Models for Antigen Receptor Gene Rearrangement. I. Biased Receptor Editing in B Cells: Implications for Allelic Exclusion

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Recent evidence suggests that lymphocyte Ag receptor gene rearrangement does not always stop after the expression of the first productively rearranged receptor. Light chain gene rearrangement in B cells, and α-chain rearrangement in T cells can continue, which raises the question: how is allelic exclusion maintained, if at all, in the face of continued rearrangement? In this and the accompanying paper, we present comprehensive models of Ag receptor gene rearrangement and the interaction of this process with clonal selection. Our B cell model enables us to reconcile observations on the κ:λ ratio and on κ allele usage, showing that B cell receptor gene rearrangement must be a highly ordered, rather than a random, process. We show that order is exhibited on three levels: a preference for rearranging κ rather than λ light chain genes; a preference to make secondary rearrangements on the allele that has already been rearranged, rather than choosing the location of the next rearrangement at random; and a sequentiality of J segment choice within each κ allele. This order, combined with the stringency of negative selection, is shown to lead to effective allelic exclusion. The Journal of Immunology, 1999, 163: 1793–1798.
In addition to explaining the high \(k:l\) ratio, the ability of a B cell to make secondary rearrangements on a single \(k\) allele explains why \(70\%\) of mouse splenic \(k\) B cells have only one rearranged \(k\) locus, the other one remaining unrearranged (19); and why, in mice that have only one functional \(k\) locus, \(k\) B cell production is \(70\%\) (rather than one-half) of that in wild-type mice (13). Furthermore, it has been suggested (13, 20, 21) that rearrangement may proceed sequentially rather than stochastically. That is, that \(5'Jk\) segments are used before \(3'Jk\) segments. Recent observations on receptor editing in mice transgenic for autoreactive Abs (6–8) have also hinted at the possibility of order in the rearrangement process. Hence, the following question arose: can ordered rearrangement account for the observations on allele bias, \(Jk\) usage, and the \(k:l\) ratio?

The present study is an attempt to address this question. We identify the degree of order in BCR gene rearrangement as a primary mechanism ensuring allelic exclusion. Our methodology is to perform computer simulations of receptor gene rearrangement, incorporating different assumptions, and to compare the results to the available experimental data. Thus, we assess the relative ability of each hypothesis in turn to account for the experimental observations. Our model leads us to conclude that: 1) secondary BCR gene rearrangements are negative-selection driven, in the sense that a cell has a limited time window in which it can edit its receptor and be rescued from deletion; and 2) light chain rearrangement is an ordered process on three levels: a preference for rearranging \(k\) rather than \(l\) light chain genes; a preference to make secondary rearrangements on the allele that has already been rearranged, rather than choosing the location of the next rearrangement at random; and a sequentiality of rearrangement within each \(k\) allele, such that \(Jk1,2\) are preferentially used before \(Jk4,5\). This order, combined with the stringency of negative selection, is shown to lead, with high probability, to effective allelic exclusion. That is, the likelihood of a cell producing two productive rearrangements on two light chain alleles, within a limited time window and under the constraints of ordered rearrangement, is extremely small.

### A Simulation of BCR Gene Rearrangement

We have created a stochastic simulation of BCR gene rearrangement. The general structure of the simulation is given in Fig. 1. Individual modules can be described as follows.

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4 The program is available from the authors upon request.
1) Cell birth: cells are “born” into the simulation having all light chain genes in the unarranged (germline) state.

2) Light chain selection: at each rearrangement step, a preliminary selection of either $\kappa$ or $\lambda$ is made, according to the selection probability $P_{\text{sel}}$, which is a parameter of the program.

3) BCR rearrangement: if $\kappa$ is selected, then one or the other allele is randomly selected for rearrangement. Subsequent selections of J$\kappa$ segments for rearrangement may either rearrange the previously rearranged allele or switch to the opposite allele, depending on the probability parameter $P_{\text{switch}}$. For example, if $P_{\text{switch}} = 0$, then the cell rearranges the previously rearranged allele, provided there are J$\kappa$ segments remaining on it. Once a $\kappa$ allele is selected, one of the four J$\kappa$ segments is chosen using the probability parameters $P_1, P_2, P_3, P_4$. Rearrangements are followed by renormalization of the allele in question, that is, a rearranged J$\kappa$ segment is deleted (as are all unarranged J$\kappa$ segments 5’ relative to the rearranged segment), and cannot be chosen again by the program. For example, if we assume a strictly random J$\kappa$ usage, the initial values of the usage probabilities will be $p_1 = p_2 = p_3 = 1/3$; after rearrangement to, say, J$\kappa 1$, these probabilities will be changed to $p_1 = 0, p_2 = p_3 = 1/3$; etc.

4) BCR rearrangement: if $\lambda$ is selected, then one of the two $\lambda$ alleles is chosen at random, with equal probabilities for the two alleles. Rearrangement at $\lambda$ has been simplified to two rearrangements per allele. Thus, one of two J$\lambda$ segments on the current allele is chosen at random. Failures at $\lambda$ lead to deletion of only the failed segment and have no effect on any other segments, either $\kappa$ or $\lambda$.

5) If a cell has run out of J$\kappa$ segments on one allele, then it automatically switches to the other allele. If a cell is entirely out of J$\kappa$ segments on both alleles, then it switches to $\lambda$ segments until all segments are exhausted. The cell dies if there are no more light chain segments available for rearrangement.

6) Selection: once a V-J rearrangement is made, the program determines whether the rearranged chain is in frame, with a probability $P_{\text{product}}$. If it is in frame, the program determines whether the resulting light chain can pair with the existing heavy chain, with a probability $P_{\text{H/L match}}$. If so, the program determines whether the resulting BCR is autoreactive (anti-self), with a probability $P_{\text{auto}}$. Rearrangement is repeated until a productive, H/L matched, nonauto-reactive BCR is produced, or until the cell dies.

7) Cell fate after selection: Results of rearrangement are classified into one of three categories: 1) cells containing an anti-self rearrangement. These cells are assigned a high death probability, denoted by $P_{\text{death}}$. If, however, a cell does not die after such a rearrangement, it may try another rearrangement (receptor editing); 2) cells containing only out-of-frame V-J joins, H/L mismatched heavy-light chain pairs, or germline $\kappa$ and $\lambda$. These cells are assigned a moderate death probability, $P_{\text{m}}$. If such cells do not die, they also continue rearranging their light chain genes; and 3) cells containing one in-frame, H/L matched, nonauto-reactive rearrangement, are allowed to mature.

Parameters
Table I summarizes the default parameter values used in our simulations. The program follows each new cell until it either matures or dies, and repeats the process for a predetermined number of mature cells produced. We usually simulate $10^5$ viable cells, having found that, in most cases, $10^6$ cells are sufficient for simulation variability to stabilize. The program then generates as output the distribution of genotypes among the cells that have matured. The simulation does not include cell divisions, because developing B cells do not divide while light chain rearrangement and selection are going on.

Table I. B cell simulation parameters with our “default” set of values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition parameters with our “default” set of values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N$</td>
<td>No. viable cells to be simulated</td>
</tr>
<tr>
<td>K1start</td>
<td>K1 start (yes/no)</td>
</tr>
<tr>
<td>K1site</td>
<td>K1 site (which J$\kappa$)</td>
</tr>
<tr>
<td>Klexp</td>
<td>K1 site expression</td>
</tr>
<tr>
<td>$P_{\text{auto}}$</td>
<td>P(BCR is auto-reactive)</td>
</tr>
<tr>
<td>$P_{\text{prod}}$</td>
<td>P(rearrangement is productive)</td>
</tr>
<tr>
<td>$P_{\text{H/L}}$</td>
<td>P(H/L chains match)</td>
</tr>
<tr>
<td>$P_{\text{prod}}$</td>
<td>P(death if anti-self)</td>
</tr>
<tr>
<td>$P_{\text{prod}}$</td>
<td>P(death if out of frame or H/L mismatch)</td>
</tr>
<tr>
<td>$P_{\text{prod}}$</td>
<td>P(death if out of frame or H/L mismatch)</td>
</tr>
<tr>
<td>$P_{\text{switch}}$</td>
<td>P(switch to a different allele)</td>
</tr>
<tr>
<td>$p_i$</td>
<td>J segment probabilities (random case)</td>
</tr>
<tr>
<td></td>
<td>$p_1 = 0.25$</td>
</tr>
<tr>
<td></td>
<td>$p_2 = 0.25$</td>
</tr>
<tr>
<td></td>
<td>$p_3 = 0.25$</td>
</tr>
<tr>
<td></td>
<td>$p_4 = 0.25$</td>
</tr>
<tr>
<td>$p_i$</td>
<td>J segment probabilities (strictly sequential case)</td>
</tr>
<tr>
<td></td>
<td>$p_1 = 0.999$</td>
</tr>
<tr>
<td></td>
<td>$p_2 = 0.999 \times 10^{-3}$</td>
</tr>
<tr>
<td></td>
<td>$p_3 = 0.999 \times 10^{-6}$</td>
</tr>
<tr>
<td></td>
<td>$p_4 = 0.999 \times 10^{-9}$</td>
</tr>
<tr>
<td>$p_i$</td>
<td>J segment probabilities (quasi-sequential case)</td>
</tr>
<tr>
<td></td>
<td>$p_1 = 0.4995$</td>
</tr>
<tr>
<td></td>
<td>$p_2 = 0.4995$</td>
</tr>
<tr>
<td></td>
<td>$p_3 = 0.0005$</td>
</tr>
<tr>
<td></td>
<td>$p_4 = 0.0005$</td>
</tr>
</tbody>
</table>

* These parameters were used in all simulations unless otherwise noted. KI, knock-in.

The probability for a V-J rearrangement to be productive is one-third, because this is the probability that V will be joined to J in the correct reading frame. $P_{\text{H/L}}$ was chosen to be 0.8 because previous studies (18) have shown that this is the minimum value required to give a ratio of $\kappa:\lambda > 2$. $P_{\text{auto}}$ has been independently estimated by others (22, 23).

Results
Our goal in the first series of simulations was to evaluate the hypothesis of ordered rearrangement. We tested the hypothesis of allele bias (preference to rearrange a rearranged allele) as well as the hypothesis of sequentiality in J$\kappa$ usage. To test whether a B cell’s choice of the allele to rearrange is a random choice or biased toward the allele previously rearranged, simulations were performed with either $P_{\text{switch}} = 0.5$ or $P_{\text{switch}} = 0$. When $P_{\text{switch}} = 0.5$, the choice of allele to rearrange next is random and independent of the previous rearrangement. When $P_{\text{switch}} = 0$, rearrangement continues on the same allele until that allele is exhausted.

To test the degree of order in J$\kappa$ segment usage, we used three sets of $p_i$ probabilities. The first set represents the null hypothesis, that is, that J$\kappa$ usage is completely random, and hence the probabilities are equal for all segment choices: $p_1 = p_2 = p_3 = p_4 = 0.25$. The second set represents the diametrically opposed hypothesis, strictly sequential J$\kappa$ usage: $p_1 \gg p_2 \gg p_3 \gg p_5$ (Table I). Following Wood and Coleclough (20), we have also tried an intermediate case, which we call “quasi-sequential.” In this scenario, we assume that rearrangement to J$\kappa 1$ or J$\kappa 2$ is much more frequent than rearrangement to J$\kappa 4$ or J$\kappa 5$. Hence, we set the probabilities in this case to obey the rule $p_1 = p_2 \gg p_3 = p_5$ (Table I). The probabilities of each of the two $\lambda$ alleles, and each segment within these alleles, were always equal.

The combination of two possibilities for $P_{\text{switch}}$ and three sequentiality cases gives six possible basic scenarios of rearrangement, which test the combined contribution of sequential J$\kappa$ usage
and allele preference. The results of simulations of these six scenarios are presented in Fig. 2. A comparison of these simulation results with the published experimental data leads to the following conclusions.

Rearrangement of BCR genes is biased toward the last allele rearranged

First, we performed simulations in which there was no allele preference ($P_{\text{switch}} = 0.5$). These simulations never yielded a fraction of cells with an R/R genotype, that is, only one allele preferred. On the other hand, if we assume strictly sequential rearrangement (p1 >> p2 >> p3), we get the opposite skew of the results (toward upstream Jk5). The explanation for this is straightforward: all cells will start by rearranging to Jk1. A third will succeed in making a productive rearrangement, the rest will proceed to rearrange to Jk2. A third of these (2/9 of the total) will succeed in making a productive rearrangement, the rest will proceed to rearrange to Jk3, and so on. The probability that each productive rearrangement leads to a functional, nonautoreactive receptor is independent of the Jk segment used, so that the final distribution of Jk usage is determined by the order of rearrangements (Fig. 2, C and D).

Finally, simulations of quasi-sequential rearrangement give an advantage to Jk1 and Jk2 over Jk4 and Jk5 (Fig. 2, E and F). This scenario best reproduces the published (20) murine Jk usage distribution, which was 40–45% each of Jk1 or Jk2 vs 5–10% each of Jk4 or Jk5. Even with an anti-self knock-in start to Jk1, there is still an advantage of Jk2 over Jk4 and Jk5 (~40% of the cells contain rearrangements to Jk2; data not shown). Thus, our conclusion from this series of simulations is that Jk rearrangement most likely proceeds sequentially or quasi-sequentially, but certainly not in a random manner.

Ordered receptor editing accounts for the k:λ ratio

Next, we proceeded to check whether ordered rearrangements as modeled above would reproduce not only the data on Jk usage and allele preference, but also a k:λ ratio larger than 10:1. We noticed that estimates of the k:λ ratio varied enormously in simulations of 10,000 cells. As a result, we increased the number of cells in our rearrangement model to at least 100,000 in simulations intended to examine the k:λ ratio. The results show that high values of the k:λ ratio are easily obtained once we incorporate ordered rearrangements into our model. The k:λ ratio is highly sensitive to Pκλ (Fig. 3A): only values of Pκλ > 0.95 give k:λ > 10. Thus, our first conclusion from this series of simulations is that rearrangement is preferential not only within and between the k alleles, but also with respect to the choice between k and λ. Our model reproduces the observation (13) that a developing B cell is likely to rearrange k first. In our model, this temporal order is not deterministic, but rather results from the higher probability of starting with k rearrangement. Cells would be much more likely to rearrange to λ only upon exhausting the rearrangement possibilities at the k locus.
We assigned the value of 0.67 to about one-fifth of the cells for the probabilities given in Table I. On changes in advantage of the allele that is rearranged first. The effect of rearrangements only on one allele, denoted by $\%R/0$, varies with $r$ rearrangement of both the BCR light chain and the TCR heavy chain. Extended in recent years by multiple observations of continued rear-
gangement of both the BCR light chain and the TCR $\alpha$-chain. How allelic exclusion is maintained, if at all, in spite of secondary rear-
garrangements, has been a matter of debate. The molecular mech-
anism responsible for Ag receptor gene rearrangement, and those linking Ag receptor signaling upon Ag binding to positive
and negative selection, are still largely unknown. However, under-
standing the dynamics of these processes, and how mechanistic
properties of the rearrangement process account for the resulting
lymphocyte repertoire, may eventually shed light on the inner
workings of rearrangement and selection. We have chosen to ad-
dress this problem using stochastic computer simulation to exam-
ine random vs ordered models of lymphocyte Ag receptor gene
rearrangement, and the interplay between this process and repen-
toire selection.

The present paper presents our results for gene rearrangement
and selection in developing B cells. We first examined the degree
of order in BCR gene rearrangement. We have found that BCR
gene rearrangement is ordered on three different levels, as follows.
First, our simulations support the hypothesis, which was recently
supported also by experimental observations (9–15), that $\kappa$ light
chain rearrangement precedes $\lambda$ light chain rearrangement in most,
if not all, cases. Second, our results strongly support the hypothesis
of allele preference, that is, once a rearrangement exists on one of
the $\kappa$ alleles, the cell is more likely to perform secondary rear-
garrangement, if necessary and possible, on the same allele rather than
switching to the other $\kappa$ allele. Allele preference fully explains the
high fractions of $\kappa$ light chain B cells that contain $\kappa$ rearrange-
ments on one allele only. Although the mechanism of allele pref-
erence is not known, the possibility that it is determined by the
DNA methylation status of light chain alleles has been suggested
by Bergman and colleagues (25). Third, our results show that ex-
perimental observations are consistent with the hypothesis of qua-
si-sequential rearrangement within each $\kappa$ allele, that is, when $J\kappa 1$
and/or $J\kappa 2$ are available for rearrangement, the cell is more likely
to choose one of these segments over $J\kappa 4$ or $J\kappa 5$ (19).

We also studied the effect of negative selection, determined by
the death probability of cells that have not succeeded in producing
a productively rearranged, H/L matched, nonauto-reactive BCR.
We found that the higher the death probability, the larger the $k: \lambda$ ratio. Cells are allowed few rearrangement attempts (large
death probability) and, hence, are likely to die before exhausting $k$ and proceed-
ing to $\lambda$ rearrangements. Our simulations show that, in order for
the results to be consistent with experimental observations, we
must assume that negative selection limits the number of rear-
garrangements to two to three per cell, in agreement with
previous results (24). Therefore, we refer to BCR rearrangement as
a negative selection-limited process.

Taken together, the above results reveal our proposed answer to
the question of allelic exclusion in B cells. We propose that B cell
allelic exclusion results from a high degree of order in gene rear-
garrangement and a stringent process of negative selection. A high
degree of order allows a B cell to maximize the number of rear-
garrangements attempts, first on one allele and then on the other.
Then, because negative selection limits the number of rear-
garrangement attempts to two to three per cell, ordered rearrangement
means it is likely that all these attempts will be on a single allele.
Thus, it is extremely unlikely for two productive rearrangements to
exist simultaneously on two light chain alleles. This results in the
almost complete absence of cells expressing two different BCRs
from the repertoire, i.e., in effective allelic exclusion.

Ordered secondary rearrangements thus maximize the cell’s
ability to make a productive, nonauto-reactive rearrangement be-
fore exhausting all $J\kappa$ segments. In an accompanying paper, we
show that, in contrast to B cells, the process of secondary rear-
garrangement in T cells does not have to be as stringently ordered;
rearrangement goes on simultaneously on both alleles, and the ob-
erved bias toward rearranging upstream $J\alpha$s first is not essential.
This difference may be explained by the fact that, while B cells
have only 4 functional $J\kappa$ segments per allele (and two $J\lambda$s) and
thus need to use them efficiently, T cells have 50 $J\kappa$s segments at
their disposal.

A more important difference between BCR and TCR rearrange-
ment is that the latter is limited by positive, rather than negative
selection; i.e., rearrangement may continue even after the expres-
sion of a productively rearranged TCR$\alpha$ gene, and stops only after
positive selection has been completed. Using a computer simula-
tion similar to our B cell simulation, we show in the second paper
that these two features of TCR$\alpha$ gene rearrangement, simultaneity
and persistence until positive selection, combine to account for the
appearance of T cells that have two productively rearranged and expressed TCR α-chains. Thus, allelic exclusion in B cells and allelic inclusion in T cells can be brought about by similar rearrangement processes, operating however on different gene structures and under different rules of selection.

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References