A Human Immunoglobulin \( \lambda \) Locus Is Similarly Well Expressed in Mice and Humans

By Andrei V. Popov, Xiangang Zou, Jian Xian, Ian C. Nicholson, and Marianne Brüggemann

Summary

Transgenic mice carrying a 380-kb region of the human immunoglobulin (Ig) \( \lambda \) light (L) chain locus in germline configuration were created. The introduced translocus on a yeast artificial chromosome (YAC) accommodates the most proximal Ig\( \kappa \) variable region (V) gene cluster, including 15 \( \kappa \)L genes that contribute to >60% of \( \lambda \) L chains in humans, all \( \lambda \)-C\( \lambda \) segments, and the 3' enhancer. HuIgLAC mice were bred with animals in which mouse Ig\( \kappa \) production was silenced by gene targeting. In the \( \kappa ^{—/+} \) background, human Ig\( \kappa \) was expressed by \(~84\)% of splenic B cells. A striking result was that human Ig\( \kappa \) was also produced at high levels in mice with normal \( \kappa \) locus. Analysis of bone marrow cells showed that human Ig\( \kappa \) and mouse Ig\( \kappa \) were expressed at similar levels throughout B cell development, suggesting that the IgL translocus and the endogenous \( \kappa \) locus rearrange independently and with equal efficiency at the same developmental stage. This is further supported by the finding that in hybridomas expressing human Ig\( \kappa \), the endogenous L chain loci were in germline configuration. The presence of somatic hypermutation in the human V\( \lambda \) genes indicated that the Ig\( \lambda \)-expressing cells function normally. The finding that human \( \lambda \) genes can be utilized with similar efficiency in mice and humans implies that L chain expression is critically dependent on the configuration of the locus.

Key words: human Ig\( \lambda \) translocus • light chain expression levels • pre-B cell activation • V gene usage • hypermutation

The light chain component of the Ig protein is encoded by two separate loci, Ig\( \kappa \) and Ig\( \lambda \). The proportion of antibodies containing \( \kappa \) or \( \lambda \) L chains varies considerably between different species (1–3); in mice the \( \kappa /\lambda \) ratio is 95:5, compared with 60:40 in humans. Two models exist to account for the dominance of Ig\( \kappa \) expression in the mouse. From the observations that murine Ig\( \lambda \)-producing myelomas have rearranged \( \kappa \) L chain genes, whereas Ig\( \kappa \)-producing cells have the \( \lambda \) L chain locus in germline configuration, it was proposed initially that \( \kappa \) rearrangement must occur before \( \lambda \) rearrangement can begin (4, 5). Although the same observation applies for human B cells, the proportions of \( \kappa \)- and \( \lambda \)-producing cells are similar (4), suggesting that other factors are involved. The second proposal is that \( \kappa \) and \( \lambda \) loci are equally available for rearrangement at the same time, but the mouse \( \kappa \) locus is more efficient at engaging the rearrangement process (for review, see reference 6). The occasional finding of cells with rearranged \( \lambda \) and the \( \kappa \) locus in germline configuration may support this (5, 7, 8). Any influence of antigen selection on the biased \( \kappa /\lambda \) ratio is discounted by the finding that the ratio is similar in fetal liver and in cells that have not encountered antigen (9–13).

L chain V-J rearrangement occurs at the transition from pre-B-II to immature B cells, where the surrogate L chain associated with membrane Ig\( \kappa \) is replaced by \( \kappa \) or \( \lambda \) (14). Although the timing of L chain rearrangement is essentially defined, the processes that activate L chain locus rearrangement are not fully understood. From locus-silencing experiments, it is apparent that \( \kappa \) rearrangement is not a prerequisite for \( \lambda \) recombination (15), but instead that \( \kappa \) and \( \lambda \) rearrangements are independent events (16), the activation of which may be affected by differences in the strength of the respective enhancers. Targeted deletion of the \( \kappa \) 3' enhancer in transgenic mice showed that this region is not essential for \( \lambda \) locus rearrangement or expression but is required for establishing the \( \kappa /\lambda \) ratio (17). A region that may regulate the accessibility of the human \( \lambda \) locus has been identified \( \sim 10 \) kb downstream of C\( \lambda \)7 (18, 19). Functional analysis using reporter gene assays identified a core enhancer region flanked by elements that can drastically reduce enhancer activity in pre-B cells (18). Although transfection studies showed that the \( \kappa \) and \( \lambda \) 3' enhancer

A.V. Popov and X. Zou contributed equally to this work.

A. V. Popov is supported by a Wolfson Research Fellowship, and X. Zou is supported by a Searle Scholar Pre-doctoral Fellowship.
regions appear to be functionally equivalent, the core enhancer motifs are flanked by functional sequences that are remarkably dissimilar. The human Igκ locus on chromosome 22q11.2 is 1.1 Mb in size and typically contains 70 Vκ genes and 7 Jκ-Cλ segments (20, 21). Approximately half of the Vκ genes and Jκ-CλA, 2, 3, and 7 are regarded as functional. The Vκ genes are organized in three clusters, with the members of a particular Vκ gene family contained within the same cluster. There are 10 Vκ gene families, with the largest, VκIII, having 23 members. In human peripheral blood lymphocytes, Vκ gene segments of families I, II, and III from the J-C proximal cluster A are preferentially rearranged, with the contribution of the 2a2 Vκ segment (2–14 using a position-based nomenclature; reference 22) being unusually high (23). All λ gene segments have the same polarity that allows deletional rearrangement (24). The diversity of the Igκ repertoire is provided mainly by Vκ-Jκ combination. Additional CDR3 diversity due to N (nonencoded) or P (palindromic) nucleotide additions at the Vκ to Jκ junction is present in human sequences, although not as extensively as in IgH rearrangement, but is absent in sequences from mice (25–28), where the TdT (terminal deoxynucleotidyl transferase) activity is downregulated (29). Expression of a Human Igκ Translocus in Mice.

Here we have introduced a 410-kb yeast artificial chromosome (YAC), which contains most of the Vκ genes of cluster A and all the Jκ-Cλ segments in germline configuration, into mice that have one or both endogenous Igκ alleles disrupted. The translocus shows high expression in both backgrounds, and is able to compete equally with the endogenous mouse κ locus.

Materials and Methods

The HuIgκYAC, Introduction into Embryonic Stem Cells, and Derivation of Transgenic Mice. The 410-kb HuIgκYAC, accommodating a 380-kb region (Vκ-Jκ-Cλ) of the human κ L chain locus with Vκ, Jκ, and C genes in germline configuration, was constructed as previously described (29). To allow selection, two copies of the neomycin resistance gene (Neo−) were site-specifically integrated into the ampicillin gene on the left (centromeric) YAC arm. YAC-containing yeast cells were fused with H-1 embryonic stem (ES) cells (a gift from D. Melton, Department of Pathology, University Medical School, Edinburgh, UK), as previously described (30), and G418-resistant colonies were picked and analyzed 2–3 wk after protoplast fusion. ES cells containing a complete HuIgκYAC copy, Breeding with Balb/c mice resulted in germline transmission. Further breeding with κ−/− mice (32) established the lines for analysis.

Southern Blot Analysis. Conventional DNA was obtained (33) or high molecular weight DNA was prepared in agarose blocks (34). For the preparation of testis DNA, tissues were homogenized and passed through 70 μm nylon mesh. Pulsed-field gel electrophoresis (PFGE) conditions to separate in the 50–900 kb range were 1% agarose, 180 V, 25 s switch time, and 3 h running time at 3.5°C. Hybridization probes were Cκ2+3 and the left YAC arm probe (LA) comprising LYS2 (29).

Hybridoma Production and ELISA Assay. Hybridomas were obtained from 3-mo-old HuIgκYAC/C+−/− animals by fusion of splenocytes with NS0 myeloma cells (35). After fusion, cells were plated on 96-well plates such as to obtain single clones. Human and mouse antibody production was determined in sandwich ELISA assays (36) on Maxisorp plates (Nalge Nunc, Denmark). For the detection of human or mouse Igκ, coating reagents were a 1:500 dilution of anti-human κ L chain mAb H-P 6054 (L 6522; Sigma Chemical Co.) or a 1:500 dilution of the 2.3 mg/ml rat anti-mouse κ L chain mAb (L 2280; Sigma Chemical Co.), respectively. Respective binding was detected with biotinylated antibodies polyclonal anti-human κ (B 0900; Sigma Chemical Co.), a 1:1,000 dilution of polyclonal anti-mouse κ (R PN 1178; Amsham International) or rat anti-κ Igκ (N.o. 021172D; Pharmingen) followed by streptavidin-conjugated horseradish peroxidase (Amersham International). Mouse IgG2a κ myeloma protein from HPC-1 (M 6034; Sigma Chemical Co.) and human serum Igκ (10414; Sigma Chemical Co.) were used to standardize the assays. To determine mouse κ L chain levels, plates were coated with a 1:1,000 dilution of rat anti-κ, κ, clone EM 34.1 (K 2123; Sigma Chemical Co.), and bound Igκ was detected using biotinylated rat mAb anti-mouse Igκ (Cat. No. 04-6640; Zymed). Mouse myeloma proteins IgG2a κ and IgG1 κ (UPC10, M 9144, and M O PC 21, M 9269; Sigma Chemical Co.) were used as standards. For detection of mouse Igκ, plates were coated with polyclonal anti-κ (μ, The Binding Site, U K) and bound Igκ was detected with biotinylated goat anti-mouse μ (R PN 1176; Amsham International) followed by streptavidin-conjugated horseradish peroxidase. Mouse plasmacytoma TEPC183, IgM κ (M 3795; Sigma Chemical Co.) was used as a standard.

Flow Cytometry Analysis. Cell suspensions were obtained from bone marrow, spleen, and Peyer's patches (PPs). Multicolor staining was then carried out with the following reagents in combinations (illustrated in Fig. 4); FITC-conjugated anti-human κ (F 5266; Sigma Chemical Co.), PE-conjugated anti-mouse c-kit (CD117) receptor (clone ACK45, cat. No. 09995B; Pharmingen), PE-conjugated anti-mouse CD25 (IL-2 receptor) (clone 3C7, P 3317; Sigma Chemical Co.), biotin-conjugated anti-human κ (clone G20-193, cat. No. 08172D; Pharmingen), biotin-conjugated anti-mouse CD19 (clone 1D3, cat. No. 09654D; Pharmingen), followed by Streptavidin-Quantum red (S 2899; Sigma Chemical Co.) or Streptavidin-PerCP (Cat. No. 340130; Becton Dickinson) and rat monoclonal anti-κ L chain (clone M R C -O X-20, Cat. M CA 152; Serotec, U K) coupled according to the manufacturer's recommendations with allopoxycyanin (APC) (P25C; ProZyme). Data were collected from 106 stained cells on a FACS Calibur® flow cytometer (Becton Dickinson) as previously described (32). Cells were first gated on forward and side scatter to exclude dead cells. To obtain accurate percentage distribution for comparison, cells from normal mice were stained in parallel. In addition, human peripheral blood lymphocytes were purified on Ficoll gradients (1.077 g/ml) and stained with PE-conjugated anti-human CD19 antibody (P 7437, clone SJ25-C1; Sigma Chemical Co.), biotinylated anti-human κ followed by Streptavidin-Quantum red, and FITC-conjugated anti-human κ antibodies as above.

For reverse transcriptase (RT)-PCR cloning of Vκ genes, PP cells were stained with FITC-conjugated peanut agglutinin (PNA)
The unique NotI restriction site is indicated. Probes to assess the integrity of the HuIg

50 mM Tris-HCl, pH 7.4, and 10 mM MgCl2, with 100

mutated) found by RT-PCR in spleen and sorted PP cells from HuIg

amplification of 5

Dickinson) as previously described (32) and 5

B220 antibodies (P 3567; Sigma Chemical Co.). Double-positive

was sorted on the FACStarPlus flow cytometer (Becton

prepared as previously described (37) and for cDNA preparation

verse transcriptase (GIBCO BRL) was used at 46

was primed with oligo(dT)22, and 100 U of Super Script II re-

1 ml G-50 equilibrated with TE (10 mM Tris-HCl, pH 7.8, and

inhibitor (Promega). The DNA/RNA duplex was passed through

8

8

5-

5

9

7

V

1613 Popov et al.

RACE of V

Pr1, 5-

V

RACE Products. Spleen RNA was

prepared as previously described (37) and for cDNA preparation

2–3

sites. Oligonucleotide for 5

five cycles with primers Pr2 and Pr4 to allow cloning into EcoRI

YAC was introduced into ES cells by protoplast fusion (30) and chimeric mice were produced by blastocyst

insertion with probes to the 3’ end of the construct, identifying the C\lambda2+3 regions, and to the left centromeric YAC

arm at the 5’ end, identifying the LYS2 gene (data not shown). Germline transmission was obtained, and PFGE analysis of testis DNA from one animal is illustrated in Fig. 2. A NotI fragment larger than 380 kb is necessary to accommodate this region of the HuIgYAC, and the 450-kb band obtained indicates random integration involving the single NotI site 3’ of J\lambda-Ca and a NotI site in the mouse chromosome. Digests with EcoRI/HindIII and hybridization with the C\lambda2+3 probe further confirmed the integrity of the transferred HuIgYAC (Fig. 2). The results indicated that one complete copy of the HuIgYAC was integrated in the mouse genome.

Human Ig\lambda Expression Is Dominant in Mouse k-/- Animals. To assess the human \lambda chain repertoire for the production of authentic human antibodies, the HuIgYAC mice were bred with mice in which endogenous Ig\kappa production was silenced by gene targeting (32). In these k-/- mice, the mouse Ig\kappa titers are elevated compared with k+/+ strains (32, 41). Serum titrations (Fig. 3) showed that human Ig\lambda antibody titers in HuIgYAC/k-/- mice are between 1 and 2 mg/ml, which is up to 10-fold higher than average mouse Ig\kappa levels. Interestingly, in the HuIgYAC/k-/- mice, the mouse Ig\kappa production returned to levels similar to that found in normal mice. High numbers of human Ig\lambda+
cells were also identified in flow cytometric analysis of splenic B cells from HuIg\textsubscript{L} YAC/\textk2/2 mice (Fig. 4 A), with human $\lambda$ expressed on the surface of ~84% of the B cells and mouse Ig\textk expressed on <5% (data not shown).

**Human Ig\textsubscript{L} Expression Equals Mouse Ig\textk Production.** Assessment of human Ig\textsubscript{L} production in heterozygous HuIg\textsubscript{L} YAC/\textk1/2 mice allowed a detailed comparison of expression and activation of endogenous versus transgenic L chain loci present at equal functional numbers. Serum analysis (Fig. 3) of mice capable of expressing both human $\lambda$ and mouse $\kappa$ showed similar titers for human and mouse L chains. Human Ig\textk levels in HuIg\textsubscript{L} YAC/\textk1/2 transgenic mice were similar to those in HuIg\textsubscript{L} YAC/\textk2/2 mice. Total Ig levels in HuIg\textsubscript{L} YAC/\textk1/2 mice were 1–2 mg/ml, with an average contribution of ~51% mouse Ig\textk, 43% human Ig\textk, and 6% mouse Ig\textl. As is also seen in human serum, the analysis of individual HuIg\textsubscript{L} YAC/\textk2/2 animals showed there were variations in the $\lambda$/k ratios. Three of the HuIg\textsubscript{L} YAC/\textk2/2 mice produced somewhat higher $\kappa$ levels, whereas in two mice the human $\lambda$ levels were higher than the Ig\textk titers. In HuIg\textsubscript{L} YAC/\textk2/2 mice, high translocus expression was also found in B220$^+$ peripheral blood lymphocytes. In HuIg\textsubscript{L} YAC/\textk2/2 mice, which carry a wild-type $\kappa$ locus, the levels of Ig\textk
are ~25% and endogenous κ levels are ~60% (Fig. 4 A). It is likely that these differences in expression levels are dependent on the number of active gene loci.

To assess the developmental stage at which the high contribution of the human λ translocus becomes established, we examined surface L chain expression by bone marrow cells of HuIg/YAC/κ<sup>+</sup>/κ<sup>+</sup> mice. For this, B cell lineage marker CD19 and specific antibodies to human λ and mouse κ were used in four-color staining with the early B cell markers c-kit (CD117) and CD25. Fig. 4 B shows that surface L chain expression (human λ or mouse κ) was detectable on a similar small proportion of B cells at each of these stages of development, which suggests that human and mouse λ chain rearrangements are simultaneous. The specificity of the staining detecting mouse κ and human λ on small numbers of early B cells, which has been reported independently (42), was verified by the absence of similar positive cells in the analysis of bone marrow from control mice (data not shown).

DNA Rearrangement and Diversification of a Highly Active Human λ Translocus. To further clarify the potential of the L chain translocus to contribute to the antibody repertoire, we analyzed human λ and mouse κ L chain production using individual hybridoma clones from HuIg/YAC/κ<sup>+</sup> mice. Results from two fusions suggest that human λ and mouse κ L chain–producing cells were present in the spleen of HuIg/YAC/κ<sup>+</sup> mice at similar frequencies. Furthermore, in the hybridomas the amounts of human Ig (2–20 μg/ml) or mouse Ig (4–25 μg/ml) were very similar. To determine whether Igκ rearrangement precedes Igλ, as found in mice and humans (4, 5), the configuration of the endogenous Igκ and the human λ translocus were analyzed in these hybridomas. Southern blot hybridization of randomly picked hybridoma clones showed that in 11 human Igκ expressers, 7 had the mouse κ locus in germline configuration, 1 clone had mouse Igκ rearranged, and 3 clones had the mouse κ locus deleted, whereas in 19 mouse Igκ expressers, all but 2 had the human Igκ locus in germline configuration. This result suggests that there is no locus activation bias and further emphasizes that the human λ translocus performs with similar efficiency as the endogenous κ locus.

The capacity of the human λ locus to produce a diverse antibody repertoire is further documented by the V-J rearrangement. Sequences were isolated from spleen and PP cells by 5′RACE PCR amplification to avoid bias from specific V gene primers. The use of individual Vλ genes is indicated by the triangles in Fig. 1, and shows that a substantial proportion of the Vλ genes on the translocus are being used in productive rearrangements, with Vλ3-1 and Vλ3-10 being most frequently expressed. In Vλ-Jλ rearrangements, Jλ2 was used preferentially and Jλ3 and 1 were used less frequently, whereas, as expected, Jλ4, 5, and 6 were not used as they are adjacent to ηCs. Extensive variability due to N or P sequence additions, which is found in human but not mouse L chain sequences (25, 27, 28), was not observed. Sequences obtained by RT-PCR from FACSort®-sorted PP germinal center B cells (B220<sup>+</sup>/PNA<sup>+</sup>) revealed that somatic hypermutation is operative in HuIgλ/YAC mice (Fig. 5). We identified 11 unique Vλ-Jλ rearrangements with two or more changes in the V region, ex-

Figure 5. Hypervaried human Vλ sequences from sorted B220<sup>+</sup> and PNA<sup>+</sup> PP B cells from HuIgλ/YAC/κ<sup>+</sup> mice. The sequences are representative selection of the functional Vλ-Jλ rearrangements (indicated by the triangles in Fig. 1) isolated from RT-PCR.
cluding the CDR 3, which may be affected by Vλ-κ rearrangement. The majority of mutations lead to amino acid replacements, but there was no preferential distribution in CDR 1 and CDR 2.

Discussion

The ratio of λ to κ L chain expression varies considerably between different species (1–3, 43, 44), and in mice the low λ L chain levels are believed to be a result of inefficient activation of the mouse λ locus during B cell differentiation (for review see reference 6). The Igλ (≈40%) to Igκ (≈60%) ratio in humans is more balanced and suggests that both λ and κ play an equally important role in immune responses. This is supported by the finding that the mouse Vλ genes are most similar to the less frequently used distal human Vλ gene families, whereas no genes comparable to the major contributors to the human Vλ repertoire are present in the mouse locus (40). With the HuIgYAC, these Vλ genes are available, and are able to make a significant contribution to the antibody repertoire. The 410-kb HuIgYAC translocus accommodates V gene region cluster A containing at least 15 functional Vλ genes (see Fig. 1). In humans, cluster A is the main contributor to the λ antibody repertoire, with Vλ 2-14 (2a2) expressed most frequently at 27% in blood lymphocytes (23). We also find expression of Vλ 2-14 in the translocus mice, but the main contributors to the λ L chain repertoire were 3-1 (the Vλ gene most proximal to the J-C region) and 3-10, both of which are expressed at ≈3% in humans. Although the validity of conclusions about the contribution of different genes is dependent on the numbers examined, the overexpression of Vλ3-1 (11 sequences) and Vλ3-10 (8 sequences) in the 31 sequences obtained may imply that rearrangement or selection preferences are different in mice and humans. Analysis of recombination signal sequences (RSS) in mouse L chains showed that κ and λ RSS differ significantly, and that those genes with the highest similarity to consensus RSS rearrange most frequently (45). The RSS of Vλ3-1 and Vλ3-10 show a 100% match with the mouse consensus sequence, which may explain their frequent expression in the translocus mice. In addition, most human Vλ R SS match the established consensus sequence significantly better than mouse Vλs (21, 45).

We found extensive somatic hypermutation of many rearranged human Igλ sequences, indicating that they are able to participate in normal immune responses. The levels of mutation in B220+/PNA+ PP cells from HuIgYAC translocus mice were similar to what has been reported for mouse L chains (46). Rather unexpected was the pattern of somatic hypermutation with similar numbers of silent and replacement point mutations found in the complementarity-determining and framework regions. Somatic hypermutation is usually associated with a higher level of replacement mutations in CDRs and more silent mutations in the framework regions, and the distribution observed here may argue against efficient antigen selection having taken place. Interestingly, however, λ L chain sequences obtained from human peripheral blood lymphocytes also showed high numbers of mutations in framework 2 (23). Part of framework 2 lies at the interface of the Vλ and Vμ domains and it has been suggested that this region may be important for optimal H and λ L chain interaction and, in particular, interaction of the human λ L chain and the endogenous mouse H chain (26).

In the mouse, unlike in humans, L chain diversification due to untemplated nucleotide addition is essentially absent, because TdT expression has been downregulated by the stage at which L chain rearrangement takes place (28, 47). This concept is challenged by the observation that mouse L chain rearrangement can occur at the same time as Vμ to DJμ rearrangements (48) or even earlier (42). Our results also show L chain rearrangement at the pre-B cell stage with a similar number of human λ- or mouse κ-expressing B cells also expressing c-kit+ or CD25+ (see Fig. 4). Although the cell numbers are small, the results suggest that there is no preferential activation of either the human λ translocus or the endogenous κ locus. However, despite this early activation, there is no accumulation of N or P nucleotide diversity in the rearranged human λ L chains, unlike rearranged λ L chains from human peripheral B cells (27). The small number (<1%) of human Igλ+/mouse Igκ+ double positive spleen and bone marrow cells may indicate that haplotype exclusion at the L chain level is less strictly controlled than is IgH exclusion (49).

In transgenic mice carrying Ig regions in germline configuration on minigene constructs, efficient DNA rearrangement and high antibody expression levels are rarely achieved. Competition with the endogenous locus can be eliminated using Ig knockout strains, in which transgene expression is usually improved (50). Poor transloci expression levels could be a result of the failure of human sequences to work efficiently in the mouse background or, alternatively, of the absence of locus-specific control regions that are more likely to be included on larger transgenic regions (51–53). Recently we addressed this question in transgenic mice by the introduction of different sized minigene- and YAC-based human κ L chain loci (53). The result showed that neither the size of the V gene cluster nor the V gene numbers present were relevant to achieving high translocus expression levels. The YAC-based loci contained downstream regions of the human κ locus, and it is possible that the presence of an undefined region with cis-controlled regulatory sequences may have been crucial in determining expressibility and subsequently L chain choice. The HuIgYAC contains equivalent regions from the human Igκ locus which may promote the use of the translocus in the L chain repertoire. Hybridomas from HuIgYAC/κ+ mice show no evidence for a bias in L chain locus selection during development, as demonstrated by the absence of rearrangement of the nonexpressed locus. This is in contrast with what is seen in Ig expressing mouse and human B cell clones (4, 5), and supports the model that λ and κ rearrangements are indeed independent (15, 54) and that poor Igκ expression levels in mice may be the result of inefficient signals acting during recombination (16). A possi-
ble signal that initiates L chain recombination has been identified through gene targeting experiments where the 3' k enhancer was deleted (17). In these mice, the k/λ ratio was reduced from 20:1 in normal mice down to 1:1, and the k locus was largely in germline configuration in λ-expressing cells, as we also see in the HuIgYAC+/k+/− hybridoma clones. The high level of human Igκ expression in the HuIgYAC+/k+/− mice could be due to the strength of the downstream enhancer of the human λ locus. An analysis of human L chain enhancer activities identified three synergistic modules at the 3' end of the λ locus which constitute a powerful pre-B cell specific enhancer that appears to be stronger than the corresponding k enhancer (55). Analysis of the mouse L 3' enhancer suggests the biased k/λ ratio in mice may be a direct result of the differences in locus specific regulation provided by the respective enhancers (19, 56). The results suggest that strength and ability of the human 3' λ enhancer to function in the mouse background may be the reason that λ and k loci can compete equally at the pre-B cell stage to initiate L chain rearrangement, resulting in the similar levels of human Igκ and mouse Igλ seen in the HuIgYAC+/k+/− mice.

We thank Drs. I. Tomlinson, G. Winter, and O. Ignatovich for access to their database of human Vκ sequences and helpful discussions. We are grateful to Drs. D. Melton for provision of the HM-1 ES cells, E. Corps for hybridoma production, B. Goyenechea for help with the Southern hybridization, and N. Miller for helping with the flow cytometry.

This work was supported by the Biotechnology and Biological Sciences Research Council and the Babraham Institute.

Address correspondence to Marianne Brüggemann, Laboratory of Developmental Immunology, Department of Development and Genetics, The Babraham Institute, Babraham, Cambridge CB2 4AT, UK. Phone: 44-1223-496-304; Fax: 44-1223-496-030; E-mail: marianne.bruggemann@bbsrc.ac.uk

Received for publication 14 December 1998 and in revised form 8 March 1999.

References

1. Hood, L., W.R. Gray, B.G. Sanders, and W.Y. Dreyer. 1967. Light chain evolution: antibodies. Cold Spring Harb Symp. Quant. Biol. 32:133–146.
2. McIntire, K.R., and A.M. Rousseau. 1970. Mouse immunoglobulin light chains alterations of k/λ ratio. Fed. Proc. 19:704.
3. Arun, S.S., W. Breuer, and W. Hermanns. 1996. Immunohistochemical examination of light-chain expression (lambda/kappa ratio) in canine, feline, bovine and porcine plasma cells. Zentralbl. Veterinarmed. A. 43:573–576.
4. Hieter, P.A., S.J. Korsmeyer, T.A. Waldmann, and P. Leder. 1981. Human immunoglobulin κ-light-chain genes are deleted or rearranged in λ-producing B cells. Nature. 290:368–372.
5. Coleclough, C., R.P. Perry, K. Kajalainen, and M. Wiegert. 1981. Aberrant rearrangements contribute significantly to the allelic exclusion of immunoglobulin gene expression. Nature. 290:372–378.
6. Selwing, E., and L.E. Datch. 1995. Immunoglobulin λ genes. In Immunoglobulin Genes. Second Edition. T. Honjo and F.W. Alt, editors. Academic Press, London, UK. 193–203.
7. Berg, J., M. McDowell, H.M. Jack, and M. Wabl. 1990. Immunoglobulin λ gene rearrangement can precede κ gene rearrangement. Dev. Immunol. 1:53–57.
8. Abken, H., and C. Buttler. 1991. Re-organization of the immunoglobulin κ gene on both alleles is not an obligatory prerequisite for Igλ lambda gene expression in human cells. Immunology. 74:709–713.
9. Takemori, T., and K. Rąjewsky. 1981. Lambda chain expression at different stages of ontogeny in C57BL/6, BALB/c and SJL mice. Eur. J. Immunol. 11:618–625.
10. McGuire, K.L., and E.S. Vitetta. 1981. k/λ shifts do not occur during maturation of murine B cells. J. Immunol. 127:1670–1673.
11. Kessler, S., K.J. Kim, and I. Scher. 1981. Surface membrane k and λ light chain expression on spleen cells of neonatal and maturing normal and immune-defective CBA/N B mice: the ratio is constant. J. Immunol. 127:1674–1678.
12. Lejeune, J.M., D.E. Briles, A.R. Lawton, and J.F. Kearney. 1982. Estimate of the light chain repertoire size of fetal and adult BALB/c and CBA/j mice. J. Immunol. 129:673–677.
13. Rolink, A., M. Streb, and F. Melchers. 1991. The k/λ ratio in surface immunoglobulin molecules on B lymphocytes differentiating from D10H-rearranged murine pre-B cell clones in vitro. Eur. J. Immunol. 21:2895–2898.
14. Omsmond, D.J., A. Rolink, and F. Melchers. 1998. Murine B lymphopoiesis towards a unified model. Immunol. Today. 19:65–68.
15. Zou, Y.R., S. Takeda, and K. Rąjewsky. 1993. Gene targeting in the Igκ locus efficient generation of λ chain-expressing B cells, independent of gene rearrangements in Igκ. EMBO (Eur. Mol. Biol. Org.) J. 12:811–820.
16. Arakawa, H., T. Shimizu, and S. Takeda. 1996. Re-evaluation of the probabilities for productive rearrangements on the κ and λ loci. Int. Immunol. 8:91–99.
17. Gorman, J.R., N. van der Steep, R. Monroe, M. Cogne, L. Davidson, and F.W. Alt. 1996. The Igκ 3' enhancer influences the ratio of Igκ versus Igλ B lymphocytes Immunity. 5:241–252.
18. Glozak, M., and B.B. Blomberg. 1996. The human immunoglobulin enhancer is controlled by both positive elements and developmentally regulated negative elements. Mol. Immunol. 33:427–438.
19. Aisenbauer, H., and H.G. Blobbeck. 1996. Tissue-specific deoxyribonuclease I-hypersensitive sites in the vicinity of the immunoglobulin C lambda cluster of man. Eur. J. Immunol. 26:142–150.
20. Fritti, J.P., S.C. Williams, I.M. Tomlinson, G.P. Cook, D. Chérit, D. LePaslier, J.E. Collins, I. Dunham, G. Winter, and...
of R NA isolation by acid guanidinium thiocyanate-phenol-
chloroform extraction. Anal. Biochem. 162:156–159.
38. Frohman, M.A., M.K. Dush, and G.R. Martin. 1988. Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. Proc. Natl. Acad. Sci. USA. 85:8998–9002.
39. Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, K. Struhl, and J.A. Smith, editors. 1995. Current Protocols In Molecular Biology. M cGraw-Hill, New York.
40. Williams, S.C., J.-P. Fritch, I.M. Tomlinson, O. Ignatovich, M.-P. Lefranc, and G. Winter. 1996. Sequence and evolution of the human germline V λ repertoire. J. Mol. Biol. 264:220–232.
41. Chen, J., M. Tronstine, C. Kurahara, F. Young, C.-C. Kuo, Y. Xu, J.F. Loring, F.W. Alt, and D. Hueser. 1993. B cell development in mice that lack one or both immunoglobulin κ light chain genes. EMBO J. (Europ. Mol. Biol. Organ.) 12:821–830.
42. N. Novobrantseva, T.I., V.M. Martin, R.M. Pelanda, W. Muler, K. Rajevesky, and A. Ehlich. 1999. Rearrangement and expression of immunoglobulin light chain genes can precede heavy chain expression during normal B cell development in mice. J. Exp. Med. 189:75–88.
43. Satta, M., A. Iavarone, N. Cappello, M.R. Bergami, G.C. Fiorucci, and F. Aguzzi. 1992. Reference values for immunoglobulin kappa and lambda light chain genes and the lambda/kappa lambda ratio in children's serum. Clin. Chem. 38:2454–2457.
44. Hood, L., W.R. Gray, and W.Y. Dreyer. 1966. On the mechanism of antibody synthesis: a species comparison of L-chains. Proc. Natl. Acad. Sci. USA. 55:826–835.
45. Ransdien, D.A., and G.E. Wu. 1991. Mouse κ light-chain recombination signal sequence mediates recombination more frequently than do those of λ light chain. Proc. Natl. Acad. Sci. USA. 88:10721–10725.
46. Gonzalez-Fernandez, A.S., S.K. Gupta, R. Pannell, M.S. Neuberger, and C. Milstein. 1994. Somatic mutation of immunoglobulin lambda chains: a segment of the major intron hypermutations as much as the complementarity-determining regions. Proc. Natl. Acad. Sci. USA. 91:12614–12618.
47. 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. 178:951–960.
48. 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. 173:1213–1225.
49. Harada, K., and H. Yagamishi. 1991. Lack of feedback inhibition of Vκ gene rearrangement by productively rearranged alleles. J. Exp. Med. 173:409–415.
50. Brüggemann, M., and M.S. Neuberger. 1996. Strategies for expressing human antibody repertoires in transgenic mice. Immunol. Today. 17:391–397.
51. Green, L.L., and A. Jakobovits. 1998. Regulation of B cell development by variable gene complexity in mice reconstituted with human immunoglobulin yeast artificial chromosomes. J. Exp. Med. 188:483–495.
52. Zou, X., J. Xian, N.P. Davies, A.V. Popov, and M. Brüggemann. 1996. Dominant expression of a 1.3 M human Igκ locus replacing mouse light chain production. FASEB J. 10:

M. P. Lefranc. 1995. Organization of the human immunoglobulin lambda light-chain locus on chromosome 22q11.2. Hum. Mol. Genet. 4:983–991.
21. Kawasaki, K., S. Minoshima, E. Nakato, K. Shibuya, A. Shintani, J.L. Schmits, J. Wang, and N. Shimizu. 1997. One-megabase sequence analysis of the human immunoglobulin λ gene locus. Gendrome Res. 7:260–261.
22. Giudicelli, V., D. Chaume, J. Bodmer, W. Muller, C. Busin, S. Marsch, R. Bontrop, L. Marc, A. Mlik, and M.-P. Lefranc. 1997. IMGT, the international ImMunoGeneTics database. Nucl. Acid Res. 25:206–211.
23. Ignatovich, O., I.M. Tomlinson, P.T. Jones, and G. Winter. 1997. The creation of diversity in the human immunoglobulin V(λ) repertoire. J. Mol. Biol. 268:69–77.
24. Combriato, G., and H.-G. Klöbeck. 1991. V(λ) and J(λ)-C(λ) gene segments of the human immunoglobulin λ light chain locus are separated by 14 kb and rearrange by a deletion mechanism. Eur. J. Immunol. 21:1513–1522.
25. Foster, S.J., H.-P. Brezinschek, R.J. Brezinschek, and P.E. Lipsky. 1997. Molecular mechanisms and selective influences that shape the κ gene repertoire of IgM+ B cells. J. Clin. Invest. 99:1614–1627.
26. Ignatovich, O. 1998. The creation of diversity in the human immunoglobulin Vλ repertoire. Ph.D. thesis University of Cambridge, Cambridge, U.K.
27. Bridges, S.L., S.K. Lee, M.L. Johnson, J.C. Lavelle, P.G. Fowler, W.J. Koopman, and H.W. Schroeder. 1995. Somatic mutation and CDR3 length of immunoglobulin κ light chains expressed in patients with rheumatoid arthritis and in normal individuals. J. Clin. Invest. 96:831–841.
28. Victor, K.D., K. Vu, and A.J. Feeney. 1994. Limited junctional diversity in κ light chains. J. Immunol. 152:3467–3475.
29. Popov, A.V., C. Bützler, J.-P. Fritch, M.-P. Lefranc, and M. Brüggemann. 1996. Assembly and extension of yeast artificial chromosome to build up a large locus. Gene. 177:195–201.
30. Davies, N.P., A.V. Popov, X. Zou, and M. Brüggemann. 1996. Human antibody repertoires in transgenic mice: manipulation and transfer of YACs. In Antibody Engineering: A Practical Approach. J. McCaffery, H.R. Hogenboom, and D.J. Chiswell, editors. IRL Press, Oxford. 59–76.
31. Hogan, B., R. Beddington, F. Costantini, and E. Lacy. 1994. Manipulating the Mouse Embryo: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 497 pp.
32. Zou, X., J. Xian, A.V. Popov, I.R. Rosewell, M. Müller, and M. Brüggemann. 1995. Subtle differences in antibody responses and hypermutation of λ light chains in mice with a disrupted κ constant region. Eur. J. Immunol. 25:2154–2162.
33. Wurst, W., and A.L. Joyner. 1993. Production of targeted embryonic stem cell DNA. In Gene Targeting. A.L. Joyner, editor. IRL Press, Oxford. 33–61.
34. Herrmann, B.G., D.P. Barlow, and H. Lehrach. 1987. A large inverted duplication allows homologous recombination between chromosomes heterozygous for the proximal t complex inversion. Cell. 48:813–825.
35. Galfré, G., and C. Milstein. 1981. Preparation of monoclonal antibodies: strategies and procedures. Methods Enzymol. 73:3–46.
36. Tijssen, P. 1985. Practice and theory of enzyme immunoassays. In Laboratory Techniques in Biochemistry and Molecular Biology. Volume 15. R.H. Burdon and P.H. Krijnenberg, editors. Elsevier, Amsterdam.
37. Chomczynski, P., and N. Sacchi. 1987. Single-step method
1227–1232.

53. Xian, J., X. Zou, A.V. Popov, C.A. Mundt, N. Miller, G.T. Williams, S.L. Davies, M.S. Neuberger, and M. Brügge- mann. 1998. Comparison of the performance of a plasmid-based human Igκ minilocus and YAC-based human Igκ transloci for the production of a human antibody repertoire in transgenic mice. Transgenics. 2:333–343.

54. Nadel, B., P.-A. Cazenave, and P. Sanchez. 1990. Murine lambda gene rearrangements: the stochastic model prevails over the ordered model. EMBO (Eur. Mol. Biol. Organ.) J. 9:435–440.

55. Asenbauer, H., G. Combrato, and H.-G. Klobeck. 1999. The immunoglobulin lambda light chain enhancer consists of three modules which synergize in activation of transcription. Eur. J. Immunol. 29:713–724.

56. Hagman, J., C.M. Rudin, C. Haasch, D. Chaplin, and U. Storb. 1990. A novel enhancer in the immunoglobulin lambda locus is duplicated and functionally independent of NF kappa B. Genes Dev. 4:978–992.

57. Eagle, H. 1955. Propagation in a fluid medium of a human epidermoid carcinoma strain KB. Proc. Soc. Exp. Biol. Med. 89:362–364.

58. Taub, R.A., G.F. Hollis, P.A. Hieter, S. Korsmeyer, T.A. Waldmann, and P. Leder. 1983. Variable amplification of immunoglobulin λ light chain genes in human populations. Nature 304:172–174.