The Journal of Experimental Medicine

Expression of a Targeted λ1 Light Chain Gene Is Developmentally Regulated and Independent of Igκ Rearrangements

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Abstract

Immunoglobulin light chain (IgL) rearrangements occur more frequently at Igκ than at Igλ. Previous results suggested that the unrearranged Igκ locus negatively regulates Igλ transcription and/or rearrangement. Here, we demonstrate that expression of a VJλ1-joint inserted into its physiological position in the Igλ locus is independent of Igκ rearrangements. Expression of the inserted VJλ1 gene segment is developmentally controlled like that of a VJκ-joint inserted into the Igκ locus and furthermore coincides developmentally with the occurrence of Igκ rearrangements in wild-type mice. We conclude that developmentally controlled transcription of a gene rearrangement in the Igλ locus occurs in the presence of an unrearranged Igκ locus and is therefore not negatively regulated by the latter. Our data also indicate light chain editing in ~30% of λ1 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 Igλ and two Igκ 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 Igκ are 15–20 times more frequent than those expressing Igλ. 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 Igλ locus in germline configuration, while the vast majority of λ^+ B cells has inactivated its Igκ loci by either nonfunctional VκJκ-joint or deletion of the κ constant region (Cκ) gene (7–10). Cκ deletion is the consequence of a recombination event that occurs between an RSS located either in the Jκ-Cκ intron or at the 3′ end of a nonrearranged Vκ 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 Igλ locus as shown by a small fraction of κ^+ B cells that carry nonfunctional Igλ 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 Igκ being accessible for rearrangements before Igλ. The stochastic model predicts that both IgL loci are accessible at the same time with the probability of rearrangements being higher for Igκ than for Igλ. More recently, the analyses of several mouse mutants with impaired Igκ rearrangement and/or expression dem-

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

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/2003/05/1165/8 $8.00
Volume 197, Number 9, May 5, 2003 1165–1172
http://www.jem.org/cgi/doi/10.1084/jem.20030402

onstrated that inactivation of the Igκ locus causes a 10-fold increase in λ^+ B cells. Inactivation of Igκ was achieved by replacing either the intronic κ enhancer (iEκ; reference 9) or the Cκ (14) or Jκ and Cκ (15) gene segments by a *neo*^{R} gene. While the former manipulation causes complete silencing of Vκ→Jκ rearrangements, the two latter mutations exert only a mild effect on Igκ 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 Igκ locus that would actively suppress λ rearrangements and be artificially disrupted in the mutant alleles. In WT mice, inactivation of such elements upon Igκ rearrangement was suggested to increase the probability of Igλ 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 Igκ locus, the level of Vκ germline transcription needs to exceed a certain threshold before a Vκ 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 Jλ1 segment led to a substantial increase in both Jλ1 germline transcription and Vλ1→Jλ1 rearrangement (20).

Based on the coincidence of germline transcription and Ig gene rearrangement, initiation of IgL germline transcription has been analyzed to address IgL locus accessibility. The detection of sterile Jκ but not Jλ transcripts in a minor fraction of proliferating, early pre-B cells was interpreted as ordered initiation of IgL 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 Igκ is generally rearranged before Igλ and that this phenomenon may be controlled by an Igκ-derived negative regulatory signal that interferes with Igλ rearrangement. As mentioned above, this signal would be expected to also interfere with the transcriptional accessibility of the Igλ locus. In this study, we attempted to obtain evidence for such regulation by inserting a prerearranged VJλ1 gene into the Igλ locus and analyzing whether its expression depends on Igκ rearrangement.

Materials and Methods

Generation of VJλ1i Mice. A targeting vector was designed to replace 18 kb of genomic DNA containing Vλ1 and Jλ1 by a prerearranged VJλ1 gene. A 2.8 kb short arm of homology (SAH) located 5′ of Jλ1 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 Vλ1; 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 prerearranged VJλ1 gene, was excised from pA8–6λ (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 VJλ1 thereby destroying an AvaII restriction site. A 5.4 kb AccI/EcoRI fragment located 3′ of Jλ1 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 Jλ1. 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-λ1 (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 VJλ1i/+ 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 λ1. κ^+, $\lambda1^+$, and $\kappa/\lambda1^+$ 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. λ1 message was amplified using the primer pair VJλ1-int (TTG TGA CTC AGG AAT CTG CA) and Cλ1 (CTC GGA TCC TTC AGA GGA AGG TGG AAA CA), κ message was amplified using the degenerate Vκ primer Msκ (GAT ATT GTG ATG ACC CAG TCT) and CκE (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 Igκ Rearrangements. Splenocytes of WT (C57BL/6) and VJλ1i/+ 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 Vκ→Jκ rearrangements, DNA was digested with EcoICRI and hybridized to 5′Jκ-probe (27) giving rise to a 4.5 kb κ-germline fragment. Depending on the orientation of the Vκ segment, Igκ-rearrangements lead to deletion or inversion of the DNA between Vκ and Jκ. 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) thymocyte signal was defined as 100%, the fraction of unrearranged Igκ 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, D23κi/+, LN1κ/+, VJλ1i/+, 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 λ1, fixed, DNase treated, and subsequently stained for BrdU incorporation.

Results

Targeted Insertion of a Prerearranged VJλ1 Gene into the Igλ Locus. To generate Igλ-transgenic mice where the expression of the transgenic λ light chain is regulated by its physiological control elements, we targeted a prerearranged VJλ1 gene into the Igλ locus of murine embryonic stem (ES) cells. The targeting vector was designed such that the VJλ1 gene replaces 18 kb of genomic DNA between Vλ1 and Jλ1. This region contains the JCλ3 cluster but no apparent cis-regulatory elements according to DNase hypersensitivity assays in various cell lines (29) (Fig. 1). The emerging mutant Igλ allele (referred to as VJλ1i) mimics the WT Igλ allele after Vλ1→Jλ1 rearrangement. We chose the VJλ1 joint, as ~60% of λ^+ B cells in WT mice express a λ1 light chain (30).

All Mature B Cells of VJλ1i Mice Express the Inserted VJλ1 Gene. The inserted λ1 light chain is expressed in all mature B cells of VJλ1i mice (Fig. 2) and the distribution of peripheral B cell subsets appears normal (unpublished data). The total number of splenic B cells in VJλ1i mice is re-

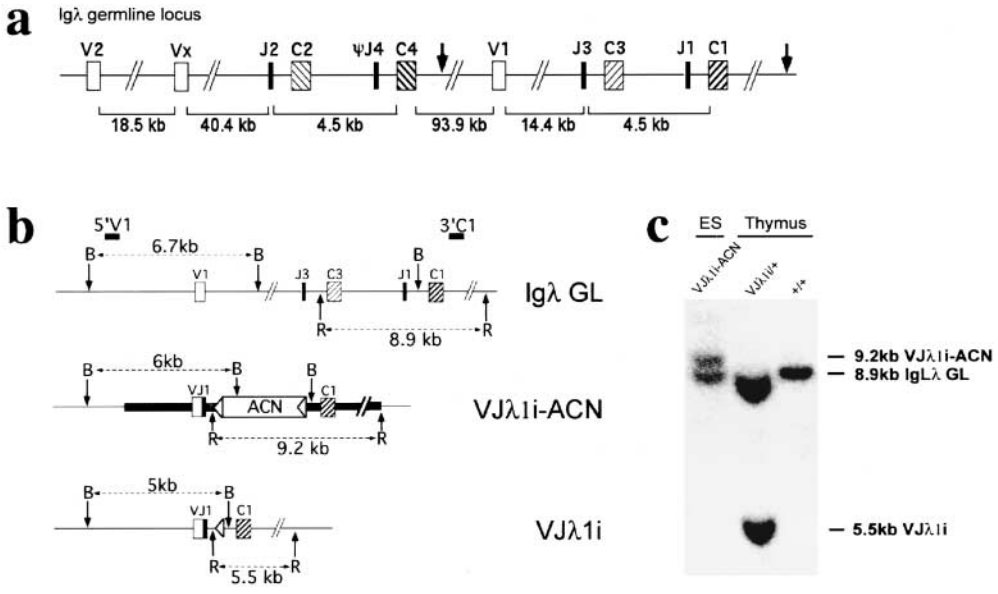

Figure 1. Targeted insertion of a prerearranged VJλ1 gene segment into the germline of the Igλ locus. (a) Overview of the genomic organization of the Igλ germline locus (reference 37). The Igλ locus is composed of three functional Jλ-Cλ clusters (JCλ1–3) and one pseudo Jλ-Cλ cluster (JCλ4). Three Vλ gene segments have been identified; Vλ1, Vλ2, and Vλx. 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 Igλ germline (Igλ GL) locus, the mutated allele after homologous recombination (VJλ1i-ACN) and the mutated allele after Cre-loxP mediated deletion of the neo^R gene containing ACN cassette (VJλ1i). Arms of homology are shown in bold in VJλ1i-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 (VJλ1i-ACN), a heterozygous mouse mutant (VJλ1i/+), 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 VJκ 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 VJλ1i mice express λ1 exclusively. However, a substantial fraction of B cells (~30%) express both λ1 and κ on the surface and 6% appear to have lost surface expression of λ1 (Fig. 2 A). Due to the organization of the Igλ locus, the VJλ1 gene cannot be deleted by "secondary" Vλ→Jλ rearrangements (see Fig. 1 A). B cells that lack surface λ1expression may represent naive B cells with inefficient heavy/λ1 light chain pairing or memory B cells that have inactivated the VJλ1 coding region through somatic hypermutation. A semiquantitative RT-PCR analysis of sorted $\lambda1^+$, $\kappa/\lambda1^+$, and κ^+ splenic B cells from VJλ1i mice confirms that the inserted gene segment is transcribed at similar levels in both surface λ1-positive and -negative subpopulations (Fig. 2 B).

Expression of a Prerearranged λ1 Light Chain Is Developmentally Controlled Like That of a Prerearranged κ Light Chain. To assess whether the prerearranged VJλ1 gene is expressed in a developmentally regulated fashion, we analyzed intracellular light chain expression in pro- and pre-B cells of VJλ1i 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. VJλ1i mice show a developmentally regulated λ1 expression pattern with three- to fourfold less λ^+ pro- than pre-B cells (Fig. 3). A comparable result was observed for κ light chain expression in mice that carry a prerearranged VJκ gene and either retain (in the case of the D23κi allele [31]) or lack (in the case of the LN1κ allele [32]) the genomic sequence between Vκ and Jκ (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 VJκi mice or λ^+ cells in VJλ1i 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 λ1 light chain gene coincides developmentally with the initiation of Igκ rearrangements in WT mice.

Igλ Rearrangement Can Occur in the Absence of Pre-B Cell Receptor Signaling. As shown in Fig. 3, VJλ1i and VJκi mice yield ~20% pro-B cells that express the inserted light chain. It has been observed previously that Igκ 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 Igλ locus. Due to their low frequency, we were unable to detect Igλ rearrangements in pro B cells of WT mice (Fig. 3

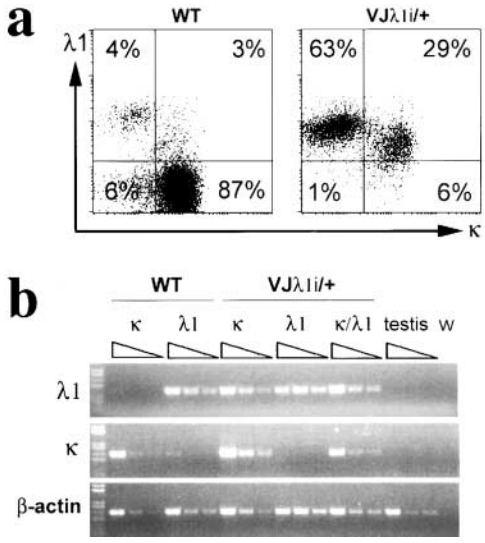

Figure 2. All mature B cells of VJλ1i/+ mice express the inserted λ1 light chain. (a) Representative staining for κ and λ1 on $CD19^+$ splenocytes from VJλ1i/+ and WT mice. Numbers indicate the percentage of cells per quadrant. (b) Semiquantitative RT-PCR analysis of sorted κ^+, $\lambda1^+$, and $\kappa/\lambda1^+$ splenic B cells from WT mice and VJλ1i/+ mice. Testis RNA from WT mice served as negative control. 1:5 serially diluted cDNA was analyzed for reverse-transcribed λ1 and κ light chain message by PCR. A β-actin PCR was performed as internal control.

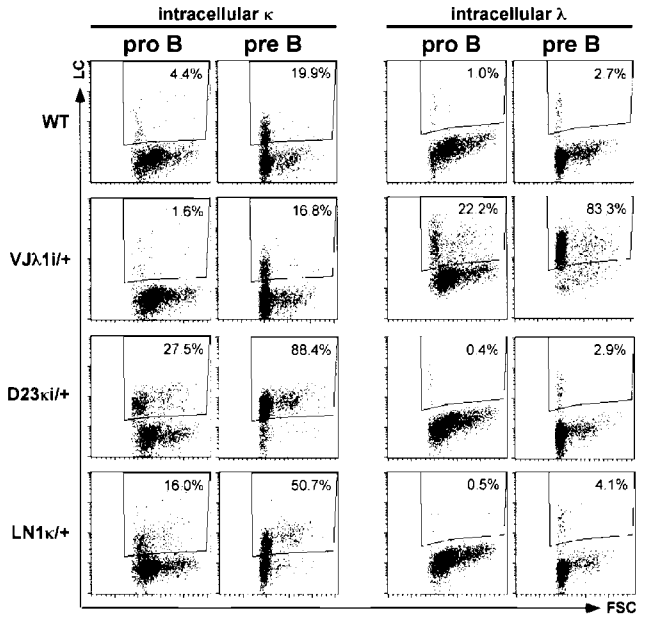

Figure 3. Intracellular light chain expression in $CD25^+$ pro-B cells and $CD25^-$ pre-B cells of light chain insertion and WT mice. Bone marrow lymphocytes were stained for surface expression of B220, IgM, and CD25 and for intracellular light chain expression. Flow cytometric analyses of intracellular κ and λ expression in $CD25^-$, $B220^+$, IgM^- pro-B cells and $CD25^+$, $B220^+$, IgM^- pre-B cells are shown for WT, VJλ1i, D23κi, and LN1κ 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 I. *Cellularity of Bone Marrow and Splenic B Cell Compartments from WT, λ5$^{-/-}$, and λ5$^{-/-}$ Cκ$^{-/-}$ Mice*

	Bone marrow					Spleen			
	Total × 10^7	Lymphocytes × 10^6	IgM$^+$ × 10^6	Pre-B × 10^6	Pro-B × 10^6	Total × 10^7	Lymphocytes × 10^7	IgM$^+$ B220$^+$ × 10^7	IgM$^+$ IgD$^+$ × 10^7
WT	2.3	6.9	1.5	1.4	0.6	6.5	5.2	3.1	2.3
λ5$^{-/-}$	2.1	3.8	0.1	0.1	0.3	4.4	3.1	0.7	0.3
λ5$^{-/-}$ Cκ$^{-/-}$	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 Cκ (14) and the surrogate light chain component λ5 (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 Igλ rearrangements that occur independently of pre B cell receptor signaling. The fact that λ5$^{-/-}$ Cκ$^{-/-}$ double mutants are able to generate B cells supports the idea that the Igλ 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 λ5$^{-/-}$ single mutants (Table I), suggesting that, also in the absence of λ5, Igκ rearrangements occur more frequently than Igλ rearrangements.

The Majority of Mature B Cells in VJλ1i Mice Carries the Igκ Locus in Germline Configuration. It has been shown previously, that a prerearranged VJκ gene efficiently drives pre- to immature B cell differentiation without allowing Igκ rearrangements to occur (35). Simultaneous transcriptional accessibility of Igκ and Igλ would imply that the same is true for a prerearranged λ1 light chain gene. To determine the extent of recombination at the Igκ locus in B cells from VJλ1i mice, κ/λ1$^+$ and λ1$^+$ B cells were sorted and analyzed for Vκ→Jκ 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 Jκ 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 λ1$^+$ B cells of WT mice has rearranged both Igκ alleles (9, 13). In contrast, more than 75% of λ1$^+$ B cells in VJλ1i mice retain the Igκ locus in germline configuration (Fig. 4 B). RS recombination is not detectable above background in VJλ1i mice. In λ1$^+$ B cells of WT mice, on the other hand, more than 60% of Igκ alleles have undergone RS recombination (Fig. 4 C), which is in accordance with published results (13). Together, these data imply that, in VJλ1i mice, the majority of pre-B cells express the inserted λ1 light chain gene and subsequently enter the immature B cell compartment before endogenous Igκ rearrangements have occurred.

Coexpression of κ and λ1 Light Chains in VJλ1i Mice Is Likely to be the Consequence of Receptor Editing. The appearance of κ/λ1$^+$ mature B cells in VJλ1i mice indicates that a fraction of λ1$^+$ B cells has undergone Igκ rearrangements and thus escaped isotype exclusion. This could be explained either by Igκ rearrangements occurring in a subpopulation of pro-B cells (see above, and references 33 and

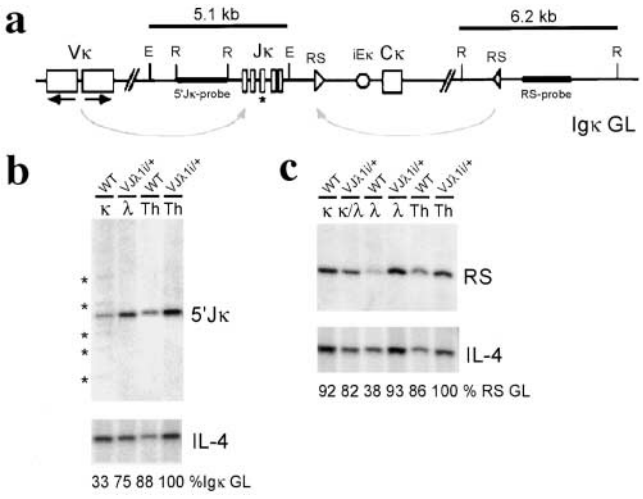

Figure 4. Only low levels of Igκ gene rearrangements are detected in λ1$^+$ B cells of VJλ1i/+ mice. (a) Partial restriction endonuclease map of the germline Igκ locus (not drawn to scale). V, J, and C gene segments, the 5′Jκ probe and the RS-probe are shown as boxes, the asterisk indicates a pseudo-J segment. The internal κ enhancer (iEκ) 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′Jκ and RS, respectively. Grey arrows visualize Vκ→Jκ and RS recombination events. E, EcoIRCI; R, EcoRI. (b and c) Genomic DNA of sorted κ$^+$, κ/λ1$^+$, and λ1$^+$ CD19$^+$ B cells from VJλ1i/+ and WT mice was analyzed by Southern blotting. To detect Vκ→Jκ rearrangements, DNA was digested with EcoIRCI and hybridized to 5′Jκ 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 Igκ germline (Igκ 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 Igκ rearrangements in a fraction of $\lambda1^+$ pre-B cells. In VJκi 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 prerearranged light chain gene (37). We thus compared the kinetics of pre-B to immature B cell transition for $\lambda1^+$ and $\kappa/\lambda1^+$ B cells from VJλ1i mice. WT pre-B cells were analyzed in parallel as a control for cells that undergo IgL 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 VJλ1i mice. Two conclusions can be drawn from this analysis. First, in VJλ1i mice, $\kappa/\lambda1^+$ B cells exit the pre-B cell compartment ~12 h later than B cells that express only λ1 and thus appear to have undergone secondary Igκ rearrangements. Second, WT B cells exit the pre B cell compartment with kinetics similar to $\kappa/\lambda1^+$ B cells from VJλ1i mice. The delay with respect to B cells that carry an inserted light chain is likely to reflect the kinetics of Igκ 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 VJκi mice (37). If expression of prerearranged κ and λ1 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 VJκi or Vλ1i mice (Fig. 5 C).

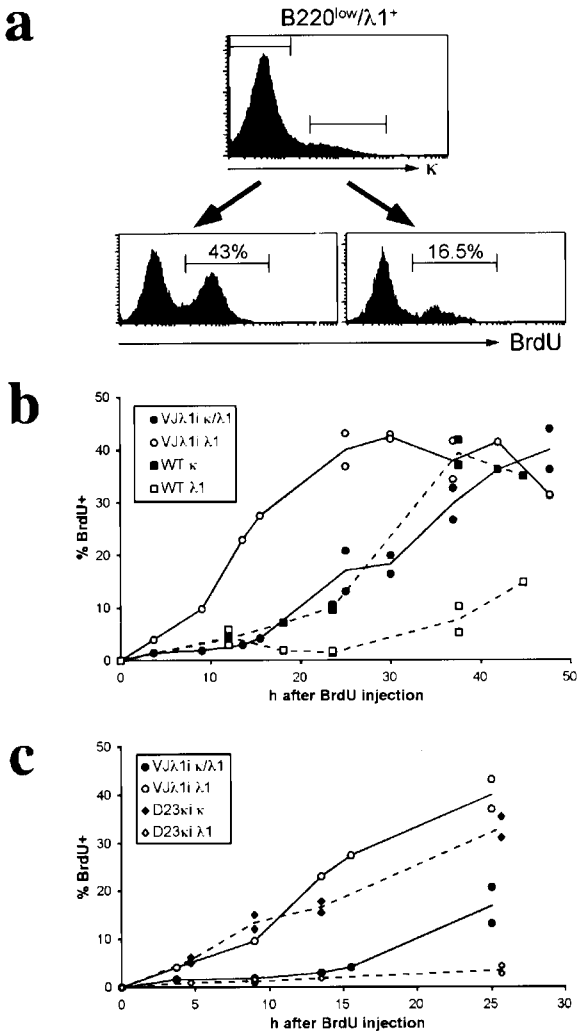

Figure 5. BrdU incorporation in immature B cells of VJλ1i/+, D23κi/+, and WT mice. Mice were intraperitoneally injected with BrdU and analyzed at different time points thereafter. Immature B cells were defined as $B220^{low}/\lambda1^+$ and were subdivided according to κ expression. Panel a shows representative 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. VJλ1i/+ mice (circles) were compared to either WT mice (squares) (b) or D23κi/+ mice (diamonds) (c). Open symbols correspond to $\lambda1^+$ immature B cells, closed symbols depict κ^+ immature B cells in WT and D23κi/+ mice or $\kappa/\lambda1+$ immature B cells in VJλ1i/+ mice. Each pair of symbols represents one animal.

Discussion

Transcription of an Inserted VJλ1 Element Is Developmentally Controlled like that of VJκ Rearrangements and Is Independent of the Latter. The predominance of Igκ over Igλ rearrangements in mice and humans has been subject of extensive research over the last decades. There is suggestive evidence that in B cell development, Igλ may become accessible for V(D)J recombination later than Igκ (7, 13, 21). More specifically, the analysis of targeted mutations in the Igκ locus suggested the existence of a negative regulatory signal that originates from an unrearranged Igκ locus and suppresses Igλ 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 VJλ1 rearrangement inserted into its physiological position in the Igλ locus. The results of this analysis were clear-cut: expression of the inserted VJλ1 element was developmentally controlled and coincided with the developmental stage at which Vκ and Jκ gene segments are rearranged and functional VJκ-rearrangements are expressed (Fig. 3); and VJλ1 expression did not depend upon Igκ rearrangement (Fig. 4). Thus, at the level of expression of a gene rearrangement in the Igλ locus there is no evidence for sequential accessibility of Igκ and Igλ over developmental time and a signal originating from a nonrearranged Igκ locus that interferes with the transcription of a rearranged Igλ locus can be excluded. This in turn restricts a possible developmental program of successive accessibility of Igκ and Igλ loci to the control of the initiation of IgL gene rearrangements. Such a developmental program would further have to assume differential accessibility of rearranged versus nonrearranged Igλ loci during B cell development, which could be due to juxtaposition of promotor

and enhancer elements and/or the loss of cis-regulatory elements upon Vλ-Jλ 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 Igκ over Igλ rearrangements to a competition between the two loci, in which the Igκ locus is at an advantage. This model is also consistent with our analysis of λ5-deficient mice which suggests that both Igκ and Igλ rearrangements can occur in a small fraction of pro-B cells, yet with a lower efficiency for the latter (Table I).

Inefficient Igλ rearrangements could be due to differences in the quality of Igκ- and Igλ-specific RSSs with respect to their affinity for the RAG1/2 complex. Indeed, it has been demonstrated earlier that a representative pair of Igκ RSSs rearranges more efficiently than a pair of Vλ1 and Jλ1 RSSs in vitro (38). Moreover, RSSs appear to be an important factor in determining the order of V(D)J recombination in the TCRβ locus (39, 40). To test whether Igλ rearrangements are intrinsically inefficient, Igκ-specific RSSs will have to be analyzed in the context of the mouse Igλ locus.

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

Receptor Editing in VJλ1i Mice. While most pre-B cells do not undergo Igκ rearrangements in VJλ1i mice, 30% of mature B cells express both a λ1 and a κ light chain on the surface (Fig. 2 A). In VJκi 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 VJκ 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 VJλ1i mice (Fig. 3) is consistent with the idea of secondary rearrangements in a fraction of $\lambda1^+$ pre-B cells. We further demonstrate that $\kappa/\lambda1^+$ pre-B cells take ~12 h longer to exit the pre-B cell compartment than their $\lambda1^+$ 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 $\lambda1^+$ B cells in VJλ1i mice have undergone receptor editing in order to reduce the surface density of a self-reactive IgH/Igλ1 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 λ1 light chains.

We thank F. Alt, C. Bassing, and M. Kraus for critical reading of the manuscript; C. Goettlinger and T. Schneider for cell sorting; B. Hampel, A. Egert, V. Dreier, D. Gerlich, A. Roth, and C. Uthoff-Hachenberg for technical help; S. Casola and M. Maruyaina for helpful discussion; U. Storb for providing Igλ cosmid DNA; S. Weiss for L22.18.2 mAb.

This work was supported by grants from the Volkswagen Stiftung and the National Cancer Institute (POI CA92625-0).

Submitted: 13 March 2003
Revised: 13 March 2003
Accepted: 23 March 2003

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