

Special Feature

Modelling the impact of antigen kinetics on T-cell activation and response

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Summary Cytotoxic T lymphocyte (CTL) responses are thought to be important for the control of many viral and other infections. Qualitative aspects of the CTL response, including the epitope specificity, affinity, and clonal composition, may affect the ability of T cells to mediate infection control. Although it is clear that the mode of introduction and the dose of antigen can affect these qualitative aspects of the response, little is understood of the mechanisms. We have developed an *in silico* model of the CTL response, which we use to study the impact of antigen dose, antigen kinetics and repeated antigen delivery on the response. The results suggest that recent observations on differences in response to killed antigen can be explained simply by differences in timing of T-cell activation. These findings may provide insight into how different vaccination strategies can quantitatively and qualitatively affect the outcome of the immune response.

Key words: antigen kinetics, computer modelling, cytotoxic T lymphocytes, T-cell activation, vaccination.

Introduction

The CTL response plays an important role in the control of infectious agents, particularly chronic viral infections such as HIV, EBV, and hepatitis C virus (HCV).^{1–3} A large number of vaccination strategies are currently being developed, aimed at eliciting CD8⁺ T-cell responses to these viruses.⁴ Analysis of CTL responses to infection or vaccination demonstrates that they are focused on a small number of immunodominant epitopes, and that responses to epitopes are often dominated by a few T-cell clones.^{5,6} Both the affinity and breadth of the CTL response appear to play an important role in determining outcome. Subdominant or low-affinity T-cell responses have been shown to be less efficient at controlling viral infection^{7–9} and studies with cancer vaccines have suggested that despite the ability of vaccination to induce CD8⁺ T-cell responses to cancer antigens, these cells appear unable to recognize physiological levels of antigen *in vivo*.¹⁰ Therefore, an optimal vaccine should elicit a high-affinity response to optimize viral control. However, *in vitro* and *in vivo* evidence suggests that there is a reciprocal relationship between antigen concentration and affinity of responding T cells.^{11–13} Therefore, one might assume that there is a trade-off between the magnitude and affinity of the CTL response¹² and that administration of high-dose vaccines could elicit low-affinity responses. Separate from the affinity of the T cell response, the breadth of the CTL response could also be important in limiting the ability

of rapidly mutating viruses to ‘escape’ immune recognition by CTL, since a virus might more easily evade a highly focused response.^{14–16}

Currently, very little is understood about how the mode of antigen delivery affects the outcome of vaccination. Immunological simulation provides the opportunity to make quantitative predictions of the effects different methods of immune stimulation have on the CTL response. We have developed an *in silico* model of CTL responses that explicitly considers the kinetics of T cells and focuses attention on the importance of antigen kinetics. In this paper, we use the model to study the impact of antigen dose, antigen kinetics, and repeated antigen delivery on the CTL response. The results provide insights into the effects of timing of T-cell proliferation on the diversity and affinity of the response. They also suggest that recent observations on differences in response to killed antigen¹⁷ can be explained simply by antigen kinetics.

Methods

We use a previously described stochastic model of CTL responses (D. L. Chao *et al.* in press). The model follows the dynamics of individual T-cell clones, keeping track of the number of cells in different states and at different differentiation stages (e.g. activation, division, and memory). CTL are represented as discrete entities in a stochastic framework, in which, for example, each cell has a probability of proliferating based upon its affinity for antigen, the amount of antigen present, and other factors. This stochasticity generates a distribution of outcomes from a given set of initial conditions, not just the expected outcome, and thus variability amongst experimental animals can be accounted for. Stochastic effects can affect the outcome of immune responses, especially in primary responses, which start with small numbers of precursor T cells.

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Received 6 November 2003; accepted 6 November 2003.

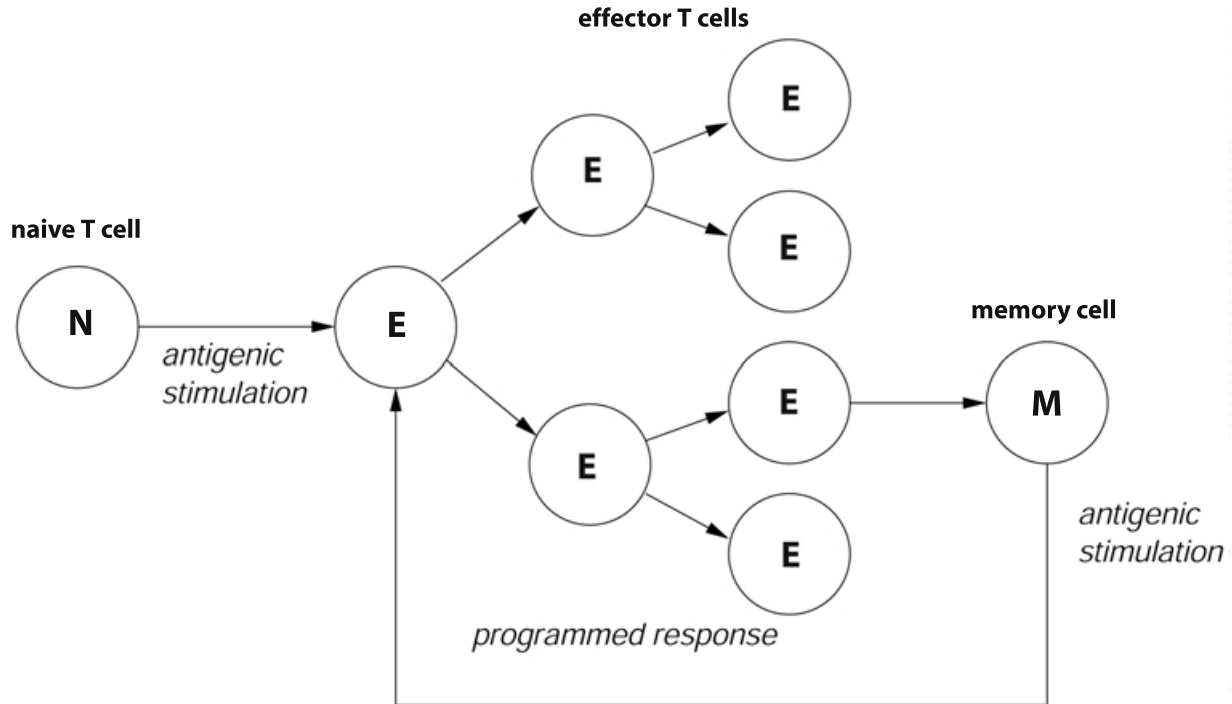


Figure 1 CD8⁺ T-cell model. T cells are initially in a quiescent naive state. Upon being stimulated by antigen, they begin a programmed response, during which they proliferate and acquire effector functions that allow them to eliminate infected cells. During the response, about 5% of the effector cells convert to long-lived memory cells. After the programmed response, which is of fixed duration, effector cells rapidly die. Memory cells, like naive cells, are quiescent until antigenic stimulation, after which they begin a programmed response.

In addition to T cells, the model also includes a representation of viral infection. Virus infects uninfected cells, converting them to infected cells, which in turn produce more virus.^{18–20} A constant source replenishes the pool of uninfected cells, allowing the body to recover after infection. In the absence of an immune response, the population of a replicating virus tends to rise rapidly then decline to an equilibrium level. Effector CD8⁺ T cells can eliminate infected cells and clear the infection.

Our model of CD8⁺ T-cell populations is summarized in Fig. 1. Naive T cells are quiescent until they are stimulated by the presence of antigen, at which time they start to proliferate according to a *programmed response*, which occurs even in the absence of continuing antigenic stimulation.^{21–23} During the programmed response, cells proliferate in an antigen-independent manner, eliminate infected cells, and stochastically convert to long-lived memory cells. From the moment they are stimulated, T cells are subject to a high death rate²⁴ which is compensated for by the high proliferation rate during the programmed response. At the end of the response, cells stop proliferating, and the constant death rate causes the population to decline rapidly, leaving only quiescent memory cells. Like naive cells, memory cells are activated by antigenic stimulation. Memory cells of a particular clonotype respond to the same level of antigen as their naive counterparts,^{25,26} but whereas naive cells experience a delay prior to activation, memory cells are rapidly activated.²⁵ Once stimulated, memory cells have a programmed response similar to that of naive cells but with a lower death rate²⁷ so their net population growth is faster. The model does not include other components of the immune response, such as CD4⁺ helper T cells and dendritic cells; their roles in assisting the CD8⁺ response are assumed to be implicit.

Each T-cell clone has a defined affinity for antigen. The level of stimulation T cells receive is determined both by the affinity a cell

has for an antigen and by the number of virally infected cells. The degree of antigenic stimulation in turn affects the rate of conversion from naive to effector status, and affinity determines the rate at which effector cells clear infected cells. Thus, higher affinity cells are recruited faster and clear infected cells more efficiently than low-affinity ones. Each clone is initially composed of 10 identical naive cells. In response to infection, this small population can expand to hundreds of thousands of effector cells.

We define the dissociation constant (K_d) of a T cell for an antigen to be the amount of antigen that is required to induce half-maximal stimulation of the T cell. Operationally, since we only deal with infected cells and not MHC–peptide complexes, if $K_d = 10^4$ for some clone, a T cell from this clone would be half-maximally stimulated in the presence of 10^4 infected cells. One could consider K_d to be the ‘threshold’ for antigenic stimulation of different clones. At a given level of infection, only clones with sufficiently low values of K_d will receive significant stimulation. The function that translates infected cell numbers and the dissociation constant of a clone to a stimulation level for a CTL is saturable so, as the number of infected cells increases, the stimulation level of a particular T cell clone approaches a maximum value. We define a T cell’s affinity to be the inverse of its dissociation constant.

In the model, competition amongst T cells occurs only through the killing of infected cells. Higher-affinity T cells are stimulated by lower levels of antigen and can eliminate infected cells before low-affinity T cells are recruited. Because proliferation is antigen-independent, once low-affinity cells are recruited they no longer need to compete with high-affinity cells for survival.

The simulation results described in the following sections are drawn from sample runs of our stochastic model. The behaviour of the model can differ between runs with identical parameters because

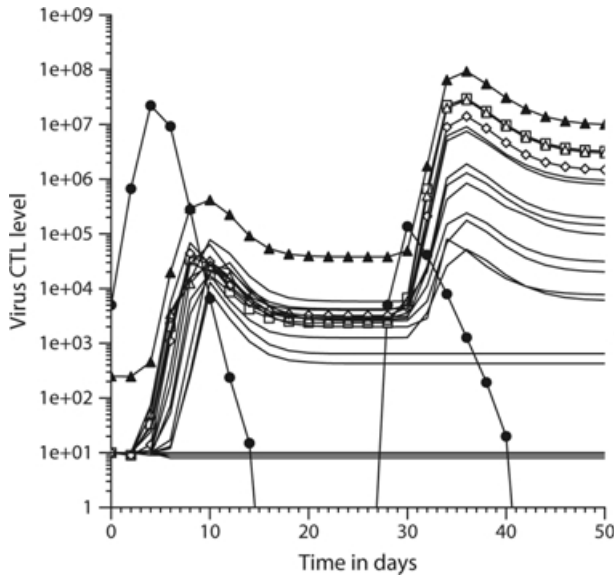


Figure 2 Primary and secondary CTL responses to a viral infection. 5000 viral units were injected on days 0 and 28. The virus levels are indicated by (●), the number of CTL in the three highest-affinity clones by (□), (△) and (◇) in decreasing order of affinity, and the total number of CTL by (▲). Lower-affinity clones are represented by lines with no markers. Each CTL clone initially has 10 unstimulated naive cells.

the model is stochastic. For all of these runs, the same set of 25 CTL clones with dissociation constants between $K_d = 5.3 \times 10^3$ and $K_d = 4.5 \times 10^7$ was used. These dissociation constants were determined by a simulation representing the thymic selection of randomly generated CTL clones with different specificities. The result of this process is a set of CTL clones with a wide distribution of affinities for antigen.

Results

The primary response

We simulated the primary CTL response to a viral infection. Early in infection, antigenic levels were too low to stimulate T-cell proliferation, so the naive T-cell population was stable. As the virus infected cells, the higher-affinity CTL were stimulated and their probability of entering the response increased. Low-affinity CTL were later stimulated to join the immune response when antigen reached sufficiently high levels (Fig. 2). Thus, the entry of clones into the response was staggered, with progressively lower affinity clones tending to enter the response later. A similar observation has been made in murine systems: the contribution of a T cell clone to an immune response is largely determined by the time of its entry into the response.²⁸ Low-affinity clones sometimes responded more quickly than high-affinity ones because the simulation is stochastic. With a more slowly growing virus, this occurred less often because the more gradual rise in antigen levels led to a greater delay between the times of stimulation of high- and low-affinity T cells (data not shown).

Even among syngeneic mice, the CTL involved in a primary response can have a variable mix of affinities for

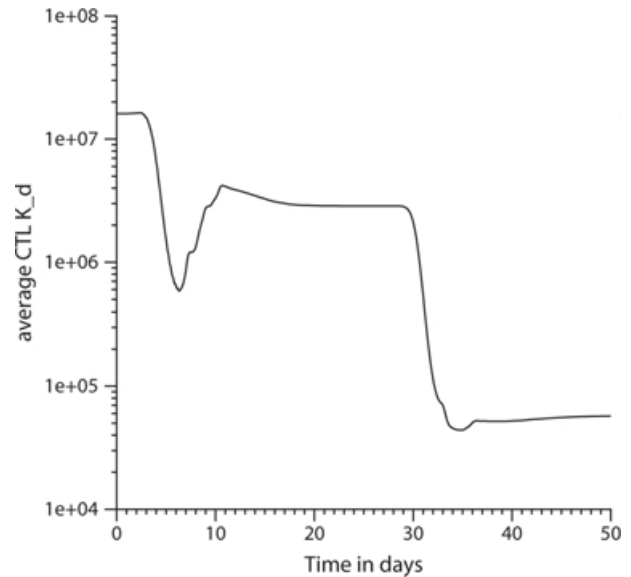


Figure 3 The average CTL K_d during primary and secondary responses to antigen. The dissociation constant K_d is defined as the amount of antigen required to induce half-maximal stimulation in a CTL and is the inverse of the affinity. Thus, a low value of K_d in the graph corresponds to a high affinity to antigen. 5000 viral units were injected on days 0 and 28. The data plotted are the average values from three experiments.

antigen.²⁹ In our model, different runs with identical initial parameters had different responding clones. Because the initial number of cells in a single clone is small, stochastic effects play a large role in the composition of the primary response. In the model, a newly stimulated naive T cell must survive a high death rate between the time of antigenic stimulation and the beginning of its programmed response, so that on average only 6 of the 10 cells from a particular T cell clone survive to proliferate. Because the model is discrete and assumes that proliferation is antigen-independent, a response that begins with 1–6 proliferating cells will peak between 60 000 and 360 000 effector cells. This agrees with the estimate that only 1–6 cells per clone initiate CTL responses in mice and that individual clones produce between 4×10^4 and 3.7×10^5 cells at the peak of the response.²⁸ As a consequence of the antigen-independent proliferation of CTL, memory levels formed by the primary response in our model are proportional to the initial number of cells that successfully enter proliferation because a constant fraction of effector cells formed convert to memory (about 5%).

The average affinity of T cells changed dramatically during the response to infection in the model. We define the average affinity of the response to be the inverse of the average K_d value of all CTL. Three days after infection, the average affinity rose (i.e. the average K_d fell) rapidly as high-affinity clones expanded (Fig. 3). The rising antigen levels progressively crossed the stimulation threshold of lower and lower affinity cells and recruited them into the response. As the T-cell response peaked, the average affinity dropped (i.e. K_d rose) as the contribution of low-affinity clones to the overall response increased and the programmed expansion of high-affinity

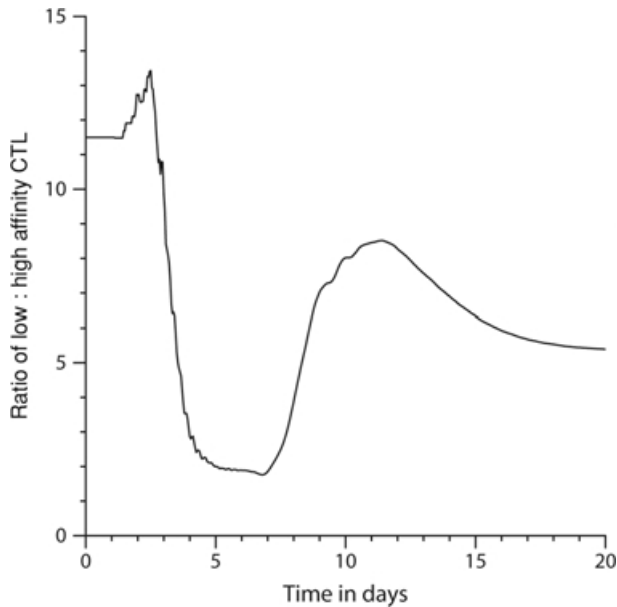


Figure 4 The ratio of low- to high-affinity T cells during a primary response to antigen. 5000 viral units were injected on day 0. The data plotted are the ratios of the number of cells of the 23 lower-affinity clones to the 2 highest-affinity clones averaged over three experiments.

cells ended. The average affinity stabilized after day 10 as memory cells formed and dominated the population. These trends agree with observations made during experimental infection of mice with paramyxovirus simian virus 5: high-affinity CD8⁺ T-cell clones were exclusively detected early in the CTL response at day 3, but low-affinity clones comprised ~50% of the response by day 5 postinfection.³⁰ Similarly in the model, low-affinity clones comprised half of the response after day 7 postinfection. We also measured the affinity of the response as the ratio of low-affinity to high-affinity CTL. This ratio rapidly dropped at the beginning of the CTL response then rose after day 7 (Fig. 4), which agrees qualitatively with observations in mice following infection with recombinant vaccinia expressing a well-characterized peptide antigen from ovalbumin. This ratio was initially high, dropped by day 6 postinfection, and returned to a high value in the memory population after the primary response.¹²

The secondary response

We modelled a secondary response to antigen by simulating the injection of additional virus into the system 28 days after a primary challenge. The T-cell clonal hierarchy in the secondary response was more consistent across different simulation runs than that observed in the primary response. In our simulations of the secondary response to virus, we found that the five highest-affinity T-cell clones were dominant, while a variable mix of lower-affinity clones comprised a small fraction of the response. The effective recruitment of high-affinity memory cells drove a second increase in average T-cell affinity for antigen (Fig. 3).

The model results agree with observations that the clonal composition of the secondary response in mice varies less

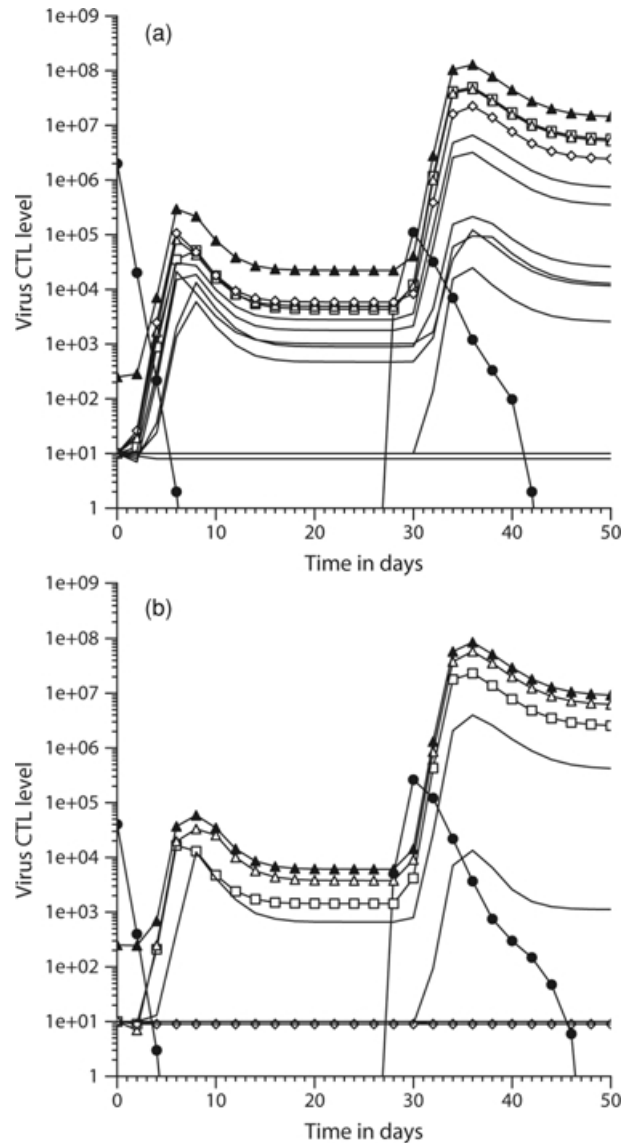


Figure 5 CTL responses to non-replicating virus followed by replicating virus challenge. The non-replicating virus dose on day 0 was (a) 2×10^6 units and (b) 4×10^4 units. The replicating virus challenge of 5000 viral units was administered on day 28 in both experiments. Virus levels are indicated by (●), the number of CTL in the three highest-affinity clones by (□), (△) and (◇) in decreasing order of affinity, and the total number of CTL (▲). Lower affinity clones are represented by lines with no markers.

than the primary amongst syngeneic animals,^{29,31–33} that the secondary response is composed of a smaller set of responding clones³⁴ and that while the primary response recruits a mix of high- and moderate-affinity clones, the secondary preferentially recruits high-affinity clones.³⁵ In our simulations, this apparent consistency of the secondary response compared to the primary occurs because of the larger number of cells involved. As discussed above, precursor frequencies are low in the primary response, allowing stochastic effects to determine whether the first cell to proliferate will come from a high- or low-affinity clone. By contrast, there is a large

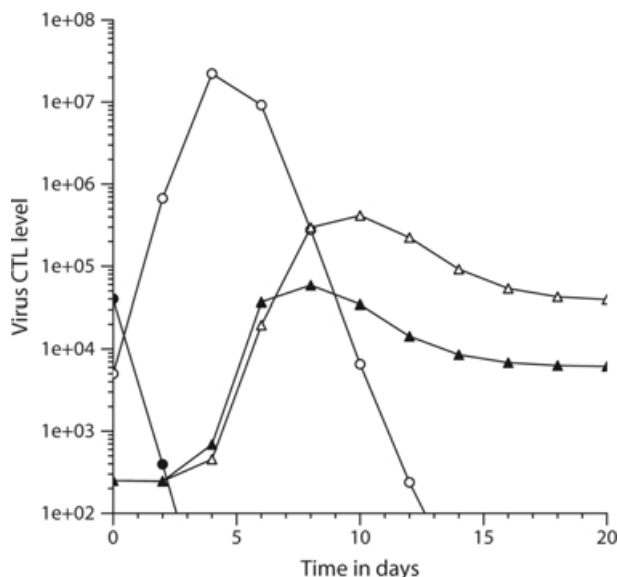


Figure 6 Comparison of responses to replicating and non-replicating virus challenges. For the replicating virus infection, the virus levels are indicated by (○) and the total number of CTL by (Δ). For the non-replicating antigen, the antigen levels are indicated by (●) and the total number of responding CTL by (▲). The data in this figure are drawn from the experiments shown in Figs 2 and 5b.

number of cells per clone in the secondary response, and the hierarchy of responding cells is therefore much more stable among simulation runs.

Non-replicating antigen

We simulated immunization with 2×10^6 viral units of non-replicating antigen. This immunization created a sharp spike in the antigen level that rapidly decayed. The high initial antigen load maximally stimulated all T cells with an affinity above a certain threshold (dependent on the antigen dose). This is in contrast to infection with replicating antigen, in which the gradually increasing antigen stimulates high-affinity clones first and gives them a time advantage over the lower-affinity clones. If these high-affinity clones clear the infection quickly, then low-affinity clones receive insufficient antigenic stimulation to be recruited into the response. This time advantage is not a factor in infection with non-replicating antigen, in which the sharp spike in antigenic stimulation caused clones of different affinities to peak simultaneously (Fig. 5a). Because our model features antigen-independent proliferation, the high-affinity clones do not interfere with the proliferation of low-affinity clones that have already been stimulated. Therefore, non-replicating antigen creates a more even distribution of high- and low-affinity clones, with the average affinity being dependent on the antigen dose. The decay phase of antigen provides some period during which the stimulation of high- and low-affinity clones can be differentiated. That is, as antigen levels progressively decline, high-affinity cells will be stimulated for the longest time. This occurs for both replicating and non-replicating antigen, as both undergo a decay phase. However, this effect probably

makes only a small contribution to differentiating high- and low-affinity cells for two reasons: (i) it might occur during the phase of antigen-independent proliferation; and (ii), if antigen decay is very rapid, there is little time difference between when the thresholds of high- and low-affinity cells are crossed.

A variety of experiments suggests that higher-affinity clones can be recruited with lower doses of antigen.^{11,12} Presumably, low doses of antigen cannot stimulate low-affinity clones, but can stimulate high-affinity ones. These high-affinity clones appear to be better for infection control.^{7,36} To investigate this phenomenon, we simulated inoculation with a smaller dose of 4×10^4 viral units of non-replicating antigen. Fewer clones responded to the low dose (Fig. 5b) than the high dose (Fig. 5a). The low dose produced memory cells with a higher average affinity for antigen than the high dose. However, because the low dose recruited small numbers of T cells, systematic differences in affinities recruited by the different antigen doses were sometimes obscured by stochastic effects. When used as a vaccine, the smaller antigen dose afforded less protection against subsequent infection by virus, allowing the virus to peak at levels three times higher than in the trial with the larger antigen dose. The large number of memory cells of various affinities formed in response to the high-dose vaccine provided better protection than the small number of high-affinity cells from the low-dose vaccine. The lack of increased protection using low doses might be because our simulation does not include direct competition between clones. Thus, the same set of high-affinity clones are stimulated with high- and low-dose antigens in the simulation and grow equally well while, in an animal vaccinated with a low dose, these high-affinity clones may expand more due to a lack of competition with low-affinity clones for resources.

Comparing the dynamics of the CTL responses to replicating and non-replicating virus infection yielded results similar to those found in mice responding to a killed bacteria vaccine.¹⁷ In both the computer model and the mouse experiments, the CTL levels in the replicating and non-replicating virus scenarios were indistinguishable on day 5 (Fig. 6). However, the responses soon diverged, with the response to the replicating virus peaking days later and the response to the non-replicating declining. The final memory cell level induced by the replicating virus infection was about an order of magnitude larger than that from the non-replicating antigen.

Discussion

We used a computer model to study the effects of antigen kinetics on CTL responses. Our results suggest that a large dose of antigen will recruit a larger and broader CTL response than a smaller dose. The clonal hierarchy of the primary response is obscured by stochastic effects because the number of T cells per clone is initially small. These stochastic effects are less evident in the secondary response because the memory cell populations established by the primary infection are relatively large. Thus, the clonal hierarchy in the secondary response and in the memory T-cell population established by this response is consistent across different stochastic runs of the same antigenic challenges. Nearly all of the cells involved in the simulated secondary responses were derived

from memory cells established in the primary response, indicating that the clonal composition of the primary response constrained the composition of subsequent responses. Although naive cells can be recruited into a secondary response (as shown in Fig. 5a,b), memory cells usually eliminated antigen before this occurred to any noticeable extent. The inhibitory effect pre-existing memory cells have on the recruitment of new clones has been reported in murine experiments.³⁷

Antigen kinetics played a role in shaping the clonal composition of CTL responses. The rising level of antigen created by replicating antigen stimulated high-affinity clones first, giving them a time advantage over low-affinity clones. In the primary response, this effect was evident in the changing average affinity of T cells involved in the response, which rose as high-affinity clones entered the response and dropped as low-affinity clones were stimulated. However, the number of cells recruited into the response from each clone was limited by the small number of cells per clone. Therefore, additional stimulation did not recruit more high-affinity naive cells. Because T cells in our model follow an antigen-independent programmed response, the time advantage experienced by high-affinity clones did not translate to greater representation in the memory pool. Antigen kinetics played a larger role in the secondary response. The population of high-affinity memory cells is not necessarily exhausted by recruitment, so early stimulation of high-affinity clones could translate to greater numbers of cells entering the secondary response. In addition, the high-affinity clones from the large memory T-cell population cleared replicating antigen before lower-affinity clones could receive stimulation. Thus, replicating antigen can lead to an accentuated clonal hierarchy in the secondary response, while non-replicating antigen recruits a more balanced number of higher- and lower-affinity cells.

Our model of CTL response omits many features of actual T cells and makes only a modest number of assumptions about CTL behaviour, in particular, the existence of a programmed response to antigen and the lack of explicit competition among T-cell clones. Despite these simplifications, the model reproduces many of the phenomena seen in CTL responses. We view model-building as a tool for hypothesis testing. One can validate one's assumptions about T-cell behaviour by comparing a model's results with real-world experiments. Modelling also allows one to perform experiments that are difficult or even impossible to perform in the laboratory. For example, in a computer model one can replicate experiments exactly or choose to allow stochastic fluctuations to influence the outcome. In biological systems, achieving this level of control is impossible. We encourage those interested to use and modify the CTL model used to produce the results in this paper. The computer source code is available at <http://www.cs.unm.edu/~dlchao/imm>.

Acknowledgements

Dennis L. Chao and Dr Stephanie Forrest gratefully acknowledge the partial support of the Office of Naval Research (N00014-99-1-0417) and the Defense Advanced Research Projects Agency (AGR F30602-00-2-0584). Dr Forrest also acknowledges the partial support of the National Science Foundation (ANIR-9986555 and DBI-0309147), the National Institutes of Health (RR-P20RR018754-010002), and the Intel

Corporation. Dr Miles P. Davenport is supported by the James S. McDonnell Foundation (21st Century Research Awards/Studying Complex Systems). Dr Alan S. Perelson acknowledges the support of the National Institutes of Health (grants R37 AI28433 and R01 RR06555). Portions of this work were done under the auspices of the US Department of Energy and supported under contract W-7405-ENG-36.

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