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

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Abstract

Immunoglobulin light chain (IgL) rearrangements occur more frequently at Iγκ than at Igλ. Previous results suggested that the unrearranged Iγκ locus negatively regulates Igλ transcription and/or rearrangement. Here, we demonstrate that expression of a VJλ1-joint inserted into its physiological position in the Iγκ locus is independent of Igλ 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 Igκ 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 Igκ and two Igλ 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 Vλκ-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 Igκ locus as shown by a small fraction of κ+ B cells that carry nonfunctional Igκ 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-
shown 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 (δκκ; reference 9) or the Ck (14) or Jκ and Cκ (15) gene segments by a neo<sup>k</sup> gene. While the former manipulation causes complete silencing of Vκ→Jκ rearrangements, the 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 IgA 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, Nussen-zweig 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)-promoter driven neo<sup>k</sup> gene 5′ of the Jα1 segment led to a substantial increase in both Jα1 germline transcription and Vα1→Jα1 rearrangement (20).

Based on the coincidence of germline transcription and Ig gene rearrangement, initiation of Igλ germline transcription has been analyzed to address Igλ locus accessibility. The detection of sterile Jκ but not Ja transcripts in a minor fraction of proliferating, early pre-B cells was interpreted as ordered initiation of Igλ 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 Igα rearrangement. As mentioned above, this signal would be expected to also interfere with the transcriptional accessibility of the Igκ locus. In this study, we attempted to obtain evidence for such regulation by inserting a prerearranged VJκ1 gene into the Igλ locus and analyzing whether its expression depends on Igκ rearrangement.

**Materials and Methods**

*Generation of VJκ1i Mice.* A targeting vector was designed to replace 18 kb of genomic DNA containing Vα1 and Jα1 by a prerearranged VJκ1 gene. A 2.8 kb short arm of homology (SAH) located 5′ of Jα1 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, phase clone KX39 (covering 15 kb upstream of Vα1; gift from Ursula Storb, University of Chicago, Chicago, IL) was PCR-amplified using the primer pair 5′/NXF1 (TGG CAG AGC GCC CGC TGCC 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 VJκ1 gene, was excised from pA8–6α (gift from Sigfried Weiss, German Research Centre for Biotechnology, Braunschweig, Germany) with EcoRI and Accl. PCR was used to introduce a silent GTC→GTG (codon 36) mutation in framework region 2 of VJκ1 thereby destroying an AvalI restriction site. A 5.4 kb AccI/EcoRI fragment located 3′ of Jα1 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<sup>k</sup> gene and the cre recombinase gene under the control of the sperm-specific ACE promoter (excised with EcoRI and Xhol from pACN [23]) was cloned into an intronic AccI site downstream of Jα1. The ACN-cassette is deleted in chimeras during spermatogenesis. To select against random integration, a thymidine kinase (TK) gene (excised with Xhol and SalI from pBl-S-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′ internal 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); primers for the 3′ internal probe (3′C1) were 5′Xbal/Xhol (CAG AAA TGC AAG CCC AGG AAG) and 3′Xbal/Xhol (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 (PC61.5) and anti-IgM (1B4B1) (from eBioscience); anti-B220 (RA3–6B2), anti-CD19 (1D3), anti-CD43 (S7), FcBlock (PC61.5) and anti-IgM (1B4B1) (from eBioscience); anti-κ (R33–18–10) mAb.

In intracellular stainings, cells were subsequently fixed in PBS/2% formaldehyde for 20 min at room temperature. Intracellular staining was performed with F(ab′)<sub>2</sub>-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 (PC61.5) and anti-IgM (1B4B1) (from eBioscience); 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 VJκ1i/+ mice were enriched for B lymphocytes using CD19 beads and the MACS technology (Miltenyi Biotec) according to the manufacturer’s protocol. The CD19+ splenocytes were cultured in 10% fetal bovine serum (FBS) RPMI medium with 10 ng/ml recombinant mouse IL-4 and 10 ng/ml recombinant mouse IL-7 for 4 days and RNA was isolated for subsequent RT-PCR analysis.

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fraction was subsequently stained for CD19, κ, and λ1. κ*, λ1*, and κ/λ1* B cells were sorted and total RNA was isolated using TRIzol (Invitrogen) following the manufacturer’s protocols. 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 VJa1-int (TTG TGA CTC AGG AAT CTG CA) and CA1 (CTC GGA TCC TTC AGA GGA AGG TGG AAA CA), κ message was amplified using the degenerate Vκ primer Msk (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-actin (CTT AAG GCC AAC CGT GAA GAG) and m-β-actinB (TCT TCA TGG TGC TAG GAG CCA). All primer pairs were intron-spanning.

Southern Blot Analysis of IgK Rearrangements. Splenocytes of WT (C57BL/6) and VJa1i/+ 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 recombinant, 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→κ rearrangements, DNA was digested with EcoRIK and hybridized to 5’κ→κ-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 EcoRIK digest. The signal intensities of each sample were quantified using a Storm 860 Molecular Dynamics scanner and ImageQuant software (Amerham 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 unarranged 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, D23ki/+ , LN1k/+ , VJa1i/+ , 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 VJa1 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 VJa1 gene into the Igλ locus of murine embryonic stem (ES) cells. The targeting vector was designed such that the VJa1 gene replaces 18 kb of genomic DNA between Vλ1 and Jκ1. This region contains the JCA3 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 VJa1i) mimics the WT Igλ allele after Vα1→Jκ1 rearrangement. We chose the VJa1 joint, as ~60% of λ+ B cells in WT mice express a λ light chain (30).

All Mature B Cells of VJa1i Mice Express the Inserted VJa1 Gene. The inserted λ1 light chain is expressed in all mature B cells of VJa1i mice (Fig. 2) and the distribution of peripheral B cell subsets appears normal (unpublished data). The total number of splenic B cells in VJa1i mice is re-

![Figure 1](image_url)

Figure 1. Targeted insertion of a prerearranged VJa1 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 Jα-CA clusters (JCA1-3) and one pseudo Jα-CA cluster (JCA4). Three Vα gene segments have been identified; Vα1, Vα2, and Vα3. Constant region (C) exons are depicted as hatched boxes, V segments as open boxes, J segments as closed boxes. Arrows indicate distances between selected exons in kb (reference 38). (b) Partial restriction endonuclease map of the Igκ germline (Igκ GL) locus, the mutated allele after homologous recombination (VJa1i-ACN) and the mutated allele after Cre-mediated deletion of the neoR gene containing ACN cassette (VJa1i). Arrows of homology are shown in bold in VJa1i-ACN. V1, 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 (VJa1i-ACN), a heterozygous mouse mutant (VJa1i/+), 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 \( \text{VJ}_{\kappa} \) gene and may reflect the restricted B cell repertoire in mice that predominantly express one particular light chain.

The majority of splenic B cells in \( \text{VJ}_{\lambda1} \) mice express \( \lambda_1 \) exclusively. However, a substantial fraction of B cells (\( \sim 30\% \)) express both \( \lambda_1 \) and \( \kappa \) on the surface and 6% appear to have lost surface expression of \( \lambda_1 \) (Fig. 2 A). Due to the organization of the Ig\( \lambda \) locus, the \( \text{VJ}_{\lambda1} \) gene cannot be deleted by "secondary" \( \text{V}\lambda\rightarrow\text{J}\lambda \) 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 \( \text{VJ}_{\lambda1} \) coding region through somatic hypermutation. A semiquantitative RT-PCR analysis of sorted \( \lambda_1^+ \), \( \kappa/\lambda_1^+ \), and \( \kappa^+ \) splenic B cells from \( \text{VJ}_{\lambda1} \) 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 \( \kappa \) Light Chain. To assess whether the prerearranged \( \text{VJ}_{\lambda1} \) gene is expressed in a developmentally regulated fashion, we analyzed intracellular light chain expression in pro- and pre-B cells of \( \text{VJ}_{\lambda1} \) mice. Both pro- and pre-B cells are Ig\( ^M^+ \), 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. \( \text{VJ}_{\lambda1} \) mice show a developmentally regulated \( \lambda_1 \) expression pattern with three- to fourfold less \( \lambda^+ \) pro- than pre-B cells (Fig. 3). A comparable result was observed for \( \kappa \) light chain expression in mice that carry a prerearranged \( \text{VJ}_{\kappa} \) gene and either retain (in the case of the D23\( \kappa \) allele [32]) or lack (in the case of the LN1\( \kappa \) allele [32]) the genomic sequence between \( \text{V} \) and \( \text{J} \) (Fig. 3). Similarly, in WT mice, \( \kappa^+ \) pro-B cells are four to five times less abundant than \( \kappa^+ \) pre-B cells. The fractions of both \( \kappa^+ \) pro- and pre-B cells are reduced by a factor of \( \sim 4.5 \) when compared with \( \kappa^+ \) cells in \( \text{VJ}_{\kappa} \) mice or \( \lambda^+ \) cells in \( \text{VJ}_{\lambda1} \) 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 \( \kappa \) and \( \lambda_1 \) light chain gene coincides developmentally with the initiation of Igk rearrangements in WT mice.

Figure 2. All mature B cells of \( \text{VJ}_{\lambda1} \) mice express the inserted \( \lambda_1 \) light chain. (a) Representative staining for \( \kappa \) and \( \lambda_1 \) on CD19\( ^+ \) splenocytes from \( \text{VJ}_{\lambda1/+/} \) and WT mice. Numbers indicate the percentage of cells per quadrant. (b) Semiquantitative RT-PCR analysis of sorted \( \kappa^+ \), \( \lambda_1^+ \), and \( \kappa/\lambda_1^+ \) splenic B cells from WT mice and \( \text{VJ}_{\lambda1/+/} \) mice. Tests RNA from WT mice served as negative control. 1.5 serially diluted cDNA was analyzed for reverse-transcribed \( \lambda_1 \) and \( \lambda \) light chain message by PCR. A \( \beta\)-actin PCR was performed as internal control.

Figure 3. Intracellular light chain expression in CD25\( ^+ \) pre-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 \( \kappa \) and \( \lambda \) expression in CD25\( ^+ \), B220\( ^+ \), IgM\( ^+ \) pro-B cells and CD25\( ^- \), B220\( ^- \), IgM\( ^+ \) pre-B cells are shown for WT, \( \text{VJ}_{\lambda1}, \text{D23}\kappa, \) and LN1\( \kappa \) 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.
ceptor signaling. The fact that arrangements that occur independently of pre B cell differentiation into immature B cells relies on Ig not form a functional pre-B cell receptor, hence pro-B cells from VJ/H9261 mice. In /H9261 combination is not detectable above background in VJ/H9260 gene and subsequently enter the immature B cell compartment before endogenous Igκ rearrangements have occurred.

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 Igκ 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 Igκ 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 VJ/λ1i 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 Igκ would imply that the same is true for a prerearranged A1 light chain gene. To determine the extent of recombination at the Igκ locus in B cells from VJ/λ1i mice, κ/λ1+ and λ1+ B cells were sorted and analyzed for Vk→Jκ rearrangements and RS recombination by Southern blotting. Thymocytes served as negative control. Individual samples were assayed for the retention of a germline EcoRI CI 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 VJ/λ1i mice retain the Igκ locus in germine configuration (Fig. 4 B). RS recombination is not detectable above background in VJ/λ1i 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 VJ/λ1i 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 VJ/λ1i Mice Is Likely to be the Consequence of Receptor Editing. The appearance of κ/λ1+ mature B cells in VJ/λ1i mice indicates that a fraction of A1+ 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 36).
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, Igκ may become accessible for V(D)J recombination later than Igλ (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 VJκ1 rearrangement inserted into its physiological position in the Igκ locus. The results of this analysis were clear-cut: expression of the inserted VJκ1 element was developmentally controlled and coincided with the developmental stage at which Vk and Jκ gene segments are rearranged and functional Vκ-rearrangements are expressed (Fig. 3); and VJκ1 expression did not depend upon Igκ rearrangement (Fig. 4). Thus, at the level of expression of a gene rearrangement in the Igκ 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 Igκ 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 Igλ 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

Discussion

Transcription of an Inserted VJκ1 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 cells expressing an inserted light chain has also been observed in VJκ mice (37). If expression of pre-rearranged κ and λ light chain genes in pre-B cells was 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 VJκ or Vλi 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+/−/λ1+ and were subdivided according to λ expression. Panel a shows representative histograms from a mouse analyzed 30 h after BrdU injection. The percentage of BrdU incorporation in 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, κ/λ1+ 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 κ/λ1+ 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 VJκ mice (37). If expression of pre-rearranged κ and λ1 light chain genes in pre-B cells was 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 VJκ or Vλi mice (Fig. 5 C).

36) or by secondary Igκ rearrangements in a fraction of λ1+ 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 pre-rearranged light chain gene (37). We thus compared the kinetics of pre-B to immature B cell transition for λ1+ and κ/λ1+ 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, κ/λ1+ 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 κ/λ1+ 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.
and enhancer elements and/or the loss of cis-regulatory elements upon Vα-Jα 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 Vα1 and Jα1 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 Igα 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 Igα 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 Igα locus.

Receptor Editing in VJκ1i Mice. While most pre-B cells do not undergo Igκ rearrangements in VJκ1i 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 λ1 pre-B cells. We further demonstrate that κ/λ1+ pre-B cells take \( \sim 12 \) h longer to exit the pre-B cell compartment than their λ1+ 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 energy or clonal deletion (41, 42). We thus propose that \( \sim 30\% \) of α1+ 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.

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