

Expression of a Targeted $\lambda 1$ Light Chain Gene Is Developmentally Regulated and Independent of $Ig\kappa$ Rearrangements

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Abstract

Immunoglobulin light chain (IgL) rearrangements occur more frequently at $Ig\kappa$ than at $Ig\lambda$. Previous results suggested that the unrearranged $Ig\kappa$ locus negatively regulates $Ig\lambda$ transcription and/or rearrangement. Here, we demonstrate that expression of a $VJ\lambda 1$ -joint inserted into its physiological position in the $Ig\lambda$ locus is independent of $Ig\kappa$ rearrangements. Expression of the inserted $VJ\lambda 1$ gene segment is developmentally controlled like that of a $VJ\kappa$ -joint inserted into the $Ig\kappa$ locus and furthermore coincides developmentally with the occurrence of $Ig\kappa$ rearrangements in wild-type mice. We conclude that developmentally controlled transcription of a gene rearrangement in the $Ig\lambda$ locus occurs in the presence of an unrearranged $Ig\kappa$ locus and is therefore not negatively regulated by the latter. Our data also indicate light chain editing in $\sim 30\%$ of $\lambda 1$ expressing B cell progenitors.

Key words: gene rearrangement • B lymphocyte • light chain • receptor editing • development

Introduction

The variable region genes of Igs and the TCR comprise variable (V), diversity (D), and joining (J) segments. These gene segments are assembled during early lymphocyte differentiation by a common V(D)J recombinase that consists of the recombination activating gene products RAG1 and RAG2 (1, 2) and recognizes conserved recombination signal sequences (RSS)* flanking the V, D, and J segments. In the case of Igs, gene rearrangements occur at the genetic loci encoding Ig heavy (IgH) and Ig light chains (IgL). While IgH rearrangement can occur on two IgH alleles, Ig light chains can be generated from four different loci, two $Ig\lambda$ and two $Ig\kappa$ alleles. Any given B cell expresses only one of the two allelic IgH loci and one of the multiple IgL loci as proteins and thus carries an Ig molecule of single specificity. This phenomenon is termed allelic or (κ versus λ) isotype exclusion (for a review, see reference 3).

IgH and IgL gene rearrangements usually take place at consecutive developmental stages during B cell development. IgH rearrangements occur in pro-B cells and, if productive, promote a phase of proliferative expansion and

subsequent IgL rearrangement in pre-B cells (4). If the emerging receptor is self-reactive, its specificity can be revised by secondary IgL rearrangements, a process known as receptor editing (5, 6).

In mice, B cells that express $Ig\kappa$ are 15–20 times more frequent than those expressing $Ig\lambda$. In humans, the frequencies of κ and λ expressing B cells are similar, yet in both mice and humans, κ^+ B cells generally carry the $Ig\lambda$ locus in germline configuration, while the vast majority of λ^+ B cells has inactivated its $Ig\kappa$ loci by either nonfunctional $V\kappa J\kappa$ -joint or deletion of the κ constant region (C κ) gene (7–10). C κ deletion is the consequence of a recombination event that occurs between an RSS located either in the $J\kappa$ -C κ intron or at the 3' end of a nonrearranged $V\kappa$ gene and a downstream "rearranging" sequence called RS in mice (11) and κ -deleting element (Kde) in humans (12). However, in some cells, IgL rearrangement is initiated at the $Ig\lambda$ locus as shown by a small fraction of κ^+ B cells that carry nonfunctional $Ig\lambda$ rearrangements (10, 13, 14).

An ordered and a stochastic model were put forward to explain these findings. The ordered model proposes regulated opening of IgL loci with $Ig\kappa$ being accessible for rearrangements before $Ig\lambda$. The stochastic model predicts that both IgL loci are accessible at the same time with the probability of rearrangements being higher for $Ig\kappa$ than for $Ig\lambda$. More recently, the analyses of several mouse mutants with impaired $Ig\kappa$ rearrangement and/or expression dem-

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*Abbreviations used in this paper: ES, embryonic stem; RSS, recombination signal sequence.

onstrated that inactivation of the $Ig\kappa$ locus causes a 10-fold increase in λ^+ B cells. Inactivation of $Ig\kappa$ was achieved by replacing either the intronic κ enhancer (iE κ ; reference 9) or the C κ (14) or J κ and C κ (15) gene segments by a *neo^R* gene. While the former manipulation causes complete silencing of V κ →J κ rearrangements, the two latter mutations exert only a mild effect on $Ig\kappa$ rearrangement but abolish expression of a functional κ light chain. The drastic increase in λ -expressing B cells in these mice led to the proposal of negative regulatory elements in the germline $Ig\kappa$ locus that would actively suppress λ rearrangements and be artificially disrupted in the mutant alleles. In WT mice, inactivation of such elements upon $Ig\kappa$ rearrangement was suggested to increase the probability of $Ig\lambda$ rearrangements (14, 15).

Generally, tissue-specific and developmentally regulated Ig rearrangement is ensured by Ig locus-specific enhancers, which render the Ig locus accessible for DNA binding proteins such as transcription factors and the RAG1/RAG2 complex. Germline transcripts from unrearranged Ig loci that initiate upstream of V, D, or J segments can be detected in B cell progenitors that are in the process of rearranging the respective Ig loci (16–18). Recently, Nussenzweig and colleagues showed that, in the $Ig\kappa$ locus, the level of V κ germline transcription needs to exceed a certain threshold before a V κ segment becomes susceptible to rearrangement, thus providing evidence for a functional association of germline transcription with rearrangement (19). Similarly, the introduction of a phosphoglycerol kinase (PGK)-promotor driven *neo^R* gene 5' of the J λ 1 segment led to a substantial increase in both J λ 1 germline transcription and V λ 1→J λ 1 rearrangement (20).

Based on the coincidence of germline transcription and Ig gene rearrangement, initiation of $Ig\lambda$ germline transcription has been analyzed to address $Ig\lambda$ locus accessibility. The detection of sterile J κ but not J λ transcripts in a minor fraction of proliferating, early pre-B cells was interpreted as ordered initiation of $Ig\lambda$ rearrangement (21). However, detection of a particular germline transcript depends on its transcription rate and mRNA stability. Hence, lack of detectable germline transcripts does not necessarily reflect transcriptional inaccessibility.

Taken together, current knowledge suggests that $Ig\kappa$ is generally rearranged before $Ig\lambda$ and that this phenomenon may be controlled by an $Ig\kappa$ -derived negative regulatory signal that interferes with $Ig\lambda$ rearrangement. As mentioned above, this signal would be expected to also interfere with the transcriptional accessibility of the $Ig\lambda$ locus. In this study, we attempted to obtain evidence for such regulation by inserting a prerrearranged VJ λ 1 gene into the $Ig\lambda$ locus and analyzing whether its expression depends on $Ig\kappa$ rearrangement.

Materials and Methods

Generation of VJ λ 1i Mice. A targeting vector was designed to replace 18 kb of genomic DNA containing V λ 1 and J λ 1 by a prerrearranged VJ λ 1 gene. A 2.8 kb short arm of homology

(SAH) located 5' of J λ 1 was generated in two steps: in order to introduce a NotI site at the distal end of the SAH for linearization of the final vector, phage clone KX39 (covering 15 kb upstream of V λ 1; gift from Ursula Storb, University of Chicago, Chicago, IL) was PCR-amplified using the primer pair 5'NXF1 (TGC CAG AGC GGC CGC TGC TAG TAA CAA TAA GAG TGG) and 3'NXF-1 (GTT CTA GAG TGA CAA TAG TAA CGA). The PCR product was cut with NotI and EcoRI to obtain the distal SAH fragment. The proximal SAH fragment, which also contains the prerrearranged VJ λ 1 gene, was excised from pA8–6 λ (gift from Sigfried Weiss, German Research Centre for Biotechnology, Braunschweig, Germany) with EcoRI and AccI. PCR was used to introduce a silent GTC→GTG (codon 36) mutation in framework region 2 of VJ λ 1 thereby destroying an AvaII restriction site. A 5.4 kb AccI/EcoRI fragment located 3' of J λ 1 and excised from cosmid cos2 (gift from Ursula Storb [22]) served as long arm of homology (LAH). A *loxP* flanked ACN cassette containing the *neo^R* gene and the *cre*-recombinase gene under the control of the sperm-specific ACE promotor (excised with EcoRI and XhoI from pACN [23]) was cloned into an intronic AccI site downstream of J λ 1. The ACN-cassette is deleted in chimeras during spermatogenesis. To select against random integration, a thymidine kinase (TK) gene (excised with XhoI and SalI from pBS-TK [24]) was inserted 3' of the LAH. The targeting construct was linearized with NotI and transfected into Bruce4 C57BL/6 embryonic stem (ES) cells (25) as described (26). G418- and gancyclovir-resistant ES cell clones were screened for homologous recombination by Southern blot analysis. Probes used for Southern blotting were generated by PCR: primers for the 5' external probe (5'V1) were 5'XF-3 (TAA AAA GAA AAA AAA CAT AGG) and 3'XF-2 (CCA AGA TTG GGT TAA TGT ATC), KX39 served as template; primers for the 3' internal probe (3'C1) were 5'XbaI/XhoI (CAG AAA TGC AAG CCC AGG AAG) and 3'XbaI/XhoI (TTA CTG GGG AAC ACA CTA CAC), cos2 was used as template. 7 out of 480 double-resistant ES cell clones were homologous integrants. Two of these were injected into CB20 blastocysts and the resulting chimeric males were bred to C57BL/6 females for germline transmission.

Flow Cytometry, Cytoplasmic Staining, and Cell Sorting. Single cell suspensions from bone marrow and spleen were stained with mAbs or polyclonal Ab conjugated to FITC, phycoerythrin (PE), PerCP, or biotin. Biotin conjugates were visualized with Streptavidin-allophycocyanin (APC). For intracellular stainings, cells were subsequently fixed in PBS/2% formaldehyde for 20 min at room temperature. Intracellular staining was performed with FITC-conjugated Ab in staining buffer containing 0.05% saponin. The following mAbs were used for surface staining: anti-B220 (RA3–6B2), anti-CD19 (1D3), anti-CD43 (S7), FcBlock (2.4G2), anti- κ (187.1) (all from BD Biosciences); anti-CD25 (PC61.5) and anti-IgM (1B4B1) (from eBioscience); anti- κ (R33–18–10; generated in our laboratory) and anti- λ 1 (L22.18.2, gift from Sigfried Weiss). Intracellular light chain stainings were performed with either goat anti-mouse λ polyclonal Ab (Southern Biotechnology Associates, Inc.) or anti- κ (R33–18–10) mAb. Stained cells were acquired on FACSCalibur™ and data were analyzed with CELLQuest™ software, cell sorting was performed on FACS Vantage™ (all Becton Dickinson). All analyses were restricted to cells within the lymphocyte gate.

RT-PCR Analysis of Light Chain Transcripts. Splenocytes of WT (C57BL/6) and VJ λ 1i/+ mice were enriched for B lymphocytes using CD19 beads and the MACS technology (Miltenyi Biotec) according to the manufacturer's protocol. The CD19+

fraction was subsequently stained for CD19, κ , and $\lambda 1$. κ^+ , $\lambda 1^+$, and $\kappa/\lambda 1^+$ B cells were sorted and total RNA was isolated using TRIzol (Invitrogen) following the manufacturer's protocol. cDNA was synthesized from 20,000 cells using Thermoscript RT-PCR System (Invitrogen) according to the manufacturer's instructions. 1/10 of the cDNA template and serial dilutions thereof were subjected to PCR. $\lambda 1$ message was amplified using the primer pair VJ $\lambda 1$ -int (TTG TGA CTC AGG AAT CTG CA) and C $\lambda 1$ (CTC GGA TCC TTC AGA GGA AGG TGG AAA CA), κ message was amplified using the degenerate V κ primer M κ (GAT ATT GTG ATG ACC CAG TCT) and C κ E (ACA CTC ATT CCT GTT GAA GCT CTT). Primers for β -actin amplification were m- β -actinT (CCT AAG GCC AAC CGT GAA AAG) and m- β -actinB (TCT TCA TGG TGC TAG GAG CCA). All primer pairs were intron-spanning.

Southern Blot Analysis of Ig κ Rearrangements. Splenocytes of WT (C57BL/6) and VJ $\lambda 1i/+$ mice were enriched for B cells, stained and sorted as described above. Thymocytes served as negative control. Genomic DNA from 10^6 cells per sample was subjected to Southern blot analysis. To detect RS recombination, DNA was digested with EcoRI and hybridized to RS-probe (11) resulting in a 5.8 kb RS-germline fragment which is lost upon RS recombination. To detect V κ →J κ rearrangements, DNA was digested with EcoICRI and hybridized to 5'J κ -probe (27) giving rise to a 4.5 kb κ -germline fragment. Depending on the orientation of the V κ segment, Ig κ -rearrangements lead to deletion or inversion of the DNA between V κ and J κ . In both cases, the characteristic 4.5 kb fragment is lost. To control for DNA loading, blots were stripped and rehybridized with an IL-4 gene specific probe (28) yielding a 10 kb fragment for the EcoRI digest and a 4.8 kb fragment for the EcoICRI digest. The signal intensities of each sample were quantified using a Storm 860 Molecular Dynamics scanner and ImageQuant software (Amersham Biosciences). RS- and κ -germline band intensities were standardized using the respective IL-4 intensities. The WT (C57BL/6) thy-

mocyte signal was defined as 100%, the fraction of unrearranged Ig κ was calculated as the ratio of RS- or κ -germline intensity over the thymocyte signal.

BrdU-labeling of Immature B Cells. BrdU labeling and analysis was performed using BrdU Flow Kit (BD Biosciences) according to the manufacturer's instructions. In brief, D23 $\kappa i/+$, LN1 $\kappa/+$, VJ $\lambda 1i/+$, and WT (C57BL/6) mice were injected with 1 mg BrdU intraperitoneally and analyzed at the indicated time points thereafter. Bone marrow single cell suspensions were stained for B220, κ and $\lambda 1$, fixed, DNase treated, and subsequently stained for BrdU incorporation.

Results

Targeted Insertion of a Prerearranged VJ $\lambda 1$ Gene into the Ig λ Locus. To generate Ig λ -transgenic mice where the expression of the transgenic λ light chain is regulated by its physiological control elements, we targeted a prerearranged VJ $\lambda 1$ gene into the Ig λ locus of murine embryonic stem (ES) cells. The targeting vector was designed such that the VJ $\lambda 1$ gene replaces 18 kb of genomic DNA between V $\lambda 1$ and J $\lambda 1$. This region contains the J λ 3 cluster but no apparent cis-regulatory elements according to DNase hypersensitivity assays in various cell lines (29) (Fig. 1). The emerging mutant Ig λ allele (referred to as VJ $\lambda 1i$) mimics the WT Ig λ allele after V $\lambda 1$ →J $\lambda 1$ rearrangement. We chose the VJ $\lambda 1$ joint, as ~60% of λ^+ B cells in WT mice express a $\lambda 1$ light chain (30).

All Mature B Cells of VJ $\lambda 1i$ Mice Express the Inserted VJ $\lambda 1$ Gene. The inserted $\lambda 1$ light chain is expressed in all mature B cells of VJ $\lambda 1i$ mice (Fig. 2) and the distribution of peripheral B cell subsets appears normal (unpublished data). The total number of splenic B cells in VJ $\lambda 1i$ mice is re-

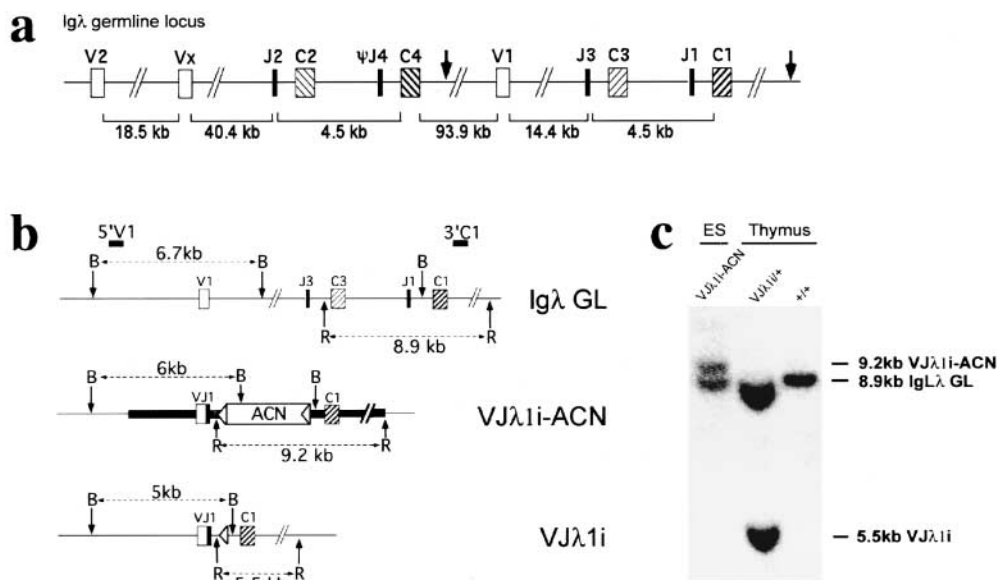


Figure 1. Targeted insertion of a prerearranged VJ $\lambda 1$ gene segment into the germline of the Ig λ locus. (a) Overview of the genomic organization of the Ig λ germline locus (reference 37). The Ig λ locus is composed of three functional J λ -C λ clusters (J λ 1-3) and one pseudo J λ -C λ cluster (J λ 4). Three V λ gene segments have been identified; V λ 1, V λ 2, and V λ x. Constant region (C) exons are depicted as hatched boxes, V segments as open boxes, J segments as closed boxes. Arrows indicate DNase hypersensitive sites (reference 22). Numbers indicate distances between selected exons in kb (reference 38). (b) Partial restriction endonuclease map of the Ig λ germline (Ig λ GL) locus, the mutated allele after homologous recombination (VJ $\lambda 1i$ -ACN) and the mutated allele after Cre-

loxP mediated deletion of the neo^R gene containing ACN cassette (VJ $\lambda 1i$). Arms of homology are shown in bold in VJ $\lambda 1i$ -ACN. V, J, and C region gene segments are indicated as described in panel a, loxP sites are shown as open triangles. Double headed arrows and associated numbers depict the indicative restriction fragments and their respective sizes as revealed by either an external probe (5'V1) or an internal probe (3'C1). B, BamHI; R, EcoRI. (c) Southern blot analysis of one injected ES cell clone (VJ $\lambda 1i$ -ACN), a heterozygous mouse mutant (VJ $\lambda 1i/+$), and a WT littermate (+/+). ES cell or thymic genomic DNA was digested with EcoRI and hybridized with 3'C1.

duced by 35% when compared with WT mice ($2.3 \times 10^7 \pm 0.6 \times 10^7$ and $3.5 \times 10^7 \pm 1.0 \times 10^7$ B cells, respectively). A similar reduction in B cell numbers has been reported for mice that carry an inserted VJ κ gene (27) and may reflect the restricted B cell repertoire in mice that predominantly express one particular light chain.

The majority of splenic B cells in VJ $\lambda 1$ i mice express $\lambda 1$ exclusively. However, a substantial fraction of B cells (~30%) express both $\lambda 1$ and κ on the surface and 6% appear to have lost surface expression of $\lambda 1$ (Fig. 2 A). Due to the organization of the Ig λ locus, the VJ $\lambda 1$ gene cannot be deleted by “secondary” V λ →J λ rearrangements (see Fig. 1 A). B cells that lack surface $\lambda 1$ expression may represent naive B cells with inefficient heavy/ $\lambda 1$ light chain pairing or memory B cells that have inactivated the VJ $\lambda 1$ coding region through somatic hypermutation. A semiquantitative RT-PCR analysis of sorted $\lambda 1^+$, $\kappa/\lambda 1^+$, and κ^+ splenic B cells from VJ $\lambda 1$ i mice confirms that the inserted gene segment is transcribed at similar levels in both surface $\lambda 1$ -positive and -negative subpopulations (Fig. 2 B).

Expression of a Prerearranged $\lambda 1$ Light Chain Is Developmentally Controlled Like That of a Prerearranged κ Light Chain. To assess whether the prerearranged VJ $\lambda 1$ gene is expressed in a developmentally regulated fashion, we analyzed intracellular light chain expression in pro- and pre-B cells of VJ $\lambda 1$ i mice. Both pro- and pre-B cells are IgM $^-$, express low levels of the B cell marker B220 and can be distinguished using either CD25 (Fig. 3) or CD43 (data not depicted) as additional markers. VJ $\lambda 1$ i mice show a developmentally regulated $\lambda 1$ expression pattern with three- to fourfold less λ^+ pro- than pre-B cells (Fig. 3). A compar-

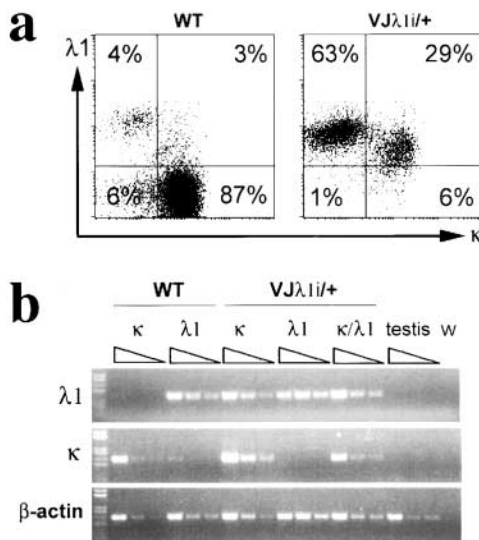


Figure 2. All mature B cells of VJ $\lambda 1$ i/+ mice express the inserted $\lambda 1$ light chain. (a) Representative staining for κ and $\lambda 1$ on CD19 $^+$ splenocytes from VJ $\lambda 1$ i/+ and WT mice. Numbers indicate the percentage of cells per quadrant. (b) Semiquantitative RT-PCR analysis of sorted κ^+ , $\lambda 1^+$, and $\kappa/\lambda 1^+$ splenic B cells from WT mice and VJ $\lambda 1$ i/+ mice. Testis RNA from WT mice served as negative control. 1:5 serially diluted cDNA was analyzed for reverse-transcribed $\lambda 1$ and κ light chain message by PCR. A β -actin PCR was performed as internal control.

able result was observed for κ light chain expression in mice that carry a prerearranged VJ κ gene and either retain (in the case of the D23 κ i allele [31]) or lack (in the case of the LN1 κ allele [32]) the genomic sequence between V κ and J κ (Fig. 3). Similarly, in WT mice, κ^+ pro-B cells are four to five times less abundant than κ^+ pre-B cells. The fractions of both κ^+ pro- and pre-B cells are reduced by a factor of ~4.5 when compared with κ^+ cells in VJ κ i mice or λ^+ cells in VJ $\lambda 1$ i mice. As only one third of newly formed rearrangements in WT B cell progenitors is expected to be productive, the fractions of pro- and pre-cells that undergo IgL rearrangement in WT mice correspond approximately to the fraction of pro- and pre-B cells that express the prerearranged light chain in IgL insertion mice. We thus conclude that transcription of both an inserted κ and $\lambda 1$ light chain gene coincides developmentally with the initiation of Ig κ rearrangements in WT mice.

Ig λ Rearrangement Can Occur in the Absence of Pre-B Cell Receptor Signaling. As shown in Fig. 3, VJ $\lambda 1$ i and VJ κ i mice yield ~20% pro-B cells that express the inserted light chain. It has been observed previously that Ig κ rearrangements can occur independently of IgH rearrangements and pre-B cell receptor signaling in 15–20% of pro-B cells in WT mice (33). However, it is still unclear whether a similar phenomenon can take place at the Ig λ locus. Due to their low frequency, we were unable to detect Ig λ rearrangements in pro B cells of WT mice (Fig. 3

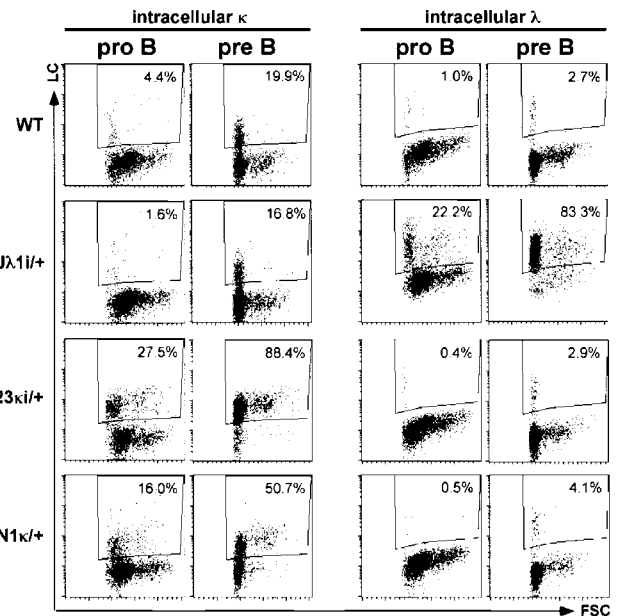


Figure 3. Intracellular light chain expression in CD25 $^+$ pro-B cells and CD25 $^-$ pre-B cells of light chain insertion and WT mice. Bone marrow lymphocytes were stained for surface expression of B220, IgM, and CD25 and for intracellular light chain expression. Flow cytometric analyses of intracellular κ and λ expression in CD25 $^-$, B220 $^+$, IgM $^-$ pro-B cells and CD25 $^+$, B220 $^+$, IgM $^-$ pre-B cells are shown for WT, VJ $\lambda 1$ i, D23 κ i, and LN1 κ mice. Light chain (LC) expression is plotted against cell size (forward scatter, FSC). Numbers indicate the percentage of light chain expressing cells. Similar results were obtained in three or more independent experiments.

Table 1. Cellularity of Bone Marrow and Splenic B Cell Compartments from WT, $\lambda 5^{-/-}$, and $\lambda 5^{-/-}$ $C\kappa^{-/-}$ Mice

	Bone marrow					Spleen			
	Total $\times 10^7$	Lymphocytes $\times 10^6$	IgM ⁺ $\times 10^6$	Pre-B $\times 10^6$	Pro-B $\times 10^6$	Total $\times 10^7$	Lymphocytes $\times 10^7$	IgM ⁺ B220 ⁺ $\times 10^7$	IgM ⁺ IgD ⁺ $\times 10^7$
WT	2.3	6.9	1.5	1.4	0.6	6.5	5.2	3.1	2.3
$\lambda 5^{-/-}$	2.1	3.8	0.1	0.1	0.3	4.4	3.1	0.7	0.3
$\lambda 5^{-/-}$ $C\kappa^{-/-}$	2.1	4.0	0.05	0.1	0.6	2.4	1.4	0.1	0.05

In each group at least four animals at the age of 8 to 20 wk were analyzed. Bone marrow was isolated from two femurs. Bone marrow cells were stained for B220, CD43, and IgM. Splenocytes were stained for B220, IgM, and IgD. Numbers were determined based on the total numbers and percentages of the population in flow cytometric analysis.

and unpublished data). We thus analyzed mice that lack $C\kappa$ (14) and the surrogate light chain component $\lambda 5$ (34). These mice are unable to express a κ light chain and cannot form a functional pre-B cell receptor, hence pro-B cell differentiation into immature B cells relies on Ig λ rearrangements that occur independently of pre B cell receptor signaling. The fact that $\lambda 5^{-/-}$ $C\kappa^{-/-}$ double mutants are able to generate B cells supports the idea that the Ig λ locus is accessible not only for transcription but also for rearrangement in a fraction of pro-B cells. However, B cell generation appears to be less efficient than in $\lambda 5^{-/-}$ single mutants (Table 1), suggesting that, also in the absence of $\lambda 5$, Ig κ rearrangements occur more frequently than Ig λ rearrangements.

The Majority of Mature B Cells in VJ $\lambda 1i$ Mice Carries the Ig κ Locus in Germline Configuration. It has been shown previously, that a prerrearranged VJ κ gene efficiently drives pre- to immature B cell differentiation without allowing Ig κ rearrangements to occur (35). Simultaneous transcriptional accessibility of Ig κ and Ig λ would imply that the same is true for a prerrearranged $\lambda 1$ light chain gene. To determine the extent of recombination at the Ig κ locus in B cells from VJ $\lambda 1i$ mice, $\kappa/\lambda 1^+$ and $\lambda 1^+$ B cells were sorted and analyzed for V $\kappa \rightarrow$ J κ rearrangements and RS recombination by Southern blotting. Thymocytes served as negative control. Individual samples were assayed for the retention of a germline EcoIRCI fragment spanning the J κ region and for the retention of a germline EcoRI fragment spanning the RS region (Fig. 4).

It has been shown previously that the majority of $\lambda 1^+$ B cells of WT mice has rearranged both Ig κ alleles (9, 13). In contrast, more than 75% of $\lambda 1^+$ B cells in VJ $\lambda 1i$ mice retain the Ig κ locus in germline configuration (Fig. 4 B). RS recombination is not detectable above background in VJ $\lambda 1i$ mice. In $\lambda 1^+$ B cells of WT mice, on the other hand, more than 60% of Ig κ alleles have undergone RS recombination (Fig. 4 C), which is in accordance with published results (13). Together, these data imply that, in VJ $\lambda 1i$ mice, the majority of pre-B cells express the inserted $\lambda 1$ light chain gene and subsequently enter the immature B cell compartment before endogenous Ig κ rearrangements have occurred.

Coexpression of κ and $\lambda 1$ Light Chains in VJ $\lambda 1i$ Mice Is Likely to be the Consequence of Receptor Editing. The appearance of $\kappa/\lambda 1^+$ mature B cells in VJ $\lambda 1i$ mice indicates that a fraction of $\lambda 1^+$ B cells has undergone Ig κ rearrangements and thus escaped isotype exclusion. This could be explained either by Ig κ rearrangements occurring in a subpopulation of pro-B cells (see above, and references 33 and

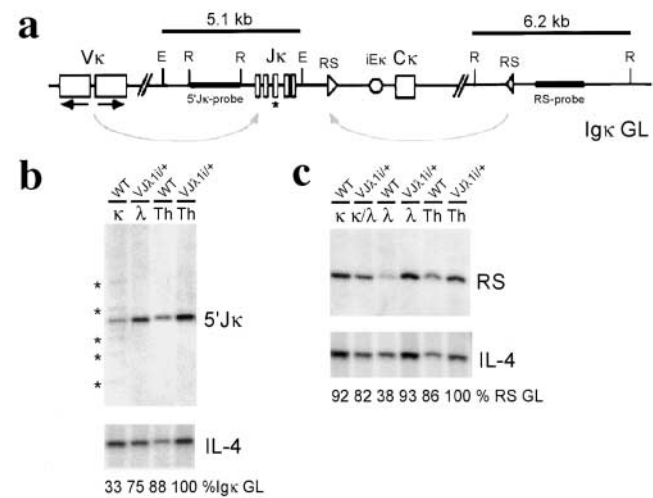


Figure 4. Only low levels of Ig κ gene rearrangements are detected in $\lambda 1^+$ B cells of VJ $\lambda 1i/+$ mice. (a) Partial restriction endonuclease map of the germline Ig κ locus (not drawn to scale). V, J, and C gene segments, the 5'J κ probe and the RS-probe are shown as boxes, the asterisk indicates a pseudo-J segment. The internal κ enhancer (iE κ) is shown as open circle, triangles depict RS recombination sites. The arrows below the V gene segments indicate their transcriptional orientation. The sizes of the germline EcoIRCI fragment and the germline EcoRI fragment are indicated as revealed by probes 5'J κ and RS, respectively. Grey arrows visualize V $\kappa \rightarrow$ J κ and RS recombination events. E, EcoIRCI; R, EcoRI. (b and c) Genomic DNA of sorted κ^+ , $\kappa/\lambda 1^+$, and $\lambda 1^+$ CD19⁺ B cells from VJ $\lambda 1i/+$ and WT mice was analyzed by Southern blotting. To detect V $\kappa \rightarrow$ J κ rearrangements, DNA was digested with EcoIRCI and hybridized to 5'J κ probe (b). RS-rearrangements were analyzed using an EcoRI digest and RS probe (c). Rehybridization with an IL-4 gene-specific probe served as internal control. The percentage of alleles retaining either the Ig κ germline (Ig κ GL) or the RS germline (RS GL) fragment is shown for each lane. Asterisks indicate fragments that originate from VJ recombination by inversion.

36) or by secondary $Ig\kappa$ rearrangements in a fraction of $\lambda 1^+$ pre-B cells. In $VJ\kappa$ mice, pre-B cells that undergo secondary light chain rearrangements were shown to take longer to exit the pre-B cell compartment than pre-B cells that express the prerrearranged light chain gene (37). We thus compared the kinetics of pre-B to immature B cell transition for $\lambda 1^+$ and $\kappa/\lambda 1^+$ B cells from $VJ\lambda 1$ mice. WT pre-B cells were analyzed in parallel as a control for cells that undergo $Ig\lambda$ rearrangements. Large, cycling pre-B cells were pulsed with BrdU in vivo and the fraction of BrdU⁺ immature B cells was determined at different time points thereafter. Immature B cells were subdivided according to light chain expression (Fig. 5 A). Fig. 5 B shows a comparison of BrdU-incorporation kinetics in WT and $VJ\lambda 1$ mice. Two conclusions can be drawn from this analysis. First, in $VJ\lambda 1$ mice, $\kappa/\lambda 1^+$ B cells exit the pre-B cell compartment ~ 12 h later than B cells that express only $\lambda 1$ and thus appear to have undergone secondary $Ig\kappa$ rearrangements. Second, WT B cells exit the pre B cell compartment with kinetics similar to $\kappa/\lambda 1^+$ B cells from $VJ\lambda 1$ mice. The delay with respect to B cells that carry an inserted light chain is likely to reflect the kinetics of $Ig\kappa$ rearrangements in WT mice.

The accelerated pre-B cell to immature B cell differentiation of cells expressing an inserted light chain has also been observed in $VJ\kappa$ mice (37). If expression of prerrearranged κ and $\lambda 1$ light chain genes in pre-B cells were initiated simultaneously, both light chains should drive this process with comparable kinetics. Indeed, no major differences were observed regarding BrdU incorporation in immature B cells of either $VJ\kappa$ or $V\lambda 1$ mice (Fig. 5 C).

Discussion

Transcription of an Inserted $VJ\lambda 1$ Element Is Developmentally Controlled like that of $VJ\kappa$ Rearrangements and Is Independent of the Latter. The predominance of $Ig\kappa$ over $Ig\lambda$ rearrangements in mice and humans has been subject of extensive research over the last decades. There is suggestive evidence that in B cell development, $Ig\lambda$ may become accessible for V(D)J recombination later than $Ig\kappa$ (7, 13, 21). More specifically, the analysis of targeted mutations in the $Ig\kappa$ locus suggested the existence of a negative regulatory signal that originates from an unrearranged $Ig\kappa$ locus and suppresses $Ig\lambda$ gene rearrangements (14, 15). Based on evidence that Ig gene rearrangement correlates and is possibly mechanistically connected with transcriptional accessibility of the target genes (19, 20), we sought to test this hypothesis through the analysis of the developmental expression pattern of a $VJ\lambda 1$ rearrangement inserted into its physiological position in the $Ig\lambda$ locus. The results of this analysis were clear-cut: expression of the inserted $VJ\lambda 1$ element was developmentally controlled and coincided with the developmental stage at which $V\kappa$ and $J\kappa$ gene segments are rearranged and functional $VJ\kappa$ -rearrangements are expressed (Fig. 3); and $VJ\lambda 1$ expression did not depend upon $Ig\kappa$ rearrangement (Fig. 4). Thus, at the level of expression of a gene rearrangement in the $Ig\lambda$ locus there is no evidence

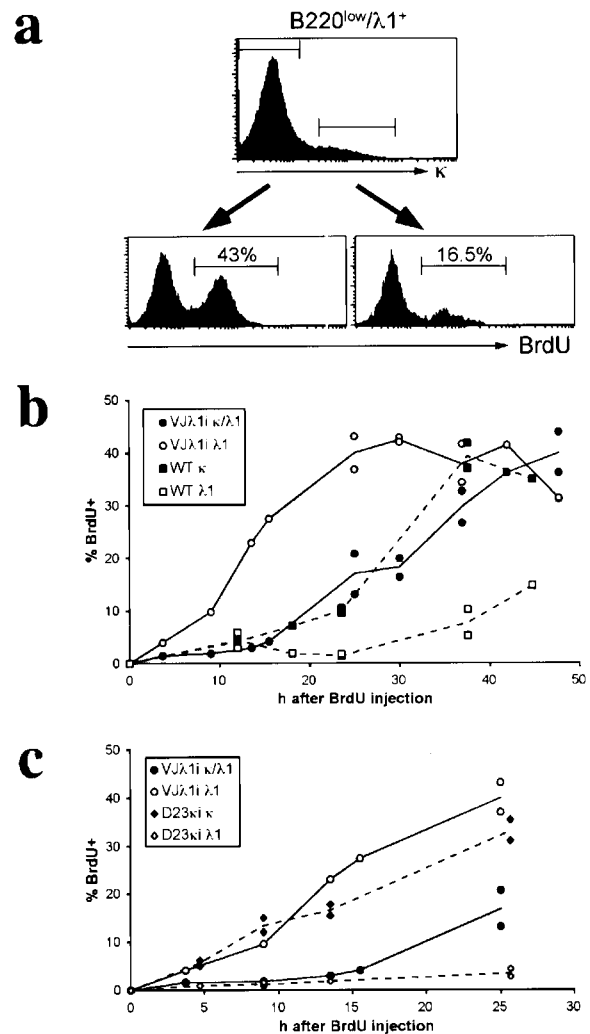


Figure 5. BrdU incorporation in immature B cells of $VJ\lambda 1$ mice, $D23\kappa i$, and WT mice. Mice were intraperitoneally injected with BrdU and analyzed at different time points thereafter. Immature B cells were defined as $B220^{low}/\lambda 1^+$ and were subdivided according to κ expression. Panel a shows representative histograms from a mouse analyzed 30 h after BrdU injection. The percentage of BrdU⁺ cells in κ^- (bottom left panel) and κ^+ (bottom right panel) B cell subpopulations was determined as shown. (b and c) The percentage of BrdU⁺ immature B cells is plotted against the time after BrdU injection. $VJ\lambda 1$ mice (circles) were compared to either WT mice (squares) (b) or $D23\kappa i$ mice (diamonds) (c). Open symbols correspond to $\lambda 1^+$ immature B cells, closed symbols depict κ^+ immature B cells in WT and $D23\kappa i$ mice or $\kappa/\lambda 1^+$ immature B cells in $VJ\lambda 1$ mice. Each pair of symbols represents one animal.

for sequential accessibility of $Ig\kappa$ and $Ig\lambda$ over developmental time and a signal originating from a nonrearranged $Ig\kappa$ locus that interferes with the transcription of a rearranged $Ig\lambda$ locus can be excluded. This in turn restricts a possible developmental program of successive accessibility of $Ig\kappa$ and $Ig\lambda$ loci to the control of the initiation of $Ig\lambda$ gene rearrangements. Such a developmental program would further have to assume differential accessibility of rearranged versus nonrearranged $Ig\lambda$ loci during B cell development, which could be due to juxtaposition of promoter

and enhancer elements and/or the loss of cis-regulatory elements upon V λ -J λ recombination. Although DNase-hypersensitive sites have not been discovered in the intervening DNA (29), such elements could nevertheless exist.

On the other hand, our results are in good agreement with models ascribing the predominance of Ig κ over Ig λ rearrangements to a competition between the two loci, in which the Ig κ locus is at an advantage. This model is also consistent with our analysis of λ 5-deficient mice which suggests that both Ig κ and Ig λ rearrangements can occur in a small fraction of pro-B cells, yet with a lower efficiency for the latter (Table I).

Inefficient Ig λ rearrangements could be due to differences in the quality of Ig κ - and Ig λ -specific RSSs with respect to their affinity for the RAG1/2 complex. Indeed, it has been demonstrated earlier that a representative pair of Ig κ RSSs rearranges more efficiently than a pair of V λ 1 and J λ 1 RSSs in vitro (38). Moreover, RSSs appear to be an important factor in determining the order of V(D)J recombination in the TCR β locus (39, 40). To test whether Ig λ rearrangements are intrinsically inefficient, Ig κ -specific RSSs will have to be analyzed in the context of the mouse Ig λ locus.

Alternatively, competition for trans-activating factors might be responsible for different rates of germline transcription at Ig κ and Ig λ . Interestingly, the rate of germline transcription has recently been shown to directly influence rearrangement in both the Ig κ (19) and the Ig λ locus (20). To address potential differences in the efficiency of Ig λ and Ig κ germline transcription, it will be interesting to analyze how Ig κ -specific enhancer elements might influence Ig λ germline transcription and rearrangement when inserted into the Ig λ locus.

Receptor Editing in VJ λ 1i Mice. While most pre-B cells do not undergo Ig κ rearrangements in VJ λ 1i mice, 30% of mature B cells express both a λ 1 and a κ light chain on the surface (Fig. 2 A). In VJ κ i mice, it has been shown that, depending on the inserted light chain, between 20 and 30% of B cells change their antigen receptor by editing, thereby generating B cells that express an endogenous VJ κ gene (37). This process is thought to be a means of revising the specificity of an otherwise self-reactive antigen receptor. The fact that we readily detect small pre-B cells that express a κ light chain in VJ λ 1i mice (Fig. 3) is consistent with the idea of secondary rearrangements in a fraction of λ 1⁺ pre-B cells. We further demonstrate that κ / λ 1⁺ pre-B cells take ~12 h longer to exit the pre-B cell compartment than their λ 1⁺ counterparts (Fig. 5 B). A similar observation has been reported previously to be the consequence of receptor editing in pre-B cells (37). Moreover, two recent reports have proposed the generation of B cells with dual receptor specificity as a way to "dilute out" the signal strength of a single, self-reactive B cell receptor, thereby circumventing anergy or clonal deletion (41, 42). We thus propose that ~30% of λ 1⁺ B cells in VJ λ 1i mice have undergone receptor editing in order to reduce the surface density of a self-reactive IgH/Ig λ 1 pair. This fraction is comparable to the fraction of editing κ ⁺ B cells in WT mice (37). We extrapolate

from this result that a maximum of two thirds of the IgH repertoire generated in WT mice can be expressed in combination with λ 1 light chains.

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References

- Oettinger, M.A., D.G. Schatz, C. Gorka, and D. Baltimore. 1990. RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination. *Science*. 248:1517-1523.
- Schatz, D.G., M.A. Oettinger, and D. Baltimore. 1989. The V(D)J recombination activating gene, RAG-1. *Cell*. 59: 1035-1048.
- Bassing, C.H., W. Swat, and F.W. Alt. 2002. The mechanism and regulation of chromosomal V(D)J recombination. *Cell*. 109(Suppl):S45-S55.
- Rajewsky, K. 1996. Clonal selection and learning in the antibody system. *Nature*. 381:751-758.
- Tiegs, S.L., D.M. Russell, and D. Nemazee. 1993. Receptor editing in self-reactive bone marrow B cells. *J. Exp. Med.* 177:1009-1020.
- Gay, D., T. Saunders, S. Camper, and M. Weigert. 1993. Receptor editing: an approach by autoreactive B cells to escape tolerance. *J. Exp. Med.* 177:999-1008.
- Hieter, P.A., S.J. Korsmeyer, T.A. Waldmann, and P. Leder. 1981. Human immunoglobulin kappa light-chain genes are deleted or rearranged in lambda-producing B cells. *Nature*. 290:368-372.
- Brauninger, A., T. Goossens, K. Rajewsky, and R. Kuppers. 2001. Regulation of immunoglobulin light chain gene rearrangements during early B cell development in the human. *Eur. J. Immunol.* 31:3631-3637.
- Takeda, S., Y.R. Zou, H. Bluethmann, D. Kitamura, U. Muller, and K. Rajewsky. 1993. Deletion of the immunoglobulin kappa chain intron enhancer abolishes kappa chain gene rearrangement in cis but not lambda chain gene rearrangement in trans. *EMBO J.* 12:2329-2336.
- Yamagami, T., E. ten Boekel, J. Andersson, A. Rolink, and F. Melchers. 1999. Frequencies of multiple IgL chain gene rearrangements in single normal or kappaL chain-deficient B lineage cells. *Immunity*. 11:317-327.
- Durdik, J., M.W. Moore, and E. Selsing. 1984. Novel kappa light-chain gene rearrangements in mouse lambda light chain-producing B lymphocytes. *Nature*. 307:749-752.
- Siminovitch, K.A., A. Bakhshi, P. Goldman, and S.J. Korsmeyer. 1985. A uniform deleting element mediates the loss of kappa genes in human B cells. *Nature*. 316:260-262.
- Coleclough, C., R.P. Perry, K. Karjalainen, and M. Weigert. 1981. Aberrant rearrangements contribute significantly to the allelic exclusion of immunoglobulin gene expression. *Nature*. 290:372-378.

14. Zou, Y.R., S. Takeda, and K. Rajewsky. 1993. Gene targeting in the Ig kappa locus: efficient generation of lambda chain-expressing B cells, independent of gene rearrangements in Ig kappa. *EMBO J.* 12:811–820.
15. Chen, J., M. Trounstein, C. Kurahara, F. Young, C.C. Kuo, Y. Xu, J.F. Loring, F.W. Alt, and D. Huszar. 1993. B cell development in mice that lack one or both immunoglobulin kappa light chain genes. *EMBO J.* 12:821–830.
16. Yancopoulos, G.D., and F.W. Alt. 1985. Developmentally controlled and tissue-specific expression of unrearranged VH gene segments. *Cell.* 40:271–281.
17. Schlissel, M.S., L.M. Corcoran, and D. Baltimore. 1991. Virus-transformed pre-B cells show ordered activation but not inactivation of immunoglobulin gene rearrangement and transcription. *J. Exp. Med.* 173:711–720.
18. Corcoran, A.E., A. Riddell, D. Krooshoop, and A.R. Venkataraman. 1998. Impaired immunoglobulin gene rearrangement in mice lacking the IL-7 receptor. *Nature.* 391:904–907.
19. Casellas, R., M. Jankovic, G. Meyer, A. Gazumyan, Y. Luo, R.G. Roeder, and M.C. Nussenzweig. 2002. OcaB is required for normal transcription and V(D)J recombination of a subset of immunoglobulin kappa genes. *Cell.* 110:575–585.
20. Sun, T., and U. Storb. 2001. Insertion of phosphoglycerine kinase (PGK)-neo 5' of Jlambda1 dramatically enhances VJlambda1 rearrangement. *J. Exp. Med.* 193:699–712.
21. Engel, H., A. Rolink, and S. Weiss. 1999. B cells are programmed to activate kappa and lambda for rearrangement at consecutive developmental stages. *Eur. J. Immunol.* 29:2167–2176.
22. Miller, J., S. Ogden, M. McMullen, H. Andres, and U. Storb. 1988. The order and orientation of mouse lambda-genes explain lambda-rearrangement patterns. *J. Immunol.* 141:2497–2502.
23. Bunting, M., K.E. Bernstein, J.M. Greer, M.R. Capecchi, and K.R. Thomas. 1999. Targeting genes for self-excision in the germ line. *Genes Dev.* 13:1524–1528.
24. Thomas, K.R., and M.R. Capecchi. 1986. Introduction of homologous DNA sequences into mammalian cells induces mutations in the cognate gene. *Nature.* 324:34–38.
25. Kontgen, F., G. Suss, C. Stewart, M. Steinmetz, and H. Bluethmann. 1993. Targeted disruption of the MHC class II Aa gene in C57BL/6 mice. *Int. Immunol.* 5:957–964.
26. Pasparakis, M., and G. Kollias. 1995. Production of cytokine transgenic and knockout mice. In *Cytokines: A Practical Approach*. F.R. Balkwill, editor. Oxford University Press, Oxford. 297–324.
27. Pelanda, R., S. Schaal, R.M. Torres, and K. Rajewsky. 1996. A prematurely expressed Ig(kappa) transgene, but not V(kappa)J(kappa) gene segment targeted into the Ig(kappa) locus, can rescue B cell development in lambda5-deficient mice. *Immunity.* 5:229–239.
28. Kuhn, R., K. Rajewsky, and W. Muller. 1991. Generation and analysis of interleukin-4 deficient mice. *Science.* 254:707–710.
29. Hagman, J., C.M. Rudin, D. Haasch, D. Chaplin, and U. Storb. 1990. A novel enhancer in the immunoglobulin lambda locus is duplicated and functionally independent of NF kappa B. *Genes Dev.* 4:978–992.
30. Sanchez, P., D. Rueff-Juy, P. Boudinot, S. Hachemi-Rachedi, and P.A. Cazenave. 1996. The lambda B cell repertoire of kappa-deficient mice. *Int. Rev. Immunol.* 13:357–368.
31. Novobrantseva, T.I. 2000. B Cells Expressing a Natural Autoreactive Immunoglobulin Receptor In Vivo. Mathematisch-Naturwissenschaftliche Fakultät. University of Cologne, Cologne, Germany.
32. Hochedlinger, K., and R. Jaenisch. 2002. Monoclonal mice generated by nuclear transfer from mature B and T donor cells. *Nature.* 415:1035–1038.
33. Novobrantseva, T.I., V.M. Martin, R. Pelanda, W. Muller, K. Rajewsky, and A. Ehlich. 1999. Rearrangement and expression of immunoglobulin light chain genes can precede heavy chain expression during normal B cell development in mice. *J. Exp. Med.* 189:75–88.
34. Kitamura, D., A. Kudo, S. Schaal, W. Muller, F. Melchers, and K. Rajewsky. 1992. A critical role of lambda 5 protein in B cell development. *Cell.* 69:823–831.
35. Pelanda, R., S. Schwers, E. Sonoda, R.M. Torres, D. Nemazee, and K. Rajewsky. 1997. Receptor editing in a transgenic mouse model: site, efficiency, and role in B cell tolerance and antibody diversification. *Immunity.* 7:765–775.
36. Ehlich, A., S. Schaal, H. Gu, D. Kitamura, W. Muller, and K. Rajewsky. 1993. Immunoglobulin heavy and light chain genes rearrange independently at early stages of B cell development. *Cell.* 72:695–704.
37. Casellas, R., T.A. Shih, M. Kleinewietfeld, J. Rakonjac, D. Nemazee, K. Rajewsky, and M.C. Nussenzweig. 2001. Contribution of receptor editing to the antibody repertoire. *Science.* 291:1541–1544.
38. Ramsden, D.A., and G.E. Wu. 1991. Mouse kappa light-chain recombination signal sequences mediate recombination more frequently than do those of lambda light chain. *Proc. Natl. Acad. Sci. USA.* 88:10721–10725.
39. Wu, C., C.H. Bassing, D. Jung, B.B. Woodman, D. Foy, and F.W. Alt. 2003. Dramatically increased rearrangement and peripheral representation of vbeta14 driven by the 3'beta1 recombination signal sequence. *Immunity.* 18:75–85.
40. Bassing, C.H., F.W. Alt, M.M. Hughes, M. D'Auteuil, T.D. Wehrly, B.B. Woodman, F. Gartner, J.M. White, L. Davidson, and B.P. Sleckman. 2000. Recombination signal sequences restrict chromosomal V(D)J recombination beyond the 12/23 rule. *Nature.* 405:583–586.
41. Li, Y., H. Li, and M. Weigert. 2002. Autoreactive B cells in the marginal zone that express dual receptors. *J. Exp. Med.* 195:181–188.
42. Kenny, J.J., L.J. Rezanka, A. Lustig, R.T. Fischer, J. Yoder, S. Marshall, and D.L. Longo. 2000. Autoreactive B cells escape clonal deletion by expressing multiple antigen receptors. *J. Immunol.* 164:4111–4119.