1 **Contribution of secondary Igkappa rearrangement to primary immunoglobulin**
2 **repertoire diversification**

3
4 **Shufang Li¹, Wei Liu², Yinghui Li¹, Shaorong Zhao¹, Can Liu¹, Mengyun Hu³, Wei**
5 **Yue⁴, Yanhua Liu¹, Yue Wang¹, Rongcun Yang¹, Rong Xiang¹*, Feifei Liu¹***

6
7 ¹Department of Immunology, School of Medicine, Nankai University, Tianjin 300071, China

8 ²Tianjin Entry-Exit Inspection and Quarantine Bureau, Tianjin 300308, China

9 ³Collage of Life Science, Nankai University, Tianjin, 300071, China

10 ⁴Department of Neurology, Huanhu Hospital, Tianjin 300060, China

11
12 * Address correspondence and reprint requests to liufeifei@nankai.edu.cn;
13 rxiang@nankai.edu.cn

14
15
16 **Abbreviations used in this article:** RSS, recombination signal sequence; BCR, B-cell
17 antigen receptors; Ag, antigen; cRS, cryptic recombination signal; FWR, framework region;
18 SE, signal end; CDR3, complementarity-determining region 3; Ab, antibody. SHM, somatic
19 hypermutation; AID, activation-induced cytidine deaminase.

20
21 **Running Title**

22 Secondary Igκ rearrangement in central B cells
23

Introduction

There is a series of important events in the process of B cell maturation from a bone marrow hematopoietic stem cell to a mature B lymphocyte (Spanopoulou, Roman et al. 1994). The most important event is the process known as V(D)J recombination, which is characterized as the somatic recombination of individual variable (V), diversity (D), and joining (J) gene segments mediated by DNA motifs known as recombination signals (RS) (Jung, Giallourakis et al. 2006, Matthews and Oettinger 2009). This complex and ordered process originates with the recombination of D to J_H, then V_H to D-J_H genes at the heavy chain (*Igh*) locus in pro-B cells (Hardy and Hayakawa 1991). Once a functional heavy chain has been generated, a similar process initiates the rearrangement at the light chain locus in small pre-B cells, eventually resulting in the production of Abs. V(D)J recombination would generate a highly diverse repertoire (more than 10⁹ specificities) of distinct B-cell antigen receptors (BCR) capable of responding to various types of foreign antigens (Ag) (Jankovic, Casellas et al. 2004). However, this recombination generates a considerable fraction ($\geq 20 \sim 55\%$) of late small pre-B and immature B cells which could recognize self-Ags with high avidity (Wardemann, Yurasov et al. 2003, Merrell, Benschop et al. 2006).

In order to avoid the responsiveness to self-antigens, B cells develop the self-tolerance mechanism which is defined as unresponsiveness to self-antigens (Jankovic, Casellas et al. 2004, Shlomchik 2008). In the autoimmune disease systemic lupus erythematosus, B cells produce high-titers of somatically-mutated Abs reactive to self Ags such as DNA or chromatin (Radic and Weigert 1994, Fields and Erikson 2003). B cells with autoreactive receptors would be deleted or inactivated or change their specificity. These three types of approaches are respectively known as deletion, anergy and receptor editing (Goodnow, Crosbie et al. 1988, Nemazee and Burki 1989, Gay, Saunders et al. 1993, Tiegs, Russell et al. 1993). Among the three methods of immune tolerance, receptor editing gives the autoreactive B cells a second chance to survive and change the BCR specificity (Gay, Saunders et al. 1993, Tiegs, Russell et al. 1993, Chen, Prak et al. 1997). Receptor editing occurs at both the H and L chain loci, but the secondary light chain rearrangement is more frequent than heavy chain V_H replacement (Casellas, Shih et al. 2001).

In previous studies, receptor editing has been viewed as an important mechanism of B cell tolerance (Halverson, Torres et al. 2004). In the process of receptor editing when autoreactive BCR recognize self-antigens in progenitor B cells, the expression of recombination activating gene (RAG-1 and RAG-2) proteins as V(D)J recombinase holoenzyme is enhanced (Gay, Saunders et al. 1993, Tiegs, Russell et al. 1993, Chen, Prak et al. 1997). The autoreactive BCR specificity is altered via V_H replacement at *Igh* locus, or secondary, *de novo* V_κ→J_κ rearrangements which replace or invert primary V_κJ_κ joints, or more rarely, inactivating recombination with RS which is located ~25kb downstream of the C_κ exon (Retter and Nemazee 1998). Secondary V_κ→J_κ rearrangements are highly efficient due to the *Igκ* locus organization: 12-RS and 23-RS respectively flank the ends of the V_κ and J_κ gene segments to facilitate repeated V_κJ_κ rearrangements by following the “12/23 rule” (Gay, Saunders et al. 1993, Tiegs, Russell et al. 1993). In addition, V_κ genes exist in both orientations, and inversion rearrangements would conserve V_κ gene segments for subsequent recombinations. As a result, a new Ig light chain is generated and the B cell obtains a different B cell receptor which may not be self-reactive. In this process, receptor editing potentially creates additional specificities and increases the efficiency of B cell development. Therefore, we hypothesize that secondary Ig_κ rearrangements may be an additional mechanism of increasing the antibody repertoires. Furthermore, in order to increase the efficiency of B cell generation, the frequency of receptor editing is high in normal B cells (Casellas, Shih et al. 2001, Halverson, Torres et al. 2004).

Unconventional Ig gene rearrangements at the light chain locus such as V_κ-V_κ cryptic RS (cRS) fusion may be responsible for Abs with diversified specificities (Langerak, Nadel et al.

2004, Lieberman, Kuraoka et al. 2009). V_{κ} - V_{κ} cRS fusion results from the joining of two V_{κ} genes and possibly creates a dramatic change in the amino acid sequence of the light-chain complementarity-determining region 3 (CDR3) (Radic and Weigert 1994). In comparison, functional cRSs have been reported in the V_H gene segment to mediate $V_H \rightarrow V_H D_H J_H$ replacement and have been proposed to facilitate self-tolerance (Klonowski and Monestier 2000). The presence of positively charged amino acid residues, such as arginine, in the heavy chain increases the affinity for Ab binding to DNA complexed with nuclear proteins such as histones (Shlomchik, Mascelli et al. 1990, Radic and Weigert 1994).

In the present study, we sought to determine the patterns of light chain secondary, *de novo* rearrangements and the property of replaced primary rearrangements in the small pre-B and immature B cell repertoires of lupus-prone Fas^{lpr} and control mice. It was shown that the Fas^{lpr} mice spontaneously developed a syndrome with anti-DNA antibodies, interstitial nephritis, and tissue inflammation which histopathologically mimics lupus in humans (Radic and Weigert 1994, Fields and Erikson 2003). Therefore, we acquired the ongoing light chain gene secondary rearrangement which is the reaction intermediates of receptor editing through a ligation-mediated PCR (LM-PCR) in $B220^+IgM^-$ pre-B and $B220^+IgM^+$ immature B cells from MRL/MpJ- Fas^{lpr} , B6.MRL- Fas^{lpr} and C3H mice, then we analyzed the productivity of the replaced primary Ig rearrangement. Interestingly, we observed that the small pre-B and immature B cell repertoires for the C3H and Fas^{lpr} strains demonstrated similar 1:2 ratios of productive vs nonproductive replaced Ig light chain rearrangements. Importantly, the presence of autoreactive anti-DNA or chromatin BCR during B-cell development in the Fas^{lpr} mice did not seem to impose additional replacement of functional Ig light chain rearrangement. In addition, by using LM-PCR, we detected V_{κ} cRS cleavage in purified small pre-B cell populations recovered from MRL/MpJ- Fas^{lpr} and C3H strains. These V_{κ} cRS are conserved at sites within V_{κ} orthologous to the location of the 3' V_H cRS (Lieberman, Kuraoka et al. 2009). Therefore, functional V_{κ} cRS may possibly be maintained to facilitate the generation of V_{κ} - V_{κ} cRS fusion. Together, our findings support the hypothesis that secondary Ig κ rearrangements and V_{κ} cRS function to increase the diversity of BCR repertoire.

Materials and Methods

Mice

Lupus-prone B6.MRL- Fas^{lpr} and MRL/MpJ- Fas^{lpr} mouse strains have spontaneous mutation in *Fas/lpr* loci and show systemic autoimmunity (Watanabe-Fukunaga, Brannan et al. 1992). B6.MRL- Fas^{lpr} (stock number 000482) and MRL/MpJ- Fas^{lpr} mice (stock number 000485) on the C57BL/6 or MRL background were obtained from the Jackson Laboratory (Bar Harbor, ME). All B6.MRL- Fas^{lpr} or MRL/MpJ- Fas^{lpr} mice were genotyped according to the methods from Jackson Lab. In addition, MRL/MpJ- $Fas^{+/+}$ (MRL $^{+/+}$) mice were not used as control, for the reason that this strain also exhibits autoimmune disorders (Radic and Weigert 1994, Fields and Erikson 2003). The autoimmune disease-free C3H/HeJ (C3H) strain was purchased from Beijing Vital River Animal Co. (Beijing, China). C3H were chosen as a control for MRL/MpJ- Fas^{lpr} because both MRL and C3H strains share the same *Igh J* allotype and MRL strain is partly derived from C3H (Klonowski and Monestier 2000). The animals were sacrificed at 2-3 months of age. The animal experiments were approved by Institutional Animal Care and Use Committee (IACUC) of Nankai University and conformed to the animal guidelines of Nankai University.

Flow cytometric analysis and cell sorting

Single-cell suspensions from bone marrow were obtained as described in previous papers (Davila, Liu et al. 2007, Ueda, Liao et al. 2007). Briefly, a single-cell suspension was obtained by flushing femurs and tibias from four-to-five mice with ice-cold staining media (phosphate buffer saline containing 5% FBS, and 100U/ml penicillin/streptomycin). Red

blood cells were lysed in ACK buffer (0.15 M ammonium chloride, 10 mM potassium bicarbonate and 0.1 mM EDTA). Freshly prepared bone marrow cells were stained with antibodies. Firstly, bone marrow cells were preceded by a 20-min incubation with a purified anti-mouse CD16/32 Ab (Fc gamma RII/III block; 2.4G2) at 4°C. Secondly, bone marrow cells were stained with FITC anti-IgD (11-26c.2a), PE anti-CD43 (S7), PE-Cy7 anti-IgM (R6-60.2), APC anti-B220 (RA3-6B2), and lineage antibodies as biotin anti-CD4 (GK1.5), biotin anti-CD8a (53.6.72), biotin anti-Mac1 (M1/70), biotin anti-Gr1 (RB6-8C5) and biotin anti-Ter119 (Ly-76) on ice for 30 min, and then washed with staining media. Thirdly, bone marrow cells were incubated with streptavidin APC-Cy7. After washing, stained BM samples were sorted as pro-B (lin⁻ B220⁺ CD43⁺ IgM⁻ IgD⁻), pre-B (lin⁻ B220⁺ CD43⁻ IgM⁻ IgD⁻), and immature B (lin⁻ B220⁺ CD43⁺ IgM⁺ IgD⁻) cells on a FACSaria II sorter (BD Biosciences). **For cell cycle analysis, stained BM samples were resuspended in Hoechst 33342 solution at a final concentration of 10 µg/ml. The cells were incubated in a H₂O bath maintained at 37°C for 1 h, and were subjected to FACS analysis.** All antibodies and markers were purchased from BD Biosciences (San Jose, CA) except for biotin conjugated antibodies (Tianjin Sungene, Tianjin, China).

Ligation reactions

A 3'-overhang artificial linker should be prepared before the ligation reactions. Two complementary oligos BW-1 (5'-AGCAACTGACGTGGAATCGCCAGAC-3') and BW-2 (5'-GTCTGGCGATTCCAC-3') were annealed by mixing 2 nmoles of each in a volume of 100 µl Tris (250 mM, pH 7.7) (Schlissel, Constantinescu et al. 1993, Davila, Liu et al. 2007). The mixture was heated to 90°C for 5 min, incubated at 60°C for 5min, and gradually cooled to room temperature. This linker mixture was stored frozen and thawed on ice before use.

Genomic DNA was isolated from freshly sorted cells using the QIAamp DNA Micro kit (56304; Qiagen). The size of purified genomic DNA varied between 30-50 kb. Ligation reactions were performed as previously described (Schlissel, Constantinescu et al. 1993, Constantinescu and Schlissel 1997). DNA (1~2 µg) in solution was subjected to linker ligation for 5h at 16°C in a 50 µl reaction mixture containing ligation buffer, 40 pmol of BW linker and 2.5 U of T4 DNA ligase (Promega, Madison WI).

LM-PCR

Ligation-mediated PCR (LM-pcr) is consisted of primary amplification and secondary amplification (Schlissel, Constantinescu et al. 1993, Constantinescu and Schlissel 1997). In the primary amplification, 100~200 ng of ligated genomic DNA was incubated in a 25 µl reaction with V_κ family outside primer (V_κ out) and J_κ BW-LCH primer, using GoTaq G2 Hot Start Polymerase (error rate = approximately $8 \times 10^{-6} \sim 1 \times 10^{-5}$ mutation/bp) (Promega). The reaction was mediated by the program of 95°C for 2min, 95°C for 3min followed by 12 cycles at 95°C for 45s, 68°C for 3min and end with 72°C for 10min. In the secondary amplification, 3~5 µl DNA from the primary reaction was amplified with a V_κ family inside primer (V_κin) and J_κ BW-LCH primer. The amplification utilized the program of 95°C for 2min, 95°C for 3min followed by 36 cycles at 95°C for 45s, 68°C for 3min and end with 72°C for 10min. After the second (internal) PCR, amplification products were separated on a 2% agarose gel and bands corresponding to a 500- to 650-bp product was excised and isolated with the QIAquick Gel Extraction Kit (28704; Qiagen). The purified products were directly (TA) ligated into the pGEM-T Easy Vector (Promega) and transformed into E. coli DH5α competent cells. After blue/white selection and confirmation by PCR, positive colonies were grown in 3 ml Luria-Bertani/Amp for plasmid purification.

The inserts were sequenced using the ABI 3730xl DNA analyzer (Sangon Biotech) with sequencing primers SP6 (5'-TAATACGACTCACTATAGGG-3') and T7 (5'-ATTTAGGTGACACTATAG-3'). Virtually the majority of plasmids contain a V_κJ_κ-J_κSE rearrangement and their sequences were analyzed using the vector NTI program (Release 11.5, Life Technologies) by comparison with the sequences of J_κ germline genes and the

downstream J κ intron region.

Repertoire analysis using IMGT/V-QUEST and IMGT/Junction Analysis

The final “~470 bp-output” nucleotides containing primary V κ J κ rearrangement were submitted online to IMGT/V-QUEST and IMGT/Junction Analysis (<http://www.imgt.org/>) (Brochet, Lefranc et al. 2008, Giudicelli, Brochet et al. 2011).

Primers:

The germline genes of mouse V κ gene families were aligned using Vector NTI (Release 11.5, Life Technologies) according to the category of subgroups listed as potential mouse IGKV repertoire in [www.IMGT.org](http://www.imgt.org). The semi-nested 5' primers for LM-PCR were designed based on the conserved framework region (FWR) 1 for outside primers, and FWR2 for inside primers, respectively. The degenerate primers were utilized to increase amplification efficiency and repertoire pool. The nucleotide sequences for primers are listed. V κ 4A out, 5'-CAA CCT GGC TTC TGG AGT CCC-3' (159-179 bp); V κ 4A in, 5'- GCT CGC TTC AGT GGC AGT GG-3' (181-200 bp). V κ 4C out, 5'-YTG GCT YCT GGA GTC CC-3' (163-179 bp); V κ 4C in, 5'- TCG CTT CAG TGG CAG TGG G-3' (183-201 bp). V κ 12 out, 5'-TCT CCT CAG CTC CTG RTC TAT-3' (127-147 bp); V κ 12 in, 5'- AGG TTC AGT GGC AGT GGA TC-3' (181-200 bp). V κ 19A out, 5'-TGG AGT CCC TGA TCG CTT CAC-3' (168-188 bp); V κ 19A in, 5'-GGC AGT GGA TCT GGG RCA GAT-3' (208-228 bp). V κ 23A out, 5'-CTC TGG GAT CCC CTC CAG G-3' (165-183 bp); V κ 23A in, 5'-TGG CAG TGG ATC AGG GAC AG-3' (189-208 bp). V κ RF out, 5'-GGA TCC ACT TTG CAA TCT GGA ATT CC-3' (151-176 bp); V κ RF in, 5'-TCA AGG TTC AGT GGC AGT GGA TC-3' (178-200 bp). J κ BW-LCH, 5'-ACT GAC GTG GAA TCG CCA GAC CAC AST-3'. The numbers in parenthesis indicate the annealing position of each primer on the germline V κ gene segments based on ncbi Ig repertoire. PCR of CD14 was performed as previously described (Han, Dillon et al. 1997).

Statistical analyses:

The analyses were conducted with the Prism (release 6.01, GraphPad, San Diego, CA) and SPSS (Release 17.0, SPSS Inc., Chicago, IL) softwares.

Results

LM-PCR approach to amplify ongoing Ig κ replacement segments from sorted early B-cell compartments of B6.MRL-Fas^{lpr}, MRL/MpJ-Fas^{lpr} and C3H mice

B6.MRL-Fas^{lpr} mice have previously been reported to have a mild autoimmune disease which is associated with Fas^{lpr} defect, whereas the MRL/MpJ-Fas^{lpr} mouse strain manifests even stronger autoimmune disease with a spectrum of autoantibodies (Shlomchik, Euler et al. 2003). In order to address whether Fas^{lpr} expression impacted the B cell development, FACS was used to analyze and acquire pro-B (B220⁺ CD43⁺ IgM⁻ IgD⁻ Lin⁻), small pre-B (B220⁺ CD43⁻ IgM⁻ IgD⁻ Lin⁻ FSC^{small}), immature-B (B220⁺ CD43⁻ IgM⁺ IgD⁻ Lin⁻) and circulating mature B cells (B220⁺ CD43⁻ IgM⁺ IgD⁺ Lin⁻) in the bone marrow of MRL/MpJ-Fas^{lpr}, B6.MRL-Fas^{lpr}, and C3H mice (**Figs. 1A-C**) (Hardy, Carmack et al. 1991, Hardy, Li et al. 2000). [Cell cycle analysis by Hoechst 33342 was performed on these B cell compartments \(Supplementary Figs 1A-C\)](#). Consistent with the cell size difference, a greater proportion of large pre-B cells were in the cell cycle (Kline, Hayden et al. 2001, Wang, Stephan et al. 2002). In contrast, small pre-B cells exit the cell cycle to undergo B-cell differentiation (Ehlich, Schaal et al. 1993).

Previous studies have estimated that a total of 140 germline V κ segments in the murine Ig repertoire are available for rearrangements to four functional J κ segments (J κ 1, 2, 4 and 5) (Thiebe, Schable et al. 1999, Brekke and Garrard 2004). These V κ segments belong to at least 19 V κ gene families. Conventional light chain gene rearrangements result from the combination of single V κ and J κ genes in the appropriate orientation by either inversion or

deletion, whereas during secondary Ig κ rearrangements the intermediate products containing the genomic region from the signal end (SE) of downstream J κ to the replaced primary V κ J κ joints are generated (**Fig. 2**). Furthermore, secondary Ig κ rearrangements originate from the presence of four functional J κ genes, flanked by RSS with a 23-bp spacer, which are available for recombination with upstream V κ flanked by 12-bp RSS in either orientation (Brekke and Garrard 2004).

Receptor editing is thought to conduct via “leap-frogging” in which unrearranged upstream V κ combines with downstream J κ elements (Gay, Saunders et al. 1993, Tiegs, Russell et al. 1993, Radic and Weigert 1994, Halverson, Torres et al. 2004). As a result, the primary V κ -J κ joints were excised by the RAGs (**Figs. 2A-B**). Presumably, when the primary Ig κ rearrangement encodes self-reactive BCR or immunoglobulin, the primary V κ J κ segments could be biased toward productive rearrangements, and be replaced via receptor editing (Retter and Nemazee 1998, Retter and Nemazee 1999, Aoki-Ota, Torkamani et al. 2012). In addition, nonproductive primary rearrangements generate “absence of BCR-signal”, which would initiate ongoing recombination to rescue the early B cells (Lutz, Muller et al. 2006). However, another possibility exists that if the ongoing Ig κ replacement is random and non-selected, then two thirds of primary V κ J κ joints would be out-of-frame junction. Furthermore, in-frame rearrangements may contain termination codons and V κ pseudo genes. Therefore, the ratio of functional to nonfunctional joints is 1:2 or is close to 1:2.

Here, in order to test the properties of the Ig κ repertoire undergoing replacement, we amplified the intermediate products of ongoing secondary Ig κ rearrangements which contain primary V κ J κ joints, by means of ligation-mediated PCR (LM-PCR). We amplified the V κ 4, V κ 5, V κ 6, V κ 8, V κ 12, and V κ 16 family to test ongoing secondary V κ J κ rearrangements. Genomic DNA was purified from sorted BM B220⁺ CD43⁺ pro-B, B220⁺ IgM⁻ small pre-B, and B220⁺ IgM⁺ immature B cells of B6.MRL-Fas^{lpr}, MRL/MpJ-Fas^{lpr} and C3H mice, respectively. In addition, we adapted the LM-PCR approach to acquire ongoing secondary rearrangements using a semi-nested 3' primer for the artificial linker ligated to blunt breaks of J κ signal ends (**Fig. 2C**). LM-PCR products were run on agarose gel. It was shown that ongoing secondary Ig κ rearrangements were present in both small pre-B and immature B cells, but absent in pro-B cells (**Figs. 3A-D**). [In accordance with the cell cycle analysis \(Supplementary Figs. 1A-C\) and re-expression of RAG1/2 recombinases at small pre-B cell stage \(Ehlich, Schaal et al. 1993, Kline, Hayden et al. 2001, Wang, Stephan et al. 2002\), ongoing secondary Ig \$\kappa\$ rearrangements preferentially occurred after cell-cycle division of the large pre-B cells.](#)

Rearrangement library of ongoing replaced primary V κ J κ joints in small pre-B and immature B cells of B6.MRL-Fas^{lpr}, MRL/MpJ-Fas^{lpr} and C3H mice

Ongoing secondary Ig κ rearrangements were sequenced and the replaced primary V κ J κ joints were analyzed on the IMGT/V-QUEST. By virtue of the analysis on the IMGT/V-QUEST, the outputs we acquired were the V-gene and allele, the J-gene and allele, the junctional region sequences, the unproductive Ig κ rearranged sequence (the V is a pseudogene, or contains stop codons or out-of-frame junction), or the productive Ig κ rearranged sequence (no stop codon and in-frame junction) (**Supplementary Tables 1-3**) (Brochet, Lefranc et al. 2008, Giudicelli, Brochet et al. 2011).

A total of 180 MRL/MpJ-Fas^{lpr}, 129 B6.MRL-Fas^{lpr}, and 120 C3H unique ongoing secondary V κ -to-J κ rearrangement clones were sequenced and analyzed for this study (**Fig. 4A**). A high proportion of nonfunctional replaced primary V κ J κ rearrangements were identified in pre-B and immature B of the MRL/MpJ-Fas^{lpr}, B6.MRL-Fas^{lpr} and C3H mice. Furthermore, the ratios of productive (P) vs nonproductive (nP) replaced primary V κ J κ joints for either small pre-B or immature-B cells within two autoimmune FAS^{lpr} strains were close to 1: 2 (**Fig. 4A**). For small pre-B cells, P vs nP rearrangements equaled 1:1.96 ($p=0.94$) in the MRL.FAS^{lpr} mice and 1:2.12 ($p=0.83$) in the B6.MRL-Fas^{lpr} mice. Interestingly, immature B cells also exhibited 1:1.78 (P vs nP) ($p=0.63$) rearrangements in

autoimmune MRL/MpJ-Fas^{lpr} mice and 1:2.18 ($p=0.79$) in B6.MRL-Fas^{lpr} mice. As a control, the ratios of the P vs nP joints were 1:2.0 ($p=1$) in the small pre-B cells and 1:2.16 ($p=0.80$) in the immature B cells of C3H. Statistical analysis indicated that these P: nP ratios were not different from the theoretic ratio of 1:2. These sequences are available from GenBank under accession numbers KT998908 to KT999336 ([Supplementary Tables 1-3](#)).

Previous studies have demonstrated the correlation of anti-DNA reactivity and V κ gene usage in the repertoire of 3H9 transgenic mice, carrying a site-directed transgene 3H9 which codes for an anti-DNA H chain derived from a diseased MRL/MpJ-Fas^{lpr} mouse (Erikson, Radic et al. 1991, Gay, Saunders et al. 1993, Chen, Prak et al. 1997, Li, Jiang et al. 2001). The 3H9 V_H gene is repeatedly used in anti-DNA antibodies from the autoimmune MRL/MpJ-Fas^{lpr} strain where it is associated with various light chains including members of the V κ 4, V κ 8, V κ 9, and V κ 23 groups. In contrast, editor κ chains, V κ 12/13, V κ 20, V κ 21D, and V κ 38c in association with the 3H9 heavy chain efficiently veto DNA binding and effectively edit anti-DNA B cells (Radic, Erikson et al. 1993, Li, Jiang et al. 2001). Therefore, we grouped the samples of the replaced primary V κ J κ rearrangements by V κ families which were either permissive or non-permissive for DNA-binding activity (**Fig. 4B**). The P:nP ratios of V κ J κ replacements from the small pre-B cells and immature B cells ranged between 1:1.40 ($p=0.26$) and 1:2.25 ($p=0.72$) for MRL/MpJ-Fas^{lpr}, 1:1.40 ($p=0.41$) and 1:3.29 ($p=0.27$) for B6.MRL-Fas^{lpr}, and 1:1.60 ($p=0.60$) and 1:2.78 ($p=0.42$) for the C3H mice (**Fig. 4B**). These P:nP ratios were not different from theoretic ratio of 1:2. In addition, all three strains contained the recombination of a particular V κ gene member, V κ 16-104, in secondary light chain rearrangements. The presence of the V κ 16-104 gene is interesting in that it encodes the Rheumatoid Factor and has frequently been observed in Abs with autoreactive specificities (Silverman, Goldfien et al. 1988). Notably, both productive and nonproductive primary rearrangements for V κ 16-104 undergoing secondary replacement were found in early B cells from the autoimmune FAS^{lpr} mice and control C3H mice (**Supplementary Tables 1-3**).

J κ gene usage

The J κ genes at the light chain locus consist of four functional gene segments, namely J κ 1, J κ 2, J κ 4 and J κ 5, and a pseudogene J κ 3 (ψ) (Brekke and Garrard 2004). The J κ 1 gene is the most V κ -proximal J gene, mapping 19 kbp downstream of IGKV3-1, and has been observed to be frequently used in C57BL/6 mice (Martinez-Jean, Folch et al. 2001, Giudicelli, Duroux et al. 2006).

In this study, we acquired ongoing replacement products containing one J κ intron region, i.e. V κ J κ 1-J κ 2 SE, or V κ J κ 4-J κ 5 SE for about 460~490 bp. Productively rearranged light chain J κ genes can be read in one reading frame, which embodies the nucleotide TTC to encode PHE amino acid (Giudicelli, Duroux et al. 2006). Among the amplified genomic DNA replacement products, we observed a clear difference in the pattern of J κ gene usage for ongoing secondary rearrangement. For the C57BL/6 mice, both the small pre-B and immature B cells tended to replace J κ 1, and utilize J κ 2 in the majority of their secondary light chain replacement (Nishi, Kataoka et al. 1985). In the present study, there was also a significant difference in the overall distribution of the J κ genes used. For B6.MRL-Fas^{lpr}, MRL/MpJ-Fas^{lpr} and C3H small pre-B cells and immature B cells, the proximal J κ 1 gene segment was more abundantly replaced than the distal J κ 4 gene segment (**Table 1**). Although the number of unique V κ J κ 4-J κ 5 SE clones is limited, the pattern of J κ 1 gene usage for the productive and nonproductive rearrangements was similar in these progenitor B cells from the autoimmune FAS^{lpr} mice and control C3H mice (**Table 1**).

P and N nucleotides

The junctional diversity of Ab genes is reinforced by the addition of N and P nucleotides. P nucleotides are presumably generated by uneven cutting of DNA which resolves hairpins produced by the intermediate coding joints of the Ab genes (Shockett and Schatz 1999). Most of the sequences of replaced primary rearrangements from small pre-B and immature

B cells of the B6.MRL-Fas^{lpr}, MRL/MpJ-Fas^{lpr} and C3H mice exhibited rearrangements with homologous junctions (**Table 2**). Furthermore, there was no significant difference in the amount of P nucleotide additions among the three strains. The MRL/MpJ-Fas^{lpr} and B6.MRL-Fas^{lpr} mice respectively possessed an average of 0.30 (± 0.82) and 0.25 (± 0.68) P nucleotides per CDR3, and the C3H mice had an average of 0.27 (± 0.84) P nucleotides per CDR3 for small pre-B cells. The N nucleotides are added by TdT, which is up-regulated at the pro-B cell stage and downregulated in the pre-B and immature B cells (Li, Hayakawa et al. 1993). As expected, the frequency of N nucleotide additions in the small pre-B and immature B cells from all three strains was limited.

CDR3 properties

The nucleotide sequences of the junctional region and the deduced amino acid sequences for the replaced V κ J κ primary rearrangements are listed in **Supplementary Tables 1-3**. The light chain CDR3 is critical for interaction with Ag epitopes. In particular, specific amino acid residues such as arginine, have been correlated to mediating CDR3 binding of auto-Abs to DNA or DNA/histone complexes (Shlomchik, Mascelli et al. 1990, Li, Schettino et al. 2000). Overall, the CDR3 sequences deduced from the replaced productive primary rearrangements for the small pre-B or immature B cells displayed similar properties for the MRL/MpJ-Fas^{lpr}, B6.MRL-Fas^{lpr}, or C3H strains (**Table 3** and **Supplementary Tables 1-3**). For instance, there was no significant difference in length between the FAS^{lpr} and C3H CDR3 sequences (9.0 $\pm$ 0.70 amino acids for small pre-B and 8.9 $\pm$ 0.60 for immature B in MRL/MpJ-Fas^{lpr}, and 9.1 $\pm$ 0.28 amino acids for small pre-B and 9.0 $\pm$ 0.00 for immature B in B6.MRL-Fas^{lpr}). Additionally, their average arginine contents were similar in the immature B cells (0.36 $\pm$ 0.49 arginine per CDR3 for MRL/MpJ-Fas^{lpr}, and 0.37 $\pm$ 0.60 arginine per CDR3 for C3H). Taken together, these data suggest that ongoing secondary rearrangements in autoimmune FAS^{lpr} and control C3H mice occur similarly at either the small pre-B or immature B cell stage. Therefore, it is possibly the defect in the tolerance selection that leads to the presence of autoimmune antibodies.

Somatic hypermutation of replaced primary V κ J κ rearrangement

Somatic hypermutation (SHM) and V gene usage both partly account for the specificity of the autoreactive Abs. A characteristic of lupus-associated autoantibodies is somatic mutation (Guo, Smith et al. 2010). In addition, the developmental expression of activation-induced cytidine deaminase (AID) which mediates somatic mutation was first detected in the mouse pro-B cells and peaked in the immature and transitional T1 B cells from the bone marrow (Kuraoka, Holl et al. 2011). Given that MRL/MpJ-Fas^{lpr} mice develop high titers of somatically-mutated IgG autoantibodies in the periphery, we tested whether or not these replaced primary rearrangements undergo mutation in central development and whether or not this modification is relevant. The mutation analysis by IMGT/V-QUEST indicated that approximately half (40%, 32 of 80 for small pre-B; and 52%, 52 of 100 for immature B cells) of the replaced primary V κ J κ rearrangements from the MRL/MpJ-Fas^{lpr} early B cells were mutated, mostly carrying one unique point mutation (total SHM, n=50, and 75, **Fig. 5A-B**, **Supplementary Table 4**). Additionally, 19% (14 of 75) and 17% (9 of 54) of the replaced primary rearrangements from the B6.MRL-Fas^{lpr} small pre-B and immature B cells respectively carried one or four unique point mutations (total SHM, n=19 or 16). In comparison, the point mutations remained at relatively low levels in the C3H early B cells (12%, 7 of 60 for small pre-B; and 28%, 17 of 60 for immature B cells). In all cases, a majority of somatic mutation resulted in amino acid replacement (**Supplementary Table 4**).

To exclude the possible contamination of circulating mature B cells in the sorted progenitor B cells in the bone marrow, we re-analyzed the collections of secondary Ig κ rearrangements by only considering the sequences without somatic mutation (**Fig. 5C**). This restriction reduced our sample sizes by 50% - 20% in all cohorts. However, P vs nP replaced primary V κ J κ rearrangements for either small pre-B or immature-B cells within all three strains did not significantly differ from the theoretic 1:2 (**Fig. 5C**). For autoimmune MRL/MpJ-Fas^{lpr} mice, P vs nP ratios were 1:2.54 ($p=0.56$) for small pre-B cells, and 1:1.63

($p=0.59$) for immature B cells (**Fig. 5C**). For B6.MRL-Fas^{lpr} mice, small pre-B and immature B cells exhibited P vs nP ratios of 1:1.86 ($p=0.84$) and 1:2.31 ($p=0.73$), respectively. As the non-autoimmune control, C3H mice also exhibited P vs nP ratios of 1:2.57 ($p=0.53$) for small pre-B cells, and 1:2.0 ($p=0.99$) for immature B cells, respectively. Together (**Figs. 4A, B**), it seems that P vs nP ratios of replaced V κ J κ joints were not altered significantly by BCR expression that dictates self-reactivity in the central compartment.

V κ cRS cleavage *in vivo*

Canonical light chain Ab gene rearrangements originate from the combining of single V κ and J κ genes in the appropriate orientation, whereas atypical rearrangements are defined as hybrid V κ -V κ cRS fusions, V κ - κ deleting element (Kde), and intron-Kde gene rearrangements (Langerak, Nadel et al. 2004, Lieberman, Kuraoka et al. 2009). Previous studies have applied a statistical model (**RIC**) of mouse RS to identify cRS in germline mouse V κ gene segments and alleles (Lieberman, Kuraoka et al. 2009). V κ cRSs with 23-bp spacers were calculated to be conserved at multiple locations within V κ gene segments. LM-PCR is a standard assay used to test the RAG-mediated cleavage at the RS and cRS heptamers (Schlissel, Constantinescu et al. 1993). Here, by means of LM-PCR with V κ family-specific primers and linker primers, we recovered short products (~120-150bp) in sorted small pre-B cells from MRL/MpJ-Fas^{lpr}, and C3H mice (**Table 4**). Sequencing showed that these short LM-PCR amplification products represented V κ cRS gene cleavage *in vivo*. They featured blunt, double-strand cleavage events precisely at the V κ cRS heptamers. Most of these cRS are located at nucleotide position 196-265 bp within framework 3 or at conserved site flanking CDR3 (**Table 4**), a finding which is similar to that of Lieberman et al., who reported the predominant presence of V κ 23-cRS oriented opposite to the physiological 12-RS (Lieberman, Kuraoka et al. 2009). The seven unique cRS cleavage events occurred at about three different V κ nucleotide positions from four distinct V κ gene families. In contrast, we could not detect the cleavage at the V κ cRS signal ends from bone marrow pro-B, nor immature B cells in the B6.MRL-Fas^{lpr}, MRL/MpJ-Fas^{lpr}, and C3H mice, which is similar to previous reports (Lieberman, Kuraoka et al. 2009). Therefore, V κ cRS is cleaved *in vivo* in small pre-B cells, during which primary Ig κ V κ →J κ rearrangements occur, possibly to increase the Ab repertoire.

Discussion

The development of systemic lupus erythematosus in the FAS^{lpr} mouse model is characterized by the loss of tolerance of nuclear autoantigens, the presence of anti-nuclear Abs capable of binding DNA and nucleosomes, and the expansion of autoreactive B and T cell clones (Seo, Mandik-Nayak et al. 2003, Shlomchik, Euler et al. 2003). The activation of polyclonal B cells leads to hypergammaglobulinemia, increased autoantibody production, and immune complex deposition which in turn give rise to multiorgan tissue inflammations. In this study, we sought to evaluate the ongoing secondary Ig κ rearrangements amplified from adult lupus-prone MRL/MpJ-Fas^{lpr}, B6.MRL-Fas^{lpr} and control C3H B220⁺IgM⁻ small pre-B cells which have not yet undergone Ab selection, and B220⁺IgM⁻ immature B cells which are subjected to tolerance. Previous informative assays investigating B cell tolerance have focused on measuring relatively late-stage events, such as the change of the cell-surface phenotypes of transgenic autoreactive BCR, or the presence of splenic autoantibody production (Gay, Saunders et al. 1993, Tiegs, Russell et al. 1993, Radic and Weigert 1994, Halverson, Torres et al. 2004). However, the pathways which progenitor B cells in bone marrow undertake on the way of tolerance or become autoimmune have seldom been tested. The LM-PCR rearrangement assay we presented in this paper takes the pathways and offers insights into central B cell tolerance.

Indeed, the presence of these replaced primary rearrangements recovered by LM-PCR is not the final output of the B cell, and may not influence the selection events leading to the

ultimate fate determined by the expressed antibody. However, these ongoing replacements reflect the recombination history of the B cells, and indicate the initiating forces which drive secondary rearrangements in B-cell pool. Worth noting is that three research articles from Melchers (Yamagami, ten Boekel et al. 1999), Shapiro (Fitzsimmons, Aydanian et al. 2014), Harada (Harada and Yamagishi 1991) and their colleagues report a 1: 2 ratio of functional: nonfunctional V κ J κ rearrangements in central development. The first report (Yamagami, ten Boekel et al. 1999) originates from single-cell direct PCR analyses of recombination in the Ig κ repertoire at different stages of B cell development from wild-type, or J κ C κ ^{+/-} mice which carried one κ allele. An early report by Harada et al (Harada and Yamagishi 1991) also characterized multiple recombinations from circular DNAs excised by Ig κ gene rearrangements, among which 5 of 16 translational reading frames were in phase. These reports are consistent with our analysis of LM-PCR-amplified ongoing secondary Ig κ rearrangements from lupus-prone B6.MRL-Fas^{lpr} and MRL/MpJ-Fas^{lpr} mice which showed that these two strains possess a near 1:2 ratio of P vs nP replaced primary rearrangements in either small pre-B or immature B cell compartments, the same as the C3H control. Importantly, our approach captured the events of ongoing replacements by LM-PCR in the Ig κ repertoire, especially including progenitor B cells from the autoimmune lupus-prone strains.

At least two nonmutually exclusive explanations may be provided for the presence of productive and non-productive secondary Ig κ rearrangements in the primary repertoire. First, V(D)J gene accessibility to rearrangement is complex and multifactorial, as many aspects, such as chromatin condensation, histone acetylation, DNA methylation, enhancer and promoter activity, and germline transcription, can impact V(D)J recombination (Degner-Leisso and Feeney 2010, Schatz and Ji 2011, Aoki-Ota, Torkamani et al. 2012). One interpretation is that secondary rearrangements are extensive in wild-type progenitor B cells due to the high proportion of non-productive rearrangements, additional receptor editing of functional light chains as a result of autoreactivity, nonpairing with heavy chains, and the suboptimal expression of BCR (Aoki-Ota, Torkamani et al. 2012, Fitzsimmons, Aydanian et al. 2014). As a result, additional editing may impact subtly against the high background of ongoing rearrangements. These facts are supported by the observation that a considerable fraction ($\geq 20\%$) of late small pre-B undergo secondary V(D)J rearrangements (Casellas, Shih et al. 2001). It was observed that small pre-B and immature B cells have half-lives of 2 to 4 days (Rolink, Andersson et al. 1998) and thus exist long enough to allow several secondary rearrangements (Yamagami, ten Boekel et al. 1999). In addition, developmental arrest precedes the apoptosis of autoreactive B cells, to ensure that editing takes place (Chen, Nagy et al. 1995, Melamed and Nemazee 1997, Casellas, Shih et al. 2001).

Second, our data of ongoing L-chain replacements suggest that the intrinsic defects in the production of autoantibody present in FAS^{lpr} mice indeed do not result from the development of B cell precursors with incremental replacement of their productive Ig κ repertoire. This observation is revealing in that our results indicate that autoreactive BCR generated by the defective Fas apoptotic pathway has a limited impact on the process of secondary Ig κ rearrangements. Instead, secondary Ig κ rearrangements may persist [stochastically](#) to increase BCR diversification [in some development window of bone marrow B cells](#). In addition, it is believed that the acquisition of a functional IgL variable gene rearrangement can possibly prevent further recombination at the *Ig κ* locus (Langerak, Nadel et al. 2004, Brady, Steinel et al. 2010). However, we identified that functional Ig κ gene rearrangements were revised at a similar frequency when progenitor B cells from autoimmune FAS^{lpr} and control non-autoimmune C3H mice were analyzed and compared. Therefore, [it is possible that](#) secondary Ig κ gene rearrangements mostly function to diversify the pre-immune repertoire of IgL specificities, [from which the tolerized Ig repertoires are selected](#).

There is increasing evidence that some part of the secondary rearrangement at *Igh* or *Ig κ*

is possibly antigen-independent. Recently, evidence that the regulation of V_H replacement is independent of BCR specificity appeared in studies which utilized a mouse model carrying a pre-rearranged productive $V_H D_H J_H$ knock-in at the physiological IgH locus (Kumar, Bach et al. 2015, Sun, Novobrantseva et al. 2015). These data indicate that V_H replacement occurs before the pre-B cell stage of development and is not involved in the revision of autoreactive antibodies (Sun, Novobrantseva et al. 2015). Furthermore, the approach of somatic cell nuclear transfer technology to establish mice reprogrammed from the nucleus of a terminally differentiated IgA⁺ plasma cell confirm that V_H replacement is independent of pre-BCR signaling and constitutes up to 20% of the antibody V gene repertoire (Kumar, Bach et al. 2015). In addition, the earliest results demonstrated frequent V_H replacement in mice transgenic for non-autoreactive heavy chains (Taki, Meiering et al. 1993, Taki, Schwenk et al. 1995, Cascalho, Ma et al. 1996). Subsequently, we showed that V_H cRS SE were detected only in pro-B cells, including pro-B cells from μ MT mice which could not assemble functional BCR (Davila, Liu et al. 2007). Together, these results suggest that V_H gene replacement functions to increase the capacity of BCR diversification, but this is not simply driven by the recognition of self-antigens. For the light chain loci, researchers utilized anti-DNA-associated ($V_{\kappa 4}$ -J $\kappa 4$, Ig $\kappa^{V_{\kappa 4}/h}$) or innocent (anti-HEL $V_{\kappa 5-45}$ -J $\kappa 2$) light-chain knockin mice and identified a significant alternation of light-chain knockin specificities that resulted in a reduction in autoreactivity and permitted the progenitor B cells to survive tolerance (Andrews, Zhang et al. 2013). But the relative frequency of RS rearrangements was almost unaltered between these mice at the pre-B cell stage or between the edited and unedited immature B cell populations sorted from Ig $\kappa^{V_{\kappa 4}/h}$ mice. Interestingly, no significant difference in RS rearrangement levels was observed in bone marrow pre-B or immature B cells between the two genetic backgrounds on MRL/lpr or wild-type B6 crossed with Ig $\kappa^{V_{\kappa 4}/h}$ mice (Andrews, Zhang et al. 2013). These data indicate that little change in central tolerance exist in the bone marrow of these light-chain transgenic mice. Thus, consistent with our results, secondary Ig κ rearrangements possibly persist spontaneously during some developmental time frame of progenitor B cells.

Despite the capacity for repeated secondary Ig κ rearrangements, our results indicate that the V_{κ} gene segments contain embedded heptamers, which are located in the 3' region and may serve to mediate VJ recombination-type events such as V_{κ} - V_{κ} cRS fusion genes. These V_{κ} cRS are conserved in the orientation opposite to physiologic V_{κ} 12-RS, and have 23-bp spacers (Lieberman, Kuraoka et al. 2009). Our results are consistent with earlier research which utilized a computational scan for cRS in V_{κ} gene segments (Lieberman, Kuraoka et al. 2009). It has been reported that V_{κ} cRS are conserved at multiple sites in V_{κ} gene segments. Due to the limitation of our design of 5' LM-PCR primers which recognize conserved nucleotides on frameworks 1 and 2, we only detected V_{κ} cRS at positions analogous to the 3' V_H cRS which mediate V_H replacement. Interestingly, our LM-PCR demonstrated that these V_{κ} cRS are only functional in small pre-B cells, while being undetectable in immature B cells (Lieberman, Kuraoka et al. 2009). Therefore, V_{κ} cRS possibly occur to promote the increment of BCR diversity (Davila, Liu et al. 2007).

Previous research observed less-efficient receptor editing in lupus-prone MRL/lpr mice (Lamoureux, Watson et al. 2007). These articles focused on the tests of less endogenous rearrangement and the down-regulation of RAG-1/2 messenger RNA expression. However, the properties of replaced primary $V_{\kappa} J_{\kappa}$ rearrangement were not examined. In addition, one critical factor is the existence of apoptosis defects due to Fas (*lpr*) or FasL (*gld*) gene mutations during lupus (Watanabe-Fukunaga, Brannan et al. 1992). There is evidence that apoptosis is impaired in lupus individuals or humans with autoimmune lymphoproliferative or Canale-Smith syndromes which accompany Fas or FasL mutations (Elkon and Marshak-Rothstein 1996). This decrease in apoptosis efficiency may be critical to allow enough survival time for the secondary Ig κ rearrangements to initiate in order to replace the primary autoreactive BCR, whereas a normal B cell would otherwise die before being

rescued. Additionally, FAS^{lpr} mice have defect in negative selection of B cells and the resultant accumulation of autoreactive Abs (Pasqualetto, Vasseur et al. 2005). It would be interesting to test how these phenotypes impact on intrinsic Igκ rearrangements. However, in the circumstance of Fas^{lpr} mice, we could not observe the biased replacement of functional *Ig* rearrangements which presumably encode autoreactive BCR pools in progenitor B cells from B6.MRL-Fas^{lpr} or MRL/MpJ-Fas^{lpr} mice. Instead, these replacement events on *Igκ* genes exhibited no difference compared to the control C3H early B cell populations. In addition, our current results are consistent with the earlier study of secondary *Igκ* rearrangements that utilized C57BL/6, Jκ^{+/-}, H50Gμ, and 3H9 mice that carried autoreactive *Igh* knock-in (Liu, Cowell et al.).

In addition, we observed the biased preference for Jκ1 instead of Jκ4 gene segments in the primary rearrangements, although our data of *P* vs *nP* ratios involving Jκ4 primary rearrangements are limited in number. This is of special relevance during systemic autoimmunity diseases where anti-nuclear Abs often express the distal Jκ4 gene (Constantinescu and Schlissel 1997). This observation is consistent with the possibility that successive rearrangements may occur until the B cells proceed to selection for the functional BCR. Furthermore, these secondary rearrangements may include Vκ-Vκ cRS fusion which increases the diversity of the antibody repertoire.

Therefore, another model (Yamagami, ten Boekel et al. 1999, Andrews, Zhang et al. 2013, Kumar, Bach et al. 2015, Sun, Novobrantseva et al. 2015) possibly exists in which secondary light chain rearrangements occur routinely and spontaneously during the process of central B cell development and selection. These secondary Igκ rearrangements and the possible Vκ-embedded cRSs function to create combinatorial diversity beyond the expectation that they only utilize the combination of Vκ and Jκ gene segments. In addition, these four pathways may coexist as they are not mutually antithetical: secondary Igκ rearrangements induced by autoreactive BCR signals (Halverson, Torres et al. 2004), the absence of BCR signals (Heltemes and Manser 2002), tonic BCR signals (Casola, Otipoby et al. 2004, Tze, Schram et al. 2005), and the random stochastic rearrangements may coincide to encounter the central tolerance and endure the pressure of BCR diversity. These ideas form the core of immunology concepts, merit further experimental tests, and could not be resolved entirely by our experiments. However, various experimental data (Taki, Meiering et al. 1993, Taki, Schwenk et al. 1995, Cascalho, Ma et al. 1996, Yamagami, ten Boekel et al. 1999, Davila, Liu et al. 2007, Lieberman, Kuraoka et al. 2009, Andrews, Zhang et al. 2013, Kumar, Bach et al. 2015, Sun, Novobrantseva et al. 2015) elucidate that ongoing secondary rearrangements at *Igh* or *Igκ* are not absolutely driven by antigens, although self-antigens may also possibly shape the antibody repertoire (Gay, Saunders et al. 1993, Tiegs, Russell et al. 1993, Radic and Weigert 1994, Chen, Nagy et al. 1995, Melamed and Nemazee 1997, Halverson, Torres et al. 2004).

Reference

- Andrews, S. F., Q. Zhang, S. Lim, L. Li, J. H. Lee, N. Y. Zheng, M. Huang, W. M. Taylor, A. D. Farris, D. Ni, W. Meng, E. T. Luning Prak and P. C. Wilson (2013). "Global analysis of B cell selection using an immunoglobulin light chain-mediated model of autoreactivity." *J Exp Med* **210**(1): 125-142.
- Aoki-Ota, M., A. Torkamani, T. Ota, N. Schork and D. Nemazee (2012). "Skewed primary Igkappa repertoire and V-J joining in C57BL/6 mice: implications for recombination accessibility and receptor editing." *J Immunol* **188**(5): 2305-2315.
- Brady, B. L., N. C. Steinle and C. H. Bassing (2010). "Antigen receptor allelic exclusion: an update and reappraisal." *J Immunol* **185**(7): 3801-3808.
- Brekke, K. M. and W. T. Garrard (2004). "Assembly and analysis of the mouse immunoglobulin kappa

gene sequence." Immunogenetics **56**(7): 490-505.

Brochet, X., M. P. Lefranc and V. Giudicelli (2008). "IMGT/V-QUEST: the highly customized and integrated system for IG and TR standardized V-J and V-D-J sequence analysis." Nucleic Acids Res **36**(Web Server issue): W503-508.

Cascalho, M., A. Ma, S. Lee, L. Masat and M. Wabl (1996). "A quasi-monoclonal mouse." Science **272**(5268): 1649-1652.

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**(5508): 1541-1544.

Casola, S., K. L. Otipoby, M. Alimzhanov, S. Humme, N. Uyttersprot, J. L. Kutok, M. C. Carroll and K. Rajewsky (2004). "B cell receptor signal strength determines B cell fate." Nat Immunol **5**(3): 317-327.

Chen, C., Z. Nagy, E. L. Prak and M. Weigert (1995). "Immunoglobulin heavy chain gene replacement: a mechanism of receptor editing." Immunity **3**(6): 747-755.

Chen, C., E. L. Prak and M. Weigert (1997). "Editing disease-associated autoantibodies." Immunity **6**(1): 97-105.

Constantinescu, A. and M. S. Schlissel (1997). "Changes in locus-specific V(D)J recombinase activity induced by immunoglobulin gene products during B cell development." J Exp Med **185**(4): 609-620.

Davila, M., F. Liu, L. G. Cowell, A. E. Lieberman, E. Heikamp, A. Patel and G. Kelsoe (2007). "Multiple, conserved cryptic recombination signals in VH gene segments: detection of cleavage products only in pro B cells." J Exp Med **204**(13): 3195-3208.

Degner-Leisso, S. C. and A. J. Feeney (2010). "Epigenetic and 3-dimensional regulation of V(D)J rearrangement of immunoglobulin genes." Semin Immunol **22**(6): 346-352.

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**(5): 695-704.

Elkon, K. B. and A. Marshak-Rothstein (1996). "B cells in systemic autoimmune disease: recent insights from Fas-deficient mice and men." Curr Opin Immunol **8**(6): 852-859.

Erikson, J., M. Z. Radic, S. A. Camper, R. R. Hardy, C. Carmack and M. Weigert (1991). "Expression of anti-DNA immunoglobulin transgenes in non-autoimmune mice." Nature **349**(6307): 331-334.

Fields, M. L. and J. Erikson (2003). "The regulation of lupus-associated autoantibodies: immunoglobulin transgenic models." Curr Opin Immunol **15**(6): 709-717.

Fitzsimmons, S. P., A. G. Aydanian, K. J. Clark and M. A. Shapiro (2014). "Multiple factors influence the contribution of individual immunoglobulin light chain genes to the naive antibody repertoire." BMC Immunol **15**: 51.

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**(4): 999-1008.

Giudicelli, V., X. Brochet and M. P. Lefranc (2011). "IMGT/V-QUEST: IMGT standardized analysis of the immunoglobulin (IG) and T cell receptor (TR) nucleotide sequences." Cold Spring Harb Protoc **2011**(6): 695-715.

Giudicelli, V., P. Duroux, C. Ginestoux, G. Folch, J. Jabado-Michaloud, D. Chaume and M. P. Lefranc (2006). "IMGT/LIGM-DB, the IMGT comprehensive database of immunoglobulin and T cell receptor nucleotide sequences." Nucleic Acids Res **34**(Database issue): D781-784.

Goodnow, C. C., J. Crosbie, S. Adelstein, T. B. Lavoie, S. J. Smith-Gill, R. A. Brink, H. Pritchard-Briscoe, J. S. Wotherspoon, R. H. Loblay, K. Raphael and et al. (1988). "Altered

immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice." *Nature* **334**(6184): 676-682.

Guo, W., D. Smith, K. Aviszus, T. Detanico, R. A. Heiser and L. J. Wyszocki (2010). "Somatic hypermutation as a generator of antinuclear antibodies in a murine model of systemic autoimmunity." *J Exp Med* **207**(10): 2225-2237.

Halverson, R., R. M. Torres and R. Pelanda (2004). "Receptor editing is the main mechanism of B cell tolerance toward membrane antigens." *Nat Immunol* **5**(6): 645-650.

Han, S., S. R. Dillon, B. Zheng, M. Shimoda, M. S. Schlissel and G. Kelsoe (1997). "V(D)J recombinase activity in a subset of germinal center B lymphocytes." *Science* **278**(5336): 301-305.

Harada, K. and H. Yamagishi (1991). "Lack of feedback inhibition of V kappa gene rearrangement by productively rearranged alleles." *J Exp Med* **173**(2): 409-415.

Hardy, R. R., C. E. Carmack, S. A. Shinton, J. D. Kemp and K. Hayakawa (1991). "Resolution and characterization of pro-B and pre-pro-B cell stages in normal mouse bone marrow." *J Exp Med* **173**(5): 1213-1225.

Hardy, R. R. and K. Hayakawa (1991). "A developmental switch in B lymphopoiesis." *Proc Natl Acad Sci U S A* **88**(24): 11550-11554.

Hardy, R. R., Y. S. Li, D. Allman, M. Asano, M. Gui and K. Hayakawa (2000). "B-cell commitment, development and selection." *Immunol Rev* **175**: 23-32.

Heltemes, L. M. and T. Manser (2002). "Level of B cell antigen receptor surface expression influences both positive and negative selection of B cells during primary development." *J Immunol* **169**(3): 1283-1292.

Jankovic, M., R. Casellas, N. Yannoutsos, H. Wardemann and M. C. Nussenzweig (2004). "RAGs and regulation of autoantibodies." *Annu Rev Immunol* **22**: 485-501.

Jung, D., C. Giallourakis, R. Mostoslavsky and F. W. Alt (2006). "Mechanism and control of V(D)J recombination at the immunoglobulin heavy chain locus." *Annu Rev Immunol* **24**: 541-570.

Kline, G. H., T. A. Hayden and P. Riegert (2001). "The initiation of B cell clonal expansion occurs independently of pre-B cell receptor formation." *J Immunol* **167**(9): 5136-5142.

Klonowski, K. D. and M. Monestier (2000). "Heavy chain revision in MRL mice: a potential mechanism for the development of autoreactive B cell precursors." *J Immunol* **165**(8): 4487-4493.

Kumar, R., M. P. Bach, F. Mainoldi, M. Maruya, S. Kishigami, H. Jumaa, T. Wakayama, O. Kanagawa, S. Fagarasan and S. Casola (2015). "Antibody repertoire diversification through VH gene replacement in mice cloned from an IgA plasma cell." *Proc Natl Acad Sci U S A* **112**(5): E450-457.

Kuraoka, M., T. M. Holl, D. Liao, M. Womble, D. W. Cain, A. E. Reynolds and G. Kelsoe (2011). "Activation-induced cytidine deaminase mediates central tolerance in B cells." *Proc Natl Acad Sci U S A* **108**(28): 11560-11565.

Lamoureux, J. L., L. C. Watson, M. Cherrier, P. Skog, D. Nemazee and A. J. Feeney (2007). "Reduced receptor editing in lupus-prone MRL/lpr mice." *J Exp Med* **204**(12): 2853-2864.

Langerak, A. W., B. Nadel, A. De Torbal, I. L. Wolvers-Tettero, E. J. van Gastel-Mol, B. Verhaaf, U. Jager and J. J. van Dongen (2004). "Unraveling the consecutive recombination events in the human IGH locus." *J Immunol* **173**(6): 3878-3888.

Li, H., Y. Jiang, E. L. Prak, M. Radic and M. Weigert (2001). "Editors and editing of anti-DNA receptors." *Immunity* **15**(6): 947-957.

Li, Y. S., K. Hayakawa and R. R. Hardy (1993). "The regulated expression of B lineage associated genes during B cell differentiation in bone marrow and fetal liver." *J Exp Med* **178**(3): 951-960.

Li, Z., E. W. Schettino, E. A. Padlan, H. Ikematsu and P. Casali (2000). "Structure-function analysis of a lupus anti-DNA autoantibody: central role of the heavy chain complementarity-determining region 3 Arg in binding of double- and single-stranded DNA." Eur J Immunol **30**(7): 2015-2026.

Lieberman, A. E., M. Kuraoka, M. Davila, G. Kelsoe and L. G. Cowell (2009). "Conserved cryptic recombination signals in V kappa gene segments are cleaved in small pre-B cells." BMC Immunol **10**: 37.

Liu, F., L. G. Cowell, E. Heikamp and G. Kelsoe "Spontaneous Secondary Igk Rearrangements during B-cell Development." manuscript revision.

Lutz, J., W. Muller and H. M. Jack (2006). "VH replacement rescues progenitor B cells with two nonproductive VDJ alleles." J Immunol **177**(10): 7007-7014.

Martinez-Jean, C., G. Folch and M. P. Lefranc (2001). "Nomenclature and overview of the mouse (*Mus musculus* and *Mus sp.*) immunoglobulin kappa (IGK) genes." Exp Clin Immunogenet **18**(4): 255-279.

Matthews, A. G. and M. A. Oettinger (2009). "RAG: a recombinase diversified." Nat Immunol **10**(8): 817-821.

Melamed, D. and D. Nemazee (1997). "Self-antigen does not accelerate immature B cell apoptosis, but stimulates receptor editing as a consequence of developmental arrest." Proc Natl Acad Sci U S A **94**(17): 9267-9272.

Merrell, K. T., R. J. Benschop, S. B. Gauld, K. Aviszus, D. Decote-Ricardo, L. J. Wysocki and J. C. Cambier (2006). "Identification of anergic B cells within a wild-type repertoire." Immunity **25**(6): 953-962.

Nemazee, D. A. and K. Burki (1989). "Clonal deletion of B lymphocytes in a transgenic mouse bearing anti-MHC class I antibody genes." Nature **337**(6207): 562-566.

Nishi, M., T. Kataoka and T. Honjo (1985). "Preferential rearrangement of the immunoglobulin kappa chain joining region J kappa 1 and J kappa 2 segments in mouse spleen DNA." Proc Natl Acad Sci U S A **82**(19): 6399-6403.

Pasqualetto, V., F. Vasseur, F. Zavala, E. Schneider and S. Ezine (2005). "Fas receptor signaling is requisite for B cell differentiation." J Leukoc Biol **78**(5): 1106-1117.

Radic, M. Z., J. Erikson, S. Litwin and M. Weigert (1993). "B lymphocytes may escape tolerance by revising their antigen receptors." J Exp Med **177**(4): 1165-1173.

Radic, M. Z. and M. Weigert (1994). "Genetic and structural evidence for antigen selection of anti-DNA antibodies." Annu Rev Immunol **12**: 487-520.

Retter, M. W. and D. Nemazee (1998). "Receptor editing occurs frequently during normal B cell development." J Exp Med **188**(7): 1231-1238.

Retter, M. W. and D. Nemazee (1999). "Receptor editing: genetic reprogramming of autoreactive lymphocytes." Cell Biochem Biophys **31**(1): 81-88.

Rolink, A. G., J. Andersson and F. Melchers (1998). "Characterization of immature B cells by a novel monoclonal antibody, by turnover and by mitogen reactivity." Eur J Immunol **28**(11): 3738-3748.

Schatz, D. G. and Y. Ji (2011). "Recombination centres and the orchestration of V(D)J recombination." Nat Rev Immunol **11**(4): 251-263.

Schlissel, M., A. Constantinescu, T. Morrow, M. Baxter and A. Peng (1993). "Double-strand signal sequence breaks in V(D)J recombination are blunt, 5'-phosphorylated, RAG-dependent, and cell cycle regulated." Genes Dev **7**(12B): 2520-2532.

Seo, S. J., L. Mandik-Nayak and J. Erikson (2003). "B cell anergy and systemic lupus erythematosus." Curr Dir Autoimmun **6**: 1-20.

Shlomchik, M., M. Mascelli, H. Shan, M. Z. Radic, D. Pisetsky, A. Marshak-Rothstein and M. Weigert

773 (1990). "Anti-DNA antibodies from autoimmune mice arise by clonal expansion and somatic mutation."
 774 J Exp Med **171**(1): 265-292.
 775 Shlomchik, M. J. (2008). "Sites and stages of autoreactive B cell activation and regulation." Immunity
 776 **28**(1): 18-28.
 777 Shlomchik, M. J., C. W. Euler, S. C. Christensen and J. William (2003). "Activation of rheumatoid factor
 778 (RF) B cells and somatic hypermutation outside of germinal centers in autoimmune-prone MRL/lpr
 779 mice." Ann N Y Acad Sci **987**: 38-50.
 780 Shockett, P. E. and D. G. Schatz (1999). "DNA hairpin opening mediated by the RAG1 and RAG2
 781 proteins." Mol Cell Biol **19**(6): 4159-4166.
 782 Silverman, G. J., R. D. Goldfien, P. Chen, R. A. Mageed, R. Jefferis, F. Goni, B. Frangione, S. Fong and
 783 D. A. Carson (1988). "Idiotypic and subgroup analysis of human monoclonal rheumatoid factors.
 784 Implications for structural and genetic basis of autoantibodies in humans." J Clin Invest **82**(2): 469-475.
 785 Spanopoulou, E., C. A. Roman, L. M. Corcoran, M. S. Schlissel, D. P. Silver, D. Nemazee, M. C.
 786 Nussenzweig, S. A. Shinton, R. R. Hardy and D. Baltimore (1994). "Functional immunoglobulin
 787 transgenes guide ordered B-cell differentiation in Rag-1-deficient mice." Genes Dev **8**(9): 1030-1042.
 788 Sun, A., T. I. Novobrantseva, M. Coffre, S. L. Hewitt, K. Jensen, J. A. Skok, K. Rajewsky and S. B.
 789 Koralov (2015). "VH replacement in primary immunoglobulin repertoire diversification." Proc Natl
 790 Acad Sci U S A **112**(5): E458-466.
 791 Taki, S., M. Meiering and K. Rajewsky (1993). "Targeted insertion of a variable region gene into the
 792 immunoglobulin heavy chain locus." Science **262**(5137): 1268-1271.
 793 Taki, S., F. Schwenk and K. Rajewsky (1995). "Rearrangement of upstream DH and VH genes to a
 794 rearranged immunoglobulin variable region gene inserted into the DQ52-JH region of the
 795 immunoglobulin heavy chain locus." Eur J Immunol **25**(7): 1888-1896.
 796 Thiebe, R., K. F. Schable, A. Bensch, J. Brensing-Kuppers, V. Heim, T. Kirschbaum, H. Mitlohner, M.
 797 Ohnrich, S. Pourrajabi, F. Roschenthaler, J. Schwendinger, D. Wichelhaus, I. Zocher and H. G. Zachau
 798 (1999). "The variable genes and gene families of the mouse immunoglobulin kappa locus." Eur J
 799 Immunol **29**(7): 2072-2081.
 800 Tiegs, S. L., D. M. Russell and D. Nemazee (1993). "Receptor editing in self-reactive bone marrow B
 801 cells." J Exp Med **177**(4): 1009-1020.
 802 Tze, L. E., B. R. Schram, K. P. Lam, K. A. Hogquist, K. L. Hippen, J. Liu, S. A. Shinton, K. L. Otipoby,
 803 P. R. Rodine, A. L. Vegoe, M. Kraus, R. R. Hardy, M. S. Schlissel, K. Rajewsky and T. W. Behrens
 804 (2005). "Basal immunoglobulin signaling actively maintains developmental stage in immature B cells."
 805 PLoS Biol **3**(3): e82.
 806 Ueda, Y., D. Liao, K. Yang, A. Patel and G. Kelsoe (2007). "T-independent activation-induced cytidine
 807 deaminase expression, class-switch recombination, and antibody production by immature/transitional 1
 808 B cells." J Immunol **178**(6): 3593-3601.
 809 Wang, Y. H., R. P. Stephan, A. Scheffold, D. Kunkel, H. Karasuyama, A. Radbruch and M. D. Cooper
 810 (2002). "Differential surrogate light chain expression governs B-cell differentiation." Blood **99**(7):
 811 2459-2467.
 812 Wardemann, H., S. Yurasov, A. Schaefer, J. W. Young, E. Meffre and M. C. Nussenzweig (2003).
 813 "Predominant autoantibody production by early human B cell precursors." Science **301**(5638):
 814 1374-1377.
 815 Watanabe-Fukunaga, R., C. I. Brannan, N. G. Copeland, N. A. Jenkins and S. Nagata (1992).
 816 "Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis."

Nature **356**(6367): 314-317.

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**(3): 317-327.

Acknowledgements

This work was supported by grants from National Science Foundation (NSF 81202309), Tianjin City Foundation of Basic and Advanced Research (13JCQNJC11200), Initiation Program of Nankai University, and the New Teacher Fund from the Ministry of Education (20120031120057) to F. L. This work was also supported by the National Basic Research Program of China 973 Program (No. 2013CB967201) and NSF 81470354 to R. X.

Author Contributions

F. L. and S. L. designed research, performed experiments, interpreted data, prepared figures and wrote the manuscript. W. L. designed the primers for LM-PCR experiments. S. Z. and Y. L. cloned the plasmids. M. H. performed FACS sorting. W. Y. and Y. W. provided reagents. T. C., R. Y. and R. X. revised the manuscript.

Additional Information

The online version of this article contains supplemental material.

Competing financial interests:

The authors declare no conflict of interest.

Figure Legend

Fig. 1. Distribution of B cell lymphocyte subsets in the bone marrow of autoimmune MRL/MpJ-Fas^{lpr} and B6.MRL-Fas^{lpr} mice. (A-C) Bone marrow B cells with a pro-B phenotype (B220⁺CD43⁺IgM⁺IgD⁻Lin⁻), a small pre-B phenotype (B220⁺CD43⁻IgM⁺IgD⁻Lin⁻) and an immature B phenotype (B220⁺CD43⁻IgM⁺IgD⁺Lin⁻) were sorted from MRL/MpJ-Fas^{lpr} (A) and B6.MRL-Fas^{lpr} (B) mice. C3H mice (C) were used as the control. The percentage of cells in each gate is shown. The data are representative of five independent experiments.

Fig. 2. Continuous secondary rearrangement at the κ light chain loci and amplification of intermediates of replaced primary rearrangement.

Schematic diagram of the mouse κ light chain locus illustrating continuous, *de novo* V $\rightarrow$ J κ rearrangement. Two pathways of secondary V κ J κ rearrangements which contain one J κ intron are available. The first (A) involves recombination of an upstream unrearranged V κ gene segment to downstream J κ 2 to replace primary V κ J κ 1 recombination. In contrast, the second (B) utilizes downstream J κ 5 to rearrange upstream V κ gene segment. Both result in the deletion or inversion of intermediate V κ J κ 1-J κ 2 SE or V κ J κ 4-J κ 5 SE genomic fragments. Exons are indicated by boxes, 12- or 23-spacer recombination signals are indicated by white or black triangles, and folded lines with arrows illustrate the rearrangements. (C) LM-PCR assay was used to amplify the intermediate fragments which contain replaced primary V κ J κ rearrangement extending to downstream J κ SE. Artificial linker primers are indicated by parallel lines, and degenerate V κ family primers are shown by straight lines.

Fig. 3 Cleavage of ongoing secondary Ig κ rearrangement detected only in small pre-B and immature B cells from autoimmune Fas^{lpr} and control C3H mice. The LM-PCR for secondary rearrangements in V κ 12, V κ RF, V κ 4, V κ 19 and V κ 23 gene families demonstrates the lineage and developmental restriction of V κ J κ -to-J κ SEs, and confirms the purity of the sorted cell populations. The cleavages of V κ J κ -to-J κ SEs were detected only in small pre-B and immature B cells from autoimmune MRL/MpJ-Fas^{lpr} (A), B6.MRL-Fas^{lpr} (B) and control C3H mice (C). (D) CD14 PCR demonstrated the equivalence of the genomic template.

Fig. 4. Replaced primary V κ J κ joints in ongoing secondary Ig κ rearrangements from FAS^{lpr} and C3H progenitor B cells. (A) Percentages of productive (P) and nonproductive (nP) recombination products recovered in pre-B and immature B cells from FAS^{lpr} or C3H mice. (B) Percentages of P and nP replaced primary Ig κ rearrangements with permissive or non-permissive autoreactivity in pre- and immature B cells from FAS^{lpr} or C3H mice. The replaced V κ J κ rearrangements were categorized by permissive or non-permissive V κ families, which confer anti-DNA or non-self reactivity when associated with 3H9 IgH. The sample sizes are listed in parentheses, and the nP/P ratios are listed above each set of bars.

Fig. 5. Somatic mutation in FAS^{lpr} and C3H B cells. The frequencies of the mutated primary V κ J κ replacements (A), and distributions of the numbers of point mutations in each sequence (B) from the bone marrow small pre-B and immature B cells from MRL/MpJ-Fas^{lpr} (n=80, 100), B6.MRL-Fas^{lpr} (n=75, 54), and C3H mice (n=60, 60) are shown, respectively. (C) Percentages of P and nP replaced primary Ig κ rearrangements in FAS^{lpr} and C3H B progenitor cells when the recovered sequences not including somatic mutation were re-analyzed.

A **B** **C**

Figure 1 S. L.

Figure 2 S. L.

A

B

Figure 4 S. L.

A **B** **C** **Figure 5 S. L.**

1 **Table 1.** *J κ gene usage in productive, nonproductive, and total MRL/MpJ-Fas^{lpr}, B6.MRL-*
2 *Fas^{lpr} and C3H V κ J κ replaced primary rearrangements^a*
3

Strain		J κ 1			J κ 4		
		P	NP	total	P	NP	Total
MRL/MpJ-Fas ^{lpr}	small pre-B	22 (27.5%)	41 (51.3%)	62	5 (6.2%)	13 (16.3%)	18
	immature B	29 (29%)	50 (50%)	79	7 (7%)	14 (14%)	21
B6.MRL-Fas ^{lpr}	small pre-B	21 (28%)	34 (45.3%)	55	3 (4%)	17 (22.7%)	20
	immature B	15 (27.8%)	28 (51.9%)	43	2 (3.7%)	9 (16.7%)	11
C3H	small pre-B	14 (23%)	28 (47%)	42	6 (10%)	12 (20%)	18
	immature B	16 (26.7%)	24 (40%)	40	3 (5%)	17 (28.3%)	20

4
5 ^a Values in parentheses indicate the percent of productive vs nonproductive primary
6 rearrangements for J κ gene segment used.
7
8

Table 2. Frequency of MRL/MpJ-Fas^{lpr}, B6.MRL-Fas^{lpr} and C3H VκJκ rearrangements with homologous junctions and N or P nucleotide additions^a

Strains	Cell types	Total number of rearrangements analyzed	Number of rearrangements with homologous junctions	Number of rearrangements with N nucleotides	Number of rearrangements with P nucleotides
MRL/MpJ-Fas ^{lpr}	small pre-B	80	57 (71.3%; <i>p</i> =0.66)	10 (12.5%; <i>p</i> =0.30)	13 (16.3%; <i>p</i> =0.68)
	immature B	100	72 (72%; <i>p</i> =0.50)	18 (18%; <i>p</i> =0.08)	10 (10%; <i>p</i> =0.57)
B6.MRL-Fas ^{lpr}	small pre-B	75	59 (78.7%; <i>p</i> =0.95)	5 (6.7%; <i>p</i> =0.73)	11 (14.7; <i>p</i> =0.85)
	immature B	54	46 (85.2%; <i>p</i> =0.99)	6 (11.1%; <i>p</i> =0.66)	2 (3.7%; <i>p</i> =0.80)
C3H	small pre-B	60	48 (80%)	4 (6.7%)	8 (13.3%)
	immature B	60	51 (85%)	4 (6.7%)	5 (8.3%)

^a The values in parentheses indicate the percentage of homologous junctions and N or P nucleotide additions for each B-cell type from three mouse strains.

Table 3. Comparison of CDR3 sequences and arginine contents for productive primary *Igκ* rearrangements

Strains	Cell types	CDR3 length (amino acids)	Arginine content per CDR3
MRL/MpJ-Fas ^{lpr}	small pre-B	9.0 ± 0.70	0.19 ± 0.40
	immature B	8.9 ± 0.60	0.36 ± 0.49
B6.MRL-Fas ^{lpr}	small pre-B	9.1 ± 0.28	0.29 ± 0.46
	immature B	9.0 ± 0.00	0.24 ± 0.44
C3H	small pre-B	9.1 ± 0.62	0.30 ± 0.47
	immature B	8.8 ± 0.60	0.37 ± 0.60

Table 4. *V_κ cRS cleaved in small pre-B cells from MRL/MpJ-Fas^{lpr} and C3H mice*

cRS Position	V _κ gene	cRS sequence				Mouse strain
211-249	IGKV4-59	TCTGGGACCTCTTAC	TCTCTCACA	ATCAGCAGCATGGAGGCTGAAGA	TACTGCC	MRL/MpJ- C3H
231-269	IGKV4-80	CACAATCAGCAGTGT	GGAGGCTGA	AGATGCTGCCGATTATTACTGCC	ATCAGTG	MRL/MpJ- C3H
239-277	IGKV4-81	CAATCAGCAGCATGG	AGGCCGAGG	ATGCTGCCACTTATTACTGCCAG	CAGTGGA	MRL/MpJ- C3H
196-234	IGKV5-39	AGGTTCAAGTGGCAGT	GGATCAGGG	TCAGATTCACTCTCAGTATCAA	CAGTGTG	MRL/MpJ- C3H
236-274	IGKV5-43	GTATCAACAGTGTGG	AGACTGAAG	ATTTTGAATGTATTTCTGTCAA	CAGAGTA	MRL/MpJ- C3H
212-250	IGKV6-15	GATCTGGGACAGATT	TCACTCTCA	CCATCAGCAATGTGCAGTCTGAA	GACTTGG	C3H
227-265	IGKV12-46	ATTCCCTCAAGATCA	ACAGCCTGC	AGTCTGAAGATTTTGGGAGTTAT	TACTGTC	C3H

The positions and sequences of V_κ 23-cRS SEs recovered from small pre-B cells sorted from bone marrow of MRL/MpJ-Fas^{lpr} and C3H mice are listed. Recovered V_κ SE LM-PCR products were cloned and sequenced. Ligation of the BW-linker directly to the cRS heptamer confirmed RAG1/2-mediated cleavage. The sequences were analyzed by the IMGT database and NCBI Immunoglobulin Blast (<http://www.ncbi.nlm.nih.gov/projects/igblast/>) for germline gene identification. Heptamer and nonamer sequences of V_κ 23-cRS are in bold. Nucleotide positions using NCBI numbering indicate the location of the cRS site.