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Models for Antigen Receptor Gene Rearrangement. I. Biased Receptor Editing in B Cells: Implications for Allelic Exclusion[1]

Ramit Mehr,* Michele Shannon,* and Samuel Litwin[2†]

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.**

The long-accepted concept of allelic exclusion states that Ag receptor genes in B and T lymphocytes are expressed from only one of the two alleles in each cell. Combined with isotypic exclusion, allelic exclusion works to ensure that each lymphocyte expresses a single Ag receptor specificity on the cell surface. Allelic exclusion is strictly observed at both the B cell receptor (BCR)[3] heavy chain and the TCR β-chain loci. Expression of a productively rearranged heavy or β-chain in the form of the pre-BCR or the pre-TCR abrogates further heavy or β-chain gene rearrangement, respectively. Recent evidence suggests, however, that in the TCR α-chain, and in the BCR light chain (1–3), rearrangement may not stop after a productive receptor gene has been formed and expressed. In the case of the TCR α-chain (see the accompanying paper), this may lead to incomplete allelic exclusion. For the BCR light chain, there is evidence that secondary rearrangements occur after nonproductive rearrangements, and also after productive rearrangements that render a B cell autoreactive ("receptor editing", reviewed in Ref. 4). Using mice transgenic for autoreactive BCRs, receptor editing has been identified as one of the mechanisms of central tolerance (5–8). However, if the choice of allele for secondary rearrangements is random, it is (at least theoretically) possible that a cell will rearrange and then simultaneously express two different light chains. How then, if at all, is allelic exclusion maintained in the face of continued rearrangement? Using computer simulation of light chain gene rearrangement, we show that allelic exclusion in B cells can be maintained if rearrangement is an ordered, rather than a random, process.

Our model relies on experimental evidence concerning three related characteristics of light chain gene rearrangement: the κ:λ light chain ratio, the choice of κ allele for rearrangement, and the choice of $J\kappa$ segment within this allele. The observed ratio between κ light chain- and λ light chain-bearing B cells in the murine serum is ~20:1, and, in immature murine bone marrow cells, it is >10:1 (9–11). It is controversial whether the κ:λ ratio can be explained solely on the basis of the higher potential for multiple rearrangements in the κ locus, combined with immature B cell death due to negative selection, without assuming preferential expansion of κ B cells over λ B cells (12–15).

Previous studies attempted to calculate the κ:λ ratio based on the following observations. First, recombination signals at the κ locus are ~100 times more efficient than those of the λ locus (16); this is called the "branching ratio." Second, gene rearrangement at κ precedes λ gene rearrangement by ~24 h (13). Third, because there are three possible DNA reading frames, the probability that the rearrangement will be productive is at most one-third. The presence of rearrangeable but nonfunctional V "pseudo-genes," may reduce this probability, called the "fusion efficiency," even further (13). Third, evidence suggests that a B cell is allowed to live for only a limited amount of time in the bone marrow (17). After this time, the cell will die if it fails to make a functional receptor. Because of the preference to rearrange first at the κ locus, this factor, the "crash factor," would favor the survival of κ B cells over λ B cells. A model addressing only the branching ratio and fusion efficiencies, but which allows just one rearrangement attempt per chromosome (i.e., no receptor editing), gives a κ:λ ratio of at most 2.25 (12). Even taking into account the crash factor, one cannot account for a ratio of κ:$\lambda > 10$ without assuming extremely high values for the B cell death probability. Only when considering multiple rearrangements at the κ locus, in addition to the above three factors (branching ratio, fusion efficiency, and crash factor), can a stochastic model of BCR gene rearrangement produce a ratio of κ:λ that exceeds 10 (18). In the latter study, the κ:λ ratio was found to be related to the death probability, meaning that the fewer rearrangements the cell is allowed to try at the κ locus, the higher the resulting κ:λ ratio.

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[3] Abbreviation used in this paper: BCR, B cell receptor.

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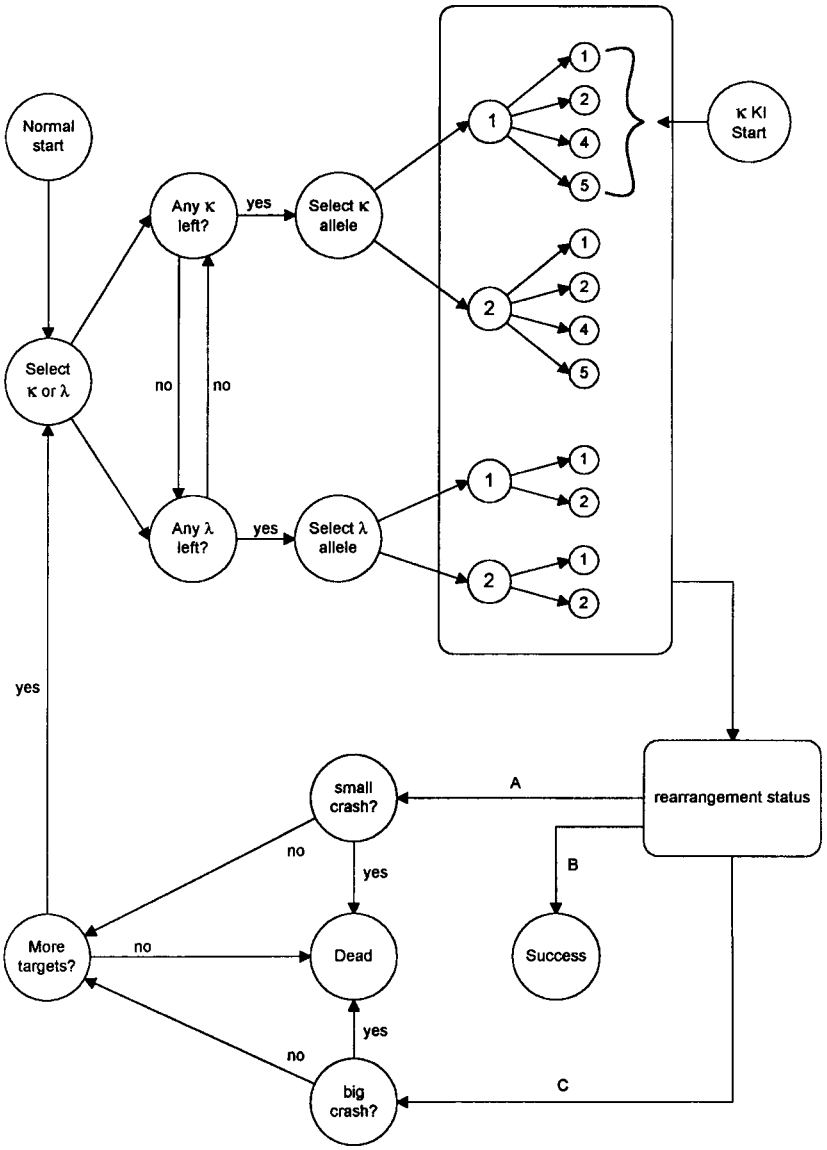

FIGURE 1. Model of B cell rearrangement. Simulation first selects either κ (with probability $P_{\kappa\lambda}$) or λ allele. An allele is randomly selected using P_{switch} and then one of the four Jκ or one of the two Jλ sites is randomly selected. Rearrangement status, success or failure, is determined by the chance of being in frame, $P_{product}$, amicable pairing with the heavy chain, $P_{H/L}$, and not being anti-self, $(1 - P_{as})$. Rearrangement success (pathway B) leads to maturation. Rearrangement failure leads to deletion of the utilized J segment, and, in the case of κ, to deletion of all upstream J segments on that allele. Presence of any anti-self rearrangement or of multiple successful rearrangements (pathway C) leads to cell death via a crash factor P_{das}. Failures due to out-of-frame rearrangements or H/L mismatches (pathway A) die with a crash factor, P_d. If a failed rearrangement does not lead to cell death, and if more J segments are available, the process is repeated. Knock-in (KI) starts may be similarly set to initiate the simulation with an existing rearrangement to any Jκ site (e.g., an anti-self rearrangement to Jκ1) on allele 1. This KI rearrangement is immediately subjected to the same success, death, or continuation decision as any cell that had been rearranged to this state. Similarly, J segments may be "knocked out" by setting the corresponding p_i to 0 from the start.

In addition to explaining the high κ:λ ratio, the ability of a B cell to make secondary rearrangements on a single κ allele explains why ~70% of mouse splenic κ B cells have only one rearranged κ locus, the other one remaining unrearranged (19); and why, in mice that have only one functional κ locus, κ 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′ Jκ segments are used before 3′ Jκ 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, Jκ usage, and the κ:λ 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 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 rearrangement within each κ allele, such that Jκ1,2 are preferentially used before Jκ4,5. 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.[4] The general structure of the simulation is given in Fig. 1. Individual modules can be described as follows.

[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 unrearranged (germline) state.

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

3) BCRκ rearrangement: if κ is selected, then one or the other allele is randomly selected for rearrangement. Subsequent selections of Jκ segments for rearrangement may either rearrange the previously rearranged allele or switch to the opposite allele, depending on the probability parameter P_{switch}. For example, if $P_{switch} = 0$, then the cell rearranges the previously rearranged allele, provided there are Jκ segments remaining on it. Once a κ allele is selected, one of the four Jκ segments is chosen using the probability parameters p_1, p_2, p_4, p_5. Rearrangements are followed by renormalization of the allele in question, that is, a rearranged Jκ segment is deleted (as are all unrearranged Jκ segments 5′ relative to the rearranged segment), and cannot be chosen again by the program. For example, if we assume a strictly random Jκ usage, the initial values of the usage probabilities will be $p_1 = p_2 = p_4 = p_5 = 1/4$; after rearrangement to, say, Jκ1, these probabilities will be changed to $p_1 = 0$, $p_2 = p_4 = p_5 = 1/3$; etc.

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

5) If a cell has run out of Jκ segments on one allele, then it automatically switches to the other allele. If a cell is entirely out of Jκ segments on both alleles, then it switches to λ 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 rearrangement is in frame, with a probability $P_{product}$. If it is in frame, the program determines whether the resulting light chain can pair with the existing heavy chain, with a probability $P_{H/L}$. If so, the program determines whether the resulting BCR is autoreactive (anti-self), with a probability P_{as}. Rearrangement is repeated until a productive, H/L matched, nonautoreactive 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_{das}. 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 κ. These cells are assigned a moderate death probability, P_d. If such cells do not die, they also continue rearranging their light chain genes; and 3) cells containing one in-frame, H/L matched, nonautoreactive 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 10^4 viable cells, having found that, in most cases, 10^4 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*[a]

Parameter	Definition	Initial Value
N	No. viable cells to be simulated	10,000
KIstart	KI start (yes/no)	n
KIsite	KI site (which Jκ)	1
KIexp	KI site expression	Anti-self
$P_{\kappa\lambda}$	P(choose κ for next rearrangement)	0.975
$P_{product}$	P(rearrangement is productive)	0.333
$P_{H/L}$	P(H/L chains match)	0.8
P_{as}	P(BCR is anti-self)	0.667
P_{das}	P(death if anti-self)	0.5
P_d	P(death if out of frame or H/L mismatch)	0.3
P_{switch}	P(switch to a different allele)	0.0
p_i	J segment probabilities (random case)	$p_1 = 0.25$ $p_2 = 0.25$ $p_4 = 0.25$ $p_5 = 0.25$
p_i	J segment probabilities (strictly sequential case)	$p_1 = 0.999$ $p_2 = 0.999 \times 10^{-3}$ $p_4 = 0.999 \times 10^{-6}$ $p_5 = 0.999 \times 10^{-9}$
p_i	J segment probabilities (quasi-sequential case)	$p_1 = 0.4995$ $p_2 = 0.4995$ $p_4 = 0.0005$ $p_5 = 0.0005$

[a] 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. $P_{H/L}$ was chosen to be 0.8 because previous studies (18) have shown that this is the minimum value required to give a ratio of κ:λ > 2. P_{as} 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 Jκ 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 $P_{switch} = 0.5$ or $P_{switch} = 0$. When $P_{switch} = 0.5$, the choice of allele to rearrange next is random and independent of the previous rearrangement. When $P_{switch} = 0$, rearrangement continues on the same allele until that allele is exhausted.

To test the degree of order in Jκ segment usage, we used three sets of p_i probabilities. The first set represents the null hypothesis, that is, that Jκ usage is completely random, and hence the probabilities are equal for all segment choices: $p_1 = p_2 = p_4 = p_5 = 0.25$. The second set represents the diametrically opposed hypothesis, strictly sequential Jκ usage: $p_1 \gg p_2 \gg p_4 \gg p_5$ (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 Jκ1 or Jκ2 is much more frequent than rearrangement to Jκ4 or Jκ5. Hence, we set the probabilities in this case to obey the rule $p_1 = p_2 \gg p_4 = p_5$ (Table I). The probabilities of using each of the two λ alleles, and each segment within these alleles, were always equal.

The combination of two possibilities for P_{switch} and three sequentiality cases gives six possible basic scenarios of rearrangement, which test the combined contribution of sequential Jκ usage

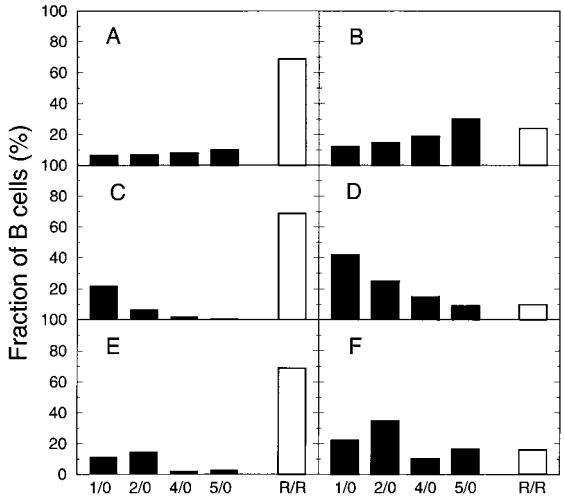

FIGURE 2. . Distribution of simulated B cells according to rearrangement status. The different genotypes were grouped here as follows. 1/0 denotes cells that contain a rearrangement to Jκ1 on one allele, leaving the other allele in the germline configuration; similarly, 2/0, 4/0, and 5/0 denote cells that ultimately mature with a final rearrangement to Jκ2, Jκ4, or Jκ5 on one allele, but no rearrangements on the other allele. R/R denotes cells that have rearrangements on both κ alleles. A and *B*, Simulations of random Jκ usage; *C* and *D*, Simulations of strictly sequential Jκ usage; *E* and *F*, Simulations of quasi-sequential Jκ usage (see text for details). *A*, *C*, and *E*, Simulations with $P_{switch} = 0.5$; B, *D*, and *F*, simulations with $P_{switch} = 0$. In all these simulations, all other parameters were assigned the default values (given in Table I). Each simulation was run until it produced 100,000 viable cells.

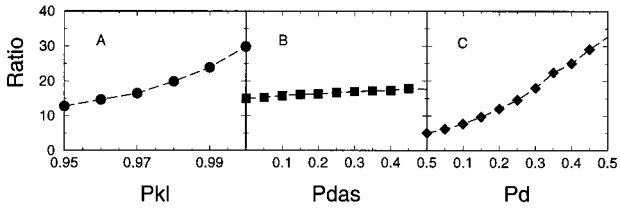

FIGURE 3. The κ:λ ratio. This ratio is plotted vs the probability $P_{\kappa\lambda}$ of choosing to rearrange κ over λ (*A*); P_{das}, the death probability for cells expressing an autoreactive BCR (B) ; P_d, the death probability for cells that failed to productively rearrange or express a functional BCR (C). All other parameters were assigned the default values; in particular, $P_{switch} = 0$ and a strictly sequential Jκ usage, which is the most conservative way to estimate the κ:λ ratio. Each point represents a simulation of 100,000 cells. With this large number of cells, the variability in each simulation is <1%, hence we did not plot the error bars.

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 ($P_{switch} = 0.5$). These simulations never yielded a fraction of cells with an R/0 genotype, that is, only one κ allele rearranged [$R/0 = 1 - R/R = (1/0 + 2/0 + 4/0 + 5/0)$], similar to the experimentally observed (13, 19) value of 70% (Fig. 2, *A*, *C*, and *E*). Rather, the fraction of R/0 cells in simulations with $P_{switch} = 0.5$ is always <30%. On the other hand, using the opposing hypothesis of strict allele preference ($P_{switch} = 0$) results in R/0 of at least 70%, as observed (Fig. 2, *B*, *D*, and *F*), regardless of order in J segment usage (see below). Hence, we conclude that experimental data is more consistent with a model of κ gene rearrangement that exhibits a high degree of allele preference.

Usage of Jκ gene segments is quasi-sequential

Second, we tested the three scenarios for Jκ usage. Simulations implementing the hypothesis of a random Jκ usage do not reconstruct the observed results. However, these simulations (Fig. 2, *A* and *B*) reveal an interesting effect. In contrast to the intuitive expectation that, within each allele, we will get a uniform distribution of Jκ usage in this case, we see that the distribution is skewed toward downstream Jκ segments: Jκ1 < Jκ2 < Jκ4 < Jκ5. This is a direct result of a cell's ability to rearrange the downstream Jκ segments either directly (deleting intermediate segments) or as a secondary rearrangement after first attempting to rearrange to more upstream segments. The more downstream the segment, the more rearrangement pathways end up with a rearrangement to that segment. Hence, a uniform probability distribution for choices of Jκ segments results in a Jκ usage distribution skewed toward 3′ Jκs; we call this the "accumulation" or "pile-up" effect.

On the other hand, if we assume strictly sequential rearrangement ($p_1 \gg p_2 \gg p_4 \gg p_5$), we get the opposite skew of the results (toward upstream Jκs). The explanation for this is straightforward: all cells will start by rearranging to Jκ1. A third will succeed in making a productive rearrangement, the rest will proceed to rearrange to Jκ2. A third of these (2/9 of the total) will succeed in making a productive rearrangement, the rest will proceed to rearrange to Jκ3, and so on. The probability that each productive rearrangement leads to a functional, nonautoreactive receptor is independent of the Jκ segment used, so that the final distribution of Jκ usage is determined by the order of rearrangements (Fig. 2, *C* and *D*).

Finally, simulations of quasi-sequential rearrangement give an advantage to Jκ1 and Jκ2 over Jκ4 and Jκ5 (Fig. 2, *E* and *F*). This scenario best reproduces the published (20) murine Jκ usage distribution, which was 40–45% each of Jκ1 or Jκ2 vs 5–10% each of Jκ4 or Jκ5. Even with an anti-self knock-in start to Jκ1, there is still an advantage of Jκ2 over Jκ4 and Jκ5 (~40% of the cells contain rearrangements to Jκ2; data not shown). Thus, our conclusion from this series of simulations is that Jκ rearrangement most likely proceeds sequentially or quasi-sequentially, but certainly not in a random manner.

Ordered receptor editing accounts for the κ:λ ratio

Next, we proceeded to check whether ordered rearrangements as modeled above would reproduce not only the data on Jκ usage and allele preference, but also a κ:λ ratio larger than 10:1. We noticed that estimates of the κ: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 κ:λ ratio. The results show that high values of the κ:λ ratio are easily obtained once we incorporate ordered rearrangements into our model. The κ:λ ratio is highly sensitive to $P\kappa\lambda$ (Fig. 3*A*): only values of $P\kappa\lambda > 0.95$ give κ:λ > 10. Thus, our first conclusion from this series of simulations is that rearrangement is preferential not only within and between the κ alleles, but also with respect to the choice between κ and λ. Our model reproduces the observation (13) that a developing B cell is likely to rearrange κ first. In our model, this temporal order is not deterministic, but rather results from the higher probability of starting with κ rearrangement. Cells would be much more likely to rearrange to λ only upon exhausting the rearrangement possibilities at the κ locus.

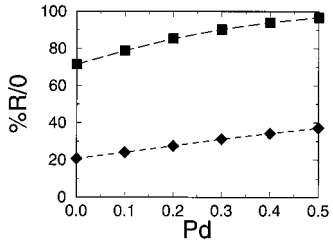

FIGURE 4. The effect of death probability on allele bias. The fraction of κ B cells containing rearrangements only on one allele is plotted vs P_d, the death probability for cells that failed to productively rearrange or express a functional BCR. Two Jκ usage cases are shown: strictly sequential (■) or random (◆). All other parameters were assigned the default values; in particular, $P_{switch} = 0$. Each point represents a simulation of 100,000 cells.

Next, we examined the effect of negative selection on the κ:λ ratio. We assigned the value of 0.67 to P_{as} (22, 23) and varied the values of each of the two death probabilities. Fig. 3, *B* and *C*, contains plots of the resulting κ:λ ratio as a function of P_{das} or P_d. As predicted above, the higher the probability of death, the larger is the κ:λ ratio. Higher values of P_{das} and/or P_d corresponds to allowing the cells to perform fewer rearrangement attempts: the average number of rearrangements in our simulations was between two and three per cell for the parameter values given in Table I. This is in agreement with previous results (24). In this case, cells are likely to die before exhausting κ and proceeding to λ rearrangements.

Note that the effect of changing P_{das} is much smaller than the effect of changing P_d, because P_{das} affects a much smaller fraction of the cells. P_{das} affects only those cells with productive, but self-reactive, rearrangements, that is, $P_{product} \times P_{H/L} \times P_{as}$, which is about one-fifth of the cells for the probabilities given in Table I. On the other hand, P_d affects the rest of the cell death cases, that is, $(1 - P_{product}) + P_{product}(1 - P_{H/L})$, which amount to about three-quarters of the total number of cells.

We also examined the sensitivity of allele usage to changes in death probabilities. Fig. 4 shows how the percent of cells with rearrangements only on one allele, denoted by %R/0, varies with P_d. Again, the higher the probability of death, the stronger is the advantage of the allele that is rearranged first. The effect of changes in P_{das} is again much smaller than the effect of changes in P_d (data not shown).

Discussion

The widely accepted concept of allelic exclusion has been challenged in recent years by multiple observations of continued rearrangement of both the BCR light chain and the TCR α-chain. How allelic exclusion is maintained, if at all, in spite of secondary rearrangements, has been a matter of debate. The molecular mechanisms 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, understanding 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 address this problem using stochastic computer simulation to examine random vs ordered models of lymphocyte Ag receptor gene rearrangement, and the interplay between this process and repertoire 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 κ light chain rearrangement precedes λ 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 κ alleles, the cell is more likely to perform secondary rearrangement, if necessary and possible, on the same allele rather than switching to the other κ allele. Allele preference fully explains the high fractions of κ light chain B cells that contain κ rearrangements on one allele only. Although the mechanism of allele preference 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 experimental observations are consistent with the hypothesis of quasi-sequential rearrangement within each κ allele, that is, when Jκ1 and/or Jκ2 are available for rearrangement, the cell is more likely to choose one of these segments over Jκ4 or Jκ5 (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, nonautoreactive BCR. We found that the higher the death probability, the larger the κ:λ ratio. Cells are allowed few rearrangement attempts (large P_{das} and P_d) and, hence, are likely to die before exhausting κ and proceeding to λ 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 rearrangement attempts 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 rearrangement and a stringent process of negative selection. A high degree of order allows a B cell to maximize the number of rearrangements attempts, first on one allele and then on the other. Then, because negative selection limits the number of rearrangement 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, nonautoreactive rearrangement before exhausting all Jκ segments. In an accompanying paper, we show that, in contrast to B cells, the process of secondary rearrangement in T cells does not have to be as stringently ordered; rearrangement goes on simultaneously on both alleles, and the observed bias toward rearranging upstream *Jα*s first is not essential. This difference may be explained by the fact that, while B cells have only 4 functional Jκ segments per allele (and two Jλs) and thus need to use them efficiently, T cells have ~50 J*α* segments at their disposal.

A more important difference between BCR and TCR rearrangement is that the latter is limited by positive, rather than negative selection; i.e., rearrangement may continue even after the expression of a productively rearranged TCR*α* gene, and stops only after positive selection has been completed. Using a computer simulation similar to our B cell simulation, we show in the second paper that these two features of TCR*α* 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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