alpha+1}\,(1-f).$$

Similarly, for the next division

$$X_{I_\alpha+2} = N\,2^{I_\alpha+2}\,(1-f)^2,$$

and by induction

$$X_{I_\alpha+i} = N\,2^{I_\alpha+i}\,(1-f)^i.$$

If $I = I_\alpha + i$, where $I_\alpha \leq I \leq I_\delta$ then

$$X_I = N\,2^I\,(1-f)^{I-I_\alpha}. \tag{4.17}$$

Substituting this into Equation 4.16 it is seen that

$$A_I = N\,2^I\,(1-f)^{I-I_\alpha} f.$$

Therefore the total amount of death, A_T, between generations I_α and I_δ, is given by

$$A_T = f\,N\,2^{I_\delta - \Delta I} \sum_{i=0}^{\Delta I} (2(1-f))^i.$$

The geometric series can be condensed to give

$$A_T = f\,N\,2^{I_\delta - \Delta I}\,\frac{1-[2(1-f)]^{\Delta I+1}}{2f-1}. \tag{4.18}$$

The aim is to compare the levels of cell wastage of responses that turn on apoptosis after different numbers of divisions. To assess this a death ratio, $\mathcal{D}$, is proposed where

$$\mathcal{D} = \frac{A_T}{X_{I_\delta}},$$

which represents the number of dead cells per cell that reaches the final division. The greater the death ratio the more wasteful the response. Substituting in Equation 4.17, when $I = I_\delta$, and 4.18, this expression simplifies to give

$$\mathcal{D} = \frac{(2-(2-2f)^{\Delta I}-2f)f}{1-2f}.$$

The value $\mathcal{D}$ is then plotted for different values of ΔI (Figure 4.4) to see how turning on apoptosis early affects the response wastage. By taking values δ and α from ref.[1], f is calculated to be approximately 0.2 for both T cell subsets.

It is observed that $\mathcal{D}$ rises rapidly as the death rate is increased at an earlier division. This suggests a strong selective pressure for increasing the apoptosis rate late in the response.

4.4 Discussion

In this chapter it was proposed that of the plausible regulatory mechanisms from the previous chapter, the most plausible would be the one that minimised T cell death during the expansion and contraction of an acute response. Simple models were developed to represent the mechanisms, and the total death associated with each was

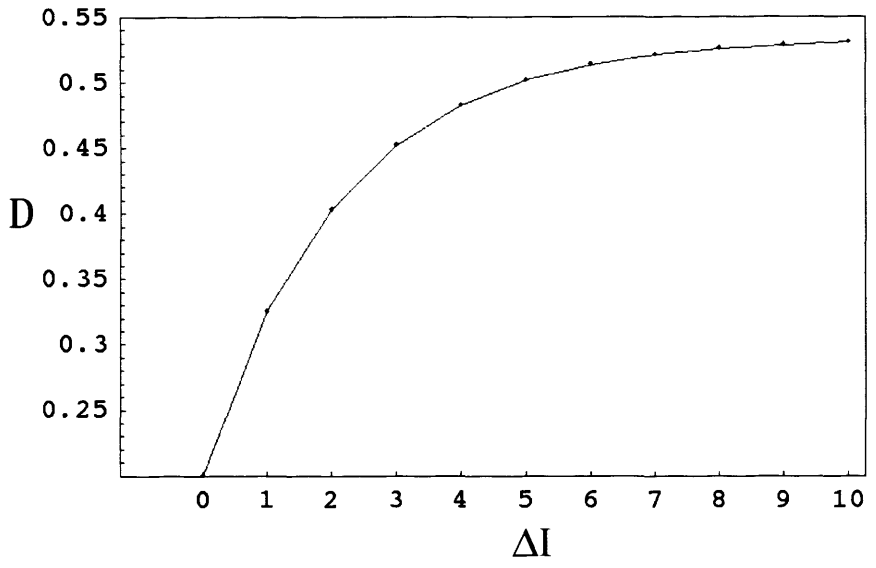


Figure 4.4: The death ratio, D, for an increasing difference, ΔI, between the maximum number of divisions, I_δ, and the division number when the apoptosis rate increases when $f = 0.2$.

calculated. A comparison of these deaths revealed that the least wasteful mechanism for both CD8 and CD4 T cells was one that increased the death rate *and* decreased the division rate. It was further shown that there is a strong selective pressure to delay the increase in the apoptosis rate until late in the period of expansion. Decreasing the division number at which the apoptosis rate rises, greatly increases the fraction of dead cells in the peak population.

For CD8 T cells the difference in total cell death between the least wasteful mechanism and the others was substantial. For CD4 T cells the differences were smaller, particularly for a comparison with a mechanism that only altered the division rate. This suggests that this optimisation strategy proposed in this chapter exerted a greater influence on the evolution of the CD8 T cell regulatory mechanism than it did for CD4 T cell mechanism. This is likely due to the far greater numbers of the former cells produced during a proliferative response relative to the latter. The great potential wastage of CD8 T cells would strongly drive the response to evolve to produce less T cell death during a response. The weaker evolutionary pressure applied by the smaller potential wastage of CD4 T cells suggests that another unknown pressure shaped the regulation for this cell type.

It is often proposed in the literature that a crucial component of any optimal strategy for controlling T cell number is the minimisation of their unintentional damage to the host [182]. This, it is argued, explains the rapid contraction of an effector CD8 T cell population following resolution of an acute response [182]. This view fails however, to recognise another essential process that occurs during the contraction phase: the gradual loss of effector function that accompanies memory formation. If the principle aim of the contraction phase was to minimise self-inflicted damage, switching off the effector function of the expanded population would be sufficient. This suggests that other considerations necessitate the rapid depletion of cell number. One such consideration may be the finite size of the entire memory pool. The entry of a new memory population into the memory pool causes the expulsion of a fraction of the total cell number to maintain its constant size. The entry of a small population produces only a small expulsion and allows the pool to maintain its diversity. The entry of a large population, however, may reduce this diversity by forcing out a sufficient

quantity of old memory to cause the extinction of some smaller memory clones. Thus the host would acquire 'amnesia' to certain previously encountered pathogens.

Another consideration may be the need for the organism to regain its responsiveness to newly arriving pathogens. A massive T cell population remaining in lymphoid tissues following a recent infection may hinder the interaction between a new antigen and its small specific naive population. The rapid contraction of the old effector population would clear the way for the elaboration of a new effector response.

In Chapter 3 the generation of memory cells during the response was ignored. This was acceptable because the number of memory T cells that appear during the timescale considered was much smaller than the number of effector T cells present. Their impact on the total kinetics is therefore negligible. For the same reason, in the present chapter it was again not necessary to accurately model the process of memory cell formation. In the following chapter however, the model presented in Chapter 3 for autoregulation is modified to explicitly incorporate the formation of memory cells. The aim is to establish the relationship between the size of the naive population and the, generally larger, memory population with a view to modulating this programmed amplification in population size.

CHAPTER 5
MODULATING THE NUMBER OF MEMORY CELLS PRODUCED DURING A PRIMARY RESPONSE

Vaunt in their youthful sap, at height decrease,
And wear their brave state out of memory;
Sonnet XV. Shakespeare (1564-1616)

During the contraction phase of the primary response a small sub-population survives the wide-spread apoptosis and can persist in the host for many years [89, 30, 102]. It is known as a memory population and is able to provide a faster and more effective response to re-encounter with its specific antigen [190, 91]. The generation of these memory cells is the final stage of the proliferative program. It acts by increasing the expression of T cell receptors for pro-survival cytokines, thereby rescuing a fraction of the effector population from apoptosis. The continued presence of these cytokines maintains the size of the generated memory population [66, 68, 18]; antigen is not required for this maintenance nor the initial formation [89, 115, 102, 104, 100, 116, 33]. The formation and maintenance of the memory population is discussed in more detail in Chapter 2.

The size of a T cell memory population is a crucial factor in its ability to respond vigorously to re-encounter with its specific antigen [89]. Re-stimulation causes memory cells to undergo another period of programmed proliferation, known as the secondary response [121, 122, 191, 93], which is able to clear specific antigen faster than can the primary. This increased effectiveness is partly due to memory cells dividing after a shorter delay and acquiring effector function more rapidly [191, 93]

than the naive cells. The main reason, however, for the superiority of the secondary response appears to be its ability to produce greater numbers of effector cells, and this is thought to be a direct consequence of having a larger initial population of resting cells [89]. The size of a memory population can be 10-100 times greater than the original naive population [35, 142].

Given its impact on subsequent immune responses, there is a keen interest in developing techniques to alter the size of the generated memory population. Much of vaccination strategy involves an attempt to increase the size of this population [106], thereby boosting the defences of the individual. Conversely, methods for the therapeutic down-modulation of an adverse immune response have also been investigated [192]. The use of cytokines and antibodies to alter T cell responses have been researched extensively. Promoting signalling by IL15 or IL7 has been shown to increase the size of the generated memory population [193, 73], while blocking their action has the reverse effect [194, 111, 73, 195]. However, the complexities of T cell regulation has often confounded efforts to develop a reliable and precise control stategy. For example, IL2 has been associated with two divergent outcomes: a promotory effect when provided at early times and a suppressive effect at late times [58]. Also it is difficult to determine the most efficient way to alter characteristics of the response. Should division, death or differentiation be targeted? A framework is required to enable accurate prediction of the effects of various signals.

By modifying the compartmental model for autoregulation of Chapter 3 to include memory formation, an expression is derived for the number of memory cells produced by the proliferative program per naive cell (the amplification). With this expression the manipulations that most efficiently modulate this amplification are determined.

It is found that increasing the amplification is most efficiently achieved by delaying the termination of division. Delaying the formation of memory cells is the most efficient way of reducing the amplification. Methods of producing a negative amplification (where the final population size is smaller than initial size), which appears to represent a form of tolerisation, are also investigated. It is found that changes to any of the kinetic properties of the T cells, except decreasing the division number at which the apoptosis rate rises, can produce this tolerance.

5.1 Deriving an expression for the final memory population size

In this section an expression is derived for the final size of the memory population when proliferating T cells change their properties with increasing division number. To describe the fold-change in the size of the activated T cell population when it changes from naive to memory phenotype, the term amplification is used.

The Autoregulatory model from the Chapter 3, where both division and apoptosis rates change with increasing division, is selected as most representative of the biology of a T cell proliferative program. Many studies show that the propensity of cells to divide and die changes during a response, and these changes appear to be linked to the number of divisions undergone by the cells [50, 53, 54]. The equal rates of contraction for populations of different sizes [35] argues against the models proposed in Chapter 3 for density-dependent regulation.

It is assumed that the effector cells can undergo not only division and apoptosis but also differentiation into the memory phenotype. As with division and apoptosis rates, the rate of memory formation increases with an increasing number of divisions undergone by a T cell population [87].

It is generally assumed that the memory population formed during a response is proportional to the peak number of effectors. To assess the biological basis for this assumption, experimental data taken from past papers is fitted, by the method of least squares, with the simple model

$$M = \beta X,$$

where M is the final memory population size, X is the peak effector population size and β is a constant of proportionality. Experimental data for both CD4 [196, 197, 51, 198, 199, 1] and CD8 [2, 51, 58, 198, 1, 200, 142, 198, 24, 122, 201, 196, 197] T cell *in vivo* responses are fitted with the model. Good fits are obtained for both CD4 (figure 5.1a; $R = 0.95$) and CD8 (figure 5.1b; $R = 0.93$) T cells and thus the memory-size assumption is considered to be valid. The formation of memory cells,

m, from effectors, x, is therefore modelled as the simple linear process

$$\dot{m} = \mu x,$$

where μ is the per capita rate of memory formation; this approach has been taken by other authors [2, 1].

A population of effector cells, x_i, that has undergone division i is considered. The *per capita* rates at which this population will divide, die and differentiate into memory are δ_i, α_i and μ_i respectively as shown in figure (5.2). Consequently

$$\dot{x}_i = -(\delta_i + \alpha_i + \mu_i)\, x_i,$$

which solves to give

$$x_i(t) = X_i\, \mathrm{e}^{-(\delta_i+\alpha_i+\mu_i)t}, \tag{5.1}$$

where X_i is the initial number of cells in the population x_i. The number of division i memory cells, m_i, increases as the division i effector cells differentiate into memory cells.

$$\dot{m}_i(t) = \mu_i\, x_i(t).$$

The number of division i memory cells at time t is given by substituting equation (5.1) into this equation and solving,

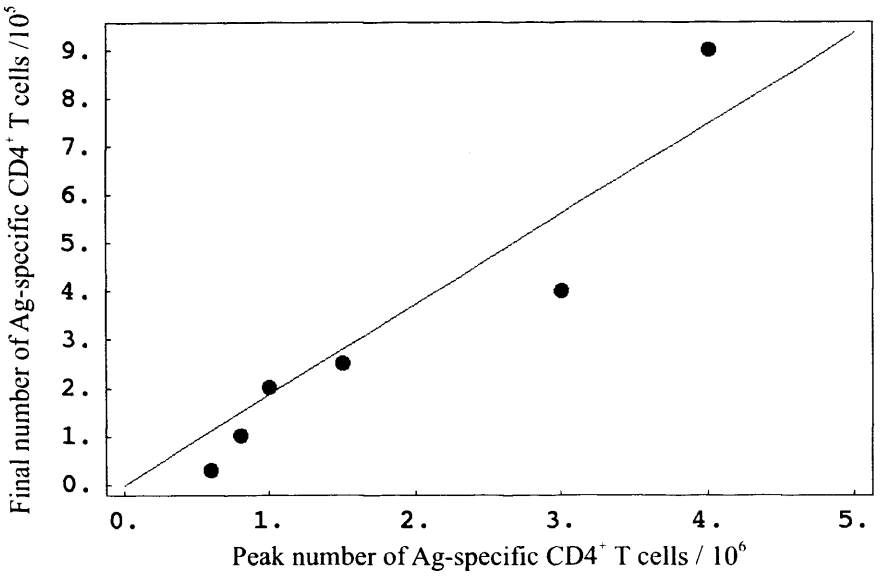
$$m_i(t) = X_i \frac{\mu_i}{\delta_i + \alpha_i + \mu_i} (1 - \mathrm{e}^{-(\delta_i+\alpha_i+\mu_i)t}).$$

Eventually, when $t >> 1/(\delta_i + \alpha_i + \mu_i)$ the number of the original X_i cells left will asymptote to zero, and the final number of division i memory cells, m_i^f, will be given by

$$m_i^f = X_i \frac{\mu_i}{\delta_i + \alpha_i + \mu_i}. \tag{5.2}$$

Similarly, of the X_i cells that enter division i, the number that divide is

$$X_i \frac{\delta_i}{\delta_i + \alpha_i + \mu_i},$$



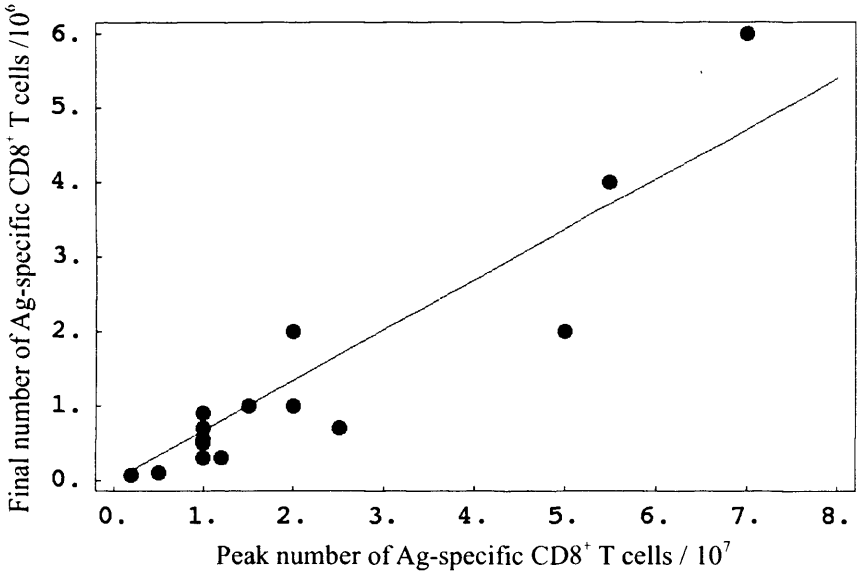


Figure 5.1: The final number of T cells for different peak numbers during a primary response. The line is the best fit of the model $M = \beta E$ to published experimental data. (a) CD4 T cells. Parameter values of best fit: $\beta = 0.066$, $R^2 = 0.91$ for 4 degrees of freedom and therefore $P = 0.0043$. (b) CD8 T cells. Parameter values of best fit: $\beta = 0.19$, $R^2 = 0.86$ for 14 degrees of freedom and therefore $P = 0.00021$.

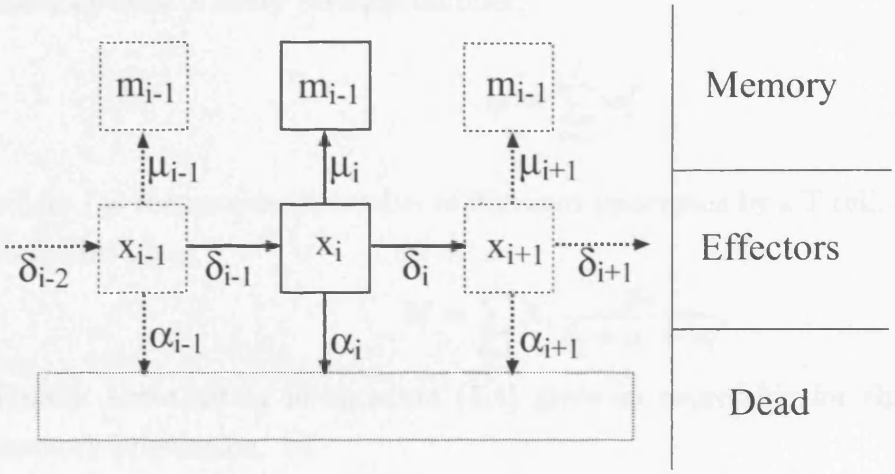


Figure 5.2: A diagrammatic representation of the model. At each division a cell population can divide again, die or differentiate into memory cells; the rate of each process is linked to the number of divisions undergone by the population

and owing to the division process the number entering the next division will be twice this value,

$$X_{i+1} = 2\,X_i\,\frac{\delta_i}{\delta_i + \alpha_i + \mu_i}. \tag{5.3}$$

Likewise, the number of cells that enter the subsequent division, X_{i+2}, will be

$$X_{i+2} = 2X_{i+1}\,\frac{\delta_{i+1}}{(\delta_{i+1} + \alpha_{i+1} + \mu_{i+1})},$$

and on substitution of equation (5.3),

$$X_{i+2} = 2^2 X_i\,\frac{\delta_{i+1}}{\delta_{i+1} + \alpha_{i+1} + \mu_{i+1}}\,\frac{\delta_i}{\delta_i + \alpha_i + \mu_i}.$$

By induction the number of cells that enter division i is

$$X_i = N\prod_{j=1}^{i}\frac{2\,\delta_{j-1}}{\delta_{j-1} + \alpha_{j-1} + \mu_{j-1}}. \tag{5.4}$$

The final memory population formed from a proliferative response will comprise the

memory cells of every division number,

$$M = \sum_{i=1}^{I} m_i^f.$$

where I is the maximum number of divisions undergone by a T cell, and from equation (5.2) this gives

$$M = \sum_{i=1}^{I} X_i \frac{\mu_i}{\delta_i + \alpha_i + \mu_i}.$$

Finally, substituting in equation (5.4) gives an expression for the final size of the memory population,

$$M = N \sum_{i=1}^{I} \left(\frac{\mu_i}{\delta_i + \alpha_i + \mu_i} \prod_{j=1}^{i} \frac{2\,\delta_{j-1}}{\delta_{j-1} + \alpha_{j-1} + \mu_{j-1}} \right). \tag{5.5}$$

By symmetry it can be seen that if A is the total number of cells that have undergone apoptosis during a response, then

$$A = N \sum_{i=1}^{I} \left(\frac{\alpha_i}{\delta_i + \alpha_i + \mu_i} \prod_{j=1}^{i} \frac{2\,\delta_{j-1}}{\delta_{j-1} + \alpha_{j-1} + \mu_{j-1}} \right).$$

Defining θ as the amplification factor of a response, and letting

$$\theta = \sum_{i=1}^{I} \left(\frac{\mu_i}{\delta_i + \alpha_i + \mu_i} \prod_{j=1}^{i} \frac{2\,\delta_{j-1}}{\delta_{j-1} + \alpha_{j-1} + \mu_{j-1}} \right).$$

equation (5.7) can be expressed as

$$M = \theta N. \tag{5.6}$$

It is believed that memory forms near the end of the expansion phase [202, 87, 203, 2] and a special case is therefore considered with the following assumptions:

1. Naive T cells divide I_δ times at a constant rate of δ.

2. Memory formation commences at a constant rate of μ when the cells have

divided I_δ times.

3. Responding cells begin to die at a constant rate of α at division I_α.

Since $\mu = 0$ for $i < I_\delta$, and $\delta = 0$ for $i > I_\delta$, equation (5.5) simplifies to

$$\theta = \left(\frac{\mu}{\alpha + \mu} \prod_{j=1}^{I_\delta} \frac{2\,\delta_{j-1}}{\delta_{j-1} + \alpha_{j-1}} \right).$$

which further simplifies to

$$\theta = \frac{\mu}{\alpha + \mu} 2^{I_\delta} \left(\frac{\delta}{\delta + \alpha} \right)^{I_\delta - I_\alpha}. \tag{5.7}$$

If apoptosis begins at the termination of division (i.e. $I_\alpha = I_\delta = I$) then the expression further simplifies to

$$\theta = \frac{\mu}{\alpha + \mu} 2^I.$$

It is observed that for fixed program parameters the size of the memory population is proportional to the number of activated naive T cells. The effect of the other parameters on the memory size is addressed in the next section.

5.2 Modulating the amplification of the response

Here the most efficient way of modulating the response amplification is determined. Firstly, a local sensitivity analysis is carried out to determine the sensitivity of the amplification to small changes in the parameter values. Then the effect of larger changes are investigated.

The analysis is restricted to the response to the well studied lymphocytic choriomeningitis virus (LCMV) that produces a rapid and extensive T cell response; values for many of the T cell kinetic parameter values have been obtained by fitting models to experimental data [1].

The effects on both the dominant CD4 and CD8 T cell responses are considered. Parameter values for the non-modulated responses are shown in table 5.1 along with

Parameters		CD4 T cells	CD8 T cells	References
δ	Maximum division rate	1.4 day^{-1}	1.9 day^{-1}	[1]
α	Apoptosis rate	0.2 day^{-1}	0.4 day^{-1}	[1]
μ	Memory T cell formation rate	0.002 day^{-1}	0.02 day^{-1}	[2]
ω	Slope factor	5	5	Chapter 3
	Division no. of most rapid:			
I_δ	decrease in division rate	10 divisions	15 divisions	[1]
I_α	increase in apoptosis rate	10 divisions	15 divisions	See text
I_μ	memory formation rate	10 divisions	15 Divisions	[87]

Table 5.1: Model parameters and their values for the dominant response to LCMV infection.

their source. The number of divisions undergone by the cells is found by dividing the duration of clonal expansion by the average doubling time ($1/\delta$). The results of Chapter 3 and 4 showed that the rate of apoptosis increased late in the response, and, similarly, experimental evidence suggests the same for the memory formation rate [87]. Therefore I_α and I_μ are first assumed to change at the same division number as the division rate. However, the effect of earlier changes are also considered. The division and apoptosis rate parameters are assumed to undergo the sigmoid changes described for the autoregulatory mechanism proposed in Chapter 3. The memory formation rate is assumed to rise in the same way as the apoptosis rate,

$$\mu_i = \mu \frac{e^{\omega(i-i_\mu)}}{1+e^{\omega(i-i_\mu)}},$$

where i_μ is the division number when the memory formation rate is half maximum, where the maximum is μ.

5.2.1 Local sensitivity analysis

Here the sensitivity of the response amplification to small changes in the kinetic parameters is determined. To achieve this, the sensitivity function, S, is often used

[204], where

$$S = \frac{V}{\theta}\frac{\partial \theta}{\partial V}.$$

The variable θ represents the system output function, here the amplification, and V denotes one of the system parameters. The function S represents the fractional change in output divided by the fractional change in input. The larger its value the more sensitive the output is to the parameter. Using the values in Table 5.1, S is calculated for each parameter and the results for CD4 and CD8 T cells are shown in figures 5.3a and 5.3b respectively.

It is observed that the response amplification is most sensitive to changes in I_δ and I_α. This is true for both CD4 and CD8 T cell subsets. This is surprising since one might assume that the most effective method of increasing the memory population would involve producing memory cells earlier or faster.

To assess the robustness of this conclusion the sensitivity analysis is performed for reasonable variations in the parameter values. Firstly, the robustness to changes in the rate parameters is considered. For the division, death, and memory formation rates, values are randomly selected within their 95% confidence interval (taken from ref.[1] and shown in table 5.2) and S is calculated for each parameter. This is repeated ten times and variation in the main result is assessed. It is found that the amplitude is always most sensitive to the same two parameters. Secondly, the robustness of the results to changes in I_α and I_μ is assessed. Both I_α and I_μ are known to increase towards the end of the expansion phase but it is possible that the most rapid change takes place a few divisions before I_δ. Sensitivity analyses are therefore performed for when I_α or I_μ are up to 5 divisions lower than I_δ. Results for CD4 and CD8 T cells are shown in figures 5.4 and 5.5 respectively. It is observed that the amplitude is always highly sensitive to changes in I_δ and is most sensitive when the apoptosis and/or memory formation rates rise earlier. The amplitude is almost always least sensitive to changes in δ.

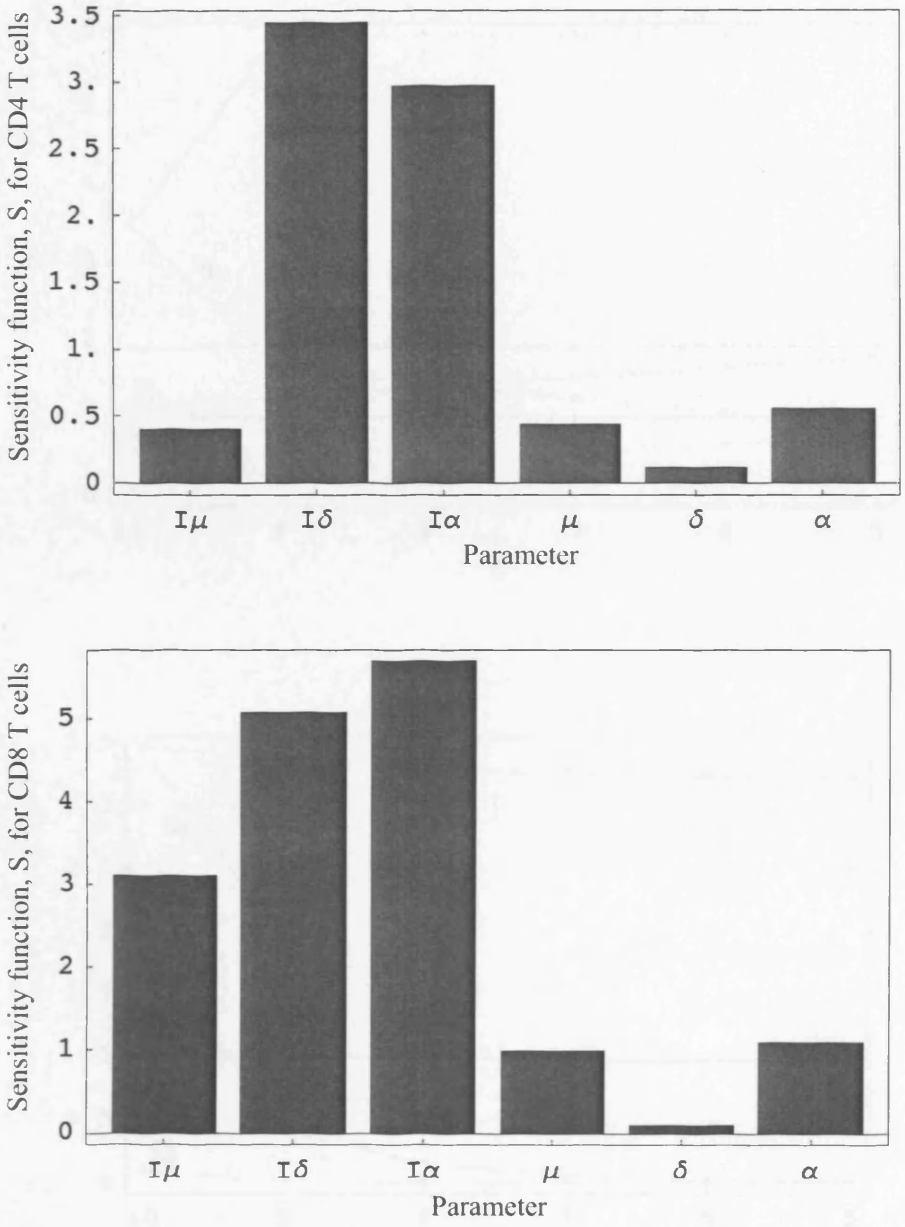
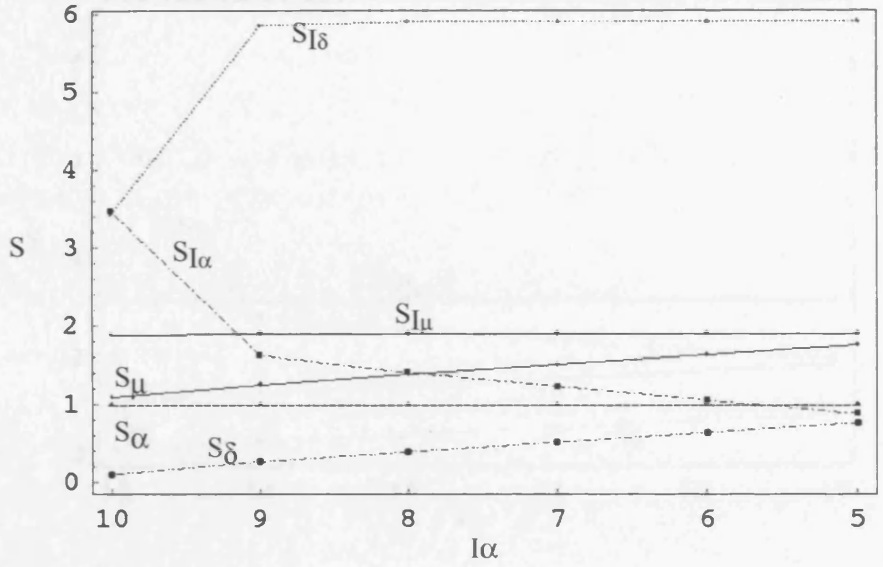


Figure 5.3: The relative sensitivities, assessed by the sensitivity function, S, of the response amplification to small changes in each parameter. The dominant CD4 and CD8 T cell responses to LCMV infection.



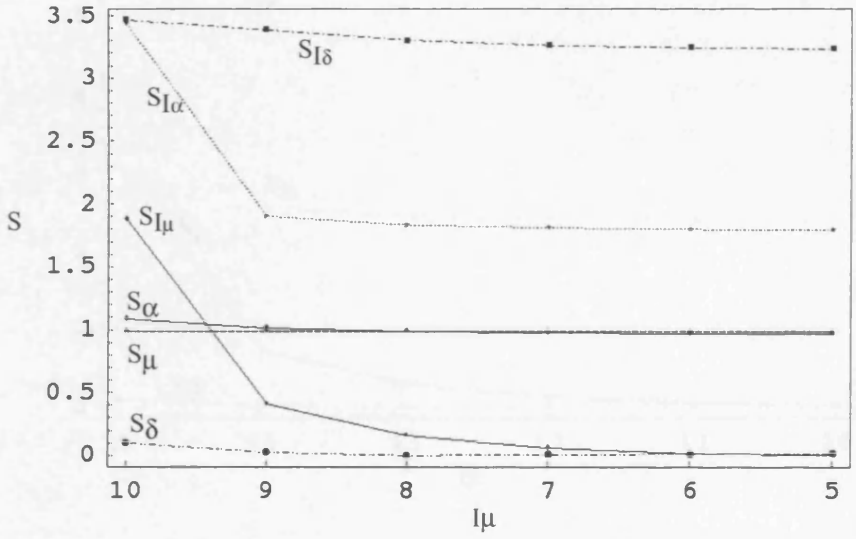


Figure 5.4: The change in the sensitivity of the output function (the response amplification) with respect to each of the parameters, for CD4 T cells, as the apoptosis or memory formation rates increase at an earlier division.

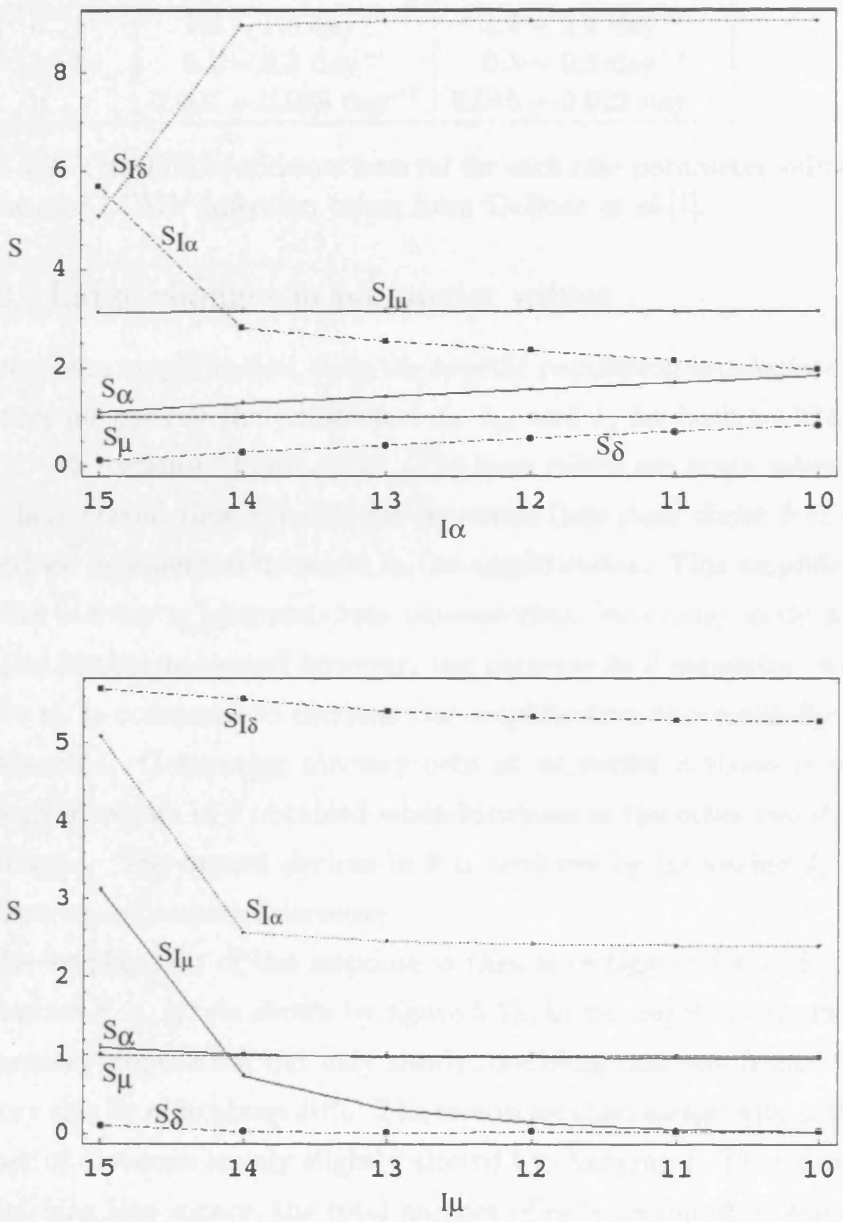


Figure 5.5: The change in the sensitivity of the output function (the response amplification) with respect to each of the parameters, for CD8 T cells, as the apoptosis or memory formation rates increase at an earlier division.

Parameter	CD4	CD8
δ	$1.2 - 1.6$ day^{-1}	$1.7 - 2.1$ day^{-1}
α	$0.1 - 0.3$ day^{-1}	$0.3 - 0.5$ day^{-1}
μ	$0.001 - 0.003$ day^{-1}	$0.015 - 0.022$ day^{-1}

Table 5.2: The 95% confidence interval for each rate parameter value for the dominant response to LCMV infection taken from DeBoer *et al* [1].

5.2.2 Large changes in parameter values

The response amplification, θ, in the specific population is calculated for a wide range of values for each of the parameters I_δ, I_α, and I_μ for both a CD4 (figure 5.6a) and CD8 T cell response (figure 5.6b). The base values are again taken from table 5.1.

It is observed that small linear increases (less than about 5 divisions) in I_α and I_δ produce exponential increases in the amplification. This amplification is increased more for a delay in apoptosis rate increase than for a delay in division rate decrease. As I_α is further increased however, the increase in θ saturates, whereas further increases in I_δ continues to increase the amplification exponentially and saturation is not observed. Generating memory cells at an earlier division is unable to produce the large increases in θ obtained when increases in the other two division parameters are delayed. The fastest decline in θ is achieved by increasing I_μ; a linear increase produces an exponential decrease.

The amplication of the response is then investigated for wide ranges of the rate parameters δ, α, μ. As shown by figure 5.7a, increasing δ always increases the size of the memory population but only slowly; doubling this rate from 1 to 2 increases the memory size by only about 10%. The reason for this insensitivity is that the maximum number of divisions is only slightly altered by changing δ. Therefore despite reaching the finishing line sooner, the total number of cells produced by the period of division will alter very little. The memory size is much more sensitive to I_δ, increasing it by less than 40% (from 5 to 7) increases the memory size by over 100%. This suggests that increasing the concentration of a pro-proliferative cytokine, such as IL2, early in a response (where it will only affect the division rate) will have a much weaker

effect than increasing it late in the response (where it will likely increase the number of divisions undergone by the cells).

As shown by figure 5.7b, increasing α always decreases the amplification. This amplification has a similar sensitivity to both apoptosis parameters; doubling I_α, or halving α increases the memory size by about 50%. As α is decreased to zero the amplification reaches a maximum value. From equation 5.7 this value is seen to be approximately 2^{I_δ}; with no apoptosis-induced contraction phase all effector cells will eventually become memory cells.

As shown by figure 5.7c, increasing μ initially increases the amplification but for $I_\mu < I_\delta$ further increases actually produce a decrease; the magnitude of this reduction is larger for lower I_μ. The increased flow of cells into the memory population reduces the number of proliferating cells and consequently the size of the peak response. This may have serious implications for therapeutic immune modulation. Using cytokines to promote memory may have the unexpected effect of reducing the final size of the memory population. For high μ, increasing I_μ initially increases the size of the memory pool but then causes a decrease as I_μ becomes higher than I_δ. This decrease in the memory population size as I_μ increases is shown in figure 5.6.

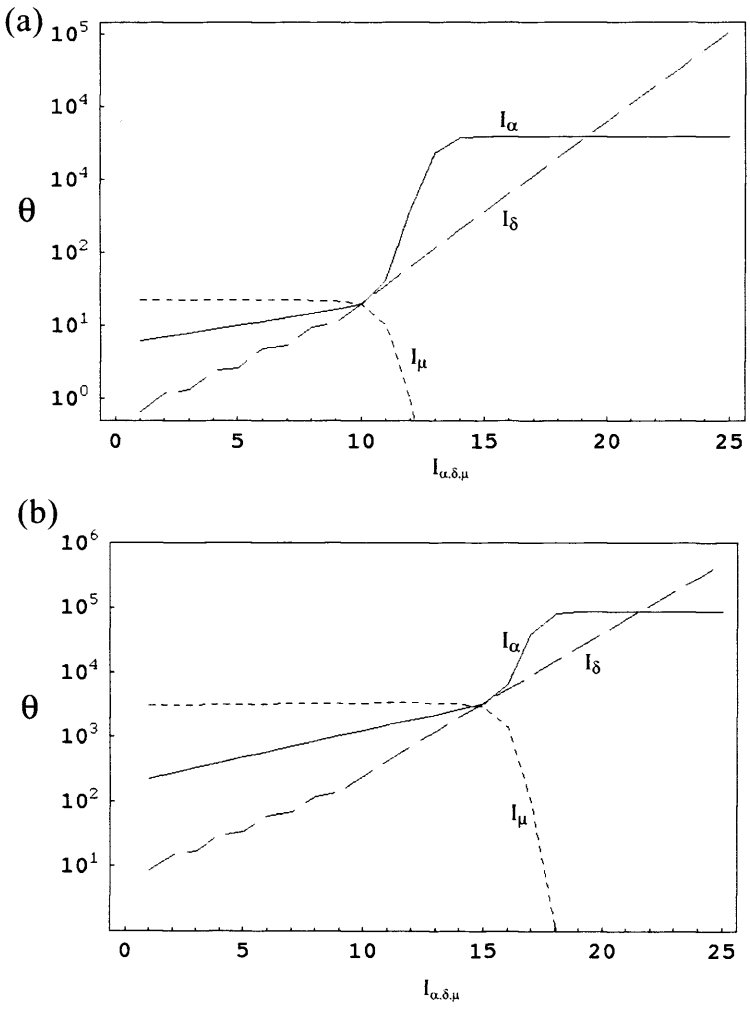


Figure 5.6: The effect of varying I_δ, I_α, and I_μ on the amplification, θ of the naive population of (a) CD4 T cells, (b) CD8 T cells.

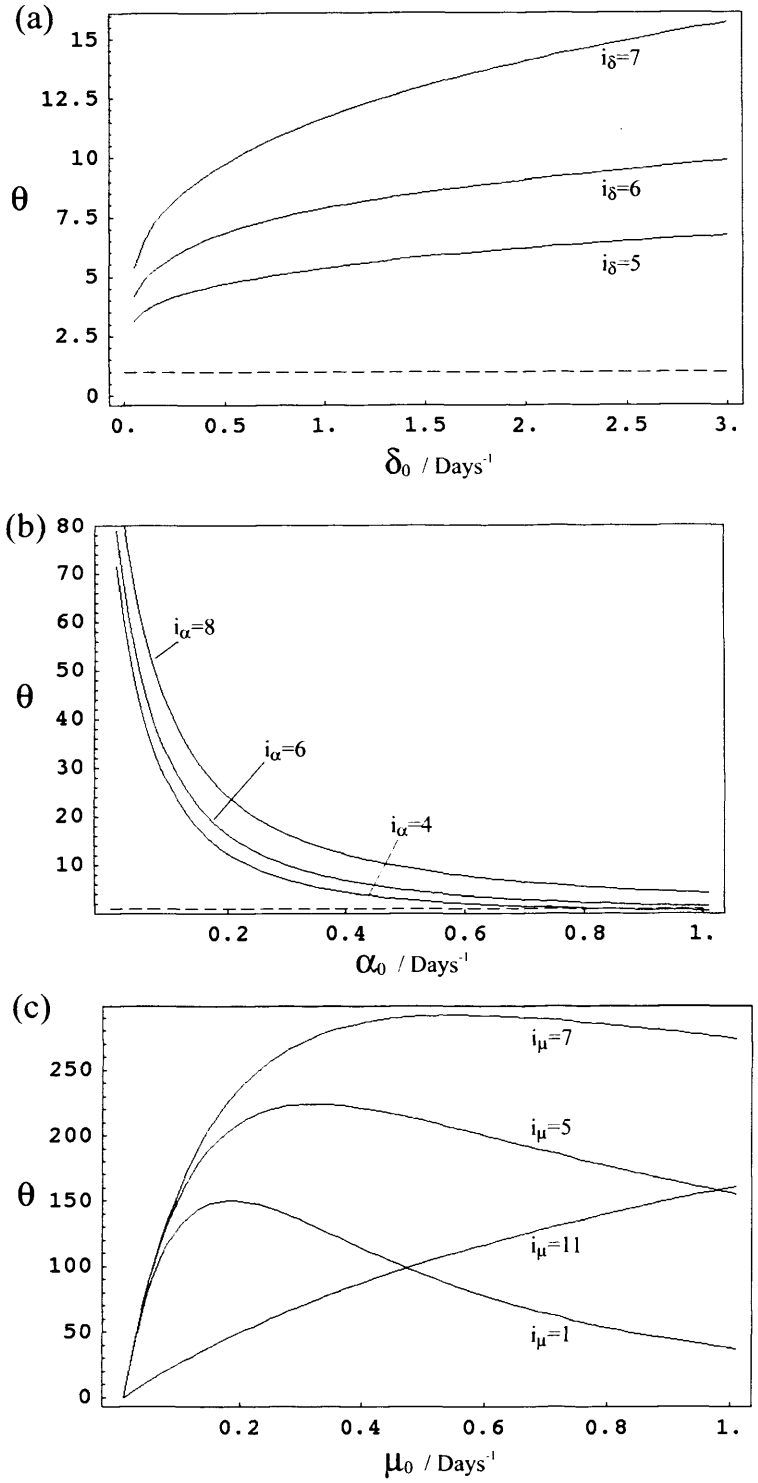


Figure 5.7: The response amplification, θ, as the rate parameters are varied. (a)The division rate, δ. Parameter values $\mu = 0.01$, $\alpha = 0.3$, $i_\mu = 8$, $i_\alpha = 8$. (b) The apoptosis rate, α. Parameter values $\mu = 0.01$, $\delta = 2$, $i_\mu = 8$, $i_\delta = 8$. (c) The memory formation rate, μ. Parameter values $\delta = 2$ day^{-1}, $\alpha = 0.3$ day^{-1}, $i_\alpha = 8$ divisions, $i_\delta = 8$ divisions.

5.3 Tolerising responses

A normal proliferative response to an antigen will produce a memory population that is larger than the original naive population. It can be said that the host has been sensitised to the antigen because a second encounter will produce a larger response. Conversely, if a second encounter produces a smaller response the host can be described as being tolerised to that antigen. Although tolerance involves a number of mechanisms [205], one very important one may simply be to decrease the number of specific memory T cells formed during a proliferative response until the final population size is smaller than the original population. It has been shown that weak antigenic stimulation (low levels of antigen, short duration of stimulus or lacking co-stimulation, for instance) can produce a response where apoptotic susceptibility rises earlier in the response, and also the ability to form memory cells diminishes [206]. This has led the authors to propose that this is the mechanism by which tolerance is achieved. It is not clear, however, whether both early death and reduced memory generation are required to produce tolerance. To address this question the apoptosis and memory parameters are manipulated to determine which can produce a shrinkage in the population size (negative amplification).

The parameters I_α, I_μ, and μ were varied, one at a time, through a large range from zero to a maximum value (shown in table 5.3) to discover which could produce a negative amplification ($\theta < 1$ or $\ln\theta < 0$) in the population size. For CD4 T cells it was found that sufficient changes in any of the three parameters could shrink the population size (figure 5.8a). For CD8 T cells, appropriate changes in either I_μ and μ could produce negative amplification but this was not so with the apoptosis parameter I_α (figure 5.8b). Every value of I_α produced positive amplification. This suggests that if a weak stimulation can produce tolerance in CD8 T cells then the reduction in memory formation is a vital component of the mechanism - this form of tolerance cannot be manufactured by increasing the apoptosis rate at an earlier division. Tolerance of CD4 T cells could be achieved by altering any of their memory generation or apoptosis properties.

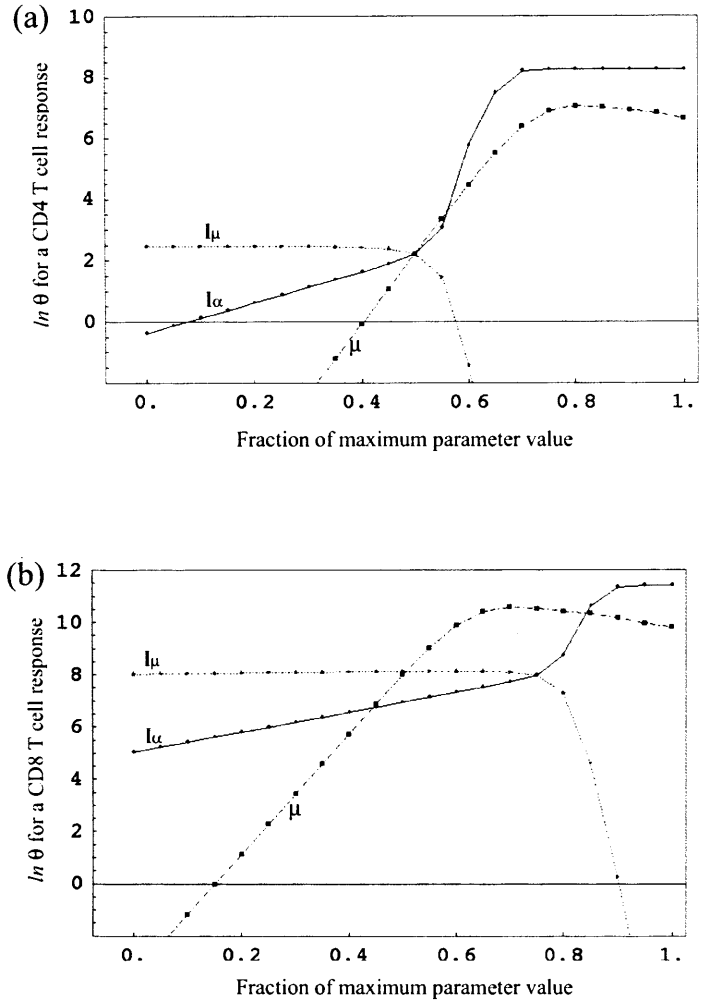


Figure 5.8: The natural logarithm of the amplification, θ, for different values of the parameters I_μ, I_α and μ for (a) CD4 T cells, and (b) CD8 T cells. The maximum values are given in table 5.3

Parameter	CD4	CD8
I_α	$0 - 20$ divisions	$0 - 20$ divisions
I_μ	$0 - 20$ divisions	$0 - 20$ divisions
μ	$0 - 0.002$ day^{-1}	$0 - 0.02$ day^{-1}

Table 5.3: The large ranges over which the parameters are varied to assess the potential of each to produce tolerance.

Whether the programmed response is geared to produce positive or negative amplification has profound implications for the outcome of repeated stimulation. If M_j is the memory population size after j successive identical stimulations (and identical programmed responses) then from equation (5.6)

$$M_j = \theta^j N.$$

It can be observed that for $\theta > 1$ the memory population will exhibit geometric expansion for increasing numbers of responses, and $M_j \to \infty$ as $j \to \infty$. Conversely, if $0 < \theta < 1$ then M will undergo a geometric decay for increasing numbers of responses and as $j \to \infty$, $M_j \to 0$. If $\theta = 1$ then $M_j = N$ for all j. Therefore if a particular antigen produces a negative amplification in the specific T cell population (by only eliciting T cells to divide a small number of times for instance) then repeated encounters with this antigen will eventually reduce the memory population size to such a low level that a specific immune response can no longer be elaborated.

5.4 Discussion

The aim of this chapter was to investigate how the nature of the antigen-induced developmental program influenced the size of the memory population, and to determine the most efficient methods of modulating the response amplification. Based on the autoregulatory compartmental model of Chapter 3, an expression was derived and used in the investigation. It was found that the memory size increased linearly with the number of activated naive T cells, and had a varying sensitivity to all the program

parameters.

To increase the size of the memory population, increasing the division number at which division decreased most rapidly, I_δ, was found to be the most consistently effective manipulation. The memory size was highly sensitive to small changes in this parameter. Changing the division number at which the apoptosis rate increased most rapidly, I_α, produced similar changes in the memory size if $I_\alpha = I_\delta$, but in the more likely case that I_α is slightly less than I_δ the sensitivity to this parameter was substantially smaller. Larger, linear, increases in I_δ produced exponential increases in the size of the memory population with no limit to the magnitude of increase. Similar increases in I_α initially produced exponential memory increases but the memory size soon saturated. This was because there was sufficient opportunity for all the cells to acquire the memory phenotype and hence be rescued from apoptosis.

The most effective method of increasing the size of the memory population formed during the response is to supply a cytokine that promotes division. One cytokine known to strongly promote division is IL2. It has also been shown to produce higher levels of death late in the response and therefore its use would be restricted to early times. The results of this chapter, however, suggest that this temporal restriction renders the cytokine unsuitable for boosting the memory population. Early promotion of division (during the first week of infection) will affect only the division rate of cells, but it has been shown here that the final memory size is insensitive to this parameter. Instead, it is crucial for division to be promoted late in the response (after about a week), exactly when IL2 becomes an agent of cell death [141]. It is therefore essential to identify a different division-promoting cytokine to enable the most efficient boosting of the generated memory population.

The most effective method of reducing the size of the generated memory population is to increase the division number at which the rate of memory formation increases. A linear increase in I_μ produces a much faster decrease in the final memory size than is achieved by changing other parameter values. One of the crucial steps by which an effector cell reaches the memory phenotype is by increasing receptor expression for the pro-survival cytokines IL7 and IL15 to allow its rescue from apoptosis.

By blocking the binding of these cytokines by the receptors, with anti-IL7 and anti-IL15 for instance, the rescue from apoptosis can be prevented. The rapid death rates during the contraction phase, particularly of CD8 T cells, means that preventing this rescue, even for a short while, will greatly reduce the number of effector cells with the potential to become memory cells. Experimental studies suggest that this is an effective method of suppressing memory formation [193, 73, 194, 111, 73, 195].

Unexpectedly, increasing the rate of memory formation does not necessary increase the size of the memory population. Increasing the rate from a low value increases the amplification until it peaks and then beyond this it declines. The earlier the formation of memory the lower the rate at which this peak occurs. The decrease in amplification is caused by the cells exiting the proliferating population to enter the memory population thereby reducing the total amount of proliferation. This reveals that the application of cytokines that promote memory formation will not necessarily increase the size of the memory population. Over rapid memory formation at too early a stage will cause too great a depletion of the proliferating population.

It is intriguing to observe that sufficient changes in most of the parameter values can produce a memory size that is smaller than the size of the activated naive population. This would represent a form of tolerance because the secondary response to a stimulus would be smaller than the primary response. This suggests a fascinating conclusion: sensitisation and tolerance may be two different outcomes of a single mechanism. Generally, the two processes are thought to be controlled by distinct mechanisms. The process of sensitisation is associated with the priming of T cells (through proliferation and memory formation) so that they respond more vigourously to a second encounter with their specific antigen. The process of tolerance is associated with the de-sensitising of a T cell population, through cell deletion and induction of anergy (non-responsiveness), to reduce or annul their response to subsequent encounters with their specific antigen. Simply by changing the parameter values, the model analysed here can switch between these processes. This suggests that moving between a tolerising and sensitising response may be much easier than has previously been appreciated. Complete tolerance could potentially be achieved by a single strong negative amplification or by a weak negative amplification repeated many times.

It has been suggested that a weak antigenic stimulus can produce a premature increase in the susceptibility of responding T cells to apoptosis and an inhibition of memory formation [206]. It was hypothesised that this mechanism allows a weak stimulus to produce tolerance. However, it is not known whether the apoptosis effect alone or the memory effect alone are sufficient to achieve tolerance. In this chapter it was shown that tolerance, defined as a smaller memory than naive population, could be achieved by the inhibition of memory formation alone but for the CD8 T cell response *not* solely by the early increase in apoptotic susceptibility. Therefore reducing expression of survival promoting genes, such as Bcl-2, without altering memory formation, would be unlikely to produce tolerance.

The results of this chapter have been based on parameter values for a particular infection, LCMV. As discussed in Chapter 2 the similarity of many aspects of T cell kinetics for responses to different stimuli in different hosts suggests that proliferating T cells are always regulated by the same mechanism. It is therefore likely that the results of this chapter would be applicable to responses to most other infections. However, to determine the generality of the results further analysis is required. Presently, precise parameter values for infections other than LCMV are not available, but with the present pace of research no doubt they soon will be.

CHAPTER 6
THE ABILITY OF THE PROGRAMMED T CELL RESPONSE TO CLEAR INFECTION

> The art of medicine consists of amusing the patient while nature cures the disease
>
> Voltaire (1694-1778)

Although the primary response is normally able to resolve an infection within a couple of weeks, many types of pathogen can persist and prolong the T cell proliferative response [207, 208, 4]. It is not clearly understood what pathogen properties allow an infection to become chronic. Generally, it has been assumed that a high replication rate and a robust resistance to clearance, by inhibiting the immune activity or hiding in sites inaccessible to T cells for instance [207], prolongs the duration of infection and the associated T cell response. However, the relationship may not be so obvious. Recent evidence suggests that low, rather than high, replication rates may produce persistence [209, 210, 132]. Also, T cell expansion is observed to terminate at the same time for a wide variation in pathogen properties [35]. This suggests that the time of infection resolution is insensitive to certain variation in the pathogen.

It would be of great benefit to understand the relationship between the properties of a pathogen and the duration of infection. Such knowledge may enable improved clearance strategies to be developed, or, conversely, analysing protracted T cell responses may reveal important information about the nature of a pathogen. Also it is not known whether infection chronicity occurs because the pathogen is invulnerable to the immune system or because it is cleared only very slowly by it. This is important because only if the latter is true would a patient benefit from immune boosting

therapy. Numerous experimental studies have focused on this area but work is slowed by the difficulties of simultaneously monitoring *in vivo* several cell populations and the pathogen load.

Theoretical approaches have attempted to clarify this relationship. Some studies predict that infections always persist indefinitely, with a dynamic equilibrium set-up between the replicating pathogen and the responding T cells [7, 211]; chronic symptoms would only be observed if the stable state of the pathogen load was sufficiently high. In contrast another study predicts that permanent persistence is not possible and clearance will always occur [3]. A few have suggested that persistence only occurs with certain conditions, when the pathogen replicates slowly [130, 132], or when multiple infections co-exist [212]. The majority of work, however, has not incorporated a program into the T cell model and may therefore have missed out on important relationships or made incorrect preditions. Models that do include a program have not considered the effect of antigen persistence on the entire T cell programmed response [213, 15, 1, 2].

In this chapter the relationship between a pathogen's properties and its ability to persist and prolong the T cell response is explored. Unlike other theoretical studies with similar aims, here the idea of programmed proliferation by T cells is incorporated, the details being based on recent experimental observations. The compartmental model of Chapter 3 for Autoregulation of the response is extended, in a similar way as Chapter 5, to represent memory formation and, furthermore, to allow the activation of these memory cells.

Within the framework of the model, it is found that the pathogen, regardless of its properties, *will* be cleared from the host if it *always* elicits a T cell program with positive amplification (as described in Chaper 5). Very low pathogen replication rates can, however, produce a quantitatively different and inferior T cell response that enables the pathogen to persist for a long, but finite, time. The results suggest that a pathogen can only produce a lifelong infection by altering the T cell proliferative program, not by changing its own kinetic parameters.

6.1 The Model

The system is modelled by considering three distinct populations of T cell: effector cells, memory cells, and precursor memory cells (pre-memory cells). The effector cells are naive or memory cells that have entered a period of intensive proliferation and acquisition of effector function following activation by antigen. Pre-memory cells are formed from a small fraction of effector cells in the later stages of a proliferative response. Memory cells are formed in turn from pre-memory cells during the weeks following a proliferative response. A cartoon of the model is shown in Figure 6.1.

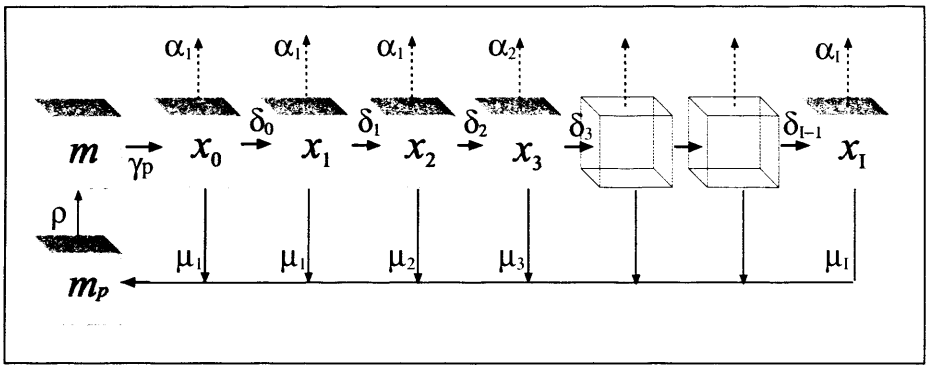


Figure 6.1: Cartoon representation of the model. The variables x, m, and m_p are the effector, memory and memory precursor cells respectively. The parameters δ, α, and μ are the division, apoptosis, and memory formation rates respectively.

6.1.1 Effector T cells

Antigen stimulates naive and memory T cells, causing them to undergo a period of proliferation and differentiate into effector cells.

From the total number of naive T cells, N, that can be activated by a particular pathogen the number recruited increases with pathogen load [214] but at high loads further increases are not observed [36]. The number of naive cells recruited into the

response, $x_0(0)$, is therefore modelled with the saturating function [2, 131]:

$$x_0(0) = N \frac{P}{\eta + P},$$

where P is the initial pathogen load encountered by the naive T cells and η is the pathogen load that produces activation of half of these cells. Because the pathogen load can varying by many orders of magnitude during a response, to avoid computational errors the pathogen load that produces almost full activation if chosen to equal 1. This is achieved by assigning η the value 0.05, which produces approximately 95% recruitment when the pathogen load is 1.

The activation of memory T cells has been modelled in previous studies with a *per capita* rate of activation that is proportional to the pathogen load [213, 3]. Here a similar approach is taken except the rate is made a saturating function of the pathogen load,

$$\frac{\gamma p}{\eta + p},$$

where p is the pathogen load and γ is the rate of activation, to represent the reasonable assumption that the activation rate would saturate at high loads.

Once activated the naive or memory T cells enter a period of programmed proliferation. As in Chapter 3, a compartmental model is used to represent this proliferation of effector cells, x_i, where the i-th compartment contains cells that have divided i times. The rate at which cells divide and die changes with each division. The *per capita* division rate, δ_i, decreases with increasing division number, i, whereas the *per capita* rate of apoptosis, α_i, increases. When a cell reaches the final compartment, $i = I$, it stops dividing. During the response effector cells begin to differentiate into memory cells. In the model the *per capita* rate at which cells leave the effector population is μ_i.

Although there is a delay of about a day [145] between antigenic stimulation and the first division, for simplicity it is not incorporated into the model because its only effect will be to shift the kinetics by an amount that is highly insensitive to the properties of the pathogen.

The proliferation of effector T cells is therefore described with the following system

of equations:

$$\dot{x}_i = \begin{cases} -(\delta_0 + \alpha_0 + \mu_0)x_0 + \gamma\, m\, p/(\eta + p) & i = 0 \\ 2\delta_{i-1}x_{i-1} - (\delta_i + \alpha_i + \mu_i)\, x_i & i = 1 \ldots I-1 \\ 2\delta_{I-1}x_{I-1} - (\alpha_I + \mu_I)x_I & i = I \end{cases} \tag{6.1}$$

The division, death and memory formation rates change as described in chapters 3 and 5.

6.1.2 Memory T cells

The size of a memory population increases with the differentiation of effector cells and decreases through activation by encounter with specific antigen. In the absence of these processes the size of a memory population is fixed by a homeostatic mechanism. This control is simulated by setting the division and death rates equal to each other for both precursor and normal memory cells; this approach for modelling memory cells has been taken by other authors [2, 213].

After leaving the effector pool it can take a T cell several weeks to acquire the full memory phenotype [106], and during this time it is thought unable to respond to antigenic stimulation [213]. In this chapter these incompletely-formed memory cells are referred to as precursor memory, m_p, and are represented with a separate compartment in the model. Their numbers increase through entry from the effector pool and decrease by a gradual differentiation into memory cells at *per capita* rate ρ. An alternative model could include a fixed time delay between entry into the pre-memory pool and exit into memory but experimental observations that small numbers of fully developed memory cells already exist at the peak of the T cell response [215] suggest that this would be a less realistic approach.

As discussed in the last section, in the presence of specific antigen, memory T cells will be recruited into the effector population at a rate

$$\frac{\gamma\, p}{\eta + p}.$$

These assumptions give the rates

$$\dot{m}_p = -\rho\, m_p + \sum_{i=0}^{I} \mu_i\, x_i, \tag{6.2}$$

and

$$\dot{m} = \rho\, m_p - \frac{\gamma\, p}{\eta + p} m. \tag{6.3}$$

6.1.3 Pathogen

The pathogen replicates at *per capita* rate ψ, and is cleared by effector T cells at the rate constant κ multiplied by the total number of effector cells. The pathogen load has carrying capacity $\psi\, p_c$. The equation for the rate of change of the pathogen load can therefore be written

$$\dot{p} = \left(\psi - \frac{p}{p_c} - \kappa \sum_{i=0}^{I} x_i\right) p, \tag{6.4}$$

with the initial pathogen load $p(0) = P$. Similar equations have often been used to represent pathogen kinetics [216, 210, 3].

For very low pathogen loads the ODE structure of the model is unable to realistically represent the biological processes. During a real infection it is possible for the pathogen to be completely eradicated, but this complete disappearance will not occur with ODE's. Therefore an addition to the model is made that applies when the pathogen load falls below a certain threshold value, p_T, where $p_T << P$. An outcome representative of clearance is engineered by setting $\psi = 0$ and $\gamma = 0$ if $p < p_T$. Therefore, if the pathogen load drops below the threshold value it will no longer be able to grow or stimulate memory cells. An alternative method would be to include an Allee effect [217], where the effective replication rate of the pathogen at low loads decreases so that the replication term would be, for example,

$$\psi \frac{p}{p_A + p},$$

where p_A is the pathogen load at which the replication rate is half-maximum. This has previously been used in a T cell model to represent pathogen clearance [3]. Another

method would be to use a stochastic framework, which could model the possible extinction of a pathogen population. However, In the present study, for simplicity, a fixed threshold is used.

6.2 Investigating the model

The ability of the T cell response to clear a pathogen, and the variation in its kinetics for different pathogen properties are investigated with the model. The effects of varying three parameters are considered:

1. The rate at which the pathogen can be cleared, κ
2. The pathogen replication rate, ψ,
3. The initial pathogen load P.

As these parameters are altered the change in two key characteristics of the response are analysed:

1. The time and height of the peak T cell response.
2. The time taken to reduce the pathogen load to the threshold level, p_T. This allows the extent of pathogen persistence to be quantified.

6.2.1 Selection of the parameter values

The values for the CD8 T cell response in the previous chapter were chosen (shown in Chapter 5, Table 5.1). Changing these values was found not to change the nature of the results (see Appendix C). Other parameter values, together with their source, are shown in Table 6.1. For parameter values not simply taken from past experimental studies, further explanation is provided below:

1. A pathogen carrying capacity is chosen so that $p_C >> P$. During a non-fatal infection the pathogen load appears never to increase more than about seven orders of magnitude above the limit of detection [120, 210], and therefore in this model $p_C = 10^7$.

	Rate Parameters	**Value** day^{-1}	**References**
ρ	Memory T cell formation rate	0.03	[2]
γ	Activation rate of memory T cells	1	[213, 2]
ψ	Pathogen replication rate	0 - 5	[210, 209]
κ	Pathogen clearance rate	$10^{-3} - 10^{-7}$	[114]
	Other Parameters	**Value**	
N	Initial number of T cells	200	[201]
p_0	Initial pathogen load	1	-
p_C	Pathogen carrying capacity	10^7	[120, 210]
p_T	Pathogen load threshold	10^{-5}	[114, 122]

Table 6.1: Parameters in the model and their values.

2. A pathogen load threshold is chosen so that $p_T << p_0$. Experimental *in vivo* data suggests that during a T cell response a reduction in the pathogen of approximately 5 orders of magnitude is sufficient to produce clearance [114, 122].

3. An approximate value for the rate constant of pathogen clearance, κ, can be calculated from an experiment in which effector T cells were transferred to the spleen of a virally infected mouse and the time required to reduce the viral load of the spleen by 50% was measured [218]. The parameter is varied over four orders of magnitude around this value.

4. Different pathogens exhibit different doubling times. They can range from minutes to days [210, 209].

From the Equations 6.1-6.4 the kinetics of x_i, for $i = 1 \ldots 20$, m_p, m, and p are calculated numerically. Only 20 division compartments are considered because T cells are thought not to divide more than this during the program [27]. The kinetics of the whole antigen-specific T cell population, w, where

$$w = m_p + m + \sum_{i=0}^{20} x_i,$$

is calculated for for different values of κ, ψ and P.

6.3 Results

It is found that if the pathogen always elicits a programmed response with a positive amplification then clearance will always be achieved. The T cell kinetics at the time of this clearance can take one of two quantitatively different forms: an exponential or linear expansion of the T cell population. The plots that follow are generated from the model; they are not experimental data.

6.3.1 Clearance by Exponential Response

Following the initial primary response, which involves exponential expansion and contraction, a secondary T cell response can immediately occur. Its appearance, or otherwise, and the magnitude of this second response is determined by how successful the primary response was at clearing the pathogen. Following initial encounter with antigen, the activated T cell population expands, peaks, and then contracts according to the proliferative program. If this is sufficient to push the pathogen load below the threshold, p_T, then a stable memory population will form and the response will terminate; the T cell kinetics in Figure 6.2a. But if the initial response fails to clear the pathogen then persisting antigen will trigger the reactivation of fully formed memory cells and produce a continuation in the proliferative response (an expression for the number of memory cells at a given time after the peak is derived in Appendix D). This proliferation will be maintained, fed by new memory cells encountering the persisting antigen, until the pathogen load is pushed sub-threshold by the armed effector T cells. Once this occurs memory cells are no longer activated and the T cell population contracts and acquire a stable memory state. This subsequent period of T cell proliferation produces the second peak predicted by the model (Figure 6.2b). This second period of expansion has been observed experimentally in response to pathogens known to persist [114, 208, 198].

The model predicts that for pathogens that are very resistant to T cell mediated clearance, the time (Figure 6.3a) and magnitude (6.3b) of the largest peak in T cell

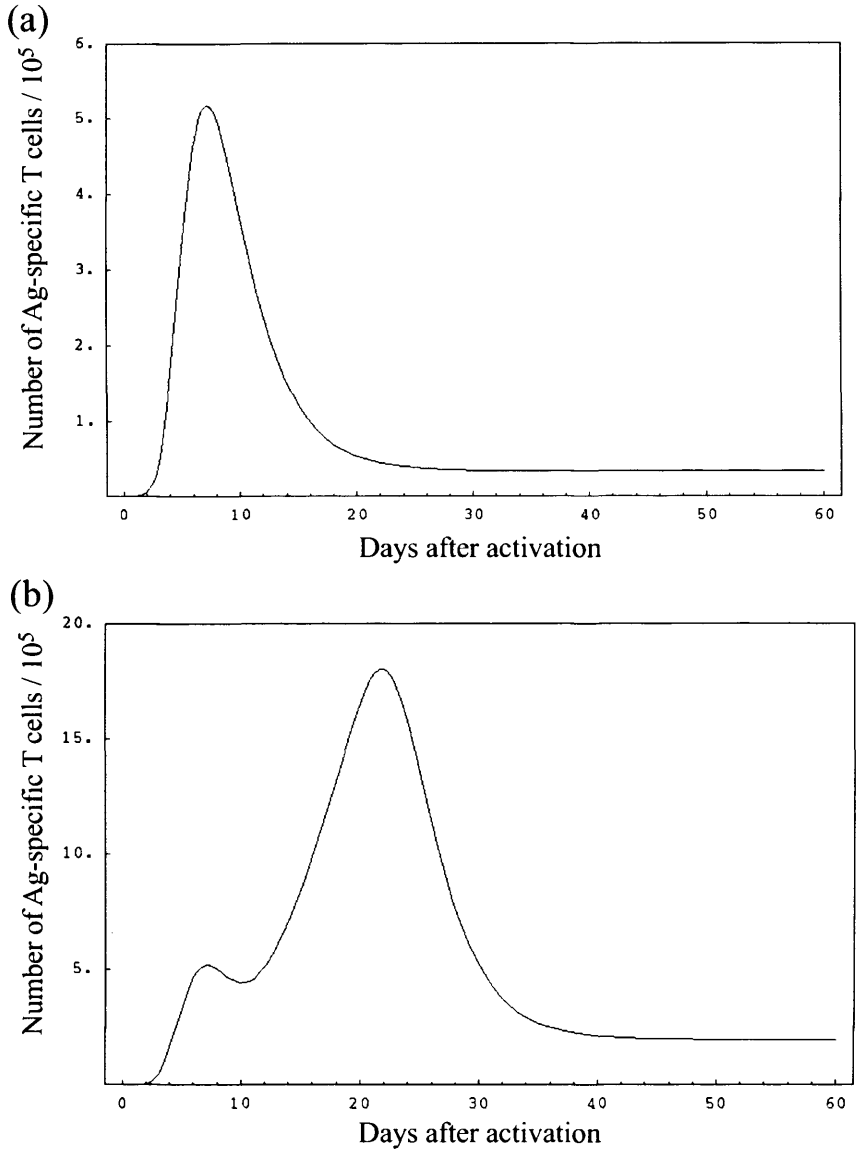


Figure 6.2: The number of antigen-specific T cells during the days after activation where the rate of pathogen clearance is (a) high, or (b) low. Pathogen parameter values: $\psi = 4/\text{days}, \kappa = 10^{-6}/\text{days}, P = 1$. The other parameter values are as given in table 6.1

number correlates with this resistance. Interestingly, this predicts that the observation of a very large T cell response following infection may be indicative *not* of a naturally strong resistance to the pathogen, but of a natural weakness. If the host's immune response was more potent, a smaller effector T cell population would suffice.

When the pathogen is not highly resistant to T cell mediated clearance, the model predicts an insensitivity of the time (Figure 6.3a) and magnitude (6.3b) of the largest peak in T cell number to the clearance rate, κ. The reason for this switch in sensitivity results from the way in which κ influences the number of memory T cells activated. A high κ results in rapid clearance of the pathogen (driving the pathogen load below the threshold). Given that memory begins to form late in the primary response, the infection duration is too short to elicit the activation of many memory cells. A lower κ however, may allow pathogen to remain following the first period of proliferation and some of the newly formed memory cells will therefore be activated. These activated memory cells, which are referred to here as 'new effectors', x_n, will undergo a second period of programmed proliferation and then contraction. If the rate of increase in x_n is greater than the rate of decrease of the 'old effectors', x, then there will be net expansion. This expansion will continue while the pathogen load is large enough to activate memory cells at a sufficient rate to allow the effector kinetics to be dominated by the proliferating T cells. When the pathogen load is finally driven below the threshold p_T the entry of new effectors will cease and soon the T cells that have stopped dividing and become susceptible to apoptosis will dominate the effector kinetics and a contraction will commence. As κ is decreased it takes longer to clear the pathogen so the expansion continues for longer creating a later and larger T cell peak. When sufficient activation occurs to produce a peak number of new effectors that is greater than the first peak this second peak will adopt the status of 'largest' peak and the plots of time and size of peak in figures 6.3a and 6.3 will refer to this second peak. This explains the biphasic form of Figure 6.3b. If $\dot{x}_n < \dot{x}$ then there will be net contraction and no second peak will be observed.

For rapidly replicating pathogens, the time and size of the largest peak (Figures 6.4 correlates with the replication rate. A more rapidly replicating pathogen will be cleared at a slower rate and the extended duration of infection allows more memory

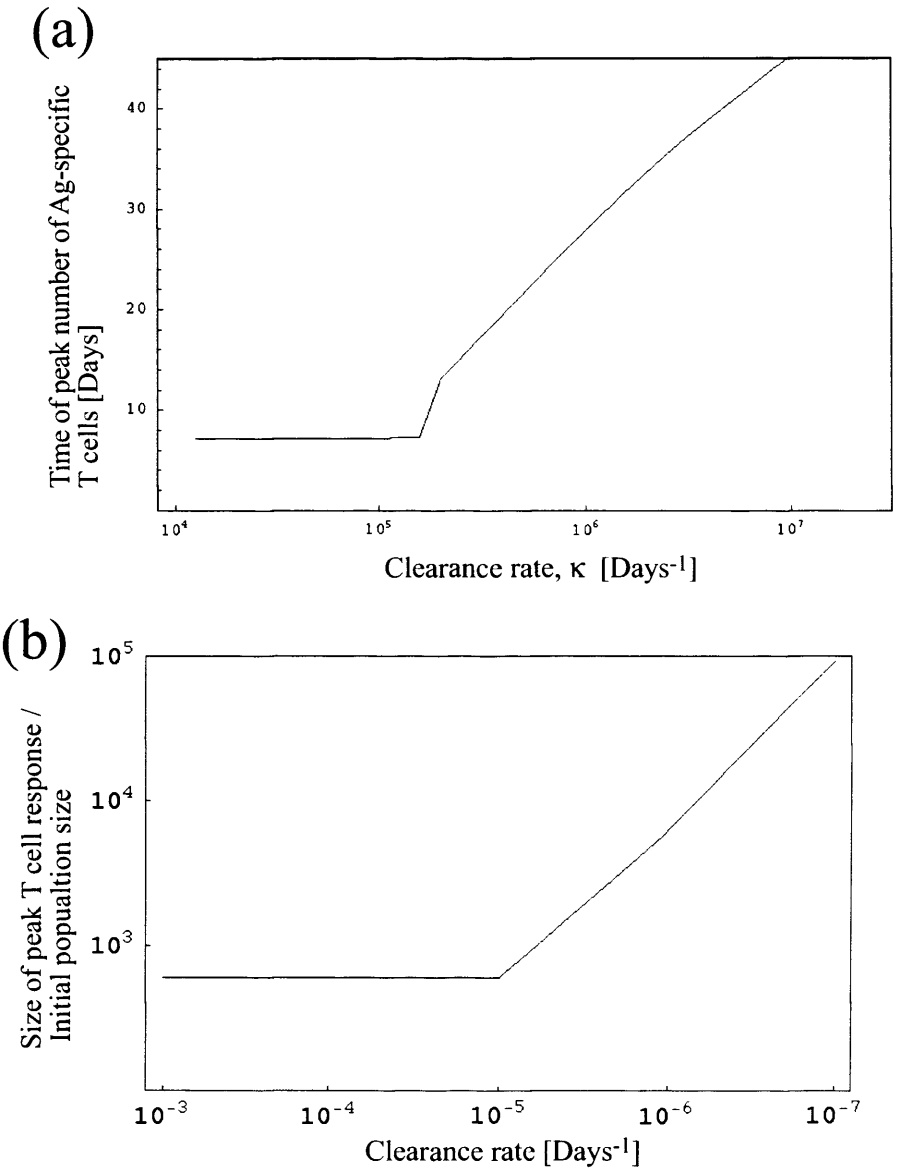


Figure 6.3: Changes in the characteristics of the T cell kinetics as the clearance rate is varied. (a) The peak time. (b) The peak size/initial size. Parameter values: same as Figure 6.2 except κ varies.

cells to be activated. The proliferation of these newly activated cells prolongs the expansion of the effector population, thereby delaying and increasing the size of the final peak in T cell number. This correlation will only exist if the replication rate is sufficient to prolong the persistence of pathogen beyond the time when memory cells begin to form. For lower replication rates the peak size and time are insensitive to this rate because the pathogen is always cleared before it can activate any memory cells.

The effect of the initial pathogen load, P, on the T cell response is surprising. It might be expected that the size of the peak T cell response always positively correlates with the initial load. A number of experimental studies demonstrate this to be true for large variations in this load, with the peak size saturating for very high initial loads (for example see ref.[35]). For high initial loads, the model presented here does agree with this observation (Figure 6.5b), and also predicts the insensitivity of peak time to initial load as shown in experiment [35]. However, counterintuitively, for low initial loads the size and time of the T cell peak correlate *negatively* with this load (Figure 6.5b). The smaller the initial pathogen dose, the larger and later the largest peak in the T cell response

The reason for this change from a negative to a positive correlation between the initial load and the peak size can be understood by considering a simple model of the primary programmed response and ignoring the formation and reactivation of memory T cells. If the program produces proliferation at the *per capita* rate δ for t_p days and then contraction at the *per capita* rate $\alpha + \mu$ then during the expansion phase the T cell number will increase exponentially,

$$x(t < t_p) = x(0)\, e^{\delta t}, \tag{6.5}$$

peaking at

$$x_p = x(0)\, e^{\delta t_p}, \tag{6.6}$$

and during the contraction phase decrease exponentially,

$$x(t > t_p) = x(t_p)\, e^{-(\alpha+\mu)(t-t_p)}. \tag{6.7}$$

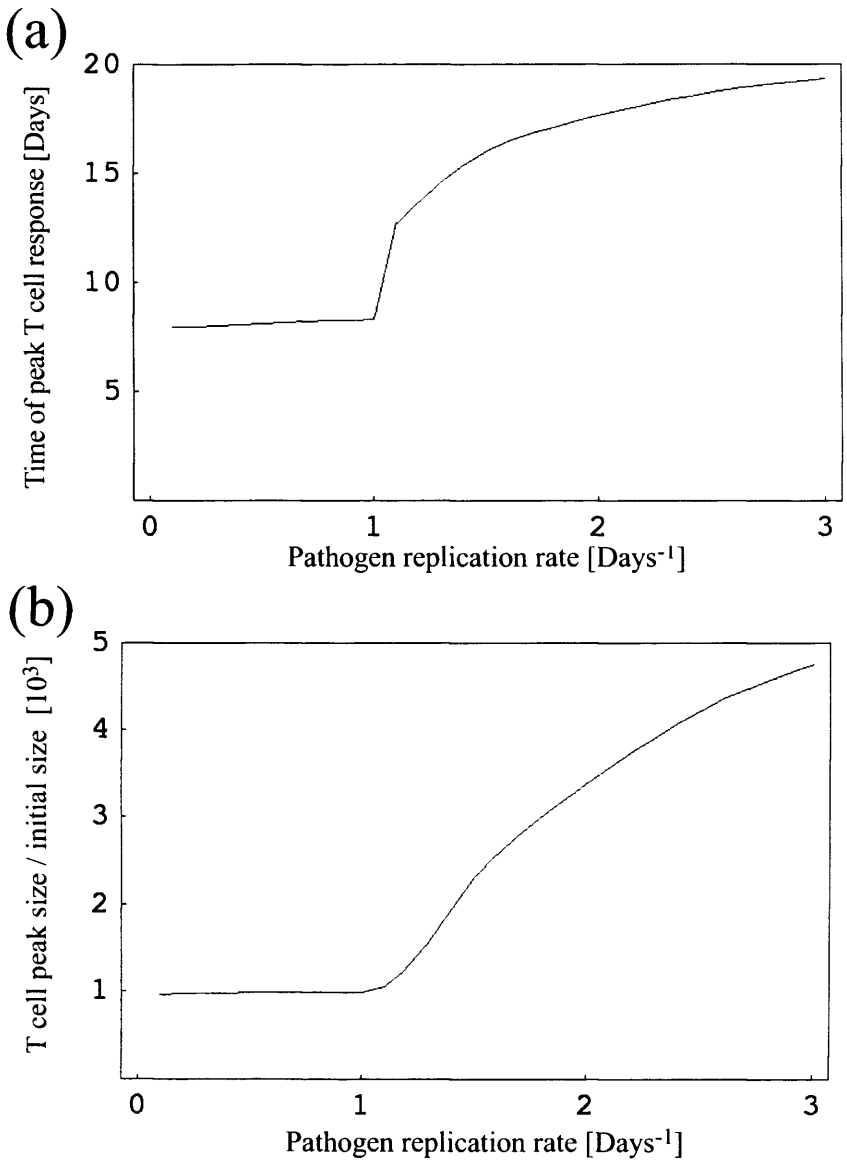


Figure 6.4: Changes in characteristics of the T cell kinetics as the replication rate is varied. (a) The peak time. (b) The peak size / initial size. Parameter values: same as Figure 6.2 except ψ varies.

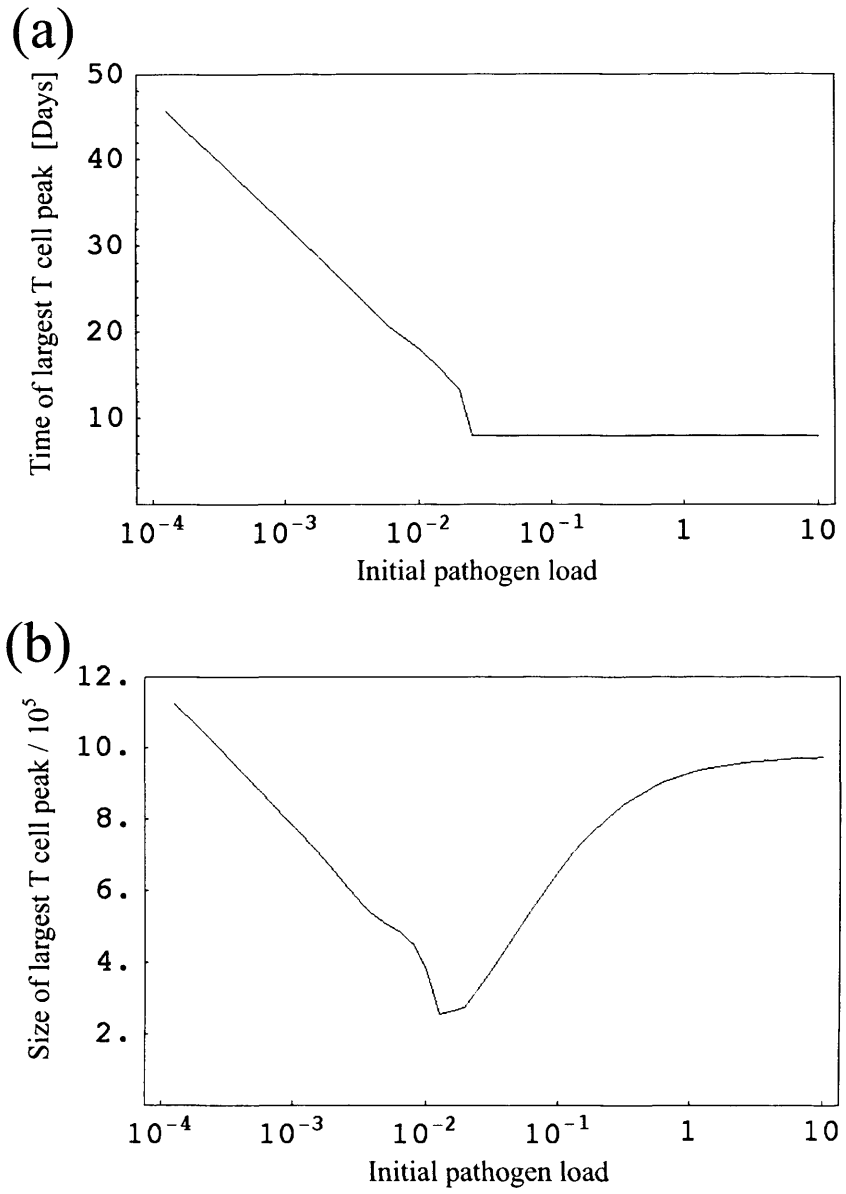


Figure 6.5: Changes in characteristics of the T cell kinetics as the initial pathogen load is varied. (a) The peak time. (b)The peak size/initial size. Parameter values: same as Figure 6.2 except P varies.

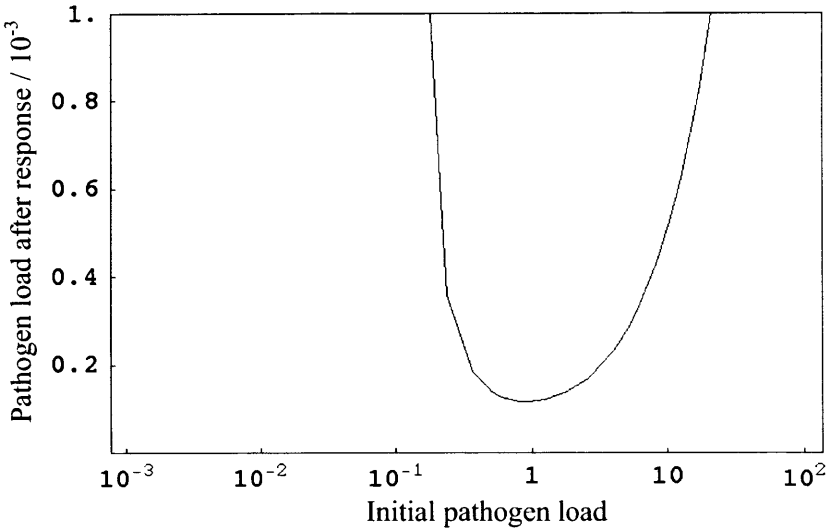


Figure 6.6: The pathogen load after the response verses the pathogen load before. A minimum is observed. Parameter values: same as Figure 6.2

Throughout the response the pathogen load will change at the following rate

$$\dot{p} = (\psi - \kappa\, x)\, p. \tag{6.8}$$

Substituting in equation (6.5) and solving allows the determination of the pathogen load at the end of the T cell expansion phase,

$$p_p = P \exp\left(\frac{(x_0\kappa(1 - \mathrm{e}^{\delta\, t_p}) + \delta\, \upsilon\, t_p)}{\delta}\right). \tag{6.9}$$

Next, substituting equation (6.7) into equation (6.8) and solving gives the pathogen load after T days, where $T > t_p$

$$p(T) = p_p \exp\left(t\psi - \frac{x_p\, \kappa\, (\mathrm{e}^{(\alpha+\mu)\, t} - 1)(\mathrm{e}^{-(t-t_p)(\alpha+\mu)})}{\alpha + \mu}\right).$$

After substituting in the values for x_p and p_p (equations (6.6) and (6.9)), the equation

takes the form

$$p(T) = P \exp(\Gamma_1 \psi - \Gamma_2 x(0)\kappa),$$

where

$$\Gamma_1 = t_p + T,$$

and

$$\Gamma_2 = \frac{e^{t_p \delta} - 1}{\delta} + \frac{e^{t_p(\alpha+\delta+\mu)-T(\alpha+\mu)}(e^{T(\alpha+\mu)} - 1)}{\alpha + \mu}.$$

The positive expressions Γ_1 and Γ_2 depend entirely on properties of the proliferative program and not on the pathogen properties. If the fraction of memory T cells recruited into the response is modelled as a saturating function of the initial pathogen load, as discussed previously, then if P' is the pathogen load after the response,

$$P' = P \exp\left(\Gamma_1 \psi - \Gamma_2 N \kappa \frac{P}{\eta + P}\right). \tag{6.10}$$

To determine stationary points the equation is first simplified by letting $A = \exp(\Gamma_1 \psi)$ and $B = \Gamma_2 N \kappa$ so that

$$P' = A P \exp\left(-B \frac{P}{\eta + P}\right).$$

Solving for P when

$$\frac{dP'}{dP} = 0,$$

gives two stationary points,

$$P_1^* = \frac{\eta}{2}(B - 2 + \sqrt{B^2 - 4B}).$$

and

$$P_2^* = \frac{\eta}{2}(B - 2 - \sqrt{B^2 - 4B}),$$

Both points are only real and positive when $B \geq 4$. To determine the nature of P_1^*,

P' is differentiated a second time w.r.t. P,

$$\frac{\mathrm{d}^2P'}{\mathrm{d}P'^2} = A\,B\,\exp\left(-\frac{B\,P}{P+\eta}\right)\frac{((B-2)P-2\eta)\,\eta^2}{(P+\eta)^4}.$$

When $P = P_1^*$ the RHS> 0 when $B > 4$, and the second stationary point is therefore a minimum when $\Gamma_2\,N\,\kappa > 4$. The presence of this minimum is shown graphically in Figure 6.6. The same method reveals P_2^* to be a maximum for $B > 4$.

Since the pathogen load after the response, P', passes through a minimum as the initial pathogen load, P, is increased, and given that the size of the second proliferative response decreases with decreasing P' (fewer memory cells would be activated), it is evident why the peak size of the proliferative response passes through a minimum as the initial pathogen load is increased.

Time until clearance

It is analytically difficult to determine the time at which the pathogen is driven below the threshold, p_T. Instead the time until the pathogen load peaks is determined. Experimental observations suggest that this is a close approximation to the actual time of clearance since the pathogen is generally cleared soon after [209]. A simplified version of the main model is considered.

During the exponential expansion the T cells can be considered to be spread between three stages:

1. Expansion. The T cells undergo about I_δ divisions. The average time spent in each division is $1/\delta$, so the approximate total time in this stage will be $\Lambda_E \approx I_\delta/\delta$.

2. Contraction. T cells stay in this stage until they differentiate into memory, which gives $\Lambda_C \approx 1/\mu$.

3. Recovery. Cells will first spend a mean time of $1/\rho$ days as pre-memory cells. The time spent as a memory cell will depend on the pathogen load but assuming a high pathogen load the rate of activation can be approximated as γ, the time

spent as a full memory cell will be $\approx 1/\gamma$. Therefore the mean time spent in this 'recovery stage' is $\Lambda_R \approx 1/\rho + 1/\gamma$.

The rate of change of the entire T cell population, w, will therefore be

$$\dot{w} = \frac{\Lambda_E}{\Lambda}\,\delta\, w - \frac{\Lambda_C}{\Lambda}\,(\alpha + \mu)w$$

where Λ is the total average time it takes cells to move through all the stages ($\Lambda_E + \Lambda_C + \Lambda_R$). Because the cells in the third stage have homeostatically balanced division and death rates their behaviour will not affect the size of the total population. Solving this equation gives

$$w = W \exp(F\,t),$$

where W is the initial size of the population and

$$F = \frac{I_\delta - (\alpha + \mu)/\mu}{\Lambda}.$$

It is seen that the population can expand or contract depending on the parameter values. If

$$I_\delta - (\alpha + \mu)/\mu > 0. \tag{6.11}$$

exponential expansion will occur, whereas if the LHS$<$ 0 the population size will decline exponentially. The amplification of the response determines whether expansion or contraction occurs. The peak size of the effector population, X, can be written

$$X = N\,\mathrm{e}^{I_\delta},$$

because the rate of expansion is δ but the time of expansion is I_δ/δ. The memory size can be expressed as

$$M = X \exp\left(-\frac{\alpha + \mu}{\mu}\right),$$

because the rate of contraction is $\alpha + \mu$ and the average time before entering the

memory population is $1/\mu$. The amplification of the response, $\theta = M/N$, is therefore

$$\theta = \exp\left(I_\delta - (\alpha + \mu)/\mu\right).$$

It can then be seen that the condition for an expanding T cell population, Equation 6.11, is equivalent to the condition for a positive amplification ($\theta > 1$). Therefore if $\theta > 1$ the T cell population will continue to expand exponentially and the pathogen will be cleared with increasing rapidity. An expression for the time until the pathogen load peaks is next calculated.

Generally, a rapid decline follows the peak in pathogen load and clearance is soon achieved by the continuing exponential expansion of the T cell effector population; this appears to be supported by experimental observations [209, 2]. Therefore the peak time, t_{peak}, is calculated and used as an approximation for clearance time. At the pathogen peak, $\dot{p} = 0$, and letting $x = w\,\Lambda_E/\Lambda$, Equation 6.8 gives

$$\psi - \kappa \frac{\Lambda_E}{\Lambda} W \exp\left(F\,t\right) = 0,$$

which rearranges to give

$$t_{peak} = \frac{1}{F} \ln \frac{\Lambda\,\psi}{\Lambda_E\,W\,\kappa}.$$

It can be seen that increasing the pathogen replication rate and/or decreasing the clearance rate delays the time of pathogen clearance.

If $\theta < 1$ (i.e. $I_\delta - (\alpha + \mu)/\mu < 0$) then pathogen clearance may not occur. The declining specific T cell population will continue to destroy pathogen but its power will diminish with its size. If it shrinks too far before the pathogen is pushed sub-threshold it will no longer be able to control the pathogen growth.

The relationship between the pathogen peak time and the parameters was then investigated through simulations. For sufficiently high replication rates and low clearance rates the simulation results agreed well with the analytical expresssion. A logarithmic relationship was observed between t_{peak} and ψ and $1/\kappa$. For low replication rates, or high clearance rates, however, the pathogen load peak time was relatively insensitive to changes in these rate parameters. This may be expected because the

pathogen would be sufficiently "weak" for the T cells to drive their numbers down almost immediately following the commencement of proliferation.

In summary, the model predicts that pathogen persistence can induce a continuous exponential expansion in the T cell population which forces down the pathogen load. Generally this response this will rapidly clear the infection but if the pathogen load becomes very low, and there is a deficit of effector cells, the characteristics of the T cell response can alter substantially. This is discussed in the next section.

The relationship between the pathogen properties and the T cell kinetics were also established. For rapidly replicating pathogens with strong resistance to clearance the time and size of the largest peak in T cell number correlates with both the replication and clearance rates. In contrast, for slowly replicating pathogens with a weak resistance to clearance, the T cell peak time and size is insensitive to these pathogen properties. For large initial pathogen loads, the T cell peak size correlates with this load (whereas the peak time is insensitive to it). For low initial loads however, the T cell peak size and time actually *decrease* with increases in the load.

6.3.2 Clearance by Linear Response

The model predicts that persisting pathogen can also elicit a quantitatively different T cell response. After an initial period of exponential expansion and contraction the cell number can begin to increase linearly (Figure 6.7a). During this linear increase the pathogen load decreases with a decreasing rate (Figure 6.8) resulting in a very slow rate of decline at late times. When the pathogen load falls below the threshold load and clearance has been accomplished the T cell population terminates its steady expansion (Figure 6.7b).

The reason for this change in the nature of the response is a change in the rate limiting step of T cell activation at low pathogen loads. At high loads the limiting steps are the slow transits from the effector pool to the pre-memory pool and from the pre-memory pool to the memory pool. At low pathogen loads, however, the limiting step becomes the activation of memory cells because the rate of activation is effectively proportional to the load. When the pathogen load is high the rate of

memory activation is high and is not a rate limiting step, so the resulting steady flow of cells leaving the effector pool, and then re-entering, will allow exponential expansion of the T cell population. The large number of effectors produced will drive down the pathogen load until it becomes sub-threshold. However, if the number of effector cells falls sufficiently low while the pathogen load is still above its threshold value, p_T, the pathogen load may begin to increase. The pathogen response is again modelled with Equation 6.8. Therefore if $x < x_T$, where

$$x_T = \frac{\psi}{\kappa},$$

then $\dot{p} > 0$ and the pathogen load will start to increase. This can cause a fundamental change in the T cell kinetics. The load will only increase until the number of effectors exceeds ψ/κ, and therefore the pathogen load will remain sufficiently low to maintain the activation of memory cells as the limiting step. This produces a steady linear expansion in the T cell population and decline in the pathogen load at a rate that decreases with time. This decrease results from the decrease in the rate of memory activation as the pathogen load drops.

The effect of the replication rate on the duration of persistence is shown in Figure 6.9. It is observed that as the replication rate is decreased below a critical value the persistence time suddenly rises considerably. Further decreases in the replication rate decrease the persistence time slowly at first and then rapidly, so that very low replication rates produce a low persistence time. The sudden change in persistence time at the critical value corresponds to the transition between two different outcomes of the initial exponential T cell response; this primary response either succeeds or fails to push the pathogen load below the threshold. The resulting pathogen re-growth following failure elicits only the linear, rather than exponential, T cell response which is capable of only slow clearance.

Long Term Behaviour

To investigate the long term behaviour of the system, a difference-equation model to represent the kinetics of the memory T cells and the pathogen is proposed. Although

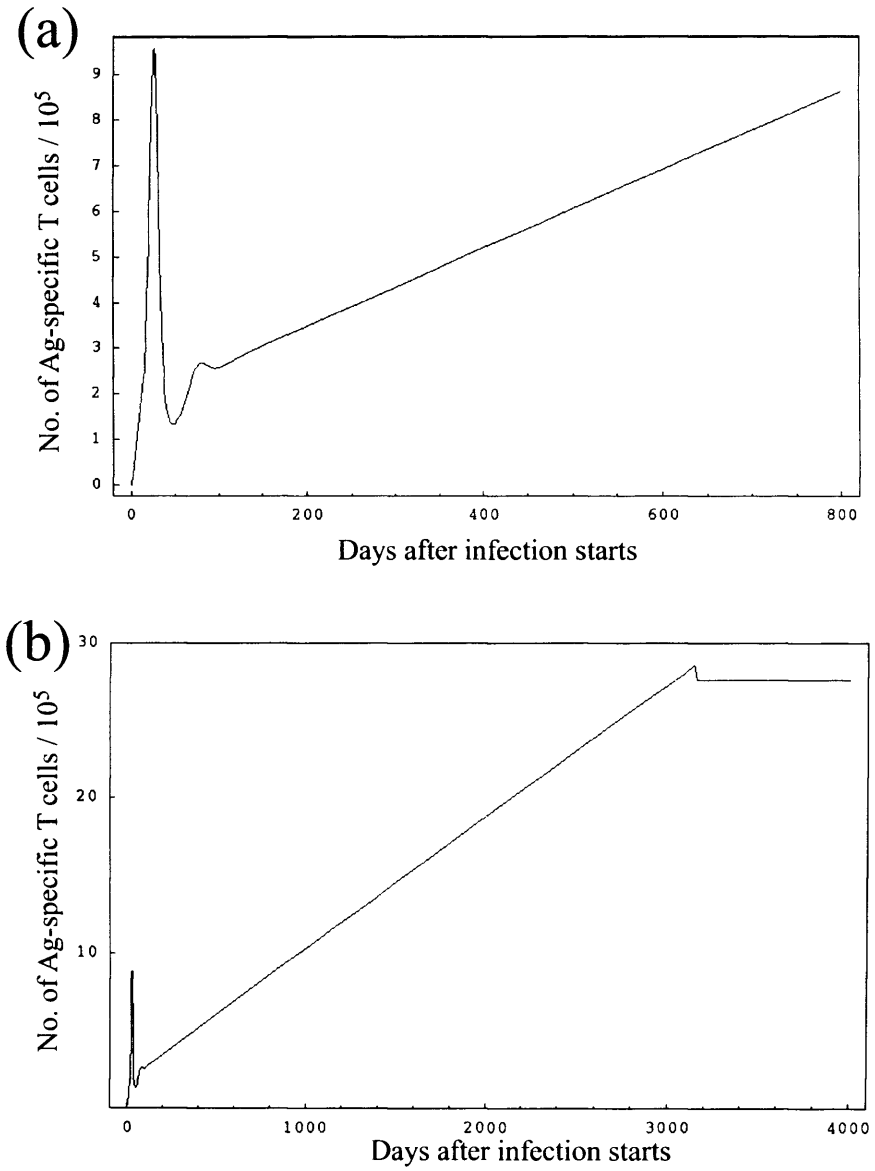


Figure 6.7: The Ag-specific T cell kinetics when pathogen persists at low level. (a) Following expansion and contraction T cell number increases linearly. (b) When the pathogen is finally cleared the specific T cell population contracts to a stable size. Parameter values : same as Figure 6.2 except $\kappa = 10^{-6}$ / day

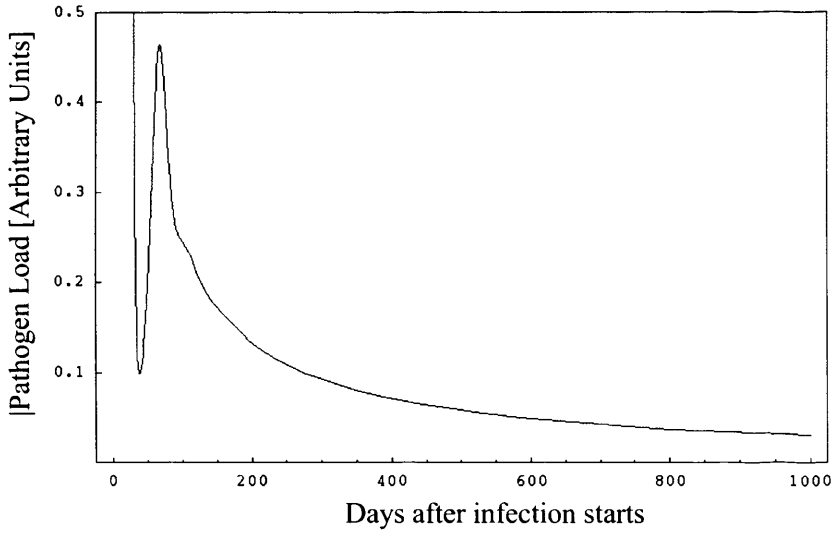


Figure 6.8: After a transient resurge in the pathogen load it declines at a decreasing rate. Parameter values : same as Figure 6.2 except $\kappa = 10^{-6}$/ day and $\psi = 0.1$ / day.

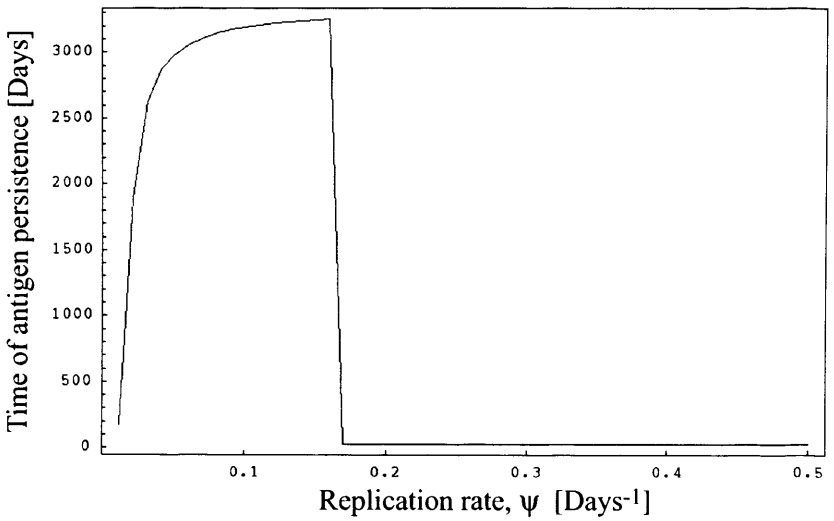


Figure 6.9: The time taken to clear the pathogen for different pathogen replication rates. Parameter values: same as Figure 6.2 except ψ varies.

memory cells will form and reactivate at different times, in this model, for simplicity, it is assumed that all memory cells form at time t_R after the initiation of the response and are immediately receptive to reactivation. At this time a new response will be initiated if persistent pathogen exists and the number of memory cells recruited into this response will be a function of the current pathogen load.

The expression previously derived for the pathogen load after the initial response, Equation 6.10, is adapted for this new purpose. Its new general form is

$$p_{j+1} = p_j \exp\left(\Gamma_1\psi - \Gamma_2\,\kappa\,\gamma\,m_j\,\frac{p_j}{\eta + p_j}\right), \tag{6.12}$$

where p_j and m_j are the pathogen load and memory size respectively after j programmed T cell responses.

The memory size at time t_R after a programmed response will equal the number of memory cells activated, $\gamma\,m_j\,p_j/(\eta + p_j)$, times an amplification factor, θ, plus the number of unactivated memory T cells,

$$m_{j+1} = m_j + (\theta - 1)\,\gamma\,m_j\left(\frac{p_j}{\eta + p_j}\right).$$

or,

$$m_{j+1} = m_j + \Theta\,m_j\left(\frac{p_j}{\eta + p_j}\right), \tag{6.13}$$

where $\Theta = (\theta - 1)\,\gamma$.

Two situations are considered: low and high pathogen loads.

1. *High pathogen load*

For a high pathogen load, when $p_j >> \eta$, the difference equations will simplify to

$$p_{j+1} = p_j \exp\left(\Gamma_1\psi - \Gamma_2\,\kappa m_j\right), \tag{6.14}$$

and

$$m_{j+1} = m_j + \Theta\,m_j. \tag{6.15}$$

From equation (6.15) it can be deduced that

$$m_j = m_0(\Theta + 1)^j, \tag{6.16}$$

and if this expression is substituted into equation (6.14)

$$p_{j+1} = p_j \exp\left(\Gamma_1\psi - \Gamma_2\,\kappa m_0(\Theta + 1)^j\right). \tag{6.17}$$

If the amplification of the response, θ, is greater than one then $\Theta > 0$ and the memory population will increase exponentially with increasing j and the pathogen load will asymptote to zero as $j \to \infty$. The pathogen behaviour when it reaches low levels is addressed in the next section.

2. *Low pathogen load*

When the pathogen load is low, $p_j << \eta$, the difference equations will simplify to

$$p_{j+1} = p_j \exp\left(\Gamma_1\psi - \tilde{\Gamma}_2\,\gamma\,\kappa\,m_j\,p_j\right), \tag{6.18}$$

and

$$m_{j+1} = m_j + \tilde{\Theta}\,m_j\,p_j, \tag{6.19}$$

where $\tilde{\Theta} = \Theta/\eta$ and $\tilde{\Gamma}_2 = \Gamma_2/\eta$.

To analyse for fixed points a new variable z is introduced where

$$z_j = p_j\,m_j,$$

and from equations (6.18) and (6.19),

$$z_{j+1} = z_j \exp\left(\Gamma_1\psi - \tilde{\Gamma}_2\,\gamma\,\kappa\,z_j\right)\,(1 + \tilde{\Theta}\,p_j),$$

and,

$$p_{j+1} = p_j \exp\left(\Gamma_1\psi - \tilde{\Gamma}_2\,\gamma\,\kappa\,z_j\right).$$

Fixed points are found by setting $p_{j+1} = p_j = p^*$ and $z_{j+1} = z_j = z^*$, to give

$$z^* = z^* \exp\left(\Gamma_1\psi - \tilde{\Gamma}_2\gamma\kappa z^*\right)(1 + \tilde{\Theta}p^*),$$

and,

$$p^* = p^* \exp\left(\Gamma_1\psi - \tilde{\Gamma}_2\gamma\kappa z^*\right),$$

which solve to give two fixed points:

$$(z_1^*, p_1^*) = (0, 0)$$

and

$$(z_2^*, p_2^*) = \left(\frac{\Gamma_1\psi}{\tilde{\Gamma}_2\gamma\kappa}, 0\right).$$

To determine the stability of these fixed points the difference equations are linearised about these points. If $z_{j+1} = f(z_j)$ then the stability at $z_j = z^*$ can be found from the modulus of $f'(z^*)$. The fixed point, z^*, is asymptotically stable if $|f'(z^*)| < 1$, and unstable if $|f'(z^*)| > 1$.

Differentiating z_{j+1} w.r.t. z at z^*,

$$f'(z_j) = \frac{\mathrm{d}z_{j+1}}{\mathrm{d}z_j} = \exp\left(\Gamma_1\psi - \tilde{\Gamma}_2\gamma\kappa z_j\right) - \tilde{\Gamma}_2\gamma\kappa z_j \exp\left(\Gamma_1\psi - \tilde{\Gamma}_2\gamma\kappa z_j\right),$$

when $p = 0$. At the first fixed point

$$f'(0) = \exp(\Gamma_1\upsilon),$$

and because Γ_1 and ψ are greater than zero this point is unstable. At the second fixed point

$$f'\left(\frac{\Gamma_1\psi}{\tilde{\Gamma}_2\gamma\kappa}\right) = 1 - \Gamma_1\upsilon,$$

and therefore the second fixed point is asymptotically stable if $0 < \Gamma_1\psi < 2$. Taking T to be the same order as t_p, about 8 days, this gives $\Gamma_1 \approx 16$. Therefore the fixed point is only stable for $\psi < 0.13$. This magnitude agrees well with results shown in

Figure 6.9 where the long persistence times, associated with the linear T cell response, only occur for replication rates less than about 0.16 day^{-1}.

In conclusion, for very low replication rates, the memory size will slowly grow while the pathogen is slowly cleared. While the pathogen load asymptotes to zero, the product of the memory population size and the pathogen load asymptotes to a constant value. The time until pathogen clearance is achieved is determined in the next section.

Time to clearance

The model predicts that for a very low pathogen replication rate, after transient behaviour, the size of the memory pool exhibits an approximately linear expansion. If during this period $m_j p_j$ is approximated to its asymptotic value,

$$m_j p_j = \frac{\Gamma_1 \psi}{\tilde{\Gamma}_2 \gamma \kappa} \tag{6.20}$$

then equation (6.19) becomes

$$m_{j+1} = m_j + \tilde{\Theta} \frac{\Gamma_1 \psi}{\tilde{\Gamma}_2 \gamma \kappa},$$

from which it can be deduced that

$$m_j = m_0 + j \tilde{\Theta} \frac{\Gamma_1 \psi}{\tilde{\Gamma}_2 \gamma \kappa}.$$

Substituting this expression into equation (6.20) gives the pathogen load after j timesteps,

$$p_j = \frac{\Gamma_1 \psi}{\tilde{\Gamma}_2 \gamma \kappa} \left(\frac{1}{j \tilde{\Theta} + m_0 \Gamma_2 \kappa / \Gamma_1 \psi} \right).$$

Given that each timestep has a duration of t_R days, $t = j t_R$, and given that the pathogen is effectively cleared when its load drops below the threshold value, p_T,

rearranging this expression reveals the time t_C of clearance,

$$t_C = \frac{t_R}{\tilde{\Theta}} \left(\frac{1}{p_T} - \frac{\tilde{\Gamma}_2 \gamma \kappa m_0}{\Gamma_1 \psi} \right)$$

The model predicts that if the pathogen is cleared by a linear T cell response then the time until clearance correlates negatively with the clearance rate and positively with the replication rate. Therefore the pathogens most able to persist are those with replication rates within a narrow range. The rate must be sufficiently low to initiate the slow linear expansion of the T cell population, yet not too low such that the linear expansion achieves clearance rapidly. It would be very interesting to experimentally determine whether the replication rates of the most persistence infections lie within this range.

A further prediction is that for a pathogen that elicits the linear T cell expansion, the time of persistence correlates negatively with the initial number of memory cells. This suggests that when several individuals infected with the same pathogen resolve their infections in varying times, the differences may be caused not by differences in the pathogen strains, or in the nature of their T cells, but simply in their initial quantity of antigen-specific T cells.

For both low and high pathogen loads, if the amplification of a proliferative response is less than one, the specific memory population size will decrease exponentially, and asymptote to zero. The same mechanism was discussed in chapter 5 in the context of repeated tolerising stimulations. While the memory population declines, the pathogen will grow exponentially at an increasing rate (tending to its maximum value ψ as $m_j \to 0$) thereby escaping clearance.

6.4 Discussion

In this chapter the relationship between a pathogen's properties and its ability to persist and prolong the T cell response is explored. Within the framework of the compartmental model, it was found that a pathogen could not achieve permanent persistence by altering its kinetic properties (i.e. replication rate, rate of clearance by

T cells, or initial load). Only a pathogen that elicited a programmed T cell response that produced negative amplification (when the final size of activated population is smaller than initial size) could persist permanently. However, within the category of pathogens that always elicit a positively amplifying T cell response, those that replicate very slowly can persist for a very long period of time. Normally infection is rapidly resolved by the fast exponential expansion in the T cell population. Very slowly replicating pathogens, however, instead elicit a slow linear expansion in the population which achieves clearance only very slowly.

Experimental data supports the existence of two distinct types of T cell response following the initial expansion. Generally, *in vivo*, exponential expansion precedes the clearance of pathogen [35, 121], but linear expansion has been observed to accompany some chronic infections [200], most noticeably in the CD8 T cell response to murine cytopathic β-herpes virus (MCMV) infection [123, 219]. The slowness of pathogen clearance by this linear response would, if unchecked by homeostatic mechanisms, result in the enormous growth of T cell clones over a period of years. This may explain the very large number of HCMV-specific CD8 T cells present in seropositive individuals long after resolution of the primary response, and the surprisingly large T cell clones possessed by some elderly people who show no symptoms of disease [220, 221].

The prediction of the model that more slowly replicating pathogens are more able to persist is supported by number of experimental observations [209, 210]. Some clinical and experimental evidence indicates that more slowly replicating mutants of HBV and HCV are observed in patients with chronic hepatitis [222, 223]. In a comparison of two patients infected with different strains of HCV, one individual cleared the infection, whereas the other, with a more slowly replicating strain, developed a chronic infection [224]. Finally, recent studies of HCV infection in chimpanzees indicate that a more slowly growing viral load correlates with longer persistence [225, 226].

The model agrees with the experimental observation that the peak time of the T cell kinetics is insensitive to substantial variation in the pathogen properties [142, 35]. In the model changing the clearance rate or initial pathogen load by several orders of magnitude has no effect on the peak time.

The effect of the initial pathogen load on the subsequent response can vary substantially. At medium initial loads there is a positive correlation between the load and the peak size, the peak time is constant. The peak size then becomes insensitive to the load as it reaches high levels. This agrees with experimental observations of a constant peak time but increasing peak magnitude as the intial pathogen dose is increased [35]. For low initial loads however, an unexpected negative correlation is observed between the initial load and both the peak size and time. The smaller the initial dose the later and larger the T cell response. There are a number of possible reasons why this surprising response has not been characterised in experimental studies. Firstly, experiments generally involve large antigen doses with the aim of eliciting a large response. Secondly, when analysing the primary response T cell numbers are not normally measured beyond the second week. The results here reveal that the peak response may not peak for many weeks. Thirdly, action of innate immunity, serving to rapidly wipeout the tiny infection, may annul this response for the pathogens commonly used in experimental studies. Many pathogens, however, are particularly resistance to the innate system and may generate substantial infection even when the initial dose is small.

CHAPTER 7
LONG TERM CHANGES IN THE PROLIFERATIVE PROGRAM

Ever fair from fair sometime declines,
By chance or nature's changing course untrimmed.
Shakespeare (1564-1616)

Following resolution of an infection, the responding T cell population contracts, homeostasis is restored, and a stable population of memory cells is established [227, 228, 229, 230, 231]. Failure to clear the pathogen, however, can lead to a disturbance in this stability. A number of infections are known to produce a gradual expansion in the specific T cell population [123, 198, 200]. Conversely, following the acute response to certain pathogens, some T cell populations have also been observed to slowly shrink. The most infamous example is the terminal decline in the entire CD4 T cell pool that accompanies HIV infection [232]. More common is the decline in just the T cell population specific for the persisting pathogen. LCMV infection can produce long term declines in both specific CD4 [51, 1, 198] and CD8 [200] T cells. Similarly, a strain of herpes virus, γHV68 produces a gradual decrease in the specific CD4 population [208]. The same outcome is also seen with a bacterial infection [199]. While a gradual expansion in T cell number is explained by repeated antigenic stimulation [123], the reason for gradual declines is not known.

There have been various explanations of why this decline occurs. A defect in the homeostatic mechanism that maintains the size of memory populations has been proposed [51, 1]. Complex cytokine-mediated control mechanisms are believed to achieve a balance between division and death but a small disruption may allow death to dominate thereby producing a net contraction. De Boer *et al* [1] showed that

this could be the mechanism by fitting a mathematical model to *in vivo* data for a CD4 T cell response to LCMV infection. This theory, however, does not explain why most T cell populations do not exhibit continuous contraction [196, 197]. Also the observation that T cells specific for different epitopes decrease at different rates [200] suggests that antigen plays a crucial role. High levels of persisting pathogen are known to cause clonal deletion through activation-induced death (AICD) [60, 152] and this may account for declining population sizes. Yet this fails to explain why a decline can occur with much lower pathogen loads than are thought necessary to produce this form of death [51, 1]. Furthermore, high levels of persisting pathogen have been observed to produce pronounced T cell activation and division [198]. Alternatively, during a chronic infection, specific T cells may repeatedly undergo a modified version of the proliferative program that exhibits a smaller expansion than contraction, and this therefore produces a net population shrinkage.

The proliferative program normally manifests a net increase in the population size, but this is not always the case. Certain modifications in the program, such as a reduced rate of memory phenotype acquisition can result in a net decrease; this was shown in Chapter 5. Such modifications can be encoded by certain antigenic signals. Low densities of antigen presented by APCs or the absence of co-stimulation reduce the receptiveness of effector T cells to cytokines, such as IL7 and IL15, that promote memory formation [206]. The consequence of repeated stimulations of this nature would therefore be a stepwise decrease in T cell population size. Persisting antigen is known to repeatedly stimulate memory T cells [233] and could therefore induce a steady decline in specific T cell number.

During chronic infections the effectiveness of specific T cells appears to diminish. It may be that this diminution results from a reduced production of effector cytokines [198], but it is not clear whether this occurs at the cell or population level. It has also been suggested that cells are born with a strict limit to their capacity for division, and once they exhaust their life-supply of divisions they die; this aging process is termed senescence [140]. But evidence suggests that this aging process is frozen during a proliferative response [234]. Another hypothesis is that the nature of the proliferative program changes through repeated stimulation. This change could be

a decrease in the amplification of the programmed proliferation, resulting from cells undergoing fewer divisions, dying sooner or faster, or differentiating more slowly into memory. Alternatively the change could be a reduction in the sensitivity of the cells to stimulation.

Most mathematical models to investigate long term decline in T cell population sizes are those concerned with HIV [6], and generally the decline has been attributed to the killing of T cells by the virus. To date, no published models have considered the proliferative program in the scenario of chronic infection. The purpose of the current study is determine firstly whether repeated stimulation of T cells can produce the observed decline in T cell number, and, secondly, to determine whether the nature of the program alters through repeated activation of the cells. Two variations on the model for chronic infection developed in the previous chapter are fitted to *in vivo* CD4 T kinetics during an LCMV infection. The model that provides the best fit is assumed to most accurately reflect the real situation. The best fit is obtained for the model in which there is a progressive decline in the sensitivity of the specific T cells to activation.

7.1 Method

A discrete-time model was derived in the previous chapter for the load of a persisting pathogen, p, and the specific memory T cell population, m after j timesteps (where each represents one programmed period of proliferation including the contraction) during a chronic infection. Its form was

$$p_{j+1} = p_j \, e^{\Gamma_1 \psi - \Gamma_2 m_j \, p_j \, \gamma \, \kappa},$$

$$m_{j+1} = \Theta \, p_j \, m_j,$$

where γ and κ are the rates of activation and clearance respectively. As previously defined, $\Theta = (\theta - 1)\,\gamma$ and γ is the rate at which memory T cells are activated, and θ represents the amplification of a T cell population that enters the proliferative program - if one cell enters the program then θ cells will exit. The parameter Θ is

the net amplification and can take negative values which signify the shrinkage of a T cell population.

To simplify the fitting of this model to data, the system is re-formulated into a system of differential equations (details in Appendix E). The first equation in the model describes the rate of change of the pathogen load,

$$\dot{p} = (\psi - \kappa\,\gamma\,m\,p)p, \tag{7.1}$$

where ψ is the replication rate, and γ and κ are the memory activation and pathogen clearance rates respectively.

The second equation in the continuous-time model describes the rate of change in the memory population,

$$\dot{m} = \Theta\,p\,m. \tag{7.2}$$

The differential equation model is a good approximation for the difference equation when the time taken to complete a proliferative program is much shorter than the time scales considered. Since the data fitted in this study spans 1-3 years and a program duration is only a few weeks, this model is deemed to be suitable.

An important initial assumption is that antigen persists in the host at a constant load during the time considered. In the model this gives $\dot{p} = 0$. In the experimental setup the viral load drops below the level which the standard technique can detect. Despite making the assumption that clearance is achieved in their analysis the authors admit that the LCMV virus may still persist. Many types of pathogen, including some strains of LCMV, have been shown to persist in the host following a T cell response normally indicative of clearance [235]. Such pathogens may persist in sites inaccessible to T cell attack, such as inside macrophages, and can enter a latent phase, suppressing its own replication. A constant pathogen load has been observed during chronic infection with some strains of LCMV [198, 200]. Alternatively, fragments of antigen may become complexed on the surface of a type of long lived APC known as a follicular dendritic cell and continuously presented to T cells in the lymphoid tissues [97]. Overall, there are a number of ways in which pathogen can persist at an approximately constant low level. This assumption is relaxed later in the chapter to

investigate the consequences of a changing pathogen load.

Three different models are considered for the change in the the program response over time:

1. **Unchanging**. All aspects of the program remain constant.
2. **Decreasing Amplification**. The average number of T cells produced from each cell that enters the proliferative program decreases exponentially with increasing time.
3. **Decreasing Activation**. The rate at which re-stimulation induces cells to enter the program decreases exponentially with increasing time.

The three models are referred to as the *Unchanging*, *Amplification* and *Activation* models respectively.

7.1.1 Unchanging

Here the situation is modelled where the nature of the program does not change during a chronic infection. As previously discussed, the load of the persisting pathogen is assumed to be fixed, i.e. $\dot{p} = 0$. If $p(t) = p_c$, then equation (7.2) takes the form

$$\dot{m} = \Theta p_c m,$$

This solves to give

$$m(t) = M_0 \, \mathrm{e}^{\Theta p_c t}, \tag{7.3}$$

where M_0 is a constant. To fit to the data the logarithm is taken of this equation to give

$$\log_{10} m = K_1 - K_2 t, \tag{7.4}$$

where

$$K_1 = \log_{10} M_0 \text{ and } K_2 = \Theta p_c \log_{10} \mathrm{e}$$

7.1.2 Decreasing Amplification

Here the amplification of cell number produced by the program declines exponentially over time. It is important to make the distinction between the amplification of the response θ, and the net amplification Θ. The parameter θ is the number of cells that enter the memory pool for every cell that enters the proliferative program, for instance if 10 cells enter the program and then produce a memory population of 100 cells then $\theta = 10$. The parameter can take values from 0 to ∞. Because $\Theta = (\theta - 1)\,\gamma$ it can take values from $-\gamma \rightarrow \infty$ and represents the net change in size of a T cell population produced by the program; positive and negative values represent expansion and contraction respectively. Here the decline in the amplification θ is modelled. Assuming exponential decline this gives

$$\dot{m} = (\theta \exp(-\beta t) - 1)\,\gamma\, p_c\, m,$$

which solves to give

$$m = M_0 \exp\left(\frac{\theta\,\gamma p_c}{\beta}\,(1 - \exp(-\beta t) - \beta t)\right). \tag{7.5}$$

To fit this equation to the data the logarithm is taken to give

$$\log_{10} m = K_3 - K_4(\mathrm{e}^{\beta t} - \beta t), \tag{7.6}$$

where

$$K_3 = \log_{10} M_0 + \frac{\theta\,\gamma p_c}{\beta}\log_{10}\mathrm{e}, \text{ and } K_4 = \frac{\theta\,\gamma p_c}{\beta}\log_{10}\mathrm{e}.$$

7.1.3 Decreasing Activation

Here the situation is modelled where, through repeated activation, the memory T cells become increasingly insensitive to activation. When activated the full program unfolds but it is assumed that the rate of activation, γ, decreases exponentially, so that

$$\dot{m} = (\theta - 1)\gamma \exp(-\beta t)\, p_c\, m, \tag{7.7}$$

which solves to give

$$m = M_0 \exp\left(\frac{\Theta p_c}{\beta}\left(1 - \exp\left(-\beta t\right)\right)\right). \tag{7.8}$$

To fit to the data the logarithm is taken of this equation to give

$$\log_{10} m = K_3 - K_4 \mathrm{e}^{-\beta t}, \tag{7.9}$$

where

$$K_5 = \log_{10} M_0 + \frac{\Theta p_c}{\beta} \log_{10} \mathrm{e}, \text{ and } K_6 = \frac{\Theta p_c}{\beta} \log_{10} \mathrm{e}.$$

7.1.4 Comparing the models

For each model the best fit to experiment data is determined using the line search algorithm from Chapter 3. The data is obtained from ref. [51], which details longitudinal studies of specific CD4 T cell numbers during LCMV infection of mice. Because the model only predicts the number of memory T cells, data for times during the primary response (less than 30 days) are not used since during this time the majority of cells are effector rather than memory cells. Data for two cell populations, one specific for the dominant epitope (GP), the other for the subdominant (NP), are independently fitted by the models to check the generality of the results and to compare differences in kinetics. In a similar manner to Chapter 3, to find the best fit to the experimental data, the algorithm minimises

$$\chi^2 = \sum_{j=1}^{J} \frac{(\log_{10} e_j - \log_{10} m(t_j))^2}{\mathcal{L}_j^2}.$$

The value e_j is the experimentally determined number of memory T cells at time t_j, where j is the j-th experimental data out of J points. The value $\mathcal{L}_j$ is the error on the mean, ϵ_j, of the j-th data point transformed to logspace. Because the error bars are non-symmetrical in logspace, the weighting factor used for each data point depends

on whether the prediction of the model is greater or less than the data point,

$$\mathcal{L}_j = \begin{cases} \log_{10}(e_j + \epsilon_j) - \log_{10} e_j & \text{If } m_j > e_j, \\ \log_{10} e_j - \log_{10}(e_j - \epsilon_j) & \text{else.} \end{cases}$$

To compare the relative likelihood of each model representing the actual mechanism, Akaike's Information Criterion (AIC) is used [236]. This method allows one to determine which of two models is more likely to be correct and quantify how much more likely. For the fit of a model to data the AIC value, $\mathcal{A}$ is calculated with the equation

$$\mathcal{A} = Z \ln \frac{SS}{Z} - 2Y,$$

where Z is the number of data points, Y is the number of parameters plus one and SS is the weighted sum of the squares of the vertical distances of the data points from the model-generated curve.

To calculate the probability, $\mathcal{P}$, that a particular model is more likely to be the correct model than another one, the difference between their AIC values, $\Delta\mathcal{A}$, is used,

$$\mathcal{P} = \frac{\mathrm{e}^{-0.5\Delta\mathcal{A}}}{1 + \mathrm{e}^{-0.5\Delta\mathcal{A}}}.$$

7.2 Results

Firstly, it is observed that good fits to the experimental data (figures (7.1a) and (7.1b)) can be obtained. This demonstrates that the decline in the size of a T cell population may result from repeated antigenic stimulation, where each stimulation produces a net decrease in population size. This is the first time that this has been shown.

Secondly, it is observed that the *Activation* model produces a better fit than do the other models for responses to both the GP epitope (figure 7.1a) and the NP epitope (figure 7.1b). To compare the likelihoods of each model being correct, AIC is used and the results are given in table 7.1. Comparing the *Unchanging* and *Activation* models for the GP epitope the test reports a 99.99% chance of the *Activation* model being

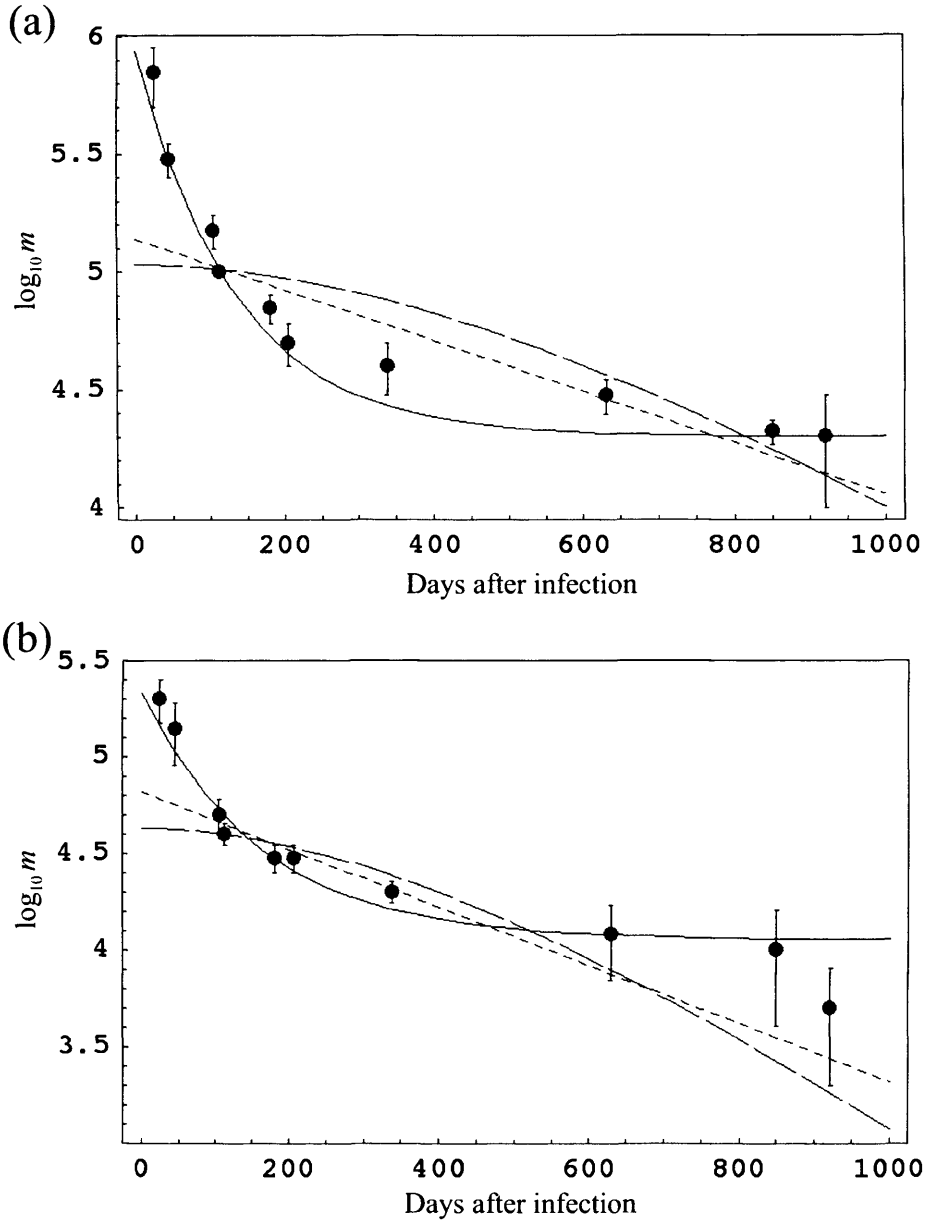


Figure 7.1: Fitting the three models to the declining CD4 T cell response for the (a) dominant (GP61), and (b) subdominant (NP309) epitopes. The *in vivo* data was sourced from Ref. [51]. The bold, small dashed and large dashed lines represent the *Activation*, *Unchanging* and *Amplification* models respectively. Parameter values are given in table (7.1)

correct compared to the *Unchanging* model given the experimental setup, and a 99.5% probability for epitope NP. Similiar probabilities, also favouring the *Activation* model, are reported when comparing the *Activation* and *Amplification* models. The results suggest that the proliferative program does progressively change through repeated stimulation and this change manifests itself as an increase in the resistance of the cells to activation.

Tested Model	**Specific Epitope**	**Parameter Values**	**Sum of Squares**	**AIC**
Unchanging	GP61	$K_1 = 5.1, \quad K_2 = 0.0011$	72.6	13.8
	NP309	$K_1 = 4.8, \quad K_2 = 0.0015$	26.5	3.7
Amplification	GP61	$K_3 = 6.7, \quad K_4 = 1.7, \quad \beta = 0.0014$	98.2	14.8
	NP309	$K_3 = 6.3, \quad K_4 = 1.7, \quad \beta = 0.0018$	45.9	7.2
Activation	GP61	$K_5 = 4.3, \quad K_6 = 1.6, \quad \beta = 0.0075$	14.1	−4.6
	NP309	$K_5 = 4.1, \quad K_6 = 1.3, \quad \beta = 0.0062$	11.2	−6.9

Table 7.1: The parameter values for the best fit of each model to data for two types of CD4 T cell each specific for a a different epitope. The sum of squares and AIC for each fit are also shown.

For the *Activation* model it is observed that the rate of activation of the memory T cell population specific for the dominant epitope declines at a faster rate than for the subdominant epitope (The parameter β is greater for the dominant epitope). This is consistent with the hypothesis that the decline in T cell number is caused by repeated activation. It would be expected that the dominant epitope would activate its specific T cell population more rapidly and hence cause a more rapid decline in population size.

An alternative interpretation of the *Activation* model is that the pathogen load, rather than the rate of re-activation, is decreasing exponentially. It is obvious from equation (7.7) that an exponentially declining pathogen load and constant activation rate would produce an equivalent model to a constant pathogen load and exponentially declining activation rate. This is not, however, a feasible scenario. It is clear from equation (7.1), for the rate of change of the pathogen load, that an exponentially

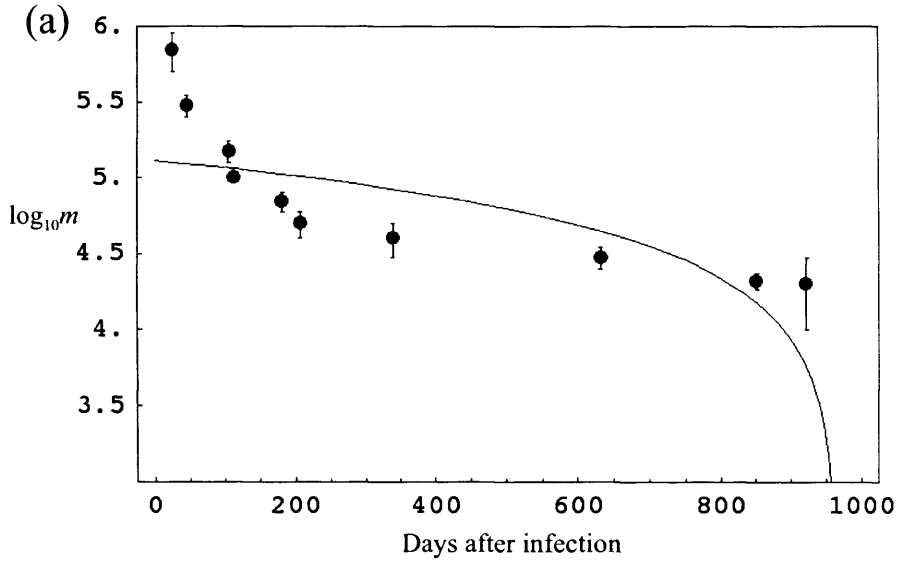
declining pathogen load cannot be produced by a process of T cell clearance. Any biologically reasonable memory kinetics would produce a rate of change in pathogen load that is not proportional to the pathogen load alone, and hence an exponential decrease would not occur. For example, if the memory population has a constant size then

$$\dot{p} = K_1 p - K_2 p^2,$$

where K_1 and K_2 are constants, and this logistic equation does not solve to give an exponential decline.

To further rule out the possibility that the memory T cell kinetics are produced by a changing pathogen load, rather than a changing program, equations (7.1) and (7.2) are together fitted to the data. Firstly, the replication rate, ψ, is allowed to take any positive value. The best fit is obtained when the replication and killing rates are approximately zero, this gives a similar fit as the *Unchanging* model for both epitopes. Fitting the model when ψ has a biologically reasonable value for a virus ($3 < \psi < 6$ represented a doubling time of several hours [210]) the fit was worse than obtained for the *Unchanging* model for both the GP-specific (figure 7.2a, $SS = 136.5$) and NP-specific (figure 7.2b, $SS = 121.2$) T cell responses.

A further observation suggests that the results for CD4 T cells can be generalised to CD8 T cells. The models were fitted to experimental data for a CD8 T cell population that exhibited a long-term decline in size after infection with LCMV [198]. The best fits are shown in figure (7.3) and the *Activation* model is again found to produce a better fit than the other models. The probability that the *Activation* model represents the correct mechanism is 93.4% when compared to the *Unchanging* model, and 98.0% when compared to the *Amplification* model. This suggests that during a chronic infection the rate at which the specific memory CD8 T cells are re-activated decreases. However, this result may not be widely generalisable because the data were obtained from a host deficient in CD4 T cells, which are known to be required during the formation of CD8 T memory cells.



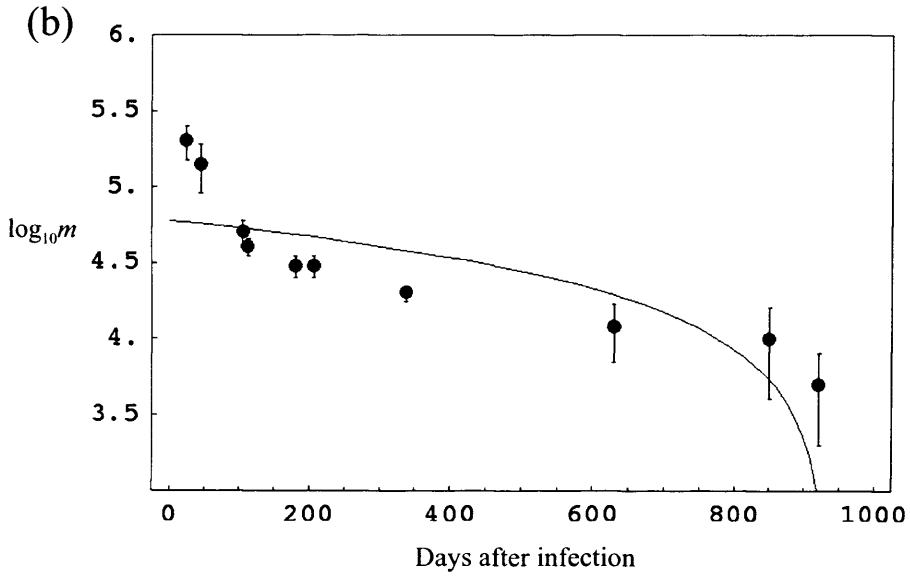


Figure 7.2: Fitting the full model, with pathogen replication, to the CD4 T cell data for both responses to two epitopes. (a) Dominant epitope (GP). Parameter values: $\psi = 3.1$, $\kappa = 1010^{-5}$, $M = 1.310^5$, $\Theta = 0.0045$, $P = 1.2$ and $SS = 136.5$. (b) Subdominant epitope (NP) Parameter values: $\psi = 3.4$, $\kappa = 2810^{-5}$, $M = 0.610^5$, $\Theta = 0.006$, $P = 1.2$ and $SS = 121.2$.

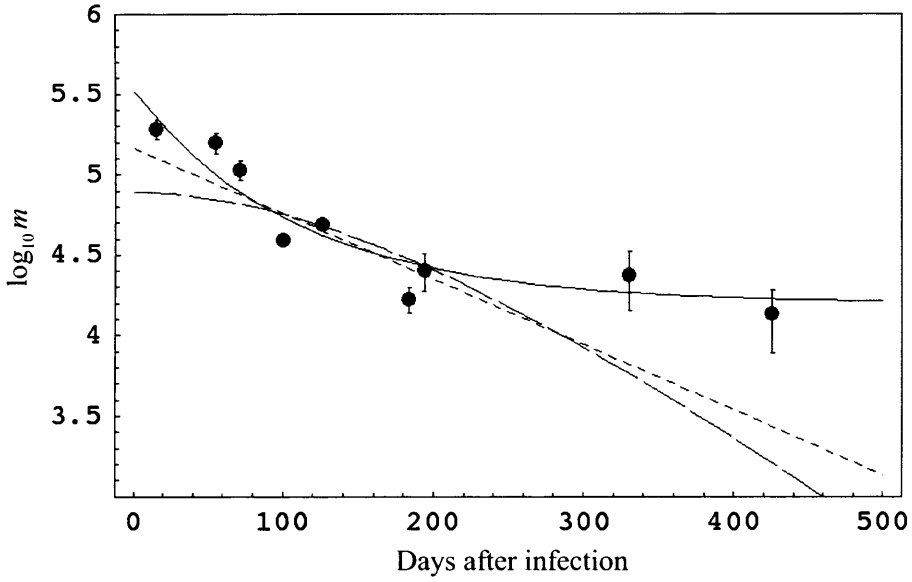


Figure 7.3: Fitting the three models to the declining GP-specific CD8 T cells response. The *in vivo* data was sourced from ref. [200]. The bold, small dashed and large dashed lines represent the *Activation*, *Unchanging* and *Amplification* models respectively.

7.2.1 Final memory size

Here the very long term kinetics of a memory population are considered given that the activation rate of the T cells decreases. It is first assumed that the pathogen load, p_c, is kept constant by another mechanism. As $t \to \infty$, it can be seen from equation (7.8) that the size of the memory population, m_c, will tend to a constant value,

$$m_c = M_0 \exp \frac{(\theta - 1)\gamma p_c}{\beta}. \tag{7.10}$$

It is observed that if the net amplification is positive then the long term size of the memory population will be larger than the initial size. This size increases exponentially with increases in Θ and p_c. The decreasing rate of activation may be a mechanism that prevents unbounded population growth of one population at the expense of the others that also require space in the memory pool.

If the net amplification is negative then the long term size of the memory population will be smaller than the initial size. The more negative the net amplification or the greater the pathogen load the smaller the final population size. The de-sensitisation of the cells to re-activation prevents a population from being completely removed by repeated weak stimulation. Possibly the cells would remain sensitive to stronger stimulation and hence still be capable of providing a defence against re-infection.

The model suggests that it is unlikely that the clonal structure of a T cell population will be conserved during a chronic infection. Each clone recognises a different epitope and these epitopes will be present in different quantities. The model predicts that during a chronic infection the final size of each memory clone will depend on the pathogen load,

$$m_i = M_i \mathrm{e}^{(\theta-1)\gamma p_i/\beta}, \tag{7.11}$$

where i denotes a particular clone and p_i is the effective pathogen load stimulating clone i. If each clone activates at a different rate (represented by a different β or γ) or undergoes a different amplification in response to a stimulus then the clonal structure will also alter. During a decrease in population size the clones that experience a

higher effective pathogen load or are activated more frequently will have a smaller final size. This prediction is supported by experimental observation that during a chronic infection the dominant clone decreases relative to the subdominant clone [131].

7.3 Discussion

In this study it was proposed that repeated activation of memory cells by a weak antigenic stimulus could explain the decline in their number exhibited following LCMV infection. It was shown in Chapter 5 that a sufficiently weak stimulus could elicit a proliferative program that produced net shrinkage in the size of the activated population and therefore repeated stimulus would produce a continuous decline. Given this effect of the pathogen on the T cell population, three variations on the model developed in the last chapter, for the memory kinetics during chronic infections, were proposed to represent changes in the nature of the proliferative program during a chronic infection. The predictions of these models were fitted to experimental data for two CD4 T cell populations. It was found that good fits to the data could be obtained for the models showing that repeated restimulation of a T cell population could account for the decline in size. Of the three models the best fit, for both epitopes, was when the rate of activation of specific T cells decreased over time. This may represent a progressive downregulation of the TCR by the cells, possibly after a fall in danger signals as inflammation subsides.

The gradual de-sensitisation of a T cell population to stimulation may have a definite function. The long term persistence of antigen that produced a positive net amplification would cause the specific T cell population to steadily grow. If unchecked this growth would squeeze out other memory clones from the finite memory pool, and the organism would lose its memory of other previously encountered pathogens. By reducing the rate of activation the total growth of a population is limited and clonal diversity preserved. Conversely, this diminution of sensitivity to stimulation may protect against the effects of continuous stimulation that elicits a negative net amplification. It might be expected that the inevitable conclusion to a shrinking

memory population is its complete disappearance. This would leave the organism unprotected against futures encounters with the specific pathogen. However, the de-sensitisation of the cells to activation by the persisting pathogen terminates the decline thus preventing the disappearance of the population. The population may still be able respond to stronger stimulation if, for instance, the pathogen load or inflammation suddenly increases, thereby allowing the population to contribute to the immune defence. Reducing the amplification, instead of the activation rate, would not lead to a stable size because when the amplification is less than one the proliferative response serves to decrease the activated population size and the decreasing population would eventually vanish from the pool.

The de-sensitisation of memory T cells may also help to maintain clonal diversity in a pathogen-specific population. If the sensitivity of cells did not change then the dominant clones would become increasingly dominant relative to the other clones. This focusing of the population may be detrimental to its ability to manage a mutating pathogen. A more diverse repertoire may be maintained by reducing the activation rates of clones.

CHAPTER 8
SUMMARY AND DISCUSSION

The proliferation of T cells in response to an antigenic stimulus is a vital component of the adaptive immune response. The standard belief of a strong coupling between the T cell and pathogen kinetics, with the T cell numbers peaking at the time of pathogen clearance, has recently been challenged. Experiments have demonstrated that, following a brief encounter with antigen, T cells enter a developmental program that can produce a full response in the absence of further antigenic stimulation. The striking similarity of T cell kinetics in different hosts in response to different stimuli demonstrates that the program strictly regulates cell number. Although T cells are receptive to a diverse range of modulatory signals, this invariance of key aspects of their kinetics suggests that regulation of cell number may be produced by a single mechanism. The first aim of this thesis was to identify the most plausible mechanism that could regulate the T cell kinetics. The second aim was to investigate how the programmed nature of the regulatory mechanism affected the outcome of infection.

Firstly, within the framework of a simple discrete structured population model of T cell proliferation, a number of potential regulatory mechanisms, which influenced T cell division and survival, were investigated to establish which could be the sole regulator of cell number. The majority of mechanisms could be rejected owing to their inability to predict experimental data. The consistent feature of all successful mechanisms was the progressive increase in death rates. This feature of the regulatory mechanism had not previously been appreciated. Instead it had been assumed that all proliferating T cells exhibited the same constant susceptibility to death. This early inhibition of death would allow cell numbers to increase more rapidly because the net proliferation rate would not be reduced by a high death rate.

Secondly, the idea that the immune system evolved according to certain optimal strategies was used to further shrink the number of plausible regulatory mechanisms.

Different control mechanisms can achieve the same T cell kinetics but with different levels of cell death. It was proposed that from a selection of plausible mechanisms the most plausible would be the one associated with the lowest wastage of T cells. The number of T cells that underwent apoptosis during a primary response were calculated for different mechanisms and the values compared. It was found that the least wasteful mechanism for CD4 and CD8 T cells was one that increased susceptibility to apoptosis *and* decreased the division rate of proliferating cells. Experimental observations further supported the existence of this mechanism and it was therefore used in subsequent models in this thesis.

The effect of the programmed nature of the regulatory mechanism on the outcome of infection was then investigated. Firstly, the effect of the program on the size of the post-primary memory population was determined. A proliferative response culminates with the formation of a memory population which is generally much larger than the initial naive population. The relationship between the number of activated naive cells and the number of memory cells formed was next established. It would be of great benefit to understand how to control this amplification of a response since the size of the memory population strongly affects subsequent responses to the specific antigen. Consequently the effects of manipulating different aspects of the response on the amplification were assessed. It was found that the manipulation that most efficiently increased the amplification was to delay the decrease in the division rate of cells. Reducing the amplification is most efficiently achieved by delaying the formation of memory cells.

The study also produced several other interesting findings. Firstly, increasing the rate of memory formation can, counterintuitively, produce a smaller memory population. This occurs because there are fewer cells left in the proliferating pool to generate the massive expansion required for a large memory population. Secondly, increasing the division rate of the cells has surprisingly little effect on the amplification. An increased rate simply means that the cells will reach the end of the program sooner, the magnitude of expansion will remain approximately the same. Thirdly, it was intriguing to observe that sufficient changes in most of the parameter values can produce a memory population that is smaller than the size of the activated naive population.

This would represent a form of tolerance because the secondary response to a stimulus may be smaller than the primary response (The main reason for the generally larger size of the secondary response relative to the primary is the greater size of the initial population). This suggests a fascinating conclusion: sensitisation and tolerance may be two different outcomes of a single mechanism. Generally, the two processes are thought to be controlled by distinct mechanisms. The process of sensitisation is associated with the priming of T cells (through proliferation and memory formation) so that they respond more vigourously to a second encounter with their specific antigen. The process of tolerance is associated with the de-sensitisation of a T cell population (through cell deletion and induction of anergy or non-responsiveness) to reduce or annul their response to subsequent encounters with their specific antigen. Simply by changing the parameter values, the model analysed here can switch between these two processes. This suggests that moving between a tolerising and sensitising response may be much easier than has previously been appreciated. Complete tolerance could potentially be achieved by a single strong negative amplification or by a weak negative amplification repeated many times. Importantly, it was observed that this mechanism of tolerance could not be achieved by modulating only the division number at which the death rate increased. This suggests that this form of tolerance requires changes in the process of memory formation.

The next investigation concerned the ability of a program-driven T cell response to clear infection. It was observed that if the cell population activated by the pathogen produced a larger memory population (positive amplification) at all stages of the infection then clearance was always achieved. This clearance could be mediated by one of two quantitatively distinct T cell responses. The first response involved the continued exponential expansion of the T cell population resulting in the rapid clearance of the pathogen. The second response, which only occurred for slowly replicating pathogens, involved the linear expansion of the population mediating a very slow clearance of the infection. The prediction of the model that more slowly replicating pathogens are more able to persist is supported by number of experimental observations [210, 222, 223, 224, 225, 226].

The existence of two distinct types of T cell response following the initial expansion

is also supported by experimental data. Generally exponential expansion precedes the clearance of pathogen [35, 2], but linear expansion has been observed to accompany some chronic infections [200], most noticeably in the CD8 T cell response to murine cytopathic β-herpes virus (MCMV) infection [123, 219]. The slowness of pathogen clearance by this linear response would, if unchecked by regulatory mechanisms, result in the enormous of growth of T cell clones over a period of years. This may explain the very large number of HCMV-specific CD8 T cells present in seropositive individuals long after resolution of the primary response and the surprisingly large T cell clones possessed by some elderly people who show no symptoms of disease [220, 221].

Traditionally, there are two main types of vaccines: live virus or bacterial vaccines and inactivated pathogen vaccines. The former have been the most successful, and this success has been attributed to the replication of the virus in the individual. The results of this study agree with this idea. It is generally observed that increases in replication rate increase the size of the largest T cell peak, which is known to be positively correlated with the final memory size [40]. The present study also makes futher predictions. Firstly, it suggests that decreasing the ability of the immune system to clear the infection would potentially boost the size of the memory pool by increasing the largest peak size. Secondly, it suggests that low or high initial pathogen doses may produce a larger memory population than a medium dose. Finally, it suggests a new form of vaccination strategy. Live attentuated pathogens with very low replication rates may be able to persist long term and produce a linear expansion in the specific T cell population. This will result in the gradual accumulation of memory T cells resulting in a very large memory population. Although this has the obvious benefit of producing very strong protection without the need for boosters, it risks generating a specific memory population so large that it squeezes other memory populations out of the the finite memory pool.

One of the findings of this study may have important implications for the early treatment of infection. The model predicts that the initial pathogen load can strongly affect the subsequent response. At low loads there is a negative correlation between the load and the peak time and size, whereas at higher loads the opposite is true. At very high initial loads the peak characteristics are insensitive to the load. The

immediate but brief administration of antibiotics may unexpectedly enhance the impact of a bacteria. The early suppression of bacterial growth will result in a low pathogen load eliciting only a small recruitment of specific-T cells. If the antibiotic is then withdrawn before clearance then the existing effector T cell population may find itself insufficient to control the now unrestrained pathogen.

The final investigation of this thesis concerned the hypothesis that repeated activation of memory cells by a weak antigenic stimulus could explain the decline in their number exhibited following LCMV infection. It was shown in Chapter 5 that a sufficiently weak stimulus could elicit a proliferative program that produced net shrinkage in the size of the activated population and therefore repeated stimulus would produce a continuous decline. Given this effect of the pathogen on the T cell population, three variations on the model developed in Chapter 6 for chronic infections were proposed to represent changes in the nature of the proliferative program during a chronic infection. The predictions of these models were fitted to experimental data for both the dominant and subdominant CD4 T cell responses. It was found that good fits to the data could be obtained for the models, showing that repeated restimulation of a T cell population could account for the decline in size. The results further suggested that the rate of activation of the specific memory cells decreased over time (a de-sensitisation).

The gradual de-sensitisation of a T cell population to stimulation may help to preserve clonal diversity in the memory pool. It would prevent the continued growth, or shrinkage, of repeatedly stimulated memory populations, which would otherwise reduce this diversity. Such a reduction would leave the organism unprotected against future encounters with new pathogens or the re-activation of those currently latent.

The results of the previous two chapters reveal that misleading, and potentially dangerous, conclusions may arise from the monitoring of T cell populations in patients. Generally, an expanding T cell population is associated with a rising threat and demands urgent treatment, whereas a shrinking T cell population suggests the system is relaxing following a successful response and that treatment is not required. The truth, however, may be the opposite. Chapter 6 demonstrates that a steadily

rising T cell population can occur when a persisting pathogen is slowly, and successfully, being cleared from the host. Even in the absence of treatment, clearance will be achieved (albeit at a slower rate). In Chapter 7 it is shown that a slowly shrinking T cell population may be associated with the failure to clear a persisting pathogen. The inevitable outcome of this decline may be the stronger re-emergence of this pathogen sometime in the future. These conclusions should be considered when treatment strategies are devised.

The work contained in this thesis could be extended in a number of ways. It has concerned the adaptive immune response alone and for simplicity the models have not included terms to represent the innate immune response. This is generally a good approximation since a fully developed adaptive response is far more effective at clearing pathogen than is the innate. It is reasonable to suggest however, that the innate system still affects the clearance of the pathogen. Firstly, in the early stages of infection the innate response will serve to limit the growth of the pathogen thereby influencing the pathogen load presented to the specific naive T cells. It is known, and demonstrated in Chapter 6, that this initial load together with the level of inflammation, again controlled by the innate system, substantially influences the T cell proliferative program. Secondly, for certain infections the strength of the innate response during the T cell contractions phase may be a critical factor in determining whether a pathogen is fully cleared. Therefore incorporating the innate system into the model may allow one to answer questions such as: In response to infection how does the immune system decide whether to initiate a full adaptive response? And what determines whether an infection is able to become chronic?

The model developed in Chapter 6 for the memory response to low levels of persisting antigen may be ideal for the study of autoimmune disease. Autoimmunity occurs when an organism's immune system mistakenly recognises its own peptides as foreign and mounts an attack against itself. Some forms of arthritis are thought to initiate by damage to a joint exposing hidden self-antigens. The presentation of these antigens in the context of inflammation triggers an immune response against them. This response can continue because damage self-inflicted by the effector CD8

T can expose more of the self-antigens The gradual worsening of autoimmune symptoms with time may result from the steady linear increase in the size of the specific memory population that, as shown in Chapter 6, can be caused by low levels of persisting antigen. This suggests a simple experiment to look at the antigen-specific T cell population over time during an autoimmune disease. Restructuring the model in terms of autoimmunity may allow one to investigate how the condition arises and suggest novel treatments.

During an immune response CD4 and CD8 T cell subsets are known to interact extensively. The presence of activated CD4 T cells appears predominantly to promote the CD8 T cell response. A full CD8 proliferative response requires externally produced IL2 which is most likely provided by the CD4 T cell population [170], also functional CD8 T cell memory can fail to form in the absence of CD4 T cell help [237]. The subsets may also interact indirectly by altering dendritic cell number or activation state. Dendritic cells can either enhance a response by re-activating newly formed memory cells, or suppress it by ligating particular T cell receptors to inhibit division [77, 173, 160]. The lifetime of dendritic cells can be prolonged by CD4 T cells, by ligating the RANK receptor [238], or shortened by CD8 T cells, by triggering death [239]. In this thesis the CD4 and CD8 T cell responses have been considered to act independently. This is a good approximation during a normal response because each subset receives all the necessary signals and this allows the proliferative programs to fully unfold. To consider situations where there is a deficiency in one subset, the precise interactions between the subsets must be modelled. This would enable conditions where the response of one subset is suppressed to be more accurately modelled. A number of infections are known to detrimentally effect a particular T cell subset. The most infamous example being the declining size of the CD4 pool caused by HIV infection.

Understanding the mechanisms that regulate T cell number during an infection is the first step towards developing precise and reliable strategies of therapeutic modulation. The boosting of weak responses may aid the clearance of chronic infections,

whereas the dampening of those that are excessive may alleviate auto-immune or allergenic symptoms. The enormous impact of vaccination on mortality and morbidity in the world demonstrates the profound benefits that can be derived from taking control of the T cell response. An experimental and theoretical unification, the sharing of data and inspiration, will maximise the pace of progress along this path and ensure that no idea is left unturned.

APPENDIX A
METHODS

In this thesis three applications, namely C++, Mathematica, and Prism, were used to obtain some of the results. Their details and where they were used are discussed below.

C++

In Chapter 3 the best-fit solutions to experimental data of the systems of coupled ordinary differential equations were determined using algorithms coded in C++. The differential equations were solved using the 4^{th}-order Runge-Kutta algorithm, which uses trial steps within the step interval to cancel out lower-order terms. The fourth-order formula for finding the function $y(x)$ is

$$\begin{aligned} k_1 &= h\, f(x_n, y_n), \\ k_2 &= h\, f(x_n + \tfrac{1}{2}h, y_n + \tfrac{1}{2}k_1), \\ k_3 &= h\, f(x_n + \tfrac{1}{2}h, y_n + \tfrac{1}{2}k_2), \\ k_4 &= h\, f(x_n + h, y_n + k_3), \\ y_{n+1} &= y_n + \tfrac{1}{6}k_1 + \tfrac{1}{3}k_2 + \tfrac{1}{3}k_3 + \tfrac{1}{6}k_4 + O(h^5), \end{aligned} \tag{A.1}$$

where h is the step size. The algorithm is known to be very accurate and well-behaved for a wide range of problems. The best fit for each model variant was found using a linear search algorithm to minimise the least-squares fit between model predictions and experimental data of the total number of T cells. The algorithm operates by fixing all but one parameter and then searching the free parameter for the value that

minimises the following

$$\chi^2 = \sum_{j=1}^{J} \frac{\left(e_j - \left(u(t_j) + n(t_j) + \sum_{i=1}^{I} x_i(t_j)\right)\right)^2}{\epsilon_j^2}. \tag{A.2}$$

The value e_j, with error on the mean ϵ_j, is the experimentally determined number of T cells at time t_j, where j denotes the j-th experimental data out of J points. The free parameter is then fixed at the value that minimises χ^2 and a previously fixed parameter is freed. This process is then repeated until a value for χ^2 is obtained that does not decrease for any parameter change. The parameter values that give this χ^2 value produce the best fit of the model's prediction to experimental data

The solution was obtained using C++ rather than a package such as Mathematica in order to reduce calculation time. This was necessary because many different models had to be investigated.

Mathematica

Mathematic 5.0 was used throughout the thesis both to verify analytical expressions, and for numerical analysis. In chapters 4, 5, 6 and 7 the function NDSolve was used to numerically solve the systems of ordinary differential equations. NDSolve is a general numerical differential equation solver that can be used with a wide range of ordinary differential equations (ODEs). In a system of such equations there can be any number of unknown functions, but all of these functions must depend on a single independent variable, which is the same for each function. An example of the required format is

$$\text{NDSolve[eqns, y[x], \{x, xmin, xmax\}],}$$

which gives solutions for y(x). It encapsulates a number of powerful methods and uses an adaptive stepsize.

Prism

Prism is a powerful data plotting and analysis application tailored towards research in the life sciences. It was used for the curve fitting in chapters 5 and 7, and for comparing the quality of different fits, using Akaike's Information Criterion, in chapter 7. The package can be obtained from the website: www.graphpad.com/prism.

APPENDIX B
MATHEMATICAL GLOSSARY

Variables		Other parameters	
		f	Falling weighting factor
m	Memory T cells	r	Rising weighting factor
m_p	Precursor memory T cells	ω	Slope factor
n	Naive T cells	i	Division number
p	Pathogen load	D	Total death caused by a mechanism
u	Unresponsive naive T cells	T	Termination time of contraction
x	Effector T cells	R_E	Expansion factor
		R_C	Contraction factor
		ϕ_a	Contact apoptosis constant
		ϕ_d	Contact div-inhibition constant
Rate Parameters		N	Initial number of naive cells
		θ	Amplification factor
		Θ	Net amplification
α	Apoptosis rate	τ	Proliferative-delay
γ	Activation rate of memory T cells	t_p	Time of peak T cell number
δ	Maximum division rate	P	Initial pathogen load
κ	Clearance rate of pathogen	p_C	Pathogen carrying capacity
μ	Pre-memory T cell formation rate	p_T	Pathogen load threshold
ψ	Pathogen replication rate	M	Final number of memory T cells
ρ	Memory T cell formation rate		Division no. of half maximum:
ζ	Rate of undergoing 1st division	I_δ	division rate
		I_α	apoptosis rate
		I_μ	pre-memory formation rate

Table B.1: Main mathematical terms used in the model.

APPENDIX C
ROBUSTNESS OF CHAPTER 5 PREDICTIONS

To be sure that the kinetic features predicted by the model in Chapter 6 are not restricted to certain parameter values a number of parameter changes are considered. Below, a representative example are shown. Figure C.1 shows that a second peak is observed for variations in several of the parameter values.

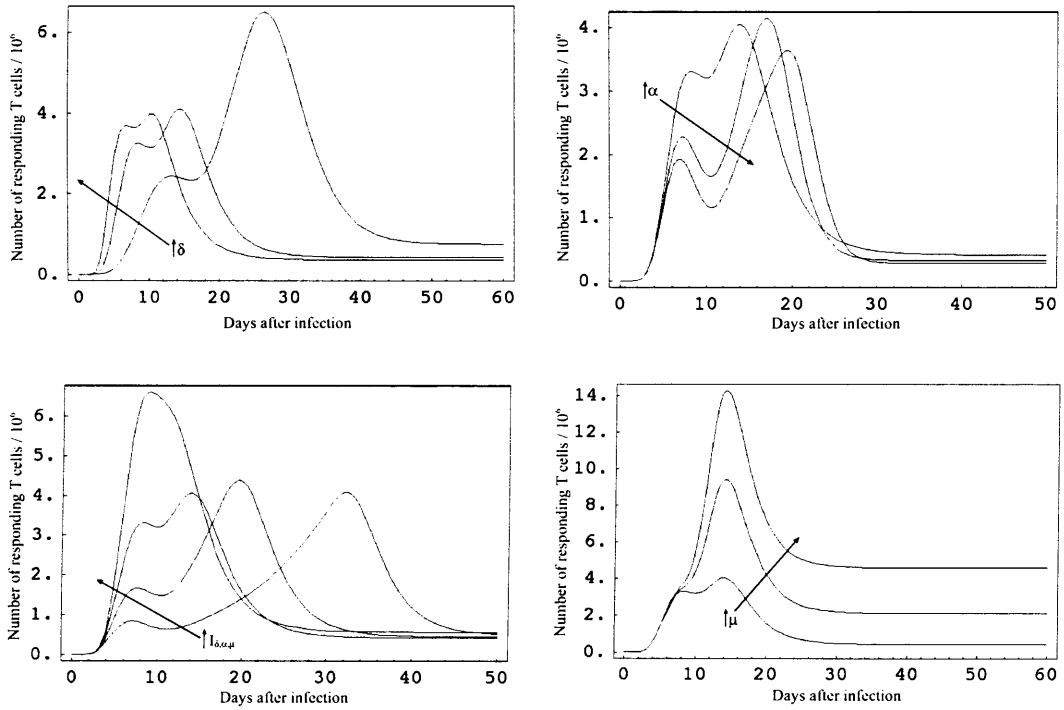


Figure C.1: The appearance of a second peak is not restricted to a particular parameter set.

Figure C.2 shows that a linear expansion is not restricted to particular parameter values.

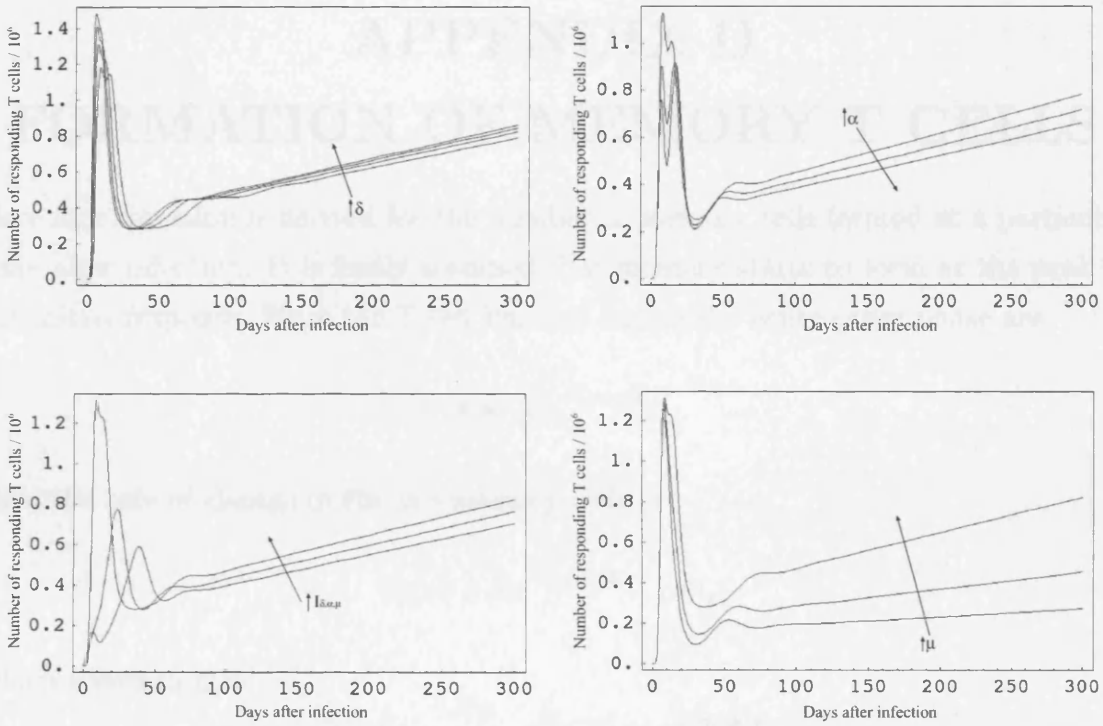


Figure C.2: The linear expansion of the the T cell population is not restricted to a particular parameter set.

APPENDIX D
FORMATION OF MEMORY T CELLS

Here an expression is derived for the number of memory cells formed at a particular time after infection. It is firstly assumed that memory starts to form at the peak of the initial response. Since the T cell kinetics during the contraction phase are

$$x = X\mathrm{e}^{-(\alpha+\mu)t},$$

then the rate of change in the pre-memory cells is

$$\dot{m}_p = \mu X\mathrm{e}^{-(\alpha+\mu)t} - \rho m_p,$$

which solves to give

$$m_p = \frac{X\mu}{\alpha+\mu-\rho}(e^{-\rho t} - e^{-(\alpha+\mu)t}).$$

From equation (6.3), in Chapter 6, it is seen that in the absence of antigenic stimulation

$$\dot{m} = \rho m_p.$$

Substituting in the expression for m_p and solving gives the number of memory cells at time t after the peak,

$$m = \frac{X\mu}{(\alpha+\mu)(\alpha+\mu-\rho)}((\alpha+\mu-\rho) - (\alpha+\mu)e^{-\rho t} + \rho e^{-(\alpha+\mu)t}).$$

APPENDIX E
DERIVING THE EQUATION FOR LONG TERM MEMORY KINETICS

The difference equation for low pathogen levels has the form

$$p_{j+1} = p_j \, e^{\Gamma_1 \psi - \Gamma_2 m_j \, p_j \, \gamma \, \kappa}, \tag{E.1}$$

where

$$\Gamma_1 = t_p + T, \tag{E.2}$$

and

$$\Gamma_2 = \frac{e^{t_p \delta} - 1}{\delta} + \frac{e^{t_p(\alpha+\delta+\mu)-T(\alpha+\mu)}(e^{T(\alpha+\mu)} - 1)}{\alpha + \mu}. \tag{E.3}$$

Firstly if the step size is Δt, a small time relative to the time of the response considered then

$$p_{t+\Delta t} = p_t \, e^{\Gamma_1 \psi - \Gamma_2 m_t \, p_t \, \kappa \, \gamma}, \tag{E.4}$$

and if $T = \Delta t$ and because $T >> t_p$, $t_p \rightarrow 0$ then

$$\Gamma_1 = \Delta t, \tag{E.5}$$

and

$$\Gamma_2 = \frac{(1 - e^{-\Delta t(\alpha+\mu)})}{\alpha + \mu}. \tag{E.6}$$

Taylor expanding the exponential term and neglecting terms containing powers of Δt greater than 1 gives

$$\Gamma_2 = \Delta t. \tag{E.7}$$

Substituting values for Γ_1 and Γ_2 into equation (E.4) gives

$$p_{t+\Delta t} = p_t \, e^{(\psi - m_t \, p_t \, \kappa \, \gamma)\Delta t}, \tag{E.8}$$

which after Taylor expanding, and again neglecting terms containing powers of Δt greater than 1 gives

$$\frac{p_{t+\Delta t} - p_t}{\Delta t} = (\psi - m_t \, p_t \, \kappa \, \gamma) \, p_t. \tag{E.9}$$

As $\Delta t \to 0$, this difference equation becomes the differential equation

$$\frac{\mathrm{d}p}{\mathrm{d}t} = (\psi - m \, p \, \kappa \, \gamma) \, p. \tag{E.10}$$

Similarly the equation for the memory size will be

$$\frac{\mathrm{d}m}{\mathrm{d}t} = \Theta \, p \, m. \tag{E.11}$$

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