Maturation of the humoral immune response as an optimization problem

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SUMMARY

Efficient immune response often depends on the production of high affinity antibodies. We show analytically that the optimal strategy for a fast production of high affinity antibodies is to utilize a step-function mutation rate, i.e. a minimal mutation rate in early stages of the immune response, followed by a discontinuous switch to the maximal possible rate when the proliferating population of B-cells exceeds a threshold value. Our results are in accordance with the biological observations concerning the time of onset of the hypermutation process, and with the mutation rate during the later stages of the primary immune response. Indeed the hypermutation process plays a crucial role in responding to a prevailing pathogen at each round of immune response, and not only for coping with future infections. Moreover, as the effect of hypermutations is shown to be crucially dependent on the number of proliferating B-cells, its onset is not expected to depend on an external signal, but rather to be related to the clone's age. This suggests that the onset is host species specific, rather than pathogen specific. Another implication of the present results is that activation of hypermutations before the B-cell population has reached the critical size may impede the efficiency of the response.

The process by which the humoral immune system copes with the task of fast recognition of an enormous spectrum of different antigens can be divided into two phases. The first phase involves the generation of a 'pre-immune' repertoire of B-cell clones, expressing predominantly low affinity antibodies (less than 10^8 different elements, (Berek & Milstein 1988)). The second phase follows antigenic challenge and involves clonal expansion and further diversification of the expanding clones, by a poorly understood process of somatic hypermutation. This process, presumably taking place in organized germinal centres (MacLennan & Gray 1986), involves an accelerated rate of point mutations along the DNA sequences encoding the variable regions of the antibody, in conjunction with a larger proliferation rate of B-cells bearing the higher affinity antibodies (Allen *et al.* 1987; Berek & Milstein 1987; Rajewsky *et al.* 1987; French *et al.* 1989). Recently it has been suggested that both the naive and the memory repertoires are modified through hypermutations and selection following each round of antigenic stimulation (Griffiths *et al.* 1984; Berek *et al.* 1985; Berek & Milstein 1987, 1988). Another view holds that cells carrying modifications that increase antibody affinity during the primary response are selected into the memory compartment, to come into action only during the secondary response (Rajewsky *et al.* 1987). Rajewsky *et al.* (1989) further suggest that the generation of somatic antibody mutants serves as a rapid adaptation by which the immune system copes with antigenically varying pathogens.

Our work focuses on the problem of response efficiency to a single antigenic challenge. Analysis of this problem seems essential for elucidating the more complex response to antigenically varying pathogens. Mathematical theory shows that, under general and reasonable assumptions about the hypermutation process, the optimal strategy for a fast production of high affinity antibodies will always be a 'bang-bang' strategy, that is, discontinuous transitions from minimum to maximum possible mutation rate. To prove this we translated into mathematical expressions the general assumptions about the hypermutation process and, by further analysis, identified the globally optimal strategy for a fast production of antibodies with the required structure. Subsequently we showed that a biologically realistic strategy is only marginally less efficient than the globally optimal strategy. The biologically realistic strategy involves a single switch from the minimal to the maximal possible mutation rate, when the number of proliferating B-cells exceeds a threshold value. We then compared laboratory estimations for the timing of onset of hypermutations, and their rate, with those calculated by our model. The implications of our results for the role of hypermutations, the time and the signal for their onset at any round of immune response, as well as for some cases of response impairment, are briefly discussed below.

The role of the hypermutation process is assumed to be a rapid production of high affinity antibodies, so as to eliminate the pathogen with minimum damage to the body. Pathogen-induced body deterioration dic-

tates an early activation of hypermutations, to minimize the time until the 'right' mutation occurs. However, as the mutation process is random and the chances of generating the right mutation are slim, activating this process when the number of B-cells that produce moderate affinity antibodies is still small may cause a random drift to low affinity antibodies, and as a consequence, a response arrest.

In this work mutations are assumed to occur at random along the DNA sequences that determine the antibody–antigen binding efficacy. To allow for the possibility that mutations occur independently of chromosomal DNA replication (Manser 1990), mutation and proliferation are taken as independent random events. We focus on the response to a specific antigen, and assume that only one specific antibody structure, not contained in the pre-immune repertoire, has a high binding affinity to this antigen. By clonal selection and hypermutation a pre-immune antibody structure may be modified to generate the required specific antibody structure (to be denoted the solution). Based on the observation that a single point mutation is required to improve antibody affinity by a factor of 10 (Allen *et al.* 1987; Berek & Milstein 1987), and that only cells expressing high affinity antibodies survive the selection process (Rajewsky *et al.* 1989), we focus attention on antibodies that are one mutation removed from the solution. For simplicity we also assume that the host's death probability per unit time is constant. The relevance of this model to the response to antigenically varying pathogens, to variable response efficacy and to variable host mortalities, will be briefly discussed below.

Denote the proliferation rate of the proliferating B-cells by λ, the probability per unit time that the pathogen kills the host by r, and the DNA length encoding the binding region by m. We are looking for a mutation rate as a function of clone size which maximizes the probability of successful response. Letting I_n and P_n stand for the probability of successful response (i.e. the probability of reaching the solution before the pathogen kills the host) and the mutation rate, respectively, when the population size of the proliferating B-cells is n, and assuming that the mutation and the proliferation processes are exponential, we obtain the probability that the next event is not the death of the host, as follows:

$$(\lambda n + mnP_n)/(\lambda n + mnP_n + r). \tag{1}$$

From (1) we can derive the recursive equation for I_n, as follows:

$$I_n = \frac{\lambda n}{\lambda n + mnP_n + r} I_{n+1} + \frac{(m-1)\,nP_n}{\lambda n + mnP_n + r} I_{n-1} + \frac{nP_n}{\lambda n + mnP_n + r}$$

$$= \frac{n}{\lambda n + mnP_n + r}(\lambda I_{n+1} + (m-1)\,P_n I_{n-1} + P_n). \tag{2}$$

From (2) we obtain:

$$\partial I_n/\partial P_n = n\frac{[(\lambda n + r)\,(m-1)\,I_{n-1} - \lambda nm I_{n+1} + \lambda n + r]}{(\lambda n + mnP_n + r)^2},$$

whose sign is independent of P_n. This means that the probability of successful response is a monotonic function of the mutation rate, or, in other words, that there is no intermediate mutation rate that maximizes the efficiency of response. Mathematical theory suggests that in such a case a discontinuous switch from minimum to maximum possible mutation rate (a 'bang-bang' strategy) will always be optimal. More formally, it follows by the Howard theorem of dynamic programming (Blackwell 1965), that any optimal strategy would use, for any size of proliferating B-cell population, either the maximal or the minimal mutation rates. We have shown analytically that the optimality of these 'bang-bang' strategies holds also for antibody structures that are more than one mutation removed from the solution.

If there is no limit on how high the mutation rates might be, then the optimal strategy will be:

$$P_n = \begin{cases} 0 & \text{for } n \leqslant n_0, \\ \infty & \text{for } n > n_0, \end{cases} \quad \text{where} \quad n_0 = \lfloor m(1 - r/\lambda) \rfloor. \tag{3}$$

That is, the mutation rate is negligible as long as the B-cell population size is below the threshold n_0, and maximal when above it. The actual value of n_0 depends on the size of the binding region and on the ratio between B-cell proliferation rate and host death probability.

To prove (3) we first identify the best value of n_0 for step-functions. Subsequently, we show that there is no n for which a Howard improvement (Blackwell 1965) of P_n increases I_n. By basic theorems of dynamic programming this is a sufficient condition for optimality of the step-function strategy. Details of this and other mathematical steps will be provided elsewhere.

For the above function we obtain:

$$I_n = \begin{cases} \dfrac{(n_0-1)!}{(n-1)!}\dfrac{\Gamma(n + r/\lambda)}{\Gamma(n_0 + r/\lambda)}\left(1 - \dfrac{r}{\lambda}\right) & \text{for } n \leqslant n_0; \\ 1 - \left(\dfrac{m-1}{m}\right)^{n-n_0}\left(\dfrac{r}{\lambda}\right) & \text{for } n > n_0; \end{cases} \tag{4}$$

where Γ is the Gamma function. As this strategy is optimal, equation (4) provides the maximal probability of successful response.

However, the globally optimal strategy (3) is biologically unrealistic in requiring an infinite rate of mutation, as well as a real-time control of the number of B-cells and of the hypermutation activation. If the hypermutation rate is very high, the activation of this process causes an immediate decrease in proliferating B-cell clone size, below the threshold, n_0, where the process of hypermutation should instantaneously be switched off, only to be switched on again upon further cellular proliferation. To avoid the need of a real-time control of the mutation process, and hence, to make the strategy biologically feasible, we limited the strategies to those that activate the hypermutation process only once. In such strategies the maximal mutation rate

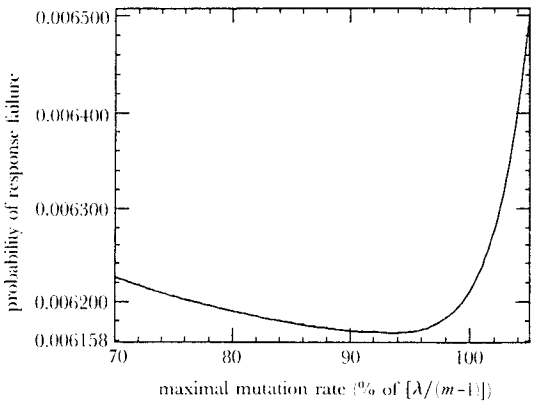

Figure 1. Probability of response failure $(1-I_1)$ for upper bounds for the mutation rate close to $\lambda/(m-1)$ and a single activation of the hypermutation mechanism. Under the optimal unbounded mutation rate strategy, the probability of response failure for the parameters $r/\lambda = 0.001$, and $m = 100$ base pairs, is 6.158×10^{-3}. Note that for mutation rates of about 95% of $\lambda/(m-1)$, the probability of response failure increases by no more than 1.2×10^{-5}. In contrast, for upper bounds greater than $\lambda/(m-1)$, that is, for rates that create a drift to low clone sizes, this probability increases significantly.

must be low enough, relative to the proliferation rate, so that the number of proliferating B-cells will not get a negative trend merely by the onset of hypermutations. For hypermutation rates smaller than $\lambda/(m-1)$, the B-cell population size still tends to increase, and thus the process may be activated only once during the infection, without too large a probability of losing all the proliferating B-cells. We have shown that for biologically realistic values for the parameters m, λ and r, the imposition of this upper bound on the mutation rate has a minor effect on the probability of successful response. For all examples calculated, the probability of successful response under such a biologically feasible policy decreases by no more than one percent of the probability of successful response when using the optimal, unbounded, strategy. Figure 1 shows numerical results of the analysis for upper bounds on the mutation rate close to $\lambda/(m-1)$.

The methodology we have followed shows that the good performance of these simple, thrifty, two-stage strategies cannot be improved by the evolution of a more complex control of the hypermutation process.

We now compare the order of magnitude of these analytical results with the results of *in vitro* and *in vivo* experiments in mice. These suggest that around the seventh day of response (Berek & Milstein 1987) or a few days earlier (Manser 1990), the mutation rate is augmented to become 0.5×10^{-3} mutations per base pair per generation, if B-cell generation time is 17 h, or 3×10^{-4} mutations per base pair per generation, if B-cell generation time is 10 h (Berek & Milstein 1988). According to our mathematical prediction, initiation of the hypermutation process should immediately follow the establishment of a clone of size $m(1-(r/\lambda))$, which, for the realistic assumption of r being much smaller than λ, roughly equals m, i.e. a few hundred nucleotides. Hence, the time until an establishment of such a clone, having initially one B-cell of that type, is nine or ten generations ($= \log_2 500, \log_2 1000$). For B-cell generation time of 17 or 10 h, this corresponds to seven or four days of proliferation, respectively, as suggested experimentally. For a sequence of 1000 base pairs and a hypermutation rate of 0.5×10^{-3} mutations per base pair per generation, the probability of generating a mutated B-cell is similar to the probability of B-cell proliferation. Thus, the upper bound for the mutation rate appears to be in agreement with laboratory estimations. Note that this conclusion remains unaltered if the silent mutations are excluded from the calculations of the mutation rate and the length of the relevant nucleotide sequence; having no effect on response efficiency, silent mutations were not incorporated in our analysis.

We showed that, under reasonable and general assumptions about the hypermutation process, the probability of successful response is a monotonic function of mutation rate. It follows by mathematical theory that the optimality of a 'bang-bang' strategy for the mutation rate is general and robust. Our results suggest that a step-function mutation rate should optimize the immune response at any round of antigenic challenge, if the response provided by the existing repertoire is insufficient. Transition from the minimal to the maximal possible rate of mutation is expected to occur once the size of the proliferating B-cell clone exceeds a certain threshold. Our results further suggest that the signal for the onset of hypermutations may be related to cellular senescence mechanisms, as these 'count' the number of generations, and consequently the clone size. It has been suggested that modulation of the rate of change in the concentrations of certain cell-cycle proteins can serve as such a 'counter' (Norel & Agur 1991).

Our results further imply that activation of the hypermutation mechanism too early during the immune response may result in a negative drift in antibody binding efficacy and, hence, in response failure. It may be interesting to check whether such a premature onset is involved in the generation of tolerance.

The correspondence between our theoretical results and laboratory estimations suggests that the timing of hypermutation onset has evolved to optimize response to a prevailing pathogen, rather than for coping with future infections. As even extreme differences in pathogenicity affect the timing of hypermutation onset by no more than one generation (check by implementing various values of r in the equation of n_0), this timing is not critically dependent on local conditions and may be species specific. For the same reason, variable host mortalities and moderate response of lower affinity antibodies, affecting this probability, are expected to have no effect on the optimal strategy, as described above.

Laboratory experiments are warranted for validating our predictions that the onset of hypermutations depends on the size and, hence, the age of the proliferating B-cell clone. Further experiment may verify whether an early activation of the hypermutation process is involved in some cases of response failure. It will also be interesting to check if the time-lag

preceding the onset of hypermutations is longest for the primary response and is progressively shortened for each subsequent response, as a result of previous mutations and selection. The mathematical tools for distinguishing between a process involving selection and mutation and that involving selection only will be described elsewhere. Such tools are essential for evaluating different assumptions about the processes that generated the observed mutations during the primary and subsequent responses. Based on the results of the present work we now analyse the immune response to multiple antigens, as well as the effect on response efficiency of competition between clones of B-cells that vary with respect to antibody affinity.

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