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Somatic Evolution in the Immune System: The Need for Germinal Centers for Efficient Affinity Maturation

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In the course of a humoral immune response, the average affinity of antibody for the immunizing antigen can increase in time. This process of affinity maturation is due to antigen-driven selection of higher affinity B cell clones and somatic hypermutation of the genes that code for the antibody variable region. Through the use of simulation models we show that the efficiency of affinity maturation is substantially improved if mutation and selection occur in germinal centers, specialized regions of lymphoid tissues, rather than in the body as a whole. We show that confining mutation and selection to germinal centers decouples the problem of affinity maturation from the problem of antigen elimination. In the germinal centers, stored antigen, high rates of B cell proliferation and apoptosis combine to create an environment where effective maturation occurs even after the primary response has eliminated much or all of the free antigen. Kepler and Perelson suggested that if hypermutation were turned on and off in a phasic manner, affinity maturation could be made more efficient under circumstances when affinity-improving mutations have a low probability of occurrence. We confirm this in the context of a stochastic model. However, even in the absence of phasic mutation, we show that affinity maturation is significantly improved when proliferation, mutation, and selection are restricted to germinal centers as opposed to occurring systemically.

Introduction

During the course of an immune response the average equilibrium binding constant or affinity of antibody for antigen typically increases with time (Eisen & Siskind, 1964; Siskind & Benacerraf, 1968; Eisen, 1991), although there are exceptions (Roost *et al*., 1995). This phenomenon, called affinity maturation, has been the subject of intense theoretical and experimental investigation for close to three decades. The molecular basis of affinity maturation involves the generation of mutations in the genes that code for the antibody variable region followed by affinity based selection (Berek & Milstein, 1987). One of the most intriguing recent findings is that somatically mutated cells appear to be generated in local regions of rapid B cell growth, called germinal centers, found in secondary lymphoid tissues such as lymph nodes, tonsils, and the spleen (Jacob *et al*., 1991; Berek *et al*., 1991; Berek & Ziegner, 1993; MacLennan, 1994). Before the role of germinal centers was elucidated, models of affinity maturation were synonymous with models of the humoral response (Bell, 1970, 1971a, b; Weinand & Conrad, 1988; Seiden & Celada, 1992) and represented events that occur within the entire immune system.

Kepler & Perelson (1993a, b) developed a differential equation model of events that occur during

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affinity maturation: B cell proliferation, mutation and affinity-based selection. The model was used to examine the question of whether B cell mutation should occur at a constant rate, or whether more complex strategies involving dynamically changing mutation rates could effect more efficient affinity maturation. Using Pontryagin's maximum principle to analyse an optimal control problem, Kepler & Perelson discovered that if mutation were turned on and off in a phasic or oscillatory manner, greater numbers of high affinity cells could be generated during an immune response than if mutation were always on. They suggested that "phasic mutation" could be implemented by having a region in the body, such as germinal centers, where mutation occurred, and then having cells repeatedly move in and out of this region. Thus, mutation being on and off would correspond to cells being inside or outside of the mutation-generating environment.

The theoretical work of Kepler & Perelson (1993a, b) suggested that germinal centers had properties that might make them particularly well suited for carrying out the mutation and selection processes underlying affinity maturation. Here we provide additional evidence that this is the case by comparing the performance of an affinity maturation model based upon an entire immune system model with a model of the events that occur within a single germinal center. Germinal centers are dynamic structures that develop due to the proliferation of a few activated B cells into a few thousand cells. Rather than "start from scratch", we modify an existing simulation model of the entire humoral response developed by Seiden & Celada (1992) and convert a differential equation model of the germinal center reaction developed by Kepler & Perelson (1993a) into a simulation model. The models that we develop are stochastic and hence can represent some of the variation in response due to the probabilistic nature of the rare events involved in the initiation of the immune response and in the somatic mutation process. The models are based on cellular automata and thus, like the Seiden & Celada (1992) model, can describe a spatially distributed system.

Seiden & Celada (1992) in their cellular automaton model of the immune system demonstrated affinity maturation by means of affinity selection. Recently, their model was expanded to include affinity maturation by somatic mutation (Celada & Seiden, 1996). In our cellular automaton model of the systemic humoral response presented below, we also find affinity maturation due to both somatic hypermutation and affinity selection. We study the behavior of the model as a function of parameters that control mutation and the probability of finding an affinity improving variant. We find that in a systemic immune system model affinity maturation occurs only for a limited range of parameters, and fails when affinity-improving mutations are rare. This is due to several reasons. First, a successful immune response eliminates the antigen rapidly and thus does not provide enough time for the generation of large numbers of somatic variants. Second, B cell clones stimulated in the response tend to survive for times comparable to the duration of the response, thus providing little opportunity for elimination of the low affinity variants involved in the response. These difficulties are overcome in the germinal center where antigen is retained for long periods of time on the surface of follicular dendritic cells (FDC). Thus, even if an immune response is successful and antigen is eliminated in most of the body, it is still retained on FDC for many months (Tew & Mandel, 1979; Tew *et al*., 1980). Moreover, germinal center B cells that are not rescued by successfully interacting with antigen die rapidly by apoptosis (Liu *et al*., 1989). Thus, the lifespan of proliferating B cells is reduced in the competitive environment of the germinal center. These features coupled with a high rate of somatic mutation of antibody V-region genes allow the germinal centers to be efficient producers and selectors of high affinity cells.

Whole Body Immune Response Model

THE MODEL

Simulations of affinity maturation in the immune system made use of a cellular automaton (CA) model based on that of Seiden & Celada (1992) but simplified to include only four classes of entities: antigen, antibodies, B cells and plasma cells*. Each B cell is assigned an affinity (probability of binding antigen) between zero and one. If it binds antigen the B cell begins to divide, producing both memory B cells and plasma cells. These B cells are copies of the original B cell, with the same affinity and lifetime. Because most of the B cells in the simulation are memory cells, the lifetime chosen for B cells (50 time steps) reflects the lifetime of a memory cell. Reproduction is assumed to continue for a fixed number of time steps, chosen to be four. As in the

*T cell help was assumed to be available and not limiting, and thus not included in this simplified model. This situation is realized in experimental models of affinity maturation that use hapten-protein conjugates as the antigen and carrier-primed mice (c.f. MacLennan *et al*., 1990).

Seiden & Celada (1992) model, each division is assumed to be asymmetric producing one B cell and one plasma cell. The plasma cells produce antibodies (here ten per time step) with the same affinity as the original B cell for a fixed number of time steps and then die. The antibodies remain in the system until they either bind antigen, which results in mutual annihilation, or until they are cleared from the body. (The lifetime of antibodies and plasma cells are taken as 10 time steps, i.e., each has a probability of elimination of 0.1 per time step.) Antigen, which is non-growing, is introduced into the system at time 0, and is removed only through binding to B cells and antibodies.

Entities diffuse through a two-dimensional, doubly periodic grid of physical compartments, taken to represent the body. This diffusion occurs in discrete time and is accompanied by interactions between the different types of entities that are present at each grid location. We used a 15×15 grid of compartments and implemented diffusion by allowing each entity at each time step to remain in place or move to one of the eight neighboring sites with equal probability.

By the convention established in the model a single time step corresponds to a B cell division time. The unit of time used throughout is the time step. This can be mapped onto days by assuming a nominal B cell division time of say 8 hr. The unit of time in turn establishes the size of a grid cell in the model as the characteristic area sampled by a B cell in the B cell division time.

The possible behaviors of the entities are executed in the following order: diffusion, interaction, reproduction, death. Thus, at any time step a typical B cell is moved, then attempts to interact with the antigen at its updated site. The probability of binding between a B cell and antigen is given by the B cell affinity. A B cell undergoes a division if it has bound antigen in the current time step or the three preceding steps. Finally, the B cell has a probability, equal to the reciprocal of its lifetime, of dying at the end of each time step.

After binding an antigen a B cell undergoes four division steps. On each division step it produces a copy of itself and a type of plasma cell (plasmablast). The plasma cells do not begin producing antibodies until the division process is complete.* Thus, after the completion of a four-step division cycle, there are eight B cells and eight plasma cells, which begin producing antibodies. Antibodies have a lifetime which is the same as that of the plasma cells. Antigens have an infinite lifetime and remain in the system until they are bound by B cells or antibodies. This model is a closer approximation to an immunization experiment, where the injected antigen is degraded and is non-reproducing, than to an infection.

*This mimics the time needed for plasmablasts to differentiate into fully developed antibody-secreting plasma cells.

THE MODEL REPRODUCES THE HUMORAL RESPONSE

The basic response of this model to the injection of antigen was evaluated for several initial conditions. The character of the response was robust to various changes in the model parameters. A typical response is shown in Fig. 1. This simulation was started with ten high affinity B cells with affinity $K = 1$, and 200 antigens placed at random in the space. (Simulations with lower affinity cells will be described in the next section.) The number of B cells and plasma cells increase almost immediately. After a delay of a few time steps antibody is produced, and the number of antigens in the system decreases rapidly. Antibodies produced initially bind with antigen and both are eliminated. Thus, the antibody concentration does not increase substantially until almost all the antigen is removed from the system. The response to the introduction of 1000 antigens is similar to that of Fig. 1 but of higher amplitude (not shown). These results resemble the response found by Seiden &

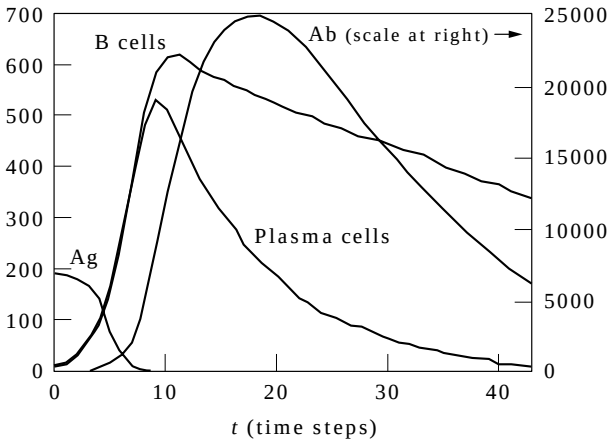

FIG. 1. Typical response of the whole body immune system model to an injection of 200 antigens. Shown are the numbers of B cells, plasma cells, antigens (Ag), and antibodies (Ab) as a function of time. The B cell, plasma cell and antigen vertical scale is on the left while the antibody scale is on the right. The immune response is initiated when B cells bind antigen. This is followed by B cell proliferation and differentiation into plasma cells. The resulting plasma cells produce antibodies that remove the antigen. When antigen removal is complete, B cell division can no longer be stimulated and the populations of B cells and plasma cells decay due to their finite lifetimes. The continued production of antibody by the plasma cell population leads to a significantly later maximum in the antibody concentration. The response for 1000 antigens is similar to that of 200 antigens, but the larger number of antigens stimulates the production of correspondingly larger numbers of B cells, plasma cells, and antibodies.

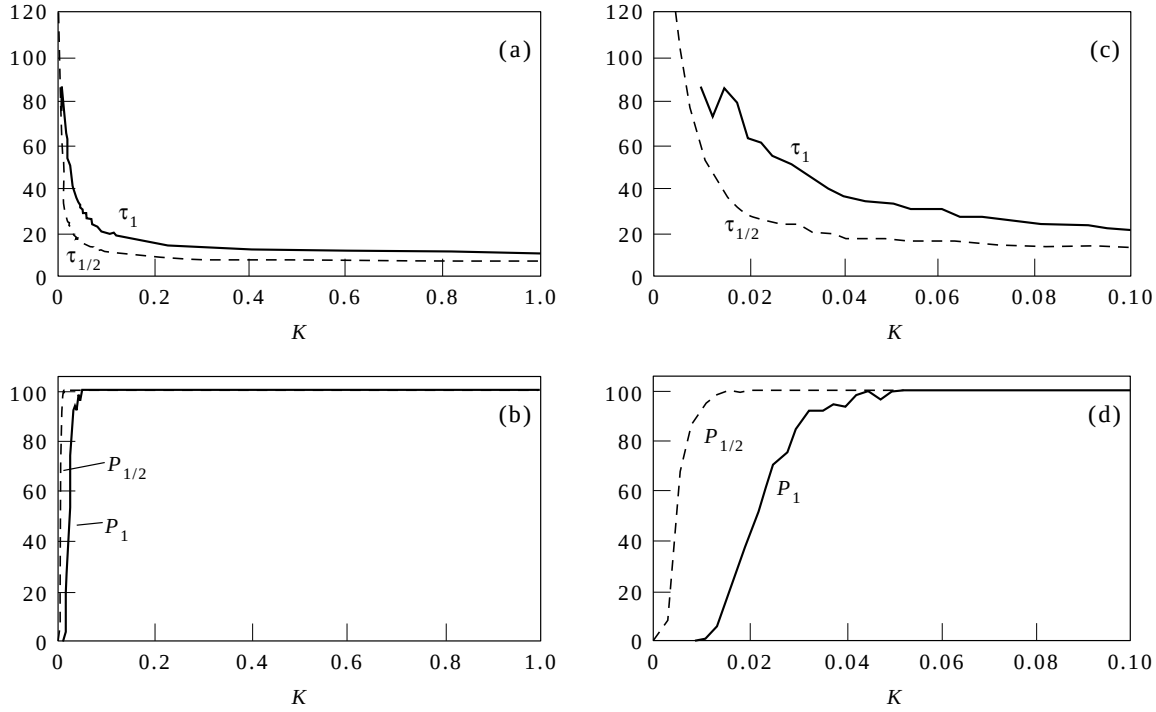

FIG. 2. Dependence of the whole body immune response on the B cell affinity K. At $t = 0$, 200 antigens were injected. The results shown are averages over 100 trials. (a) The average removal time, τ_1, and the time to remove 50% of the antigen, $\tau_{1/2}$; (b) the probability of success, P_1, and probability of 50% removal, $P_{1/2}$. Details of the low-affinity behavior are shown in (c) and (d), respectively. The removal time τ_1 is nearly constant for affinities greater than 0.1. Below this value it rapidly increases. Moreover, below $K = 0.05$ the probability of success, P_1, drops towards zero.

Celada (1992). However, since their simulations included additional lower affinity B cells, we also ran simulations that included such cells. Including these lower affinity cells had no significant effect on the total response since the high affinity cells were mainly responsible for antigen elimination (not shown).

AFFINITY DEPENDENCE OF THE RESPONSE: A MINIMUM AFFINITY IS REQUIRED TO ELIMINATE ANTIGEN

Affinity maturation relies upon the affinity dependence of B cell binding. Preliminary to the study of maturation by mutation we evaluated systematically the affinity dependence of the system response. The initial conditions for each run consisted of ten B cells of a single affinity. The effectiveness of the response was characterized by the time required to remove half, $\tau_{1/2}$, and all, τ_1, of the antigen. Averages over 100 trials are shown in Fig. 2 for an antigen load of 200. We found that the removal times were nearly constant for a wide range of affinities, $K \simeq 0.1$ to 1.0 [Fig. 2(a)]. For $K < 0.1$, however, τ_1 increased sharply, and for $K < K_{crit} \simeq 0.05$ for an antigen load of 200 the system was often unable to remove the antigen before all the B cells died and antibody was lost. Figures 2(b) and (d) show how the success rate for total antigen removal, P_1, decreases from 100%, for K just above K_{crit}, down to 0% as K is decreased. Also shown is the probability of successfully removing half of the antigen, $P_{1/2}$. For a range of low affinities, where the antigen can not be fully removed, half of the antigen can still be removed [Fig. 2(c)]. Results for an initial antigen load of 1000 are similar, except the critical affinity is reduced to 0.01.

The existence of a minimum affinity required for antigen elimination may be significant, especially since affinity thresholds have been found experimentally (Riley & Klinman, 1986; Mongini *et al*., 1991) and in other immune system models based on different premises (Sulzer *et al*., 1996). Also, according to the shape-space model of Perelson & Oster (1979), the closer the match in generalized shape between antigen and antibody the higher the affinity. The existence of an affinity threshold for effective immune responses implies that the pre-existing immune system repertoire of B cell receptors must "cover" the shape-space of possible antigens with greater than some minimum sparseness in order to have a high likelihood that any antigen will be close to at least one B cell receptor (antibody) shape. Mutation will not change this conclusion since for affinity maturation to begin B cells must first bind antigen.

The results at different antigen injection levels are consistent with the experimental observation that a higher antigen level can lead to a stronger response (Janeway & Travers, 1994). Further, as in equilibrium theories of antigen binding, the product of antigen concentration and affinity is a relevant non-dimensional quantity for characterizing system behavior. Our model exhibits an affinity threshold for effective responses, this threshold level however depends upon the antigen level. If the antigen level is lowered, the regime of little or no response increases to higher affinity. Thus, as seen *in vivo*, our model will exhibit low-zone unresponsiveness, i.e., small enough antigen levels will not trigger an effective response. Because of stochastic effects, low levels of antigen can not be totally compensated for by higher affinity, and thus the model will not behave consistently for all values of the product of affinity times antigen level.

The existence of an affinity regime ($K < K_{crit}$) where some trials were successful in eliminating the antigen while others were not demonstrates the importance of incorporating into our model the finite size of the immune system. In the $K < K_{crit}$ regime we say that "self-averaging" does not apply. The concept of self-averaging (Mezard *et al*., 1987) is important in interpreting both theoretical and experimental results. A system that self-averages has a response that is largely independent of the stochastic nature of events. This applies when all animals in a study have similar responses. Because the response of one system is the same as the average response, we use the term "self-averaging" to describe it. Self-averaging does not apply when the outcome is not uniform. Larger systems effectively contain within them a number of realizations of a smaller system and thus are generally more likely to be self-averaging.

SOMATIC HYPERMUTATION

The affinity landscape

In order to study affinity maturation by somatic hypermutation, it is necesary to set up an affinity landscape—the variation of affinity with B cell mutation (Macken & Perelson, 1989, 1995). In their model, Seiden & Celada (1992) used a shape space (Perelson & Oster, 1979; Farmer *et al*., 1986) where binary strings of 8 bits represented the shape and chemical properties of the antigen, the receptors on the B cells, and antibodies. The number of bits of a B cell receptor which match those on the antigen determined the affinity of the B cell. For eight matching bits the affinity was one, for seven matching bits the affinity was set to 0.05, while for lower numbers of matching bits the affinity was set to zero. In order to compare our results with the results of Kepler & Perelson (1993a, b), in which a wide range of affinities is studied, we assume a single type of antigen, and rather than using binary strings we use prespecified affinity classes for the B cells and antibodies. In our model, each B cell is assumed to be in one of the possible affinity classes, with the affinity of a cell in class i given by $K(i) = \min(1, K_0 r^i)$, where i is zero or any positive or negative integer. The initial condition assumes all B cells begin in class $i = 0$ with affinity K_0. K_0 is varied to evaluate its significance, along with the affinity ratio, $r \geq 1$, between neighboring classes. Classes with higher index have a higher affinity. Following a mutation, a B cell in class i is assumed to reach either class $i - 1$ or class $i + 1$. The relative probability of moving to class $i - 1$ compared with the probability of moving to $i - 1$ was set equal to $1/\Lambda^2$ for all i, where $\Lambda \geq 1$. [This notation was used to be consistent with that introduced in Kepler & Perelson (1993a).] Thus, $1/\Lambda^2$ is the ratio between the number of possible advantageous mutations and the number of disadvantageous ones. For example, in the shape space bit string representation the affinity class would correspond to B cells with a certain bit string mismatch h. The value of $1/\Lambda^2$ is given by $h/(m - h)$, since out of the m possible mutations h increase the match and $m - h$ decrease it. In the present model, and that of Kepler & Perelson, the value of $1/\Lambda^2$ is preselected and set to a constant independent of the affinity class. This is an advantage because it enables smaller values of $1/\Lambda^2$ to be simulated then in models using bit strings. For example, for strings of length 8, transitions from seven matching bits have $h = 1$, and thus $1/\Lambda^2 = 1/7$. To have $1/\Lambda^2 = 0.01$ requires bit strings longer than 100. However, a disadvantage to the current approach is that it ignores the possibility that as an antibody affinity matures it may become more difficult to find affinity-improving mutations. This phenomenon can be incorporated into our model by making $1/\Lambda^2$ a decreasing function of the affinity class.

The whole body model exhibits affinity maturation when the probability of finding an improvement mutation is high

Using our predefined affinity landscape, we ran simulations of the whole body model and measured the average antigen removal time and the ultimate affinity when antigen removal was complete. During a simulation, B cells have a probability μ of mutating when they divide. For the first set of studies, the starting affinity, $K_0 = 0.01$. Under these conditions the success rate for antigen removal without mutation is zero, but at slightly higher values of the affinity the

success rate increases. Thus, this initial value can reveal the contribution of mutation. The time dependence of the B cell populations in each affinity class are shown for a specific simulation in Fig. 3. Since all of the initial B cells are in affinity class zero, affinity maturation is evident through the appearance and eventual dominance by the higher affinity classes 1 and 2. At the final time step of this figure the antigen has been completely removed. Thus, there would be no additional maturation beyond this point and the B cells gradually die. The average B cell affinity reached at this final time step is denoted K_f. In the simulation shown in Fig. 3, the relative probability of a mutation that increases the affinity was taken to be $1/\Lambda^2 = 1/4$. This value corresponds to a quite high probability that a mutation improves the affinity. The effect of variation in the parameters μ, r, and Λ^2 is described by Fig. 4.

The model has an optimal mutation rate for affinity maturation

Figure 4, panels (a–c), show the effects of varying the mutation rate, μ. The success rate, P_1, increases with the introduction of mutation and reaches a maximum of 70% for a mutation rate of approximately 0.25/antibody/generation. It declines thereafter due to a decrease in the growth rate of higher affinity classes due to mutations that decrease B cell affinity. A similar "optimum" mutation rate has been found by Celada & Seiden (1996) and Kepler & Perelson (1993a). At the optimal mutation rate the average B cell affinity, K_f, reaches approximately 0.17, which represents a factor of 17 increase over the initial affinity of 0.01. This corresponds to an average improvement of two affinity classes. These simulations show that mutation can improve the effectiveness of the response and lead to affinity maturation. However, these results are obtained under conditions favorable to the effectiveness of mutations.

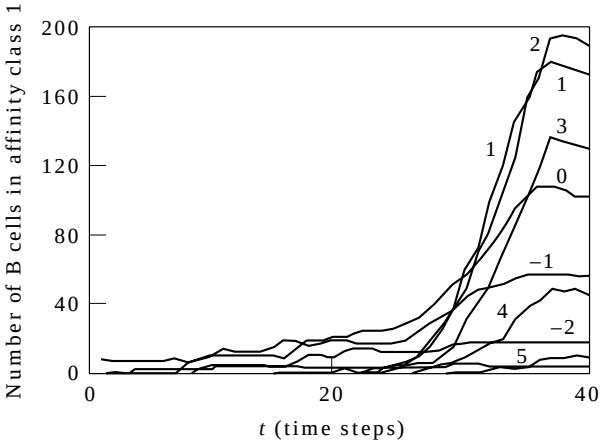

FIG. 3. Affinity maturation can occur in the whole body model. The B cell population in each affinity class is plotted as a function of time. A single representative simulation is shown. The simulation begins with ten B cells in class 0. Mutation drives cells into the adjoining affinity classes with the relative probability of generating a favorable mutation taken to be $1/\Lambda^2 = 1/4$. The affinity ratio between affinity classes, $r = 7.5$, and the mutation rate $\mu = 0.25$. At the end of the response, class 2 contains the most B cells, but only slightly more than class 1.

The effect of varying the affinity ratio between affinity classes

In this model, mutation drives a B cell from its affinity class to either a higher or lower affinity class. The affinity ratio, r, determines the affinity of the neighboring classes. Figure 4, panels (d–f), where the mutation rate was fixed at its optimum value of $\mu = 0.25$, show that when r is varied the maturation process does not improve affinity significantly above the value found at $r = 7.5$. At higher values of r the affinity saturates (the maximal affinity in this model is one). For all values of r, affinity maturation is unable to improve the affinity more than 1–2 affinity classes, and at low values of r the system fails to achieve effective maturation.

The model fails to exhibit affinity maturation when favorable mutations are rare

A more significant problem arises in this model when we consider higher values of Λ^2 so that favorable mutations are less probable. Figure 4, panels (g–i), show the behavior of the model as a function of Λ^2 for $K_0 = 0.01$ and $r = 7.5$. The effectiveness of mutation in increasing affinity and successfully eliminating antigen decreases dramatically with Λ^2. For $\Lambda^2 > 15$, we find that the probability of successfully eliminating the antigen is less than 20% and the antigen removal time is over 100 time steps or about 30 days. By contrast, Kepler & Perelson (1993a) obtained excellent maturation for $\Lambda^2 = 900$. For a constant mutation rate their germinal center model produced maturation factors of 10^2–10^3, corresponding to an improvement of approximately three affinity classes in 14 days. The removal time can be reduced, and the success rate improved, by increasing the original affinity. Increasing K_0 to 0.05 decreases the removal time for $\Lambda^2 = 15$ to 40 time steps, but there is no affinity maturation. In fact, above $\Lambda^2 = 15$ the average affinity decreases during the response due to mutation (not shown).

We conclude that the performance under mutation of this model is poor for landscapes with a low probability of affinity improvement. Although, the

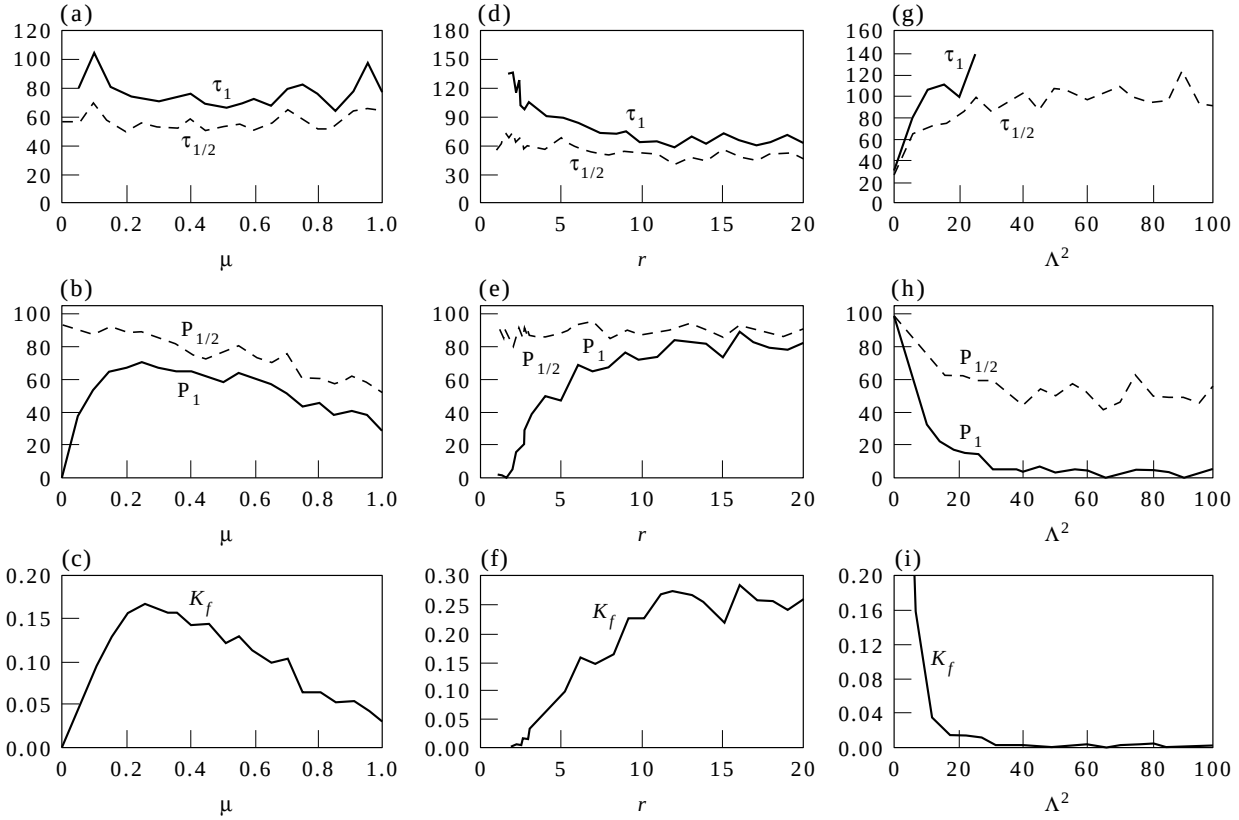

FIG. 4. Dependence of affinity maturation and successful antigen elimination on the parameters of the model. The results shown are averages over 100 trials. (top row) The removal times τ_1 and $\tau_{1/2}$, (middle row) the probabilities of successful antigen removal P_1 and $P_{1/2}$, (bottom row) K_f, the average B cell affinity at τ_1. In (a–c) the mutation rate, μ, is varied and $\Lambda^2 = 4$, $r = 7.5$ and $K_0 = 0.01$. Due to the high probability of a mutation increasing affinity and the large affinity ratio, mutation can increase affinity and improve the effectiveness of antigen removal. Note that there is an optimal mutation rate of approximately 0.25/antibody/generation. In (d–f) the affinity ratio, r is varied and $\Lambda^2 = 4$, $\mu = 0.25$ and $K_0 = 0.01$. For $r > 10$ the affinity saturates, because the maximal affinity in this model is one. For lower values of r, the affinity difference between classes is small and the maturation achieves only an average improvement of one affinity class. When r is too small mutation does not improve antibody affinity enough to eliminate the antigen. In (g–i) the relative probability of a favorable mutation, $1/\Lambda^2$, is varied, and $r = 7.5$, $\mu = 0.25$ and $K_0 = 0.01$. As Λ^2 increases, the removal time τ_1 becomes large, while the probability of antigen elimination, P_1, and affinity maturation drop sharply. The curve for τ_1 stops at $\Lambda^2 = 25$ due to lack of statistics. The results show that affinities fail to improve in the whole body model if the relative probability of a favorable mutation, $1/\Lambda^2$, is too small.

probability of affinity improvement is not well known, it is unlikely to be always as favorable as required to achieve efficient improvement in this model. In some cases, for example when DNA is the antigen, there may be many affinity improving mutations. In the case of anti-DNA antibodies the mutations that raise affinity tend to be mutations to the basic amino acids, arginine, asparagine and lysine, which have the potential for electrostatically interacting with DNA (Radic & Weigert, 1994). Since there are many mutational pathways that can generate the basic amino acids, e.g. six of the 64 codons correspond to arginine, one might expect that for anti-DNA antibodies affinity improving mutations are common. However, for systems in which the antigen is a hapten, one expects rather few mutations to improve affinity. For example, Casson & Manser (1995a) introduced random mutations into positions 58 and 59 of the variable region of the heavy chain of an anti-p-azophenylarsonate (Ars) antibody. Mutations at these positions are frequently observed in naturally isolated antibodies and account for most of the affinity improvement displayed by secondary anti-Ars antibodies (Sharon *et al*., 1989). Despite this, less than 5–10% of the mutants screened had higher affinity for Ars. In another experiment in which mutations were introduced throughout the heavy chain variable region, no mutant was found that appeared to have increased affinity for Ars (Casson & Manser, 1995b). Chen *et al*. (1992) used saturation mutagenesis to introduce point mutations into the H chain CDR2 of the anti-phosphocholine antibody T15. Of the 46 mutants analyzed none had improved binding. However, using a similar approach on another

anti-phosphocholine antibody, D16, seven out of 43 mutants with between one and four mutations in CDR2 had improved binding (Chen *et al*., 1995). In attempts to engineer antibodies to have increased affinity success has been rare. In fact, the first reported success in increasing affinity by mutagenesis was serendipitous, because the substituted residue had been predicted from a computer model to decrease affinity (Roberts *et al*., 1987). There have been other successes in increasing affinity by mutagenesis where structural models have been used to target the substitutions, but the experience has been that most mutations, particularly in antigen contact residues, do not result in increased affinity (Wong *et al*., 1995).

Factors that make the whole body model inefficient at achieving effective affinity maturation

The model simulated thus far was based on Seiden & Celada's model of the characteristic immune response occurring in the body as a whole. However, hypermutation is observed to occur in the germinal centers (Berek *et al*., 1991; Jacob *et al*., 1991b, 1993), which generate responses with different biological characteristics. Therefore, a reasonably accurate stochastic CA model of hypermutation in germinal centers may be expected to require different parameters and assumptions. The case of mutations that have only a low probability of providing affinity improvement is a difficult one for affinity optimization. We now discuss how this problem can be overcome by the design of the germinal center.

There are three factors that make the whole body model incapable of achieving effective maturation. First, the assumed long lifetime of memory B cells inhibits maturation because even when high-affinity cells are produced, the low-affinity ones are never significantly depleted. Second, the rapid decrease in the amount of antigen in the system as the primary response proceeds implies that the driving force for maturation is present over a time which is limited by the effectiveness of the response. When the response is effective there is little time for mutation to contribute. This suggests that mutation-induced maturation should be considered as primarily relevant for a second response to the antigen, as has been seen experimentally (Coico *et al*., 1983; Tsiagbe *et al*., 1992).

The third factor is the relatively small number of B cells. A mouse contains of the order of 10^8 B cells, that are divided into about 10^7 different specificities (Klinman & Press, 1975). We generally started our simulations with ten B cells, approximately the average number of cells in a virgin B cell clone, and their number typically peaked at several hundred. For $\Lambda^2 = 10$–100, the probability that a mutation increases the affinity class of any B cell is 0.1–0.01. With these parameters, a system containing only a few hundred antigen-specific B cells is not likely to produce many higher affinity cells even with a very high mutation rate.

Affinity Maturation in Germinal Centers

DESIGN ADVANTAGES OF THE GERMINAL CENTER

Germinal centers appear to be designed to overcome the problems of generating large numbers of high affinity cells encountered with the CA model of the whole body immune response. In particular: (1) Apoptosis is used to reduce the lifetime of B cells that do not bind antigen (Liu *et al*., 1989, 1992; Han *et al*., 1995b). (2) The antigen is not totally eliminated by a successful response. In mice, antigen-antibody complexes can be retained on FDC cells for many months (Tew & Mandel, 1979; Tew *et al*., 1980). (3) A large degree of clonal expansion of antigen-specific B cells occurs in germinal centers. Thus, the physiology of germinal centers appears to be well suited to the maturation of affinity by hypermutation.

MODEL OF AFFINITY MATURATION IN THE GERMINAL CENTER

To obtain a CA model of affinity maturation in the germinal center, we implemented the features of conserved antigen, a short B cell lifetime equal to the cell division time (1 time unit), and a high level of antigen (10^4) to stimulate the production of a large number of B cells. As in the model of Kepler & Perelson, we considered the germinal center as a single compartment, i.e., 1×1 grid, and did not allow B cells to differentiate into plasma cells or secrete antibody. These modifications are justified physiologically since the size of a germinal center is small and thus a B cell may be able to sample the entire germinal center in its division time. Also, throughout the primary response, germinal center B cells secrete very little antibody (Jacob & Kelsoe, 1992). Most antibody secretion occurs in antibody secreting cell foci, structures recently modeled by Oprea & Perelson (1996).

Antigen conservation was implemented by sequential (asynchronous) unbinding and rebinding of B cells in every time step. The amount of available antigen remains near its equilibrium value throughout each time step and all the B cells are subject to the same selection pressure. Since B cells that do not bind antigen perish and those that do reproduce, the

binding with antigen is analogous to fitness selection in a model of evolution.

In germinal centers B cells divide more rapidly than in other parts of the body. Cell division times as rapid as 6–7 hr have been measured in germinal centers (Zhang *et al*., 1988), and thus one time step in our germinal center simulation should be considered to be of this duration. Even though outside of the germinal center, cells may divide more slowly, the Seiden & Celada model represents the overall characteristics of the whole body response including the germinal center and therefore they considered a characteristic cell division time to be of similar duration to that in the germinal center. We therefore take an 8 hr time step for both models though we note that the shortening of the division time in the germinal center is another mechanism by which the maturation is accelerated.

THE GERMINAL CENTER MODEL ACHIEVES EFFICIENT AFFINITY MATURATION EVEN WHEN IMPROVEMENT MUTATIONS ARE RARE

Simulations of the stochastic germinal center model for the parameters used by Kepler & Perelson (1993a), $r = 7.5$ and $\Lambda^2 = 900$, were consistent with their findings. In particular, we found a similar affinity maturation factor and increase in affinity classes. Figure 5 shows the results of a single simulation of this model for $\mu = 0.1$ with $K_0 = 0.0005$. Comparing Fig. 5 with Fig. 3, we see that in the model of whole body response the increase in B cell affinity classes occurs more slowly, with little depletion of the lower classes, and significantly less affinity maturation occurs. The performance of the germinal center model is dramatically better. The B cell population moves to progressively higher affinity classes as the simulation proceeds and after 56 time steps, or 14 days, an increase of three classes has been achieved. Mutations to a higher class $i + 1$ are not significant until class i becomes highly populated and a mutation to class $i + 1$ becomes likely. Once this happens there is a rapid increase of B cells in class $i + 1$ at the expense of class i, due to the affinity difference and the effect of apoptosis.

The maturation in Figs 3 and 5 differ in character, with the latter displaying sequential appearance of affinity classes and the former displaying overlapping populations in different classes. The different character of these responses can be understood from considerations related to the different values of Λ^2. There are two time-scales that control the appearance and dominance of a new affinity class. The first is the time to achieve a single favorable mutation, which is proportional to Λ^2, and the second is the time for the affinity class to grow until it dominates the population of B cells. When the former is smaller than the latter, i.e., for small Λ^2, there are overlapping affinity classes. When the latter is smaller, i.e., for high Λ^2, then there are distinct epochs for each affinity class.

Figure 6(a) shows K_f, the average B cell affinity reached after 14 days, as a function of the mutation rate μ. Here the starting affinity $K_0 = 5 \times 10^{-4}$ and thus the peak of this curve corresponds to a maturation factor of approximately 500. This is in agreement with the results of Kepler & Perelson (1993a). Figure 6b shows the average B cell affinity, K_f, as a function of Λ^2 for $\mu = 0.1$. There is a slight decrease of the average affinity with increasing Λ^2. In contrast to the whole body model highly effective affinity maturation is achieved up to at least $\Lambda^2 = 900$. Thus, affinity maturation is effective even when improvement mutations are rare.

AFFINITY MATURATION CAN BE ENHANCED BY PHASIC MUTATION

Kepler & Perelson (1993a, b) showed, using a differential equation model, that affinity maturation can be improved in a germinal center model by using a time-pulsed or phasic mutation rate. Figure 7 shows the results of a simulation using a variable mutation

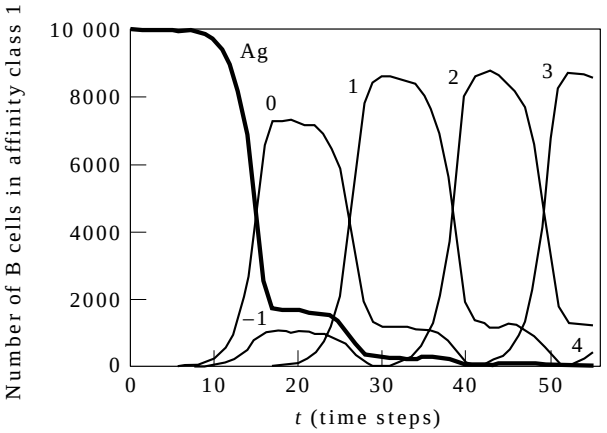

FIG. 5. Illustrative example of mutation-driven affinity maturation in the germinal center model. The B cell population in each affinity class is plotted as a function of time. The simulation begins with two B cells in class 0. The mutation rate $\mu = 0.1$, the initial affinity $K_0 = 0.0005$, and $\Lambda^2 = 900$. The highly effective affinity maturation seen is in sharp contrast to the results for the whole body model which for $\Lambda^2 = 900$ would not yield affinity maturation [see Fig. 4(i)]. The conservation of antigen and the occurrence of apoptosis in the germinal center model enables class 3 to become dominant by day 14. Even at much lower values of Λ^2 the character and effectiveness of the whole body model response is different (see Fig. 3 illustrating the results for $\Lambda^2 = 4$). In the germinal center model the appearance of affinity classes occurs sequentially with a characteristic time interval separating them.

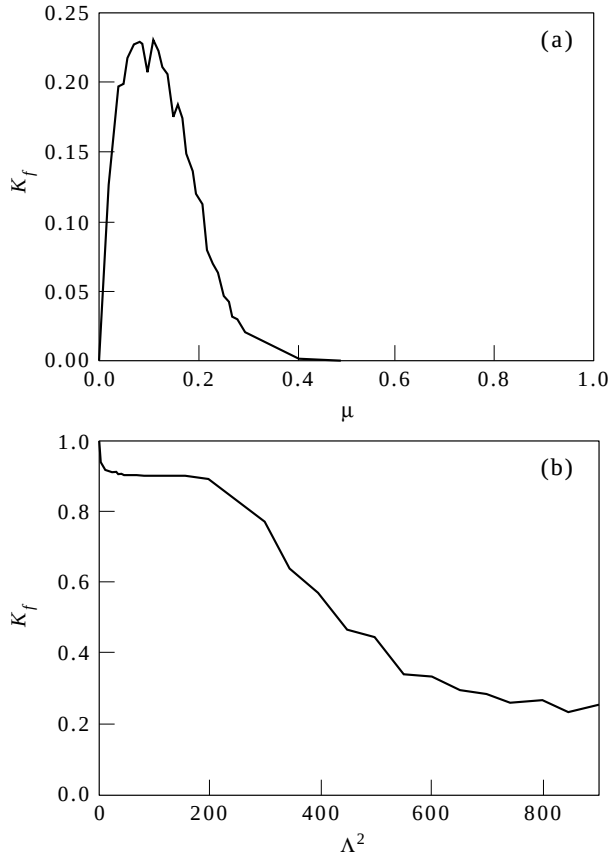

FIG. 6. The dependence of affinity maturation in the germinal center model on the mutation rate μ and the relative probability of a favorable mutation, Λ^2. Shown is the average B cell affinity, K_f, reached after 14 days. (a) The effectiveness of affinity maturation is sensitive to the mutation rate, reaching a peak in the vicinity of $\mu = 0.1$. Other parameter values are $\Lambda^2 = 900$, $r = 7.5$, and $K_0 = 5 \times 10^{-4}$. Compare with the results of the whole body model in Fig. 4(c). (b) Affinity maturation in germinal center model occurs even when the relative probability of a favorable mutation, Λ^2, is very low. K_f decreases as Λ^2 increases, but affinity maturation remains effective at the highest value studied, $\Lambda^2 = 900$. The ability to improve when the probability of advantageous mutations is low was not seen in the whole body model [compare Fig. 4(i)].

rate. Here μ was set to zero except for specific time steps, where it was set to one (gray bands in Fig. 7). This means that all B cells were simultaneously mutated, depleting the originally dominant affinity class. The B-cells after mutation populate the adjacent affinity classes below and above in the proportions Λ^2:1. In this simulation of phasic mutation affinity class 4 becomes populated after 14 days and the affinity maturation factor increases to about 2000.

We also tested the sensitivity of these results to variations in the mutation rate at the steps where mutation is triggered. The advantage of using a high mutation rate, when mutation occurs, is that the previous best affinity class is depleted. Depleting the previous best affinity class is beneficial because its coverage of the antigen on FDC slows the rate of growth of the newly emerging higher affinity class. Instead of competing with the previous best class the new improved affinity class competes with lower affinity classes (see Fig. 7). We found that decreasing the mutation rate below unity resulted in a graceful degradation of the maturation rate. The maturation was still improved over that of the best constant mutation results for episodic mutation with mutation rates above 0.5. Variation in the simulated value of μ covers a variety of biological effects including neutral mutations that do not change the affinity class and multiple mutations. These results provide some confidence that phasic mutation has a robustness that is essential for actual implementation.

Discussion

We have presented and described two cellular automaton models of affinity maturation. The first, based on the model of Seiden & Celada (1992), considered the dynamics of an immune response against antigen and included features of affinity maturation. As Seiden & Celada have already shown, the model can account for maturation of the immune response by affinity selection. However, we found that the model was rather poor at reproducing the features of affinity maturation due to hypermutation of antibody V-region genes, especially when the probability of finding an advantageous mutation was low. This we believe was not a failure of the model but rather accurately mimicked biological reality. Immune responses are designed to rid the body of antigen rapidly. However, mutation and selection processes take time, and our simulations show that by the time clones expand enough to produce high affinity mutants they are already large enough to lead to the elimination of the antigen. Thus, mutation does not seem to play an important role in the primary response, and the loss of antigen prevents the efficient selection of high affinity variants.

Our second cellular automaton model, based upon the Kepler & Perelson (1993a) differential equation model of events in germinal centers, exhibited efficient affinity maturation. Because antigen is retained in germinal centers for many months (Tew & Mandel, 1979), germinal centers provide an environment for efficient selection of high affinity variants as the antigen is removed from the remainder of the body. Thus, efficient immune responses and efficient affinity maturation are not in conflict but rather can occur independently. The immune response can generate antibody and eliminate the antigen, while the

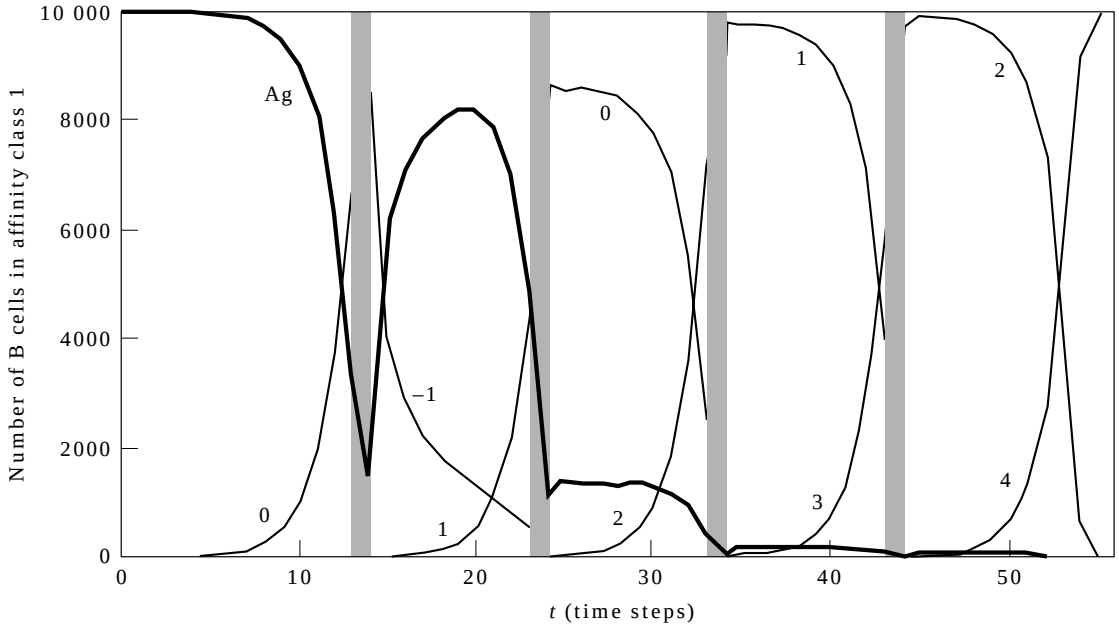

FIG. 7. Response of the germinal center model to phasic mutation. The populations of the B cells in the different affinity classes and the antigen concentration (dark line) are shown. The mutation rate μ is zero except at the times indicated by the grey bars, where the mutation rate is raised to one. Thus, during the mutation period all cells mutate. This causes all cells in affinity class 0 to disappear after the first mutation period and be replaced by cells in classes -1 and 1. Similar displacements occur at each mutation phase. Affinity maturation drives the system from affinity class 0 to affinity class 4, an increase of 4 classes, which is greater than for the optimal constant mutation rate (compare Fig. 5).

germinal center can provide a tailored environment for the generation of high affinity cells that would be useful in subsequent encounters with antigen. Thus, in our simulations germinal centers appear to be useful in the generation of immune memory and the creation of efficient secondary immune responses, but not in the generation of efficient primary immune responses.

The same conclusions have been reached from purely biological experiments. Within secondary lymphoid tissues two distinct cellular compartments are found, antibody-forming cell foci (Jacob *et al*., 1991b) and germinal centers. The foci, which are aggregates of plasmablasts and plasma cells found at the periphery of the T cell-rich areas of spleen and lymph nodes, have been found to be the major source of early circulating antibody (Miller *et al*., 1995). Thus, for example, if the germinal center reaction is interfered with early in an immune response by the use of a monoclonal antibody against the cell surface molecule B7-2, one can abolish germinal center formation and have only a modest reduction in serum antibody production (Han *et al*., 1995a). This same antibody given late in the response causes arrest of germinal center development, reduced somatic mutation, and the loss of a memory response (Han *et al*., 1995a), demonstrating the importance of germinal centers in generation of memory.

Cold-blooded vertebrates, such as *Xenopus*, appear not to have germinal centers but they still mutate their antibody V-region genes (Nahm *et al*., 1992; Wilson *et al*., 1992). Our model suggests that high affinity variants would not be efficiently selected in the absence of germinal centers, and hence cold-blooded vertebrates should have poor affinity maturation. This is, in fact, what is observed (Wilson *et al*., 1992).

Our simulation models, being stochastic, reproduce some features of immune systems that are not seen in deterministic models. For example, when the antigen level is low, only a few B cells can be stimulated to respond. With small system size, different simulation runs produce different results. This is reminiscent of observations in mice, where even though inbred strains of mice are used, different response dynamics are observed in individual animals. In physics, this is called a loss of self-averaging (Mezard *et al*., 1987). It is possible, that for larger experimental animals, with more cells, self-averaging occurs at a lower antigen level. Our simulations suggest that it might be illuminating to compare the reproducibility of immune response dynamics in animals of different sizes, e.g. mice, rats, rabbits and man. Langman & Cohn (1987) have suggested that a large animal is composed of multiple copies of a basic immunological subunit called a protecton, with each protecton

functioning in an independent and identical fashion. Our model suggests opposite, specifically that large animals might have more reliable immune systems due to self-averaging.

In addition to retaining the antigen for longer times, germinal centers also use accelerated cell division, apoptosis, and relatively low antibody concentration to generate an environment conducive to affinity maturation. All of these modifications are found to be consistent with solving the problem of finding and amplifying rare (low probability) affinity improving mutations. In order to search the set of possible antibody V-region mutations a large population of clones must be generated and mutated. In our model this occurs with a constant mutation rate (Fig. 5), but phasic mutation, as suggested by Kepler & Perelson (1993a, b) can enhance this process (Fig. 7) and lead to more rapid generation of high affinity variants. While our simulations were performed for a smooth affinity landscape with a fixed probability of finding an improvement mutation, we believe the results for a rough landscape (Macken & Perelson, 1989, 1995) would be qualitatively similar as long as the essential property—the low probability of a favorable mutation—is retained. Lastly, our germinal center was modeled as a single compartment. Extensions, using a discrete simulation approach, in which the germinal center is broken into regions representing the light and dark zones of the germinal center (MacLennan, 1994), and movement between them, are under development.

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