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Re-evaluation of the probabilities for productive rearrangements on the κ and λ loci

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Abstract

V–J rearrangements at Ig light chain (IgL) genes occur in resting small pre-B cells. In the absence of cell division, the probability of productive κ and λ rearrangements is proportional to the output of κ^+ B and λ^+ B cells in bone marrow. The kinetics and probability of productive κ or λ rearrangements was assessed in three groups of mice carrying two (wild-type), one or no intact Igκ gene, and the following conclusion are drawn. κ and λ rearrangements occur independently at different kinetics, and rearrangements are initiated at a time when κ rearrangements are stopping. The probability of productive κ and λ rearrangements per chromosome is calculated to be ~60 and ~20% respectively. Thus, a κ gene can attempt rearrangements up to three times per chromosome during B cell development. These findings explain that the observed ratio of κ^+ B/λ^+ B cell production in wild-type mice is 95/5.

Introduction

Rearrangements at Ig heavy chain (IgH) genes occur in cycling precursors, whereas subsequent rearrangements at Ig light chain (IgL) genes occur in resting small pre-B cells. There are two IgL chain isotypes encoded by separate gene families, κ and λ, which are localized on different chromosomes (1). The majority of B cell progenitors functionally rearrange either the κ or λ gene, but not both, to generate κ^+ or λ^+ B cells [isotypic exclusion (2)]. Most κ^+ B cells retain their Igλ locus in germline configuration, while most λ^+ B cells have non-functional rearrangements on both κ alleles (3–7). This observation indicates that the Igκ locus is rearranged earlier than the Igλ locus during B cell development.

To explain this sequence of rearrangements, an 'ordered' model and a 'stochastic' model have been proposed. The 'ordered' model proposes that Igκ genes rearrange first, and only if both Igκ alleles are non-productively rearranged or deleted do Igλ genes become accessible for rearrangement through an unknown mechanism (4). The 'stochastic' model postulates that κ and λ rearrangements are totally independent of each other (5,8–12). Slower activation of the Igλ locus or inefficient recombination signals in the Igλ locus (13) may be responsible for the ordered rearrangements from κ to λ. In both models, the expression of a functional IgL gene ceases IgL rearrangements (11,14).

To test these two models, we analyzed mice which cannot undergo any rearrangements at the Igκ locus due to targeted deletion of the Igκ intronic enhancer (the iEκT locus) (15,16). Consistent with other κ-deficient mice carrying deletion of J_κ and C_κ gene segments (17), mice homozygous for the iEκT locus can nonetheless generate λ^+ B cells. These findings demonstrate that recombination at the κ locus is not a prerequisite for λ recombination, arguing against the strictly 'ordered' model. However, assuming that the attempt of κ rearrangement is only once per chromosome during B cell development, the 'stochastic' model cannot explain the observed κ^+ B cell/λ^+ B cell ratio in wild-type mice or the strong increment of κ producers in κ-deficient mice for the following reason. A single attempt of V_κ–J_κ rearrangement would yield an in-frame κ rearrangements at 1/3 probability and a totally readable κ transcription unit at 2/9 probability

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due to the presence of pseudo-V_κ segments (11) We define a totally readable κ transcription unit arising after V–J recombination as *productive* κ rearrangements. Given that the probability of *productive* κ rearrangements per chromosome is 2/9, in wild-type mice $100\times(1-2/9)^2 = 61\%$ of small pre-B cells would carry *non-productive* V_κ–J_κ joints on both alleles and initiate λ recombination, while in the κ-deficient mice 100% of small pre-B cells would initiate λ recombination Compared with wild-type mice, the generation of λ^+ B cells in the κ-deficient mice increased 7- to 10-fold (16,18), which was significantly higher than the expected value of 100/61-fold, based on the assumption above. In addition, this assumption predicts that in wild-type mice 100 and 61% of small pre-B cells initiate κ and λ recombination respectively, which does not explain the observed ratio of κ^+ B cell/λ^+ B cell production, i.e. 95/5

Here, we show that (i) λ rearrangements are independent of κ rearrangements, (ii) λ rearrangements are initiated when most κ rearrangements cease and (iii) the apparent probability of productive V_κ–J_κ rearrangements per chromosome is 57%. The probability higher than 2/9 indicates that sequential κ rearrangements on one chromosome are frequent events. Also, ~80% of small pre-B cells in wild-type mice are expected to acquire productive κ rearrangements. The regulation of κ versus λ rearrangements can be explained by a scenario in which small pre-B cells failing to produce κ chain after up to three sequential κ rearrangement attempts per chromosome would proceed with λ rearrangements.

Methods

Mice

To generate the 129 strain congenic for the iEκT mutation, chimeric mice derived from embryonic stem cells of 129 origin were mated with 129 females. Mice were maintained at the animal facility at the Basel Institute under specific pathogen-free conditions Mice aged 6–8 weeks old were examined.

Antibodies for flow cytometry

The following primary antibodies were used in this study allophycocyanin-coupled RA3-6B2 [anti-B220 (19); Caltag, San Francisco, CA], biotin-conjugated LO-MM (anti-mouse IgM; Caltag), phycoerythrin-conjugated 217-170 [anti-mouse IgD^b (20); PharMingen, CA], FITC-conjugated goat anti-mouse κ (Southern Biotechnology, CA) and FITC-conjugated R26-46 [anti-mouse λ_1 and λ_2 light chain (21); PharMingen] Streptavidin–Red-613 (Gibco/BRL, NY) was used as a second-step reagent.

Immunofluoresence staining, analysis and cell sorting

For phenotypic analysis, single cell suspensions from bone marrow were stained with mAb as indicated in the figure legends. Between 1 and 3×10^6 cells were incubated with purified mAb at 5–10 μg/ml in PBS/5% FCS for 15–30 min on ice and washed once in PBS/5% FCS. Flow cytofluorometric analysis was performed on a FACScan (Becton Dickinson, Mountain View, CA) after gating on viable cells. Cells presented in the lymphocyte gate as defined by light scatter (22) were analyzed to quantitate the percentages of s-κ^+ or s-λ^+ B cells among the whole nucleated cells presented in the gate of viable cells. Fluorescence data are displayed as logarithmic dot plots using Lysys software (Becton Dickinson) The purity of cell populations after sorting was always >99%

Bromodeoxyuridine labeling in vivo and detection of $BrdU^+$ B cells in the bone marrow by flow cytometry

BrdU (Sigma, St Louis, MO) was dissolved in PBS at 2 mg/ml Fourteen- to 18-week-old mice were injected i.p. with 400 μg BrdU Three mice were killed at each time point and the bone marrow cells were isolated. FACS-sorted cells ($B220^{low}IgM^-$, $B220^{low}IgM^+$ $IgD^-\lambda_{1+2}^-$, $B220^{low}IgM^+$ $IgD^-\lambda_{1+2}^+$) were permeabilized in 500 μl 70% ethanol in water at room temperature. Cells were incubated overnight at 4°C and washed twice with PBS, before addition of 500 μl 3 N HCl, 0 5% Tween. After 20 min the cells were pelleted, supernatants removed and 500 μl 0.1 M sodium tetraborate was added. Cells were washed twice in PBS/0.5% Tween and incubated with 20 μl of FITC-labeled anti-BrdU antibody (Becton Dickinson) Incubation, washes and cytofluorometric analysis were performed as described For every FACS analysis the correct negative and positive controls ($CD45R^{low}sIgM^+sIgD^-\lambda^+$ cells without BrdU labeling and $CD45^{low}IgM^-$ cells labeled for 4 h) were performed.

Results

The kinetics of production of $BrdU^+$ $sIgM^+$ B cells represents the kinetics of productive IgL genes rearrangements

According to the state of cell cycling and the expression of $V_{pre\text{-}B}/\lambda5$ surrogate light (SL) and cytoplasmic μ chain (c-μ), $CD45R^+$ ($B220^+$) B cell precursors can be subdivided into four stages (Fig 1A): pro-B (cycling, $SL^+c\text{-}\mu^-$), large pre-B (cycling, $SL^+c\text{-}\mu^+$ or $SL^-c\text{-}\mu^+$), small pre-B (resting, $SL^-c\text{-}\mu^+$) and newly generated immature B cells (resting, surface IgM^+) (23–25). Rearrangements at the IgL loci occur mostly in the small pre-B stage (26,27).

We injected a single dose of BrdU i p. into mice of the 129 strain congenic for the iEκT mutation. Pro-B and large pre-B cells are expected to incorporate BrdU and subsequently differentiate into small pre-B cells (Fig. 1A). The small pre-B cells then undergo IgL recombination without incorporating any BrdU When *productive* κ or λ rearrangements occur, the cells become $BrdU^+$ $sIgM^+$ B cells. The probability of *productive* V_κ–J_κ or V_λ–J_λ rearrangements determines the rate of output of $BrdU^+$ κ^+ or λ^+ B cells in the bone marrow.

At various time points after a BrdU injection, we isolated the B cell progenitors ($CD45R^+sIgM^-$), κ^+ and λ^+ B cells in the newly generated B cell compartment (22) ($CD45R^+sIgM^+sIgD^-\lambda^{-/+}$) from the bone marrow by cell sorting (Fig. 1B, dot plots). These cells were stained with anti-BrdU antibody and analyzed by FACS (Figs 1B and 2) Only 4 h after the administration of BrdU, 31% of the $CD45R^+sIgM^-$ B cell progenitors already incorporated BrdU (Fig. 1B).

When pre-B cells undergo V_λ–J_λ rearrangements, most V_κ–J_κ rearrangements cease

Figure 2(A) shows representative histograms of anti-BrdU stainings and Fig. 2(B) summarizes the percentages of BrdU-labeled κ^+ or λ^+ B cells in the three types of mice: wild-type

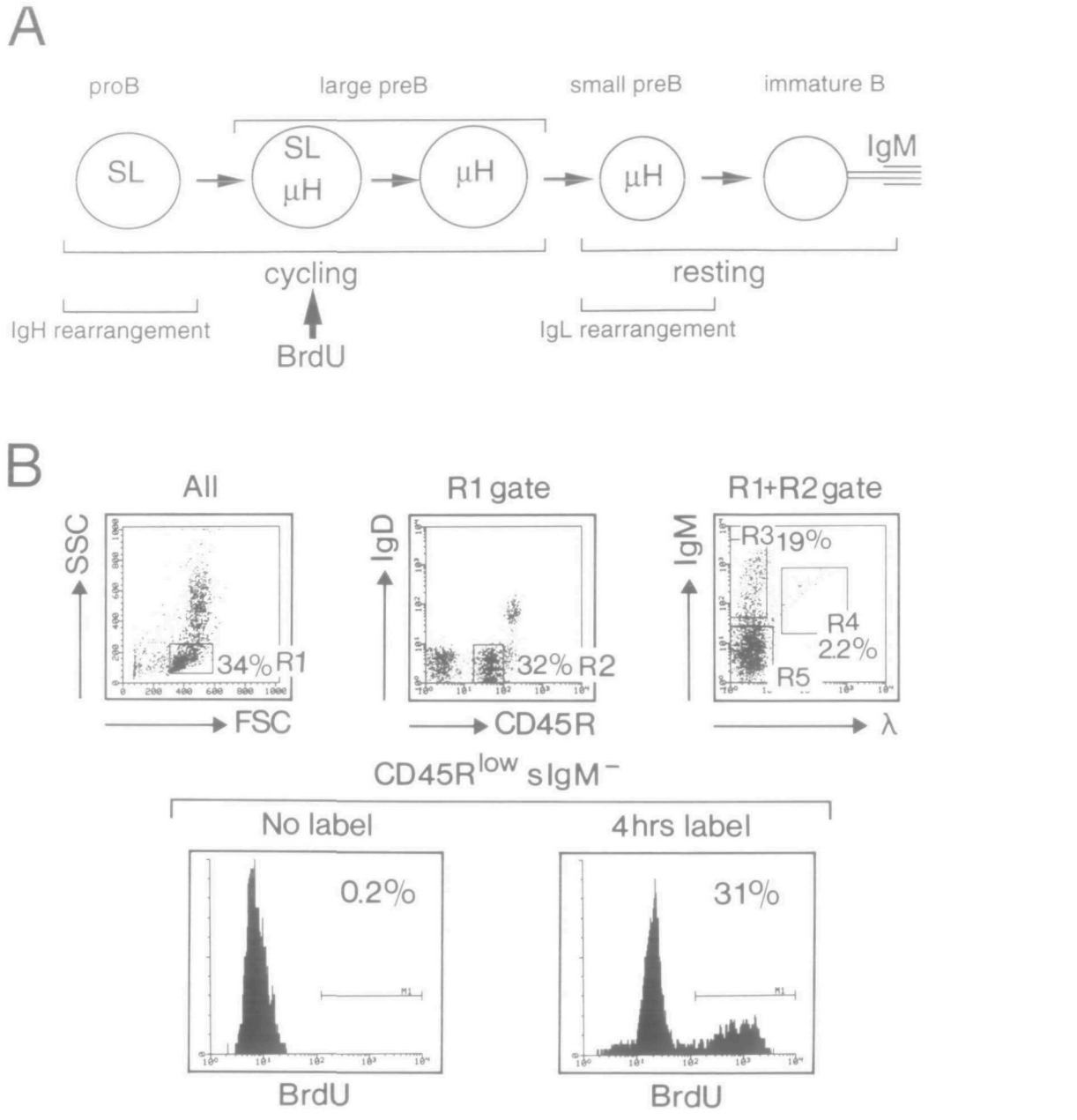

Fig. 1. Strategies to analyze the output of κ^+ or λ^+ B cells in the bone marrow by BrdU labeling (A) $CD45R^+$ B lineage cells in the bone marrow are subdivided into four stages based on the state of cell cycle and the expression of surrogate light chain (SL), μ chain and sIgM chain. Precursor cells at pro-B/large pre-B stages are expected to incorporate BrdU These cells differentiate into small pre-B cells, which undergo IgL rearrangements without cell cycling The appearance of labeled B cells is thus dependent on the kinetics of *productive* IgL rearrangements. (B) Cell sorting of bone marrow cells from mice injected with BrdU Bone marrow cells were stained with anti-CD45R + anti-IgM + anti-IgD + anti-λ_1 and -λ_2 antibodies. Cells presented in the indicated gates are displayed in the following dot plots: forward scatter versus side scatter, CD45R versus IgD and IgM versus λ. Cells presented in both lymphoid gate (R1) and the gate for B cell precursors and newly generated B cells (R2) were sorted into the following three fractions based on the staining pattern of IgM versus λ (right dot plot). $sIgM^+$ λ^- B cells (R3) and $sIgM^+$ λ^+ B cells (R4) in the newly generated B cell compartment and $sIgM^-$ B cell progenitor fraction (R5) Numbers on gates indicate percentages The histograms show the anti-BrdU staining of $CD45R^{low}sIgM^-$ bone marrow cells derived from mice without labeling (left) or from mice labeled with BrdU for 4 h (right). The horizontal bar defines the intensity of staining of the BrdU labeled ($BrdU^+$) fraction for the following studies.

(+/+), heterozygous mutant (+/iEκT) and homozygous mutant (iEκT/iEκT) mice. At 12–24 h after BrdU injection, up to 20% of κ^+ B cells are BrdU labeled, while $BrdU^+$ λ^+ B cells are hardly detectable until 36–48 h. This indicates that κ and λ rearrangements are not initiated simultaneously. By 36 h after BrdU injection, the percentage of $BrdU^+$ κ^+ B cells has

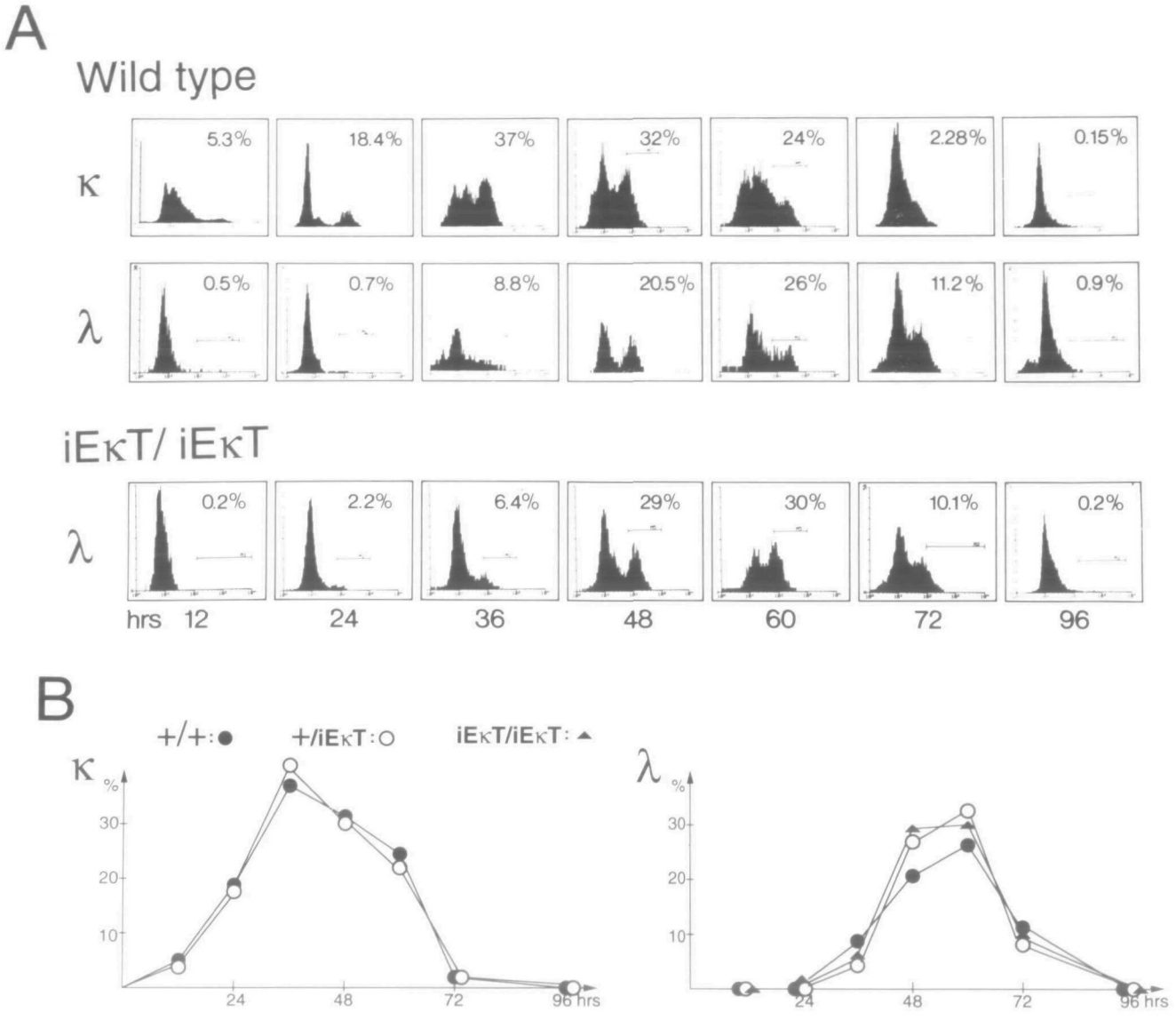

Fig. 2. BrdU$^+$ λ$^+$ B cells appear 1 day after BrdU$^+$ κ$^+$ B cells in wild-type mice and appear with the same kinetics as BrdU$^+$ λ$^+$ B cells in the κ-deficient mice. (A) Wild-type and κ-deficient (iEκT/iEκT) mice were injected with a single dose of BrdU and killed after times indicated. Bone marrow cells were sorted as shown in Fig. 1(B). The histograms of anti-BrdU staining are displayed. The numbers in the histograms show the percentages of BrdU$^+$ cells. (B) Each point represents BrdU staining of bone marrow cells pooled from three mice. The appearance of BrdU$^+$ κ$^+$ B cells (left) and BrdU$^+$ λ$^+$ B cells (right) is compared over time among wild-type, heterozygous mutant (+/iEκT) and homozygous mutant (iEκT/iEκT) mice The time point zero represents the time of BrdU injection

reached maximum levels. Given that newly generated B cells stay in the bone marrow for 50 h on average (23), BrdU$^+$ κ$^+$ B cells are not generated at the later time points In contrast, the percentages of BrdU$^+$ λ$^+$ B cells increase from 36 to 60 h. Hence, most BrdU$^+$ small pre-B cells have already ceased κ rearrangements when they undergo λ rearrangements.

Mice homozygous for iEκT undergo V_λ–J_λ rearrangements with the same kinetics as wild-type mice, although they undergo no recombination at the κ locus

Figure 2(B, right) shows that BrdU$^+$ λ$^+$ B cells are generated with the same kinetics among wild-type (+/+), heterozygous mutant (iEκT/+) and homozygous mutant (iEκT/iEκT) mice. The complete lack of rearrangements at the κ locus does not, therefore, affect the kinetics of V_λ–J_λ rearrangements. The absence of BrdU$^+$ λ$^+$ B cells in the first 24 h in mice carrying +/+ or +/iEκT genotypes indicates that the transition from κ to λ rearrangements occurs without cell division. Accordingly, B cell progenitors at the same stage in the homozygous mutant mice do not proliferate.

The output of κ$^+$ B cells in the heterozygous mutant mouse is ~70% of that in wild-type mice

Figure 2(B) shows that newly generated κ$^+$ or λ$^+$ B cells remain in the bone marrow for a similar period among the three types of mice: wild-type (+/+), heterozygous (+/iEκT)

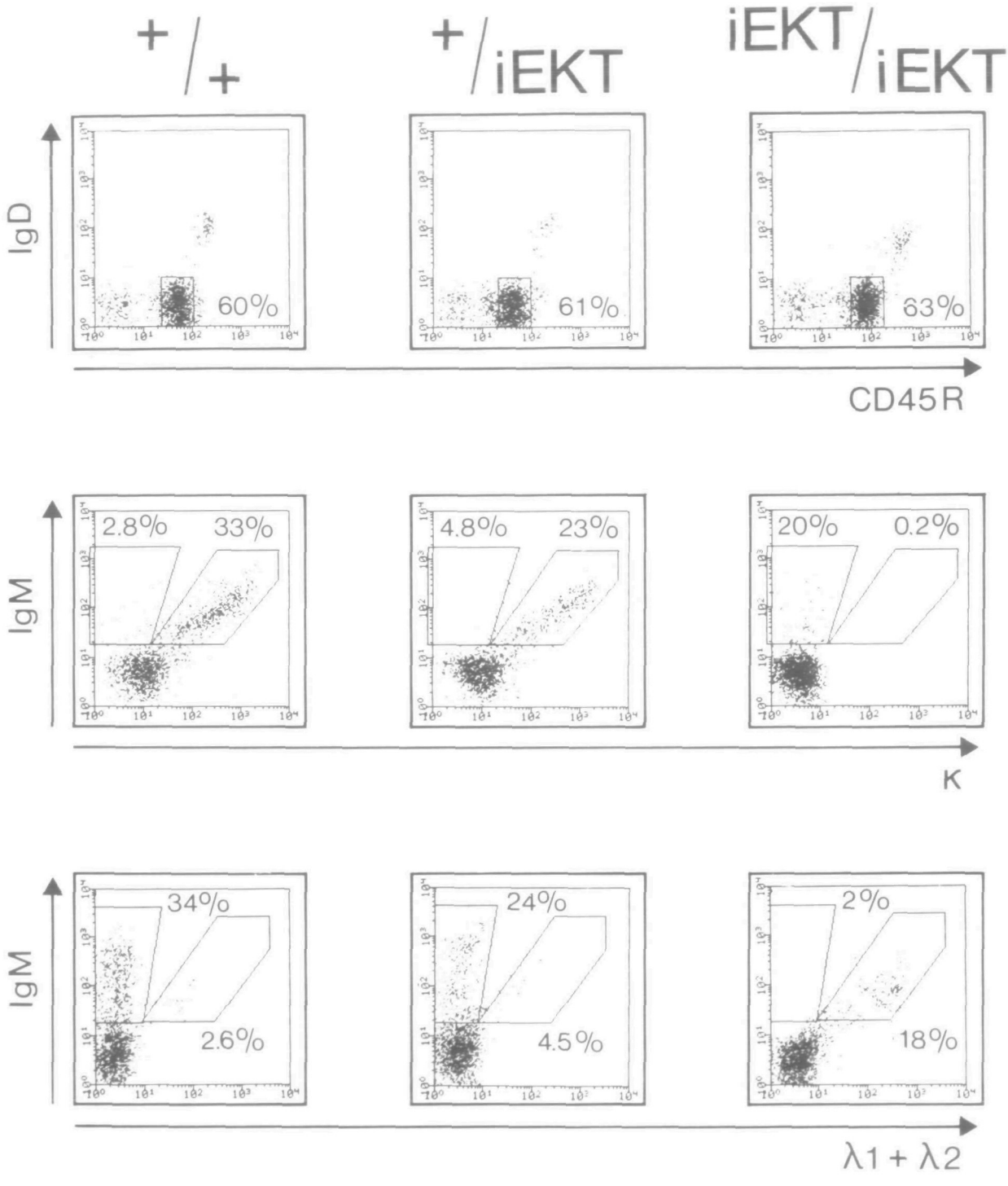

Fig. 3. The rate of output of κ^+ or λ^+ B cells in wild-type, heterozygous mutant (+/iEκT) and homozygous mutant (iEκT/iEκT) mice. Bone marrow cells derived from 6- to 8-week-old 129 strain congenic for the iEκT mutation were stained with anti-CD45R + anti-IgM + anti-IgD + anti-κ (middle row) or anti-CD45R + anti-IgM + anti-IgD + anti-λ_1 and -λ_2 antibodies (upper and lower row). Cells in the lymphocyte gate (R1 in Fig. 1B) are displayed in the upper row, and cells in both R1 and R2 gates are displayed in the middle and lower rows. Note that anti-λ_1 and -λ_2 antibody does not stain λ_3, which accounts for ~10% of the whole λ chain expression (43). Numbers given on gates indicate percentages.

and homozygous (iEκT/iEκT) mutant mice. Hence, numbers of κ^+ or λ^+ B cells in the newly generated B cell compartment are proportional to the output of κ^+ or λ^+ B cells. Since the output of κ^+ or λ^+ B cells is proportional to the probability of *productive* κ or λ rearrangements per cell, the relative probability of *productive* κ or λ rearrangements in these three

types of mice can be assessed simply by measuring the number of κ^+ or λ^+ B cells in the newly generated B cell compartment of the bone marrow

In Fig. 3, the first and third row are identical analyses as discussed in Fig. 1(B) and the second row is the analysis for κ^+ instead of $\lambda_1 + \lambda_2^+$. Table 1 summarizes the percentages of newly generated κ^+ and λ^+ B cells among the whole population of nucleated cells in the bone marrow.

The homozygous mutant (iEκT/iEκT) mice generate about six times more λ^+ B cells (and ~2.6-fold fewer total B cells) than wild-type mice (Table 1) The heterozygous (iEκT/+) mice show an approximate doubling in λ chain usage and ~68% usage of κ chain compared with wild-type mice (Table 1). Interestingly, ~70% of wild-type splenic B cells have been previously shown to carry one functional Igκ allele and one unrearranged (5,26). Thus, a single Igκ gene per cell could produce κ^+ B cells at ~70% the rate observed in wild-type mice carrying two Igκ genes, which is in accordance with the finding of heterozygous mutant mice (Table 1).

When the probability of *productive* κ rearrangement at a single Igκ locus is said to be *K* (%), the probability that both κ alleles of wild-type mice fail *productive* κ rearrangement is calculated to be $(1 - K/100)^2 \times 100\%$. Then, the probability of *productive* κ rearrangements in wild-type pre-B cells is calculated to be $100 - (1 - K/100)^2 \times 100\%$ Thus, the ratio of the calculated probability of *productive* κ rearrangements per cell in the heterozygous mice versus that in wild-type mice is $K{:}100 - (1 - K/100)^2 \times 100$ If we apply this calculated ratio to the observed ratio of κ^+ B cell production in heterozygous mutant versus that in wild-type mice, i.e. ~0.7/1.0, we can deduce the following equation, $K = 0.7 \times (100 - (1 - K/100)^2 \times 100)$. Therefore, the probability of *productive* κ rearrangement per chromosome (*K*) is calculated to be ~57% (Table 2, top middle). In wild-type mice $57/0\,7 \approx 81\%$ of small pre-B cells are expected to perform *productive* κ rearrangements (Table 2, top left).

Assuming that κ rearrangement occurs only once per chromosome during B cell development, a *productive* κ rearrangement would be performed in 2/9 = 18/81 of small pre-B cells carrying a +/iEκT genotype and in $2/9 + 2/9 \times (1 - 2/9) = 32/81$ of wild-type pre-B cells. On this assumption the ratio of κ^+ B cell production in the heterozygous mutant mice to that in wild-type mice would be $18/32 \times 100 = 56\%$, which is lower than the observed ratio, ~68% (Table 1) Even on the assumption of 1/3 probability of *productive* rearrangements after a single rearrangement, this ratio is calculated to be 60%, which is still lower than the observed ratio, ~68% Hence, this observed ratio indicates the presence of sequential attempts of κ rearrangement on one chromosome during B cell development.

The ~57% probability of productive κ rearrangement per chromosome explains a significant increase in the production of λ^+ B cells in the κ-deficient mice

In accordance with previous studies analyzing plasmacytomas and B cell hybridomas (3–7), the BrdU labeling experiments show that the initiation of λ rearrangements occurs after most pre-B cells cease κ rearrangements. Accordingly, as shown in Table 2, 19, 43 and 100% of small pre-B cells in wild-type (+/+), heterozygous mutant (+/iEκT) and homozygous mutant (iEκT/iEκT) mice respectively having failed to undergo *productive* κ rearrangements, would initiate λ rearrangements. These percentages correlate with the observed output of λ^+ B cells in these three types of mice (Table 1, second row). Hence, the regulation of κ versus λ rearrangements in these mice can be explained by a simple scenario in which small pre-B cells failing to produce *productive* κ rearrangements would proceed with λ rearrangements and acquire *productive* λ rearrangements at the same probability.

The probability of productive λ rearrangement of a single Igλ locus is ~20%

Since small pre-B cells undergoing IgL rearrangements do not proliferate, the relative generation rate of κ^+ B cells in

Table 1. Observed percentages of κ^+ and λ^+ B cells in the newly generated B cell compartment among total nucleated bone marrow cells

	+/+ (*n* = 20)	+/iEκT (*n* = 25)	iEκT/iEκT (*n* = 12)
κ^+ B cells	3.4 ± 0 3 (1.0)	2.3 ± 0 3 (0.68)	0
λ^+ B cells	0.25 ± 0 04 (0.074)	0.5 ± 0.03 (0 15)	1 4 ± 0 2 (0.41)

The percentages of newly generated B cells among nucleated bone marrow cells in mice carrying the indicated κ genotypes are displayed. Numbers given in parentheses in the upper row show numbers of analyzed mice. Numbers given in parentheses in the bottom row show yields relative to the yield of κ^+ B cells in wild-type mice. The percentages of $\mu^+ \lambda_1 + \lambda_2^+$ B cells (Fig. 3B, third row) are divided by 0.9 to calculate the percentages of total λ^+ B cells.

Table 2. Calculated percentages of cells performing indicated IgL rearrangements among small pre-B cells (100%) which initiate IgL rearrangements

Genotype of Igκ gene	+/+	+/iEκT	iEκT/iEκT
Productive κ rearrangement	81 (1.0)	57 (0.68)	0
No *productive* κ rearrangement	19	45	100
Productive λ rearrangement	6 3 (0 078)	15(0.19)	33 (0.41)

When the same number of small pre-B cells (100%) initiate IgL rearrangements in the three types of mice, the percentages of cells carrying the indicated rearrangements are calculated and displayed The percentages of pre-B cells carrying *productive* κ rearrangements (first row), those carrying no *productive* κ rearrangements (second row) and those carrying *productive* λ rearrangements (third row) in three types of mice carrying indicated κ alleles are shown The ratios of each percentage to the percentage of pre-B cells carrying *productive* κ rearrangements in wild-type mice (81%) are displayed in parentheses. Each percentage is calculated based on the following (i)–(iii) observations and on assumptions (iv) and (v). (i) The probability of *productive* κ rearrangements in the heterozygous mutant mice is 68% of that in wild-type mice. (ii) The probability of *productive* λ rearrangements in the homozygous mutant mice is 41% of the probability of *productive* κ rearrangements in wild-type mice (iii) λ rearrangements initiate after κ rearrangements cease (iv) The probability of *productive* κ or λ rearrangements is identical among the indicated three types of mice. (v) The numbers of pre-B cells which initiate IgL chain rearrangements are identical (100%) among the indicated three types of mice.

wild-type mice and that of λ^+ B cells in the homozygous mutant mice would be proportional to the probability of making *productive* rearrangements per cell at the two L chain loci The production of λ^+ B cells in homozygous mutant mice was ~41% of the observed production of κ^+ B cells in wild-type mice (Table 1, right bottom). When 81% of small pre-B cells in wild-type mice acquire *productive* κ rearrangements, 81×0.41 = 33% of small pre-B cells in the κ-deficient mice would acquire *productive* λ rearrangements. The probability of *productive* λ recombination per chromosome is calculated to be 18.1% [$(1 - 0.181)^2 = 0.67$, 67% of small pre-B cells fail to carry *productive* λ rearrangements while the other 33% carry *productive* λ rearrangements]

Since the expected percentages of small pre-B cell having failed *productive* κ rearrangements correlate (Table 2, second row) with the observed output of λ^+ B cells (Table 1, bottom row), the probability of *productive* λ rearrangements in wild-type and heterozygous mutant mice would be identical with that in homozygous mutant mice (33%). Thus, 19×0.33 = 6.3% and 43×0.33 = 14% of small pre-B cells in wild-type and heterozygous mutant mice would perform *productive* λ rearrangements respectively (Table 2, third row) The expected ratios of output of κ^+ B cells to λ^+ B cells are thus 81/6.3 in wild-type and 57/15 in the heterozygous mutant mice These ratios correlate with previous studies (16,17,28,29) as well as the observed ratio shown in Table 1. Hence the probability of *productive* λ rearrangements in wild-type mice is also ~20% per chromosome

Discussion

By comparing the probability of *productive* κ rearrangements per cell bearing two intact Igκ genes (wild-type) with that per cell bearing one intact Igκ gene (heterozygous mutant), the probability of *productive* κ rearrangements per chromosome is calculated to be ~60%. Then by comparing the probability of *productive* κ rearrangements in wild-type mice with that of *productive* λ rearrangements in homozygous mutant mice, the probability of *productive* λ rearrangements per chromosome is calculated to be ~20%.

Due to variations among individual mice, we cannot estimate very accurately the ratio of B cell generation in heterozygous mutant mice versus that in wild-type mice. In fact, we have calculated numbers shown in Table 2, given this ratio as 0.7/1 instead of the observed ratio: 0.68/1 (Table 1), because 0.7 is more consistent with the generation of λ^+ B cells. Although small effects in the comparison between wild-type and heterozygous mutant mice might lead to large errors in the calculations, our data still exclude the assumption of a single V_κ–J_κ rearrangement per chromosome during B cell development (11,14). This assumption can explain neither the κ/λ ratio in wild-type mice nor the significant increase in λ^+ B cell production of the homozygous mutant mice without assuming cell proliferation during IgL rearrangements; the BrdU labeling experiments show that there is no cell proliferation among immature κ^+ B cells in wild-type mice, immature λ^+ B cells or small pre-B cells in the homozygous mutant mice. On the other hand, the ~57 and ~20% probability of *productive* κ and λ rearrangements respectively explains not only the observed significant increase in λ^+ B cell production of the homozygous mutant mice (Table 2, third row, right versus left) but also a considerable bias towards the expression of the κ isotype over λ isotype in wild-type mice (Table 2, left, first versus third row).

The 20% probability of *productive* λ rearrangements per chromosome suggests that the life span of small pre-B cells limits the attempt of λ rearrangement to on average less than once per chromosome On the other hand, 57% probability of *productive* κ rearrangements per chromosome indicates the presence of sequential rearrangements on one chromosome. In addition, once a *productive* κ rearrangement is generated, another rearrangement on the two Igκ genes must be quickly suppressed. Since 1/3 of total V_κ segments are estimated to be in the form of pseudogenes, a single attempt of V_κ–J_κ rearrangement may generate an *productive* joint at 2/9 probability [= 1/3 (in-frame rearrangement)×2/3 (functional V_κ segments)] (11). Given the estimation of 2/9 probability, to achieve 57% probability of *productive* κ rearrangement per chromosome, a single Igκ chromosome needs to attempt V_κ to J_κ rearrangements on average up to three times during B cell development (Table 3).

The presence of sequential κ rearrangements is not inconsistent with the observed usage of J_κ segments in the V_κ–J_κ joints. If small pre-B cells could try V_κ–J_κ rearrangements up to three times per chromosome, given that there are only four J_κ segments containing intact recombination signals, most small pre-B cells should initiate V_κ–J_κ rearrangements utilizing $J_\kappa1$, which rearrangement is followed by rearrangements utilizing the more 3′ J_κ segments Mice heterozygous for the deletion of J_κ–C_κ region (18), where all B cells must carry *productive* V_κ–J_κ joints, shows the ratio of usage of J_κ segments in V_κ–J_κ joints as $J_\kappa1/J_\kappa2/J_\kappa4/J_\kappa5$ = 33/25/13/28% (30). Except for $J_\kappa5$, the more frequent usage of 5′ J_κ segments is consistent with the predicted sequence of κ rearrangements from $J_\kappa1$ to $J_\kappa4$. The higher usage of $J_\kappa5$ than $J_\kappa2$ and $J_\kappa4$ segments implies that some κ rearrangements are initiated with the $J_\kappa5$ segment while most sequential κ rearrangements occur in the order of $J_\kappa1$k–$J_\kappa2$k–$J_\kappa4$–$J_\kappa5$ segments

In addition to the 57% probability of *productive* κ rearrangement, the following observations also support the presence of sequential V_κ–J_κ rearrangements on one chromosome.

(i) At day 15/16 gestation essentially all B lineage cells are $CD43^+$ and at earlier stages than small pre-B cells, which are $CD43^-$ and undergo most IgL rearrangements (31). Among analyzed V_κ–J_κ joints, day 14 fetal liver shows 1/3 in-frame joints and 40% V_κ–$J_\kappa5$, while by day 16 there is closer to 50% in-frame joints and 51% V_κ–$J_\kappa5$ (32). The increase in the

Table 3. Calculated probability of *productive* κ rearrangements after indicated numbers of sequential rearrangements on one chromosome

After attempt	
First	$2/9 = 0.22$
Second	$2/9 + (1 - 2/9)\times2/9 = 0.40$
Third	$2/9 + (1 - 2/9)\times2/9 + (1 - 2/9)^2\times2/9 = 0.53$
Fourth	$2/9 + (1 - 2/9)\times2/9 + (1 - 2/9)^2\times2/9 + (1 - 2/9)^3\times2/9 = 0.64$

percentages of in-frame joints and V_κ–J_κ5 joints during early B cell development suggests the presence of secondary rearrangements to downstream J_κ on one chromosome.

(ii) Reciprocal products, which were generated by the back-to-back fusion of the recombination signal sequences, were found at the 5′ of V_κ–J_κ joints in several murine plasmacytomas (33,34).

(iii) If the Igκ gene can try V–J recombination only once per chromosome and perform a *productive* rearrangement at 2/9 (= 22.3%) probability (11), the ratio of the number of mature κ^+ B cells containing a *productive* V_κ–J_κ joint on one allele and germ line configuration on the other allele (κ^+/G) to the number of mature κ^+ B cells containing a *productive* V_κ–J_κ joint on one allele and an abortive V_κ–J_κ joint on the other allele (κ^+/κ^-) is expected to be 100:100 – 22.3 = 56% (κ^+/G):44% (κ^+/κ^-). Contrary to this prediction, the relative amount of unrearranged J_κ fragment in splenic B cells is ~0.35 of that in non-B cells, which means that ~70% of B cells carry a κ^+/G genotype (8,26). This observation cannot be explained by a single κ rearrangement per chromosome during B cell development. On the other hand, the presence of sequential κ rearrangements would yield 56–70% of B cells carrying a κ^+/G genotype, depending on the relative kinetics of two sequential κ rearrangements on each chromosome

(iv) The production rates (cells$\times 10^6 \times$ day^{-1}/whole body) of small pre-B and B cells in wild-type mice are 17 and 16 respectively (23), indicating that most small pre-B cells perform *productive* IgL rearrangements

With respect to the regulation of κ and λ rearrangements, our data can be summarized in the following two points: (i) the kinetics of generation of λ^+ B cells from BrdU^+ B cell precursors were identical among the three types of mice carrying +/+ , +/iEκT and iEκT/iEκT genotypes, and (ii) in these three types of mice the probability of *productive* λ rearrangement is very likely to be identical. Hence, the absence of V_κ–J_κ or RS recombination affected neither the kinetics nor probability of *productive* λ rearrangements in the bone marrow. Rearrangements at the κ and λ loci are therefore totally independent of each other, and the expression of IgL chain is solely responsible for both isotypic exclusion and allelic exclusion of κ and λ chain

Enhancer sequences have been shown to participate in the regulation of rearrangement of Ig genes (15,35). Different regulatory sequences at the κ and λ loci having different kinetics and strength of activity are likely to account for the earlier initiation of rearrangements at the κ locus during B cell development in mice. In addition, species carrying different regulatory sequences at the κ and λ loci are likely to show different kinetics of rearrangements at the two IgL loci, causing significant differences in ratios of κ^+ B cells to λ^+ B cells (36).

RS recombination, which is observed in most λ-expressing B cells, leads to the partial or complete deletion of the J_κ–C_κ region between the RS sequence located downstream of C_κ and heptamer signals located upstream of either J_κ or C_κ (37–42). Genomic sequences for 3′ κ RS recombination are well conserved between human and mouse, suggesting a physiological role of this recombination. We previously demonstrated that the κ intronic enhancer is essential for rearrangements at the Igκ locus (15) Rearrangements to the 3′ RS recombination deleting this enhancer may cause complete inhibition of subsequent rearrangements Since rearrangements at the Igκ locus are very active, even utilizing cryptic recombination signals (37), 3′ κ RS recombination may be evolved to prevent aberrant recombination like a chromosomal translocation by removing the *cis*-acting regulatory sequence for V–J recombination.

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Abbreviations

SL surrogate light chain

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