Cyclic re-entry of germinal center B cells and the efficiency of affinity maturation

Thomas B. Kepler and Alan S. Perelson

Affinity maturation of the humoral immune response by somatic hypermutation is marked by a rapid and dramatic increase in affinity for the eliciting antigen. We suggest that the optimal mutation schedule is one in which periods of rapid mutation alternate with periods of mutation-free growth. The multicompartmental structure of the germinal center, together with re-entry of positively selected B cells back into the germinal center, will naturally implement such a schedule, thereby providing an anatomical basis for the efficiency of the germinal center reaction.

Affinity maturation through somatic hypermutation of B-cell immunoglobulin (Ig) *V*-region genes[1] is a remarkably efficient process of mutation and selection by which high-affinity antibodies are generated. Its fundamental role in the immune response to foreign antigens has become increasingly clear in the past few years[2–7]. While our knowledge of the structure of the germinal centers (GCs), the loci of affinity maturation, and the kinetics of the germinal center reaction (GCR) has increased substantially[8–13], an anatomical basis for the impressive efficiency of the GCR remains unexplored. Several theories detailing events at the molecular level have been advanced to account for the efficiency of affinity maturation[14–16]. Our approach, in contrast, assumes only ordinary molecular mechanisms: diversity generated through random point mutations and darwinian selection based solely on the ability of variant Ig to bind antigen.

To explain the high efficiency of somatic mutation, we suppose that the mutation rate can be regulated[3,5,7,17,18] and we seek the mutation schedule $[\mu(t)]$ that maximizes, at the end of the GCR, the quantity:

$$A = \Sigma b_i K_i$$

where b_i is the number of B cells with affinity K_i (other reasonable criteria render very similar results). The computation is performed numerically using Pontryagin's maximum principle[19]. The optimal schedule thus found[20] calls for an alternation between periods of rapid mutation and periods of mutation-free growth. Our results suggest that the efficiency of the GCR may be accounted for by the multicompartmental structure of the GC and the re-entry of positively selected B cells, which together implement a near optimal schedule.

Modeling B-cell population dynamics

The model upon which the calculation is based (see Box 1) assigns B-cell clones to classes based on their affinity for antigen. The populations within each class grow at a rate determined by the average amount of antigen bound per cell. Mutation-mediated transitions between classes occur as a function of the mutation rate, with advantageous mutations being rarer than disadvantageous ones. Silent and lethal mutations are also considered. Antigen that is bound by proliferating B cells is no longer available for other cells to use. Cells that cannot bind enough antigen to proliferate die rapidly through apoptosis[21]. Thus, a very strong competition for antigen is realized.

The calculated optimal mutation schedule for this model is shown in Fig. 1. Periods of mutation-free growth are punctuated by bursts of high-mutation rates, and advantageous mutations are acquired sequentially rather than simultaneously. The interpretation of this optimal schedule is straightforward. At high-mutation rates, clones (or affinity classes) lose many daughter cells to lower affinity classes and lethals. Therefore, net growth within an affinity class is greatest when $\mu = 0$. Because advantageous mutants are relatively rare, a large number, N, of mutants must be generated to assure a significant likelihood that one of them will be advantageous. For example, if the probability of an advantageous mutation, p_A is 0.01, then N should be of the order of 100 to assure the generation of a higher-affinity mutant (see Box 1 and Fig. 1). In the optimal solution, the population grows as quickly as possible without mutation until there are $N = 1/p_A$ cells. Mutation is then 'switched on' at a rate consistent with the rapid production of cells with a very small number of point mutations (see also Ref. 22). After appearance of the advantageous variant, mutation is again switched off, so that the new high-affinity clone can grow as rapidly as possible and to avert the possibility that it is lost through further mutation[5]. The cycle then repeats for the duration of the GCR. This phasic schedule is robust in that the value of A reached is insensitive to small deviations from the optimal schedule. In particular, a schedule that simply switches between no mutation and a fixed constant rate can produce a value for A within 1% of its calculated optimum. Furthermore, this simple switch schedule is remarkably insensitive to significant

Box 1. B-cell population dynamic model

The B-cell population dynamic model is given by the set of differential equations:

$$\frac{db_i}{dt} = b_i\theta_i\left\{-k_d(1-h_i) + k_p h_i(2m_{ii}-1)\right\} + 2k_p\sum_{j\neq i} m_{ij}b_j h_j\theta_j$$

where b_i is the number of B cells of affinity class i. The factor θ_i, defined to be 1 when $b_i > 1$ and 0 otherwise, is a term, included as a correction to the naive continuum description, that prevents populations of less than one cell per germinal center (GC) from growing. The maximum rates for proliferation, k_p, and death, k_d, are both 4 d^{-1} (Refs 21, 29). However, the actual rates of proliferation and death are related to the antigen concentration through the function:

$$h_i = aK_i/(1 + aK_i)$$

where a is the concentration of unbound antigen in the germinal center and K_i is the affinity of B cell i. Thus, cells with higher affinity grow faster and die less frequently than cells with lower affinity. We assume that antigen is retained on the surface of follicular dendritic cells and is conserved during the GC reaction. Antigen thus obeys

$$a + \Sigma_i \sigma b_i h_i = a_0,$$

where $\sigma = 3.3 \times 10^{-4}$ μM $cell^{-1}$ is the concentration of sIg per B cell (equivalent to 10^5 receptors $cell^{-1}$) and a_0 is the total concentration of antigen in the GC. As a typical value we choose $a_0 = 3.3$ μM. For simplicity we assume the GC reaction was initiated by a single founder B cell. The founder clone is denoted by $i = 0$, and each mutant population is characterized by its affinity for the eliciting antigen, $K_i = 3^i \times 8.8 \times 10^5$ M^{-1} (Ref. 25). Thus, the unmutated clone has affinity $K_0 = 8.8 \times 10^5$ M^{-1}, and clones with $i < 0$ have decreased affinity, whereas clones with $i > 0$ have increased affinity. The rate of mutation-induced transitions from affinity class i to affinity class j is:

$$m_{ij} = \frac{\{L\mu(1-p_S)(1-p_L)\}^{|i-j|}e^{-L\mu(1-p_S)}}{|i-j|!(1+r^{i-j})}$$

where μ is the mutation rate in bp^{-1} per generation; $L = 700$ is the length (bases) of the targeted coding region; $p_S = 0.25$ is the probability that a mutation is silent; $p_L = 0.5$ is the probability that a non-silent mutation is lethal (or non-binding)[28]. Expressed, nonlethal mutations can either increase or decrease affinity. Mutations that do not change affinity are considered silent. The probability that a given expressed, nonlethal mutation is advantageous is:

$$p_A \equiv \frac{1}{(1+r)}$$

In Fig. 1, $p_A = 0.01$. The derivation of the functional form for m_{ij} is given in detail in Ref. 20.

changes in both the maximum mutation rate and the switching times.

Germinal center spatial compartmentalization and optimization

Such a near optimal phasic schedule can arise naturally from the structure of the GC if there are re-entrant pathways, so that some of the positively selected B cells exiting the GC are routed back into the GC dark zone (Fig. 2). Thus, the temporal variation of the optimal mutation schedule would be implemented by the spatial compartmentalization of the GC.

The centroblasts of the GC dark zone proliferate rapidly and bear little or no surface immunoglobulin (sIg)[12]. The centroblasts migrate to the light zone where they stop dividing but re-express sIg, and subsequently experience antigenic selection through interaction with antigen localized on follicular dendritic cells[21]. Failure to bind antigen results in apoptotic death, while success leads to migration out of the GC and differentiation into plasma cells or memory cells[13]. Therefore, irrespective of whether mutation occurs in the dark zone during replication or in the light zone in association with transcription, it is clear that mutation, proliferation and selection do not operate simultaneously on any cell cohort.

Within this context then, much circumstantial evidence points to cyclic GC re-entry. Without re-entry, selection cannot act between subsequent mutations. B cells examined in the late primary response or early secondary response can have up to nine expressed mutations per Ig gene[3,5,23] and increased affinity for the eliciting antigen of 100-fold or more. The probability of generating a functional Ig, much less a significantly higher-affinity Ig, after nine selection-free random mutations is negligible. Phylogenetic analysis of late primary response Ig *V*-region genes[8,24,25] yields a family structure consistent with sequential mutation. Site-directed mutagenesis of *V*-region genes[25,26] reveals that many of the observed mutations individually increase

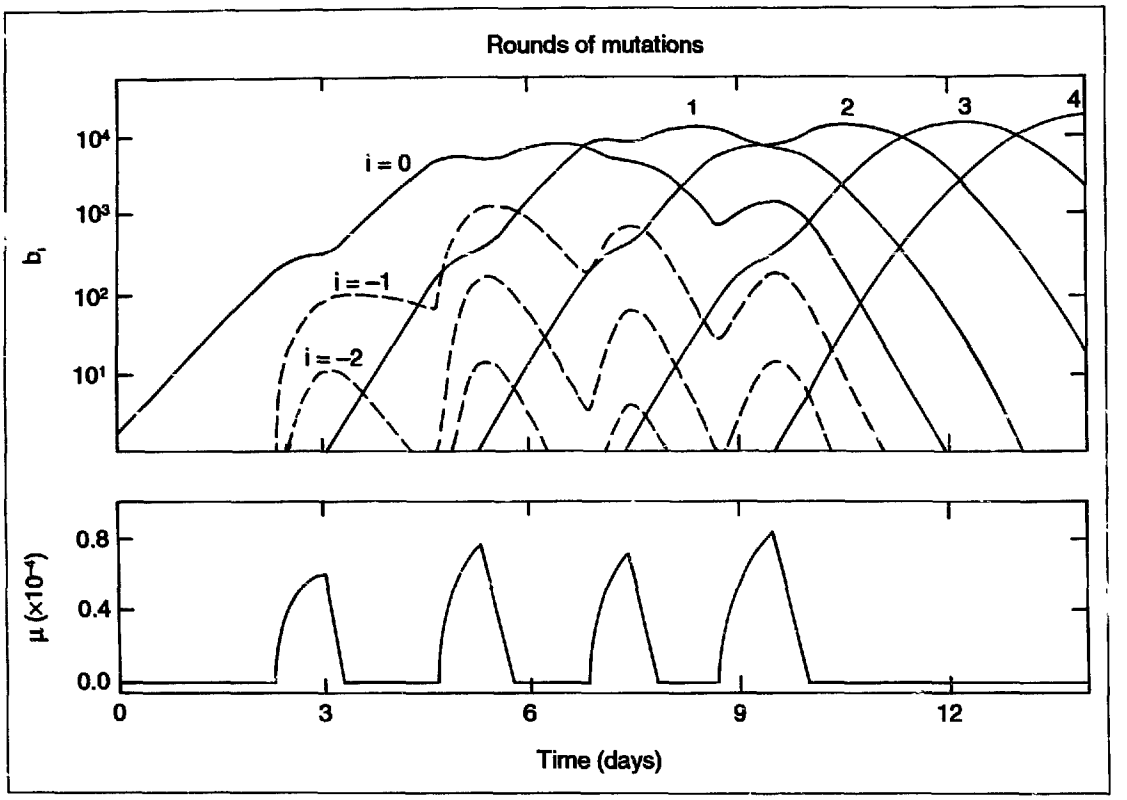

Fig. 1. *Growth and decline of model B-cell populations, in cells per germinal center, within each of the affinity classes, i (upper panel: founder class – heavy line, disadvantageous mutant classes – dotted line, advantageous mutant classes – lighter solid lines) and mutation rate, in mutations/bp/generation (lower panel) over the course of the GCR for the optimal schedule. There are four rounds of mutation, each of which produces a one-step increase in affinity. After the advantageous mutant appears, mutation is switched off to maximize its growth rate. These curves were generated by maximizing the function* $A = \Sigma_i K_i b_i$ *evaluated at day 14 as a functional of the mutation schedule* $\mu(t)$*, for the model defined in Box 1.*

the affinity for antigen, suggesting that intervening selection may in fact operate.

Briefly, then, we hypothesize that the GC enhances the efficiency of affinity maturation by allowing cohorts of B cells to cycle through specialized compartments where the steps of the reaction – mutation, selection and proliferation – are localized. In each cycle, we expect individual cells to accumulate a small number of mutations. Intervening selection will eliminate cells with significantly lowered Ig affinities and will become more stringent as the reaction progresses.

The post-selection growth implied within our scenario is evidenced by the observation that mutant clones dominate the late primary response[3,7]. Indeed, phasic mutation obviates a serious difficulty in this regard. At the mutation rate necessary to account for the frequency of mutations observed, assuming constancy of this rate (about 10^{-3}/bp per generation, Ref. 3), less than half of all daughter cells will be unmutated,

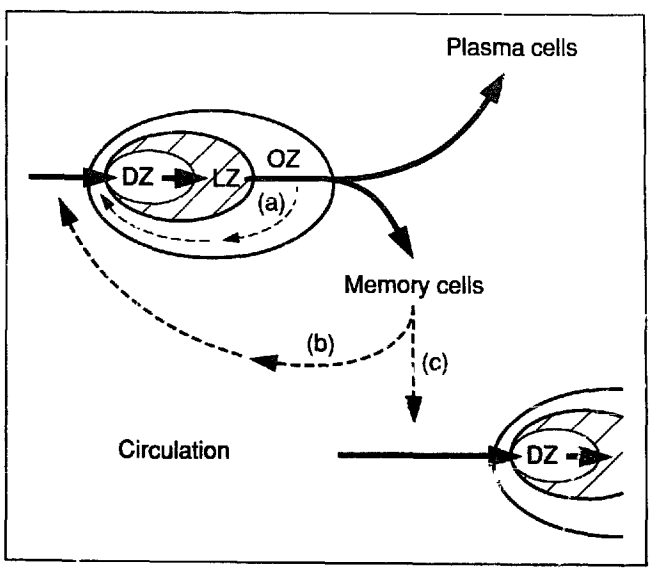

Fig. 2. *A schematic of the germinal center pathways. DZ: dark zone, site of centroblast proliferation; LZ: light zone, site of antigenic selection; OZ: outer zone. Mutation may occur in either the dark zone (if it is associated with replication) or in the light zone (if it is associated with transcription). The established pathways are shown as solid arrows. The hypothesized re-entrant pathways are shown as broken arrows. (a) Positively selected B-cell blasts from the light zone migrate through the outer zone to re-enter the dark zone[13], (b) memory cells re-enter the dark zone through the circulation, (c) memory cells sequentially colonize new germinal centers. [The results reported in Ref. 30 suggest mutation occurs in memory cells after secondary antigenic challenge and may be relevant for pathways (b) and (c).]*

so the clone cannot expand[27,28]. Finally, cyclic re-entry can explain the great cell-to-cell variation[5] in the number of mutations per gene found near the end of the GCR; some cells have simply completed more cycles through the dark zone.

Somatic mutation can generate very high-affinity antibodies in a short time. In order to understand the efficiency of this process we have asked, using the mathematical technique of optimal control theory, how to best control the mutation rate so as to optimize the process. We have shown that the optimal mutation schedule is one in which there are phases of high mutation followed by mutation-free growth. The GCR with cyclic re-entry of B cells provides a natural implementation of a phasic mutation schedule and hence is ideally suited for the affinity maturation process. Such a system should leave clear experimental signatures, and direct tests of these hypotheses should be possible.

We thank J.J. Ahouse and R.W. Anderson for helpful discussions. This work was supported by the Santa Fe Institute's program in theoretical immunology and by grants from the National Institutes of Health AI28433 and RR06555.

Tom Kepler is at the Santa Fe Institute, 1660 Old Pecos Trail, Santa Fe, NM 87501, USA and the Biomathematics Program, Campus Box 8203, North Carolina State University, Raleigh, NC 27695, USA; Alan S. Perelson is at the Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM 87545, USA.

References

1 Weigert, M., Cesari, I., Yonkovitch, S. and Cohn, M. (1970) *Nature* 228, 1045–1047
2 Griffiths, G.M., Berek, C., Kaartinen, M. and Milstein, C. (1984) *Nature* 312, 271–275
3 Berek, C. and Milstein, C. (1987) *Immunol. Rev.* 96, 23–41
4 Berek, C. and Milstein, C. (1988) *Immunol. Rev.* 105, 5–26
5 Berek, C., Berger, A. and Apel, M. (1991) *Cell* 67, 1121–1129
6 French, D., Laskov, R. and Scharff, M. (1989) *Science* 244, 1152–1157
7 Kocks, C. and Rajewsky, K. (1989) *Annu. Rev. Immunol.* 7, 537–559
8 Jacob, J., Kelsoe, G., Rajewsky, K. and Weiss, U. (1991) *Nature* 354, 389–392
9 Jacob, J., Kassir, R. and Kelsoe, G. (1991) *J. Exp. Med.* 173, 1165–1175
10 Jacob, J. and Kelsoe, G. (1992) *J. Exp. Med.* 176, 679–687
11 Leanderson, T., Kallberg, E. and Gray, D. (1992) *Immunol. Rev.* 126, 47–61
12 Liu, Y-J., Zhang, J., Lane, P.J.L., Chan, E.Y-T. and MacLennan, I.C.M. (1991) *Eur. J. Immunol.* 21, 2951–2962
13 Liu, Y J., Johnson, G., Gordon, J. and MacLennan, I. (1992) *Immunol. Today* 13, 17–21
14 Maizels, N. (1989) *Trends Genet.* 5, 4–8
15 Manser, T. (1991) *Immunol. Today* 11, 305–308
16 Steele, E., Pollard, J., Taylor, L. and Both, G. (1991) in *Somatic Mutations in V-Regions* (Steele, E.J., ed.), pp. 29–40, CRC Press
17 Manser, T. and Gefter, M.L. (1986) *Eur. J. Immunol* 16, 1439–1444
18 Siekevitz, M., Kocks, C., Rajewsky, K. and Dildrop, R. (1987) *Cell* 48, 757–770
19 Pontryagin, L., Boltyanskii, V., Gamkrelidze, R. and Mischenko, E. (1962) *The Mathematical Theory of Optimal Processes*, Wiley
20 Kepler, T.B. and Perelson, A.S. *J. Theor. Biol.* (in press)
21 Liu, Y-J., Joshua, D., Williams, G. *et al.* (1989) *Nature* 342, 929–931
22 Agur, Z., Mazor, G. and Meilijson, I. (1991) *Proc. R. Soc. London Ser. B* 245, 147–150
23 Sharon, J., Gefter, M., Wycocki, L. and Margolies, M. (1989) *J. Immunol.* 142, 596–601
24 Clarke, S.H., Huppi, K., Ruezinsky, D. *et al.* (1985) *J. Exp. Med.* 161, 6704–6887
25 Kocks, C. and Rajewsky, K (1988) *Proc. Natl Acad. Sci. USA* 85, 8206–8210
26 Brown, M., Stenzel-Poore, M., Stevens, S. *et al.* (1992) *J. Immunol.* 148, 339–346
27 Allen, D., Cumano, A., Dildrop, R. *et al.* (1987) *Immunol. Rev.* 96, 5–22
28 Shlomchik, M.J., Litwin, S. and Weigert, M. (1989) in *Progress in Immunology VII* (Melchers, F. *et al.*, eds), pp. 415–423, Springer-Verlag
29 Cohen, J., Duke, R., Fadok, V. and Sellins, K. (1992) *Annu. Rev. Immunol.* 10, 267–293
30 Rada, C., Gupta, S.K., Gherardi, E. and Milstein, C. (1991) *Proc. Natl Acad. Sci USA* 88, 5508–5512