The EMBO Journal vol.12 no.3 pp.811-820, 1993

Gene targeting in the Igκ locus: efficient generation of λ chain-expressing B cells, independent of gene rearrangements in Igκ

Yong-Rui Zou, Shunichi Takeda[1] and Klaus Rajewsky

Institute for Genetics, University of Köln, Weyertal 121, DW-5000 Köln 41, Germany and [1]Basel Institute for Immunology, Grenzacherstrasse 487, CH-4005 Basel, Switzerland

Communicated by K.Rajewsky

The production of λ chain-expressing B cells was studied in mice in which either the gene encoding the constant region of the κ chain (Cκ) or the intron enhancer in the Igκ locus was inactivated by insertion of a neomycin resistance gene. The two mutants have similar phenotypes: in heterozygous mutant mice the fraction of λ chain-bearing B cells is twice that in the wildtype. Homozygous mutants produce ~7 times more λ-expressing B cells (and about 2.3 times fewer total B cells) in the bone marrow than their normal counterparts, suggesting that B cell progenitors can differentiate into either κ- or λ-producing cells and do the latter in the mutants. Whereas gene rearrangements in the Igκ locus are blocked in the case of enhancer inactivation, they still occur in that of the Cκ mutant, although in this mutant RS rearrangement is lower than in the wildtype. This indicates that gene rearrangements in the Igλ locus can occur in the absence of a putative positive signal resulting from gene rearrangements in Igκ, including RS recombination. Complementing these results, we also present data indicating that in normal B cell development κ chain rearrangement can be preceded by λ chain rearrangement and that the frequency of κ/λ double producers is small and insufficient to explain the massive production of λ chain-expressing B cells in the mutants.
Key words: gene targeting/immunoglobulin κ and λ chains/immunoglobulin gene rearrangements/light chain isotype exclusion/RS rearrangement

Introduction

Although carrying two alleles at each of three immunoglobulin (Ig) loci [IgH for the heavy (H) chain, Igκ for light (L) chains of the κ type and Igλ for L chains of type λ], any given B cell expresses only one IgH and one IgL allele while the other alleles are phenotypically silent. This phenomenon is known as haplotype exclusion and is thought to result from a programme of variable (V) region gene rearrangements in the Ig loci during B cell development (Alt *et al.*, 1980; Tonegawa, 1983) and, perhaps, from cellular selection after gene rearrangements (for review see Cohn and Langman, 1990).

The gene rearrangements in B cell development seem to be ordered to some extent. Most B cell progenitors appear to first join a diversity (D_H) gene segment to a joining (J_H) segment in the IgH locus. This is followed by a V_H gene joining to the D_HJ_H complex. The ordered model of haplotype exclusion (Alt *et al.*, 1986) postulates that if a cell has undergone an in-frame ('productive') $V_HD_HJ_H$ recombination, further rearrangements in IgH are blocked and the cells proceed to gene rearrangements in the IgL loci. A productive rearrangement in IgL would then allow the expression of an antibody which might signal to the cell to arrest further gene rearrangements. At the level of L chain gene rearrangements there may again be an order, Igκ being first and Igλ last (Lewis *et al.*, 1982). This is supported by studies in Abelson virus transformed pre-B cell lines (Müller and Reth, 1988) which spontaneously undergo V region gene rearrangements *in vitro*, and by the observation that most κ chain-producing B cells have the λ chain genes in germline configuration, whereas in λ chain-producing B cells the κ chain genes are almost invariably rearranged (Alt *et al.*, 1980; Coleclough *et al.*, 1981; Hieter *et al.*, 1981). This rearrangement may either represent a non-productive Vκ−Jκ joint or a deletion of the κ constant region gene (Cκ) by the rearrangement of a 'rearranging' sequence [called RS in mice (Durdik *et al.*, 1984) and Kde in man (Siminovitch *et al.*, 1985; Klobeck and Zachau, 1986) and located 3′ of Cκ] to Ig recombination signal sequences (RSS) either in the Jκ−Cκ intron or at the 3′ end of a Vκ gene. The strong correlation between RS and Vλ−Jλ rearrangement *in vivo* (Durdik *et al.*, 1984; Persiani *et al.*, 1987; Nadel *et al.*, 1990) and *in vitro* (Müller and Reth, 1988) has been taken to suggest that the latter may be activated by the former through an as yet unknown mechanism.

While the order of gene rearrangements within the IgH locus seems firmly established, and while there is strong evidence that membrane-bound H chain is involved in haplotype exclusion in IgH (see Kitamura and Rajewsky, 1992), considerable variability is seen at all other levels. Thus, the analysis of Epstein−Barr virus-transformed cell lines suggests that, in the human, rearrangement in Igκ may precede IgH rearrangements (Kubagawa *et al.*, 1989). In the mouse, κ gene rearrangements have been shown not to be obligatorily dependent on the expression of membrane-bound H chain (Blackwell *et al.*, 1989; Schlissel and Baltimore, 1989; Kitamura and Rajewsky, 1992). Furthermore, cell lines have been described in which λ chains are expressed in the absence of either RS recombination or even any rearrangement in Igκ at all (Coleclough *et al.*, 1981; Berg *et al.*, 1990; Nadel *et al.*, 1990; Felsher *et al.*, 1991). Similarly, κ chains may be expressed by cells which have undergone non-productive λ gene rearrangements (Coleclough *et al.*, 1981; Persiani *et al.*, 1987; Felsher *et al.*, 1991). And finally, haplotype exclusion at the L chain level may be less strictly controlled than in IgH (Hardy *et al.*, 1986; Gollahon *et al.*, 1988; Harada and Yamagishi, 1991).

Given this complex situation it is clear that stochastic models of IgL haplotype exclusion (Coleclough *et al.*, 1981; Claverie and Langman, 1984) can by no means be ruled out. These models attribute the overall order of gene rearrange-

ments in B cell development simply to different rates at which rearrangements occur in the different loci. In the case of mouse L chain genes, the rate would be higher for Igκ than Igλ either because Igκ contains more target genes (Alt *et al.*, 1980; Coleclough *et al.*, 1981; Takemori and Rajewsky, 1981) and/or because the RSS in Igκ mediate the joining process more efficiently (Ramsden and Wu, 1991).

A problem for both ordered and stochastic models lies in the observation that the ratios of κ- to λ-expressing cells differ drastically from species to species (Hood *et al.*, 1967) and at least in mouse and human correlate both with V_L gene numbers (Coleclough *et al.*, 1981) and RSS 'efficiency' in Igκ and Igλ (Ramsden and Wu, 1991). The mouse is an extreme case with >90% κ-expressing B cells. Yet, if all cells have a chance to try out gene rearrangements in the four IgL loci with a one in three chance of a given rearrangement being in-frame, the κ/λ ratio should be close to 1 and largely unaffected by the number of V genes in a given locus (Cohn and Langman, 1990). Attempts to explain this discrepancy include sequential rearrangements within Igκ (Feddersen *et al.*, 1990; Harada and Yamagishi, 1991; Huber *et al.*, 1992), a limited time window for gene rearrangements in B cell development (Coleclough, 1990) and rapid cellular selection which could in principle explain L chain haplotype exclusion altogether (Cohn and Langman, 1990). However, while there is indeed suggestive evidence that in the mouse rearrangements may go to completion in Igκ (Coleclough *et al.*, 1981) but not Igλ (Nadel *et al.*, 1990) during B cell development, the problem of the control of the κ/λ ratio is clearly far from resolved.

In order to gain further insight into these matters, we have investigated the development of λ chain-expressing B cells in two mouse strains in which κ chain production is prohibited by the introduction of targeted mutations in the germline. The generation of one of these strains which carries a loss-of-function mutation in the Cκ gene (CκT) is described in this paper. In the other strain (iEκT), described elsewhere (S.Takeda, Y.-R.Zou, H.Blüthmann, D.Kitamura, U.Müller and K.Rajewsky, submitted), the κ intron enhancer is substituted by a neomycin resistance (*neo*^{r}) gene, resulting in a complete inhibition of gene rearrangements in the Igκ locus. We show that the generation of λ chain-expressing B cells does not require prior VκJκ or RS rearrangement and that in the absence of κ chain production surprisingly large numbers of λ chain-bearing B cells accumulate not only in the peripheral immune system, but also at the site of B cell generation, the bone marrow.

Results

Inactivation of the Igκ locus by homologous recombination

The vector used to inactivate the Cκ gene (Figure 1a) contains 5 kb of genomic DNA which includes $Jκ_{1-5}$ and the κ intron enhancer (Figure 1b). A (*neo*^{r}) gene driven by the herpes simplex virus thymidine kinase (HSV-tk) promoter and polyoma enhancer was used to replace the Cκ exon together with its 3′ untranslated region which includes the polyadenylation site. The HSV-tk gene was placed at the 5′-end of the genomic sequence permitting selection against random integration (Mansour *et al.*, 1988). Cells of the embryonic stem (ES) cell line B6III (originating from strain C57BL/6; Ledermann and Burki, 1991) were transfected with linearized vector DNA by electroporation and selected with G418 and Gancyclovir (GANC). Surviving colonies were screened for homologous recombination events using the polymerase chain reaction (PCR), and candidate clones were further analyzed by Southern blotting (Figure 1d, right panel).

Three ES cell clones containing the correct mutation, designated CκT, were injected into CB.20 blastocysts, and the chimeric mice generated were subsequently bred to C57BL/6 females. One ES cell clone transmitted the mutation into the germline (Figure 1d, left panel) giving rise to heterozygous mutant mice whose genetic background is entirely of C57BL/6 origin. Mice homozygous for the CκT mutation were obtained by intercrossing these offspring.

The second mutant mouse strain employed in the present study carries a *neo*^{r} gene under the control of a rabbit β-globin promoter and an enhancer composed of four tandem repeats of the GT-IIC+GT-I motif (Davidson *et al.*, 1988; Fromental *et al.*, 1988) instead of the κ intron enhancer in the germline, a mutation designated iEκT. The generation and characterization of this strain whose genetic background is a mix of strains C57BL/6 and 129/Sv is subject of a separate publication (see Introduction; S.Takeda *et al.*, submitted).

Mice homozygous for the CκT mutation lack κ-bearing but possess λ-bearing B cells

To verify that the CκT mutation renders the Igκ locus non-functional, spleen cells from homozygous mutant mice were examined by flow cytometry. Cells from wildtype littermates served as controls (Figure 2). As expected, the homozygous mutants are devoid of κ-producing cells in the periphery, indicating that the CκT mutation has indeed inactivated the Cκ gene (Figure 2a). Strikingly, the mutant animals harbor substantial numbers of λ-bearing B cells in the spleen, many more than their wildtype counterparts (Figure 2b, and further below, Table I).

Essentially the same result has also been obtained for mice homozygous for the iEκT mutation (Takeda *et al.*, submitted). The size of the splenic B cell compartment in the two mutants is indistinguishable (see also Table I).

κ-deficient mice generate less B cells than control mice but contain many more λ-bearing cells already in the compartment of newly generated cells

At 6−10 weeks of age, the spleens of homozygous CκT and iEκT mice contained ~30% fewer nucleated cells than those of wildtype littermates, but equal numbers of T cells (Table I). Despite the absence of κ-bearing cells (94% of the B cells in a normal mouse) B cell numbers were only reduced by a factor of <3. Accordingly, the number of splenic λ-bearing B cells (~6% of the B cells in a normal mouse) is ~7-fold higher than in normal animals.

Does this reflect an increased output of λ-bearing B cells from B cell precursors in the bone marrow of the mutant mice or the accumulation of long-lived cells in the periphery? In an attempt to resolve this question we performed a flow cytometric analysis of B cell precursors and newly generated B cells in the bone marrow of the mutants. Both cell types share the B lineage marker CD45R/B220, but are distinguished by the absence or presence of surface IgM. The data in Table I show that the ratio of lymphocytes to total nucleated cells remains normal in both mutant strains, as

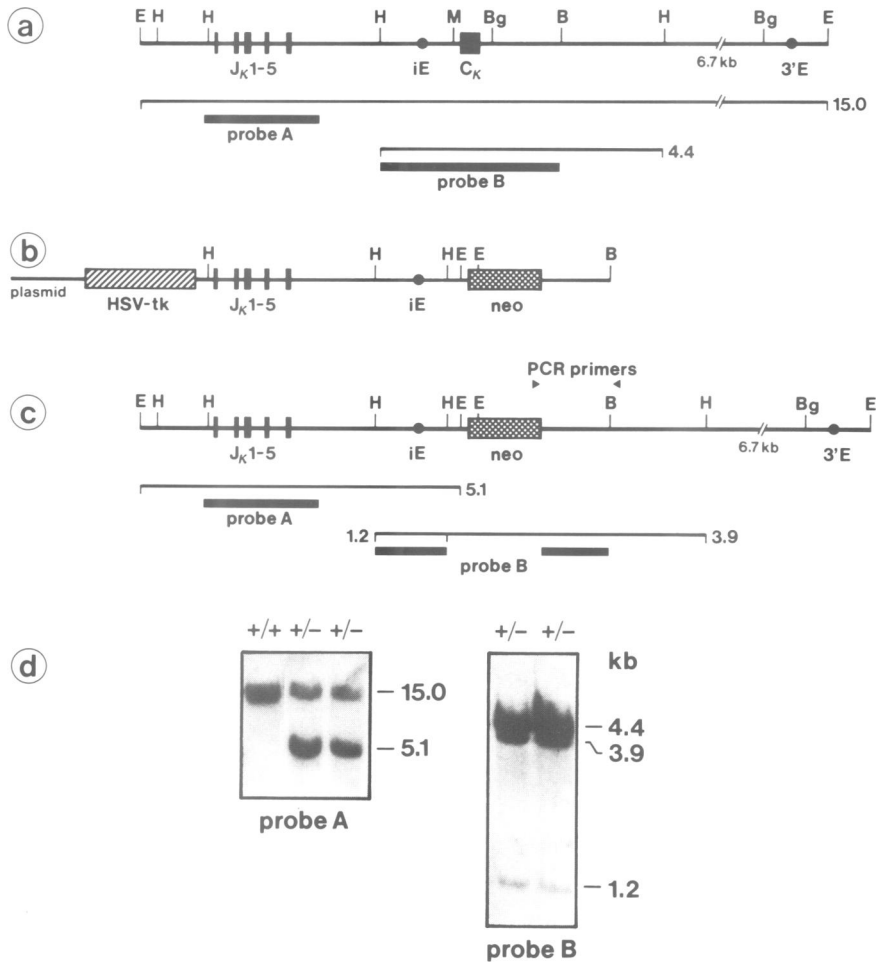

Fig. 1. Targeted disruption of the Cκ gene. (**a**) Partial restriction endonuclease map of the κ L chain locus (Lewis *et al.*, 1982; Meyer and Neuberger, 1989). Closed boxes represent exons of Jκ and Cκ. Filled circles indicate the κ enhancers located in the intron between Jκ and Cκ (iE) and 3′ of the Cκ exon (3′E) respectively. B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; M, *Mst*II; probe A, *Hin*dIII−*Xba*I fragment; probe B, *Bam*HI−*Hin*dIII fragment. The sizes of germline fragments detected by probe A and B are shown under the map. (**b**) Targeting vector. The *Mst*II−*Bgl*II fragment containing the Cκ exon (see panel a) was replaced with a 1.2 kb pMC1neo/p(A)$^+$ fragment (*neo*). The 1.7 kb HSV-tk gene was placed at the 5′-end of the genomic Jκ fragment. (**c**) Predicted structure of the CκT locus. The expected sizes of restriction fragments detected by probes A and B are shown under the scheme. (**d**) Southern blot analysis of the Cκ mutation in B6III ES cells (right panel). DNA prepared from putative targeted B6III cell lines was digested with *Hin*dIII and hybridized to probe B. Southern blots of tail DNA from offspring of germline chimeras are shown in the left panel. Black offspring from crosses between chimeric mice and C57BL/6 were analyzed. Probe A was hybridized to *Eco*RI-digested tail DNA.

compared with wildtype littermates. However, within the B lineage cells there is some change in the cellular distribution. Whereas the number of B cell precursors is about 1.4-fold higher in the mutants than in the controls, the number of newly generated B cells (all of which express λ chains; data not shown) is ~2.3-fold reduced. In agreement with these data, the ratio of newly generated to precursor B cells (which can be directly and independently determined from the flow cytometry data) was 2.5 to 3-fold lower in the mutants than in the wildtype animals (Table I). Thus, B cell production from precursor cells seems less efficient in the mutants than in the wildtype, but the difference is small considering that in a normal mouse only 6% of the B cells express λ chains. We also performed a more detailed flow cytometric analysis of the B progenitor pool in the mutants by subdividing these cells into subsets as outlined by Hardy *et al.* (1991). Some accumulation of cells was seen at the developmental stage preceding that of the B cell (fraction D). No difference between mutants and wildtype was detectable at earlier stages of development (fractions A−C′; data not shown).

Is the high number of λ chain-expressing cells in the compartment of newly generated B cells a special feature of the mutant strains? Although most earlier studies indicate that the λ/κ ratio in the mouse is low already in early phases of B cell development (Kessler *et al.*, 1981; Le Jeune *et al.*, 1981; McGuire and Vitetta, 1981; Takemori and Rajewsky, 1981; Primi *et al.*, 1986; Sauter and Paige, 1987; Rolink *et al.*, 1991), there have been conflicting reports in the literature (Haughton *et al.*, 1978; Golding *et al.*, 1980; Nishikawa *et al.*, 1984; Holmberg *et al.*, 1985) and until recently the case has been made (Cohn and Langman, 1990) that the low λ/κ ratio seen in peripheral B cells might result from antigenic selection. We have therefore reinvestigated this issue by determining the fraction of λ-expressing B cells in the bone marrow and spleen of wildtype and heterozygous mutant mice. The results of this analysis appear in Figure 3 and Table II and give a clear answer.

Although, interestingly, the fraction of λ-bearing cells in the two heterozygous mutant strains is twice that in the wildtype, the frequencies of λ chain-expressing cells are indistinguishable for newly generated and peripheral B cells in each case. These results argue against a major role of antigenic selection in the determination of the λ/κ ratio in murine B cells.

We conclude that many more λ-chain expressing B cells accumulate in the compartment of newly generated B cells in the bone marrow of homozygous mutant mice than in their wildtype counterparts. Quantitatively, since these cells range from 5 to 7% in the wildtype and since the mutants have ~40% of the newly generated B cells of the wildtype, both mutant strains have ~7 times more λ-bearing newly generated B cells in the bone marrow than the controls.

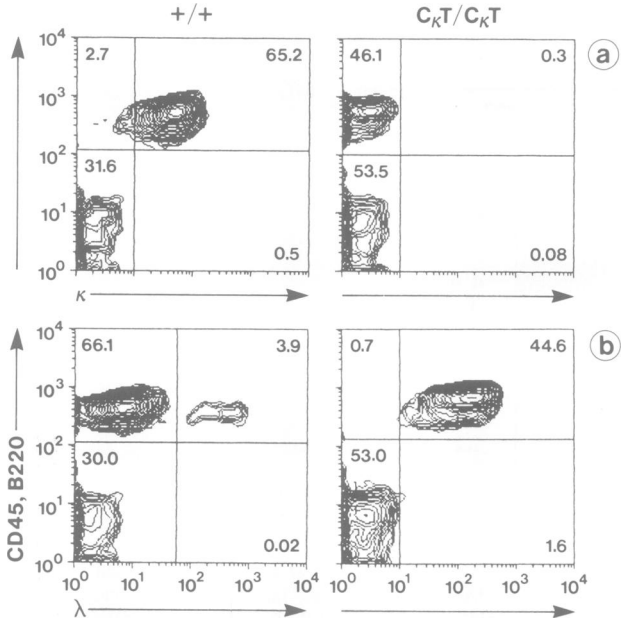

Fig. 2. Lack of κ-bearing B cells in the periphery of CκT mice. Splenic B cells obtained from 6-week-old mice were stained with PE-coupled anti-CD45R/B220 and FITC-coupled anti-κ (**a**) or anti-λ (**b**). Only data from cells in the lymphocyte gate as defined by light scatter were collected. The percentages of cells in a given quadrant are indicated in the figure.

The production of λ-bearing B cells does not require signals resulting from Vκ−Jκ or RS rearrangement

In order to analyze to what extent gene rearrangements at Igκ accompany the generation of λ-bearing B cells in the mutant animals, we isolated B cells from CκT and iEκT homozygous mutants as well as κ- and λ-producing B cells from normal mice and determined the extent of Jκ and RS rearrangements in those cells by Southern blotting. DNA isolated from T cells, in which such rearrangements do not occur, served as a control.

The strategy of this approach, the probes used and examples of Southern blots are depicted in Figure 4. Rearrangement of Jκ and of RS was quantified by measuring the disappearance of the corresponding germline bands. This was done by scanning the autoradiographs and normalizing the results through comparison with a signal given by a probe specific for a 'control' gene (the gene encoding interleukin 4). A summary of the results appears in Table III.

In total and κ-bearing B cells from control mice, 21−35% of the Jκ loci were still in germline configuration, in accord with earlier findings that a major fraction of κ-expressing B cells carry only one Igκ rearrangement (Coleclough *et al.*, 1981). In contrast, the vast majority (>90%) of the λ-bearing cells have both Jκ loci rearranged, in most cases through RS rearrangement. This is again in agreement with earlier work (Alt *et al.*, 1980; Coleclough *et al.*, 1981; Durdik *et al.*, 1984; Nadel *et al.*, 1990). In the case of the (λ-expressing) B cells of the mutants the situation is strikingly different. In iEκT homozygous mutants neither Jκ nor RS rearrangement is detectable by Southern blotting, as expected on the basis of the initial analysis by Takeda *et al.* (submitted). In contrast, in B cells from the CκT mutant, most (~90%; Table III) Jκ loci are rearranged as in normal λ-bearing B cells. However, whereas in the normal cells this rearrangement is mostly associated with (and perhaps due to) RS rearrangement (64% of the cases; Table III), in the CκT mutant RS rearrangement occurs only in 20−30% of the cases, so that most Jκ rearrangements in these cells presumably reflect Vκ−Jκ joining. The low degree of RS rearrangement in the CκT mutant emerges consistently from both the determination of RS elements in germline configuration and that of deletion of the *neo*^{r} gene in the Cκ locus (Table III).

Table I. Representation of different lymphocyte populations in mutant and control mice[a]

	Mouse genotype		
	+/+	CκT	iEκT
Spleen			
No. of nucleated cells (×10^8)	1.9 ± 0.6	1.3 ± 0.4	1.2 ± 0.4
No. of T cells (×10^7)[b]	5.1 ± 0.4	5.4 ± 0.4	5.2 ± 0.2
No. of B cells (×10^7)[c]	13.8 ± 0.3	5.5 ± 0.5	5.1 ± 0.3
Bone marrow			
No. of nucleated cells (×10^7)	2.8 ± 0.3	2.0 ± 0.2	2.3 ± 0.4
% B lineage cells	26.8 ± 3.3	31.7 ± 4.2	31.1 ± 4.9
% B cell precursors[d]	14.0 ± 2.3	26.2 ± 5.0	25.1 ± 6.1
% newly generated B cells[e]	6.0 ± 1.6	3.2 ± 0.9	3.5 ± 0.5
Ratio of newly generated B to B cell precursors	0.43 ± 0.09	0.13 ± 0.05	0.17 ± 0.06

[a]Splenocytes and bone marrow cells were isolated as described in Materials and methods. Data were derived from the analysis of 5−10 mice. Nucleated cells were recovered from two femurs. The lymphocyte gate was defined as light scatter.
[b]T cells are defined as CD3$^+$ cells.
[c]B cells are defined as CD45R/B220$^+$ cells.
[d]B cell precursors are defined as CD45R/B220^{low}, μ^- cells.
[e]Newly generated B cells are CD45R/B220^{low}, μ^+ cells.

Taken together, these results show that the two mutant strains differ drastically from each other and from control mice in the extent to which gene rearrangements occur in the Igκ loci during B cell development. Yet they are both able to produce large and equal numbers of λ-bearing B cells from equal numbers of B cell precursors in the bone marrow. This result seems to exclude a major developmental control of the Igλ locus through a positive signal generated by gene rearrangements in Igκ (see also Discussion).

In normal mice most non-productive, but very few productive Vλ−Jλ joints are carried by κ chain-expressing splenic B cells

Since the analysis of the CκT and iEκT mutants has demonstrated that λ-bearing B cells can be produced in the absence of rearrangements in the Igκ locus, one would predict that also in normal C57BL/6 mice rearrangements in Igλ can occur before rearrangements in Igκ, albeit at low frequency because of the higher rate of rearrangements in

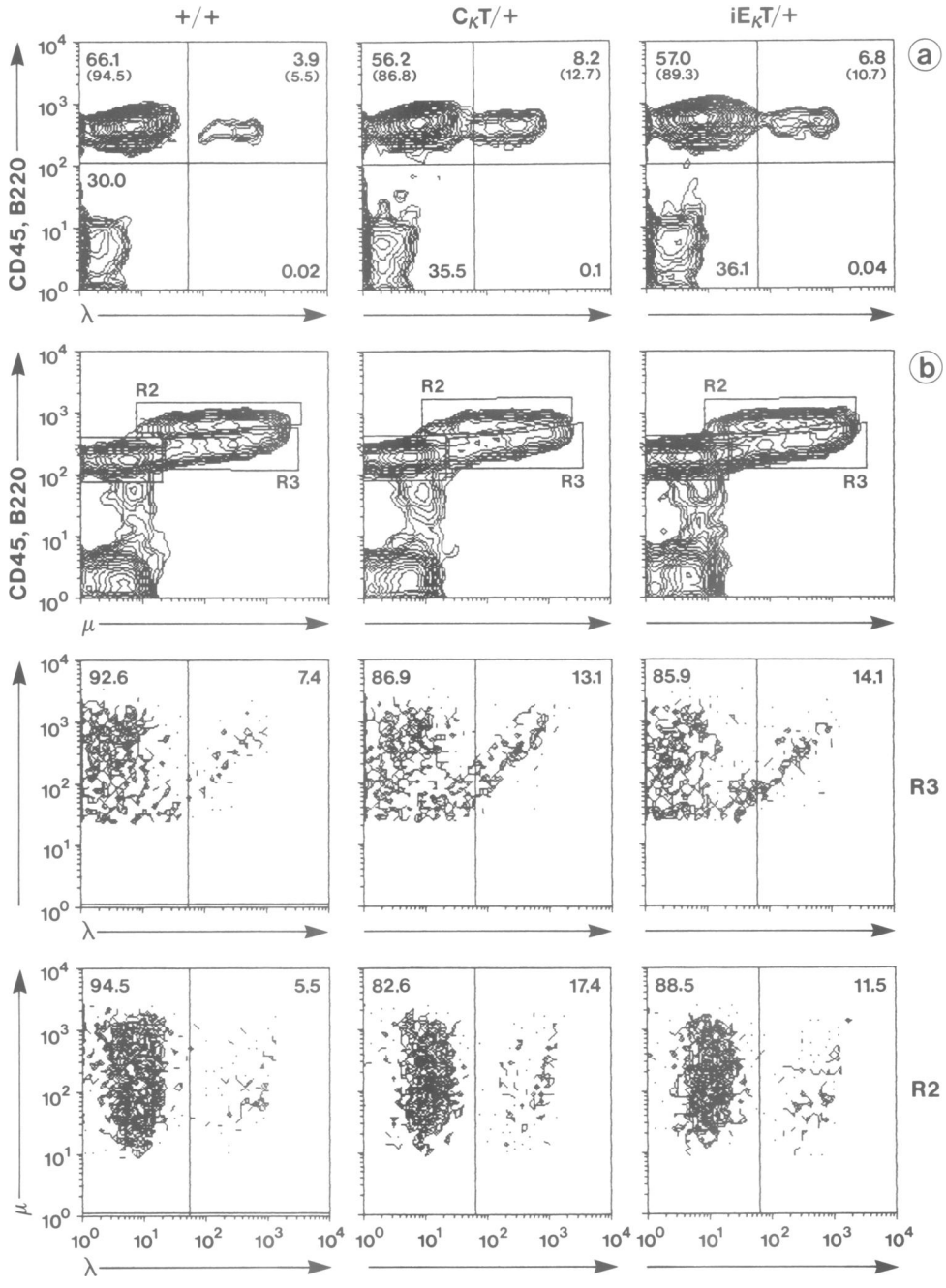

Fig. 3. Increased frequency of λ-bearing B cells in the periphery and in bone marrow of heterozygous κ-deficient mice. (**a**) Splenic B cells obtained from 6−8 week-old heterozygous CκT (+/CκT) and iEκT (+/iEκT) mice as well as control animals were stained with PE-coupled anti-CD45R/B220 and FITC-coupled anti-λ. The percentages of cells in a given quadrant are indicated in the figure. The percentages of λ^+ and λ^- B cells are given in brackets. (**b**) Bone marrow cells obtained from the two femora of 6−8 week-old +/CκT and +/iEκT mice and of wildtype littermates were simultaneously stained with PE-coupled anti-μ, FITC-coupled anti-λ and biotin-coupled anti-CD45R/B220. Biotin-coupled reagents were revealed using Cy-Chrome−streptavidin. Contour plots for anti-μ versus anti-λ are shown, respectively, for $B220^{low}/\mu^+$ (newly generated) B cells (R3) and $B220^{high}/\mu^+$ (mature) B cells (R2). The percentages of λ^+ and λ^- B cells are indicated in the figure.

Igκ. In order to test this prediction and also to resolve the issue of κ/λ double producers, we isolated and sequenced Vλ−Jλ joints from FACS-sorted B cell populations of C57BL/6 mice and determined the distribution of productive and non-productive joints in the total B cell population on the one hand and in B cells expressing κ chains or λ chains on the other. Given an independence of rearrangements at Igκ and Igλ, the prediction is that a cell carrying a non-productive Vλ−Jλ joint can proceed to further λ or κ rearrangement. Therefore, non-productive Vλ−Jλ joints should be present in κ-producing B cells—contrary to what a strictly sequential model of κ−λ rearrangement predicts. Furthermore, if isotype exclusion works faithfully at the level of L chains, productive Vλ−Jλ joints should be absent from the population of κ-bearing B cells.

The results of the experiment are in agreement with both predictions, with some qualification. When Vλ−Jλ joints were amplified by PCR, bands of the expected size were obtained for all B cell samples, and their intensity was similar for similar numbers (10^4) of κ-bearing or total splenic B cells (data not shown). The distribution of productive and non-productive joints in the various B cell populations appears in Table IV. More than 90% of the Vλ−Jλ joints isolated from the κ-bearing cells are non-productive. In contrast, in λ-bearing cells three-quarters of the joints are productive, and in the total B cell population productive and non-productive joints occur at roughly equal frequencies. It is noteworthy that we see many more non-productive joints from the λ1/λ3 sublocus than from the λ2 sublocus in our

Table II. Ratio of λ/B in the heterozygous κ-deficient and normal mice[a]

	Mouse genotype		
	+/+	CκT/+	iEκT/+
Spleen λ/B(%)[b]	5.6 ± 0.9	13.5 ± 1.7	12.8 ± 1.7
Bone marrow λ/B(%)[c]	6.6 ± 0.8	13.4 ± 2.1	11.3 ± 1.2

[a]Data were derived from the analysis of three to nine mice.
[b]Splenic B cells were stained with anti-CD45R/B220 and anti-λ antibodies. All percentages refer to λ^+ cells in CD45R/B220$^+$ population.
[c]Analysis was done as described in the legend to Figure 3. All percentages refer to λ^+ cells in the CD45R/B220^{low}, μ^+ population.

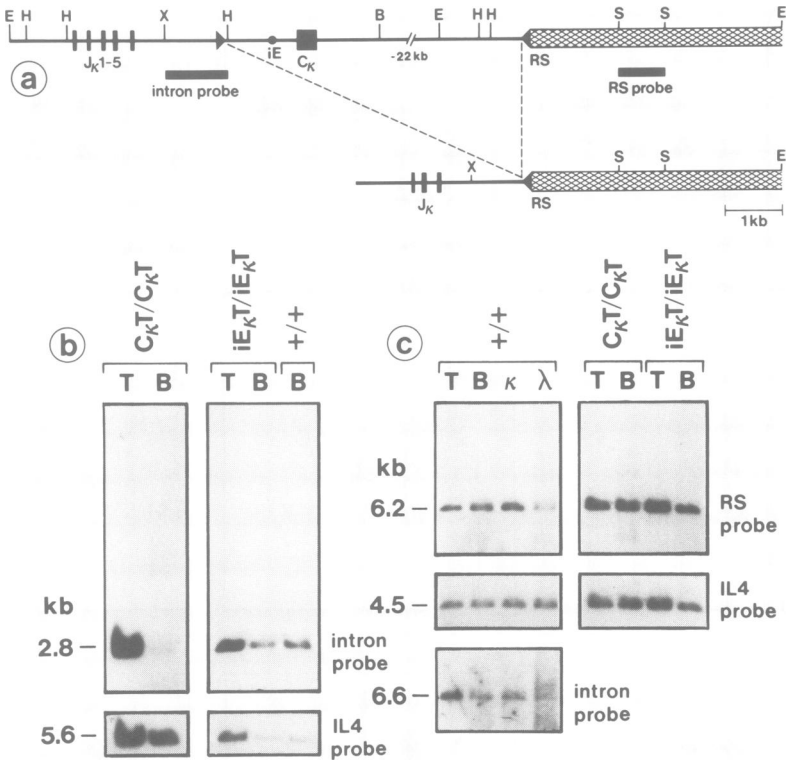

Fig. 4. Rearrangements at the Igκ locus in mutant and normal mice. (**a**) Partial restriction map of the germline Igκ locus (Durdik *et al.*, 1984; Meyer and Neuberger, 1989). In the mutant mice, the Cκ exon (CκT) or the intron enhancer (iEκT) was replaced by a *neo*^{r} gene. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; S, *Sau*3A; X, *Xba*I; intron probe, *Hin*dIII−*Xba*I fragment; RS probe, *Sau*3A fragment. Triangles represent the recombination signal sequences. The restriction map of the locus after recombination of the RS element to the isolated intron heptamer is shown under the map of the germline Igκ locus. The Cκ exon is deleted upon RS rearrangement. Thus, in the CκT mutant, RS rearrangements can also be detected by the decrease in intensity of band corresponding to the *neo*^{r} gene (Table III). (**b**) Representative Southern blot analysis of Vκ−Jκ rearrangements. DNA from splenic T cells and B cells of κ-deficient mice, as well as from splenic B cells of normal mice, was digested with *Hin*dIII and hybridized to the intron probe. The expected size of the germline band of Jκ is 2.8 kb. The same blot rehybridized to an IL-4 gene probe served as an internal control. It can be seen that a smear of genomic DNA and a few faint discrete bands appear in the case of B but not T cells from +/+ and CκT mice. This is seen only when the digested genomic DNA of B cells is probed with the intron probe, and not when an IL-4 gene probe is used. This pattern indicates Vκ−Jκ rearrangements upon which the *Hin*dIII site at the 5′ of the Jκ elements is deleted, resulting in the disappearance of the 2.8 kb germline fragment. (**c**) Representative Southern blot analysis of RS rearrangements. Cell populations were sorted as described in Materials and methods. Each sample is a pool of DNA prepared from cells of three or four 8-week-old mice. Aliquots of DNA were digested with *Eco*RI and *Bam*HI in parallel and hybridized to the RS probe. The germline RS fragment is 6.2 kb. The same blot was then rehybridized to an IL-4 gene probe which gives a 4.5 kb band indicative of a germline IL-4 locus. The digested DNA from different cell populations from normal mice was then rehybridized with the intron probe to examine the extent of Vκ−Jκ rearrangements.

collection. This effect is partly due to the fact that our amplification procedure does not extend to the VλX gene, one of the two V genes at the λ2 sublocus (Dildrop *et al.*, 1987; Sanchez and Cazenave, 1987). However, since there is no indication for a predominant usage of VλX in λ2 chain-bearing antibodies (Sanchez *et al.*, 1987), the data suggest that the λ1/λ3 sublocus may be more prone to gene rearrangements than the λ2 sublocus. This would be in line with the observation that λ1/λ3 expression prevails over λ2 expression in the B cell population (Takemori and Rajewsky, 1981; Sanchez *et al.*, 1987; Nadel *et al.*, 1990).

It is clear from these data that κ-bearing B cells indeed harbor Vλ−Jλ joints. The fact that most of these are non-productive excludes the possibility that these joints originate from contaminating λ-expressing cells and also demonstrates that the frequency of κ/λ double producers in the splenic B cell population is very low. Given that 6% of B cells express λ chains, the frequency of cells bearing a non-productive Vλ−Jλ joint should also be ~6% because the numbers of productive and non-productive joints in the total population are roughly equal. Of this 6%, approximately one-third (2%) represent λ-expressing cells [one-third of which carry a non-productive in addition to a productive Vλ−Jλ joint (Table IV)]. It follows that ~4% of the κ-bearing B cells in the spleen of a normal mouse carry a non-productive Vλ−Jλ joint and that the latter joints represent two-thirds of all non-productive joints present in the B cell population. The simplest interpretation of this result is that a major fraction of the progenitor cells in which a non-productive λ gene rearrangement occurred proceeds to κ gene rearrangement. In addition, since <10% of the Vλ−Jλ joints in κ-bearing B cells are productive (Table IV), the frequency of B cells expressing κ chains on the surface and bearing a productive Vλ−Jλ joint is <0.4%. This low frequency of potential κ/λ double producing cells could of course be partly due to counterselection. However, even in this case the frequency of double producers cannot be higher than 1−2% in the population of newly arising B cells, assuming that one in three Vλ−Jλ joints is productive. The efficient production of λ chain-expressing B cells in the CκT and iEκT mutant mice can therefore not be explained by assuming that normal mice generate equivalent numbers of κ/λ double producers.

Table III. Extent of Jκ and RS rearrangements in mutant and normal mice[a]

	*Hin*dIII digested samples				
	+/+	CκT		iEκT	
	B	T	B	T	B
% Jκ in germline configuration[b]	35	100	4	100	100
% non-deleted *neo* gene[c]	NT	100	77	NT	NT

	*Eco*RI + *Bam*HI-digested samples							
	+/+				CκT		iEκT	
	T	B	κ	λ	T	B	T	B
% Jκ in germline configuration	100	30	21	1.4	100	13	100	100
% RS in germline configuration[d]	100	100	100	36	100	72	100	100

[a]Southern blots (e.g. Figure 4b and c) were scanned with a densitometer and the signal intensity of each group was normalized to that of IL-4 gene germline band (Materials and methods). The level of signal intensity of T cells was defined as 100%. NT, not tested.
[b]Percentages of unrearranged Jκ genes were measured by hybridizing digested DNA to the intron probe (Figure 4a) and scanning the Southern blot as described above.
[c]Percentages of non-deleted *neo* gene were determined by hybridizing the samples to the *neo* gene (see legend to Figure 4a in case of the CκT mutant mice) and scanning.
[d]Percentages of unrearranged RS element were estimated by scanning the blot of samples hybridized to the RS probe (Figure 4a and c).

Table IV. Lambda light chain rearrangements in κ- and λ-bearing B cell populations and in the total splenic B cell population[a]

	Productive joints			Non-productive joints		
	λ_1,λ_3	λ_2	total λ	λ_1,λ_3	λ_2	total λ
κ-bearing B cells	1,0	1	2	15,7	4	26
λ-bearing B cells	10,1	9	20	3,2	2	7
total splenic B cells	7,0	10	17	13,1	1	15

[a]B cell populations were isolated as described in Materials and methods. Sequences are derived from two or three experiments.

Discussion

The production of λ-producing cells does not require a positive signal provided by κ gene rearrangements; role of RS recombination

The present data show that both mutant mouse strains produce large and equal numbers of λ-bearing B cells although they both differ from wildtype mice in terms of gene rearrangements in the Igκ locus. This result bears on models ascribing the control of the activation of gene rearrangements in Igλ to rearrangements in Igκ. One such model assumes that a positive control signal is generated by RS recombination (Persiani *et al.*, 1987). If such a signal exists at all, it cannot be required for Igλ rearrangement because of the phenotype of the iEκT mutant. This phenotype is identical to that of CκT mice, although in iEκT the Igκ locus is devoid of any detectable gene rearrangement. Another possibility would be a negative signal provided by the Igκ locus, resulting in a blockade of gene rearrangements in Igλ. This signal would be terminated by RS recombination, allowing initiation of gene rearrangements in Igλ (Siminovitch *et al.*, 1987; Müller and Reth, 1988). In this case the evidence from the gene targeting experiments is inconclusive. For the iEκT mutant one might argue that the 'freezing' of the Igκ locus includes the putative negative signal which could be only generated once the Igκ locus is activated. (Note that this would also be compatible with gene rearrangements in Igλ preceding rearrangements in Igκ in B cell development; see below.) In the CκT mutant, on the other hand, the Igκ locus is active in terms of Vκ−Jκ rearrangements, only RS rearrangement being partly inhibited. Since the data suggest that a major fraction of the λ chain-expressing B cells in the CκT mutant has not undergone RS recombination (Table III), a negative control of the Igλ by the Igκ locus would only be possible if the CκT mutation itself interferes with the generation of the putative negative signal. Although the CκT mutation extends only a few base pairs upstream of the Cκ exon and does not touch regulatory sequences like the intron enhancer or the MAR sequence upstream of the latter (Cockerill and Garrard, 1986), this possibility cannot be excluded at this stage considering in particular that the CκT mutation does interfere in an unknown way with the control of RS recombination.

Taken together, the mutational analysis of the Igκ locus

excludes the requirement of a putative positive signal generated through κ gene rearrangements in the activation of the Igλ locus. A negative signal from Igκ and its control by RS recombination remains a possibility. Irrespective of whether the latter mechanism operates and besides its role in L chain isotype exclusion, RS recombination would promote the production of λ-expressing at the expense of κ-expressing B cells. The efficiency of this effect is apparent from the abundance at which λ-expressing B cells are generated in the CκT and iEκT mutants.

Lambda gene rearrangements can occur before κ gene rearrangement in normal B cell development

It had already become clear from the analysis of transformed B cells that rearrangements at Igλ are occasionally seen in κ-bearing B cells (see Introduction). In addition, there is clear evidence that rearrangements among the λ subloci occur on a stochastic basis (Nadel *et al.*, 1990). Extending this kind of data, the present analysis of productive and non-productive Vλ−Jλ joints in κ- and λ-bearing B cells isolated from normal mice further underlines the mutual independence of κ and λ rearrangements in normal B cell development and indicates that at least some rearrangements in Igλ occur prior to κ rearrangements. Thus, in the total population of B cells the frequencies of productive and non-productive Vλ−Jλ joints do not differ drastically from each other, arguing against an accumulation of non-productive joints after productive κ rearrangements (through counterselection of double producers). Of the non-productive joints, the majority is carried by κ-expressing B cells. The straightforward interpretation of this result is that these joints have occurred before productive κ gene rearrangement.

Co-ordinated λ gene rearrangements on both chromosomal homologues?

λ chain-expressing B cells from normal mice were found to carry a significant load of non-productive Vλ−Jλ joints. This is in contrast to the data of Nadel *et al.* (1990) who did not see such joints in a set of λ chain-expressing hybridoma lines. We can only suggest loss of non-productive joints from the hybridoma and/or their coincidence in Southern blots with other bands in an attempt to explain the discrepancy between the two sets of data. Since in most λ-expressing cells there seems to be only one gene rearrangement per Igλ locus (Nadel *et al.*, 1990), the data in Table IV suggest that roughly one-third of the λ-producers bear a non-productive joint on the homologous chromosome. Theoretically, one would expect 40% of such cells if one in three of the joints is productive and one rearrangement is allowed per chromosome. This calculation assumes that the rearrangements in Igλ are not interrupted by gene rearrangements in Igκ, although the latter are more frequent than the former. This could be achieved by prior deletion of the Cκ gene by RS recombination. However, the presence of a major fraction of the non-productive Vλ−Jλ joints in the population of κ-bearing B cells (Table IV) suggests that co-ordinated rearrangements in both homologous Igλ loci may occur in the absence of RS recombination. This would predict that the majority of the non-productive Vλ−Jλ joints in κ-bearing cells occur pairwise and raises the intriguing possibility that an Igλ-specific signal is involved in the initiation of gene rearrangements in the Igλ locus.

Abundant production of λ-bearing cells in the κ-deficient mice and a scenario of L chain isotype control

A surprise in the present study is the efficiency by which λ chain-expressing B cells are produced in the bone marrow of the κ-deficient mice. How can the 7-fold increase in the number of such cells in the compartment of newly generated B cells in the mutants be explained?

One might first consider that the number of cells in the compartment of newly generated cells in the bone marrow does not reflect the rate of cellular production but rather the accumulation of (long-lived) cells. This appears unlikely since in normal animals the newly generated B cells do not divide and their transit time through the compartment is short (Osmond, 1991), and one would expect the mutants to be rather more prone than the wildtype to exporting B cells into the periphery because they possess less B cells in the peripheral B cell pool. However, an analysis of cell proliferation kinetics is clearly needed in order to settle this point.

If the high levels of λ-expressing B cells in the bone marrow of the mutants reflect an increased output of newly generated cells from the precursor pool, then the straightforward interpretation of the data is that a large fraction of the precursor cells which differentiate into κ-expressing B cells in a wildtype mouse become λ-expressing cells in the mutants. Although this differentiation process may take somewhat longer than in the case of κ chain production [the size of the precursor pool in the mutants being increased by a factor of 1.5 as compared with the wildtype (Table I)], the mutants seem to perform gene rearrangements in Igλ much more efficiently than the wildtype, close to the efficiency of κ gene rearrangements in the latter. Indeed, one might argue that the only reason why the mutants do not fully compensate κ by λ production may be the occurrence of sequential gene rearrangements within the Igκ but not, as the data of Nadel *et al.* (1990) suggest at least for wildtype mice, the Igλ locus. Sequential rearrangements increase the yield of productive joints.

In an attempt to fit the various pieces of evidence from the present study and earlier wisdom into a coherent picture of L chain isotype control, we arrive at the following, highly speculative scenario. In normal B cell development the cells pass through a time window in which L chain gene rearrangements occur. The rate of κ gene rearrangements is such that the majority of the cells undergoes such rearrangements during this time period. Only a minor fraction of the cells (roughly 1/10 considering the frequency of λ chain-expressing B cells and that of non-productive Vλ−Jλ joints in κ-chain bearing producers; Table IV) undergoes rearrangements in Igλ. Rearrangements in Igκ and Igλ may be initiated at any time point within this developmental period, but they require distinct signals and activation of Igκ has an inhibitory effect on the Igλ locus (and perhaps vice versa). This inhibition could be due to a specific signal from an unknown control element in the Igκ locus (subject to deletion by RS recombination) or to competition for some component which is limiting in the joining process. Both the iEκT and the CκT mutations interfere with this negative regulation so that in both cases homozygosity for the mutation results in an increase of λ gene rearrangements. If in the heterozygous mutants the negative control mechanism still

operates efficiently, then the doubling in the frequency of λ-expressing B cells would be due to the fact that only half as many κ-expressing cells are generated in the absence of one of the two (functional) Igκ loci. The production of λ-expressing cells would remain unchanged in this situation given that the size of the pool of progenitors is the same in heterozygous mutants and the wildtype.

While the latter is supported by flow cytometric analysis, a significant decrease of the fraction of newly generated cells has not been observed in any of the heterozygous mutants (see the accompanying paper by Chen *et al.*, and our own unpublished data). This either suggests that the fraction of newly generated B cells does not directly reflect cellular production (as discussed earlier) or that the increase of the fraction of λ-bearing B cells in the mutants is due to a more complex regulation, e.g. partial release of Igλ from negative control and enhancement of sequential rearrangements in the wildtype Igκ locus.

Utility of the κ-deficient mutants

The κ-deficient mice described in the present paper and the paper of Takeda *et al.* and Chen *et al.* (accompanying) will be useful not only in crosses with Ig transgenic mice but also for the analysis of the function and the repertoire of λ chain-bearing antibodies. The CκT mutant is particularly suited in the latter context since it is a genetically homogeneous strain with the genetic background of strain C57BL/6.

Materials and methods

Construction of the CκT targeting vector

A 5.6 kb *Hin*dIII–*Bam*HI fragment including $J\kappa_{1-5}$, intron enhancer and Cκ exon (Lewis *et al.*, 1982) was cloned into the pGEM-7zf vector (Promega). To destroy the Cκ exon together with its splicing signal and polyadenylation site, a 1.2 kb *Hin*dIII–*Bam*HI fragment containing the *neo* cassette [pMC1-neo/poly(A)$^+$, Stratagene; Thomas and Capecchi, 1987] flanked with one FRT site (between *Hin*dIII and *Eco*RI sites at the 5′-end of the *neo*^{r} gene, O'Gorman *et al.*, 1991) was inserted between the *Mst*II and *Bgl*II sites (Figure 1a). The HSV-tk gene which comes from pIC19R/MC1-tk (Mansour *et al.*, 1988) as a *Cla*I–*Hin*dIII fragment was ligated into the *Cla*I and *Hin*dIII sites of the construct, resulting in the targeting vector (Figure 1b). The final construct was then linearized with *Cla*I and used for ES cells transfection.

Generation of Cκ mutant ES cell line

Sixty micrograms of the linearized targeting construct was transfected into 3×10^7 B6III ES cells (Ledermann and Burki, 1991) by electroporation. Transfected cells were then selected with G418 (300 μg/ml) and GANC (2 μM) (Mansour *et al.*, 1988). The efficiency of GANC enrichment was 4-fold. Resistant colonies were screened by PCR using a 5′ primer (5′-CTCGTGCTTTACGGTATCGC) located 58 bp 5′ of the *Bam*HI site in the pMC1neo, and a 3′ primer (5′-GTAGAAGGCTCAAGGTTGTG) located 19 bp 3′ of the *Bam*HI site which is located 3′ of the Cκ exon (Figure 1c). PCR amplification was performed for 40 cycles using a Techne thermal cycler. Each cycle consisted of 1.5 min at 94°C, 2 min at 65°C and 2 min at 74°C. Putative targeted transfectants, positive for a 1.1 kb amplified fragment, were further verified by Southern blotting. For this purpose, genomic DNA was digested with *Hin*dIII and hybridized to probe B (Figure 1a). The PCR positive clones carry a 4.4 kb fragment, corresponding to the wildtype allele, and additional 3.9 kb and 1.2 kb bands, corresponding to the CκT mutation (Figure 1c and d, right hand panel) were correctly targeted cells. By this screening strategy, we examined 48 G418^r and GANC^r colonies, seven of which carried the targeted allele. Thus, the overall targeting efficiency was approximately 1 in 27 G418^r clones.

Generation of CκT mice

ES cell clones carrying the CκT mutations were injected into blastocysts of CB.20 mice and transplanted into the uteri of F_1 (Balb/c × C57BL/6) foster mothers. Male chimeras were mated to C57BL/6 females. Black offspring (indicative of germline transmission of the ES cell genome) were then analyzed by Southern blotting of the tail DNA by digesting with *Eco*RI and hybridizing to probe A (Figure 1a). Mice heterozygous for the CκT mutation carry a 15 kb wildtype fragment and an additional 5.1 kb fragment resulting from the targeted allele (Figure 1c and d, left hand panel).

Isolation and flow cytometric analysis of cells

Single cell suspensions from bone marrow and spleen were stained with monoclonal antibodies (mAbs) or polyclonal antibodies and analyzed by FACScan (Becton-Dickinson). The following mAbs were used in the flow cytometric analysis: Phycoerythrin (PE)- or biotin-conjugated RA3-6B2 (anti-CD45R/B220; Coffman, 1982); fluorescein isothiocyanate (FITC)-conjugated 145-2-C11 (anti-CD3; Leo *et al.*, 1987), R33-24-12 (anti-μ; Grützmann, 1981), goat anti-mouse λ (Southern Biotech. Assoc.). Biotin conjugates were revealed by Cy-Chrome–streptavidin (PharMingen). Cells present in the lymphocyte gate as defined by light scatter (Förster *et al.*, 1989) were analyzed. For the purpose of Southern blot analysis, splenocytes isolated from three or four 8 week-old mice were stained with FITC-coupled RA3-6B2 and PE-coupled 145-2-C11 or FITC-coupled goat-anti mouse λ antibody and PE-coupled R33-18 (Grützmann, 1981). The B220$^+$, CD3$^+$ and κ$^+$, λ$^+$ cells were sorted using FACSplus. To sort out λ-bearing B cells from normal mice, splenocytes were isolated from five 8 week-old C57BL/6 mice, the CD3$^+$ cells were depleted by magnetic cell sorting (MACS) and the CD3$^-$ cells were stained with anti-κ and anti-λ antibodies with subsequent sorting. The purity of sorted cells was ~97–99% in all cases.

Densitometry

To determine relative quantities of the germline bands of Jκ and RS elements, autoradiographs were scanned using a Quick scan Jr, TCL densitometer (model no. 1039, Helena Laboratories, USA). The signal intensities of each group were calibrated to the internal standard, the IL-4 gene. The signal intensities of the germline bands of T cells were defined as 100%.

Isolation and sequencing of Vλ–Jλ joints

Splenocytes from 8 week-old C57BL/6 mice were isolated and stained with FITC-conjugated goat anti-mouse λ antibody and PE-conjugated R33-18 followed by sorting of the κ$^+$ and λ$^+$ B cell populations. The purity of sorted cells was 99%. Vλ–Jλ joints were then amplified from the cell lysates of 10^4 sorted κ$^+$, λ$^+$ and total splenic B cells using PCR as described by Koller and Smithies (1989). PCR amplification was performed for 40 cycles using a Techne thermal cycler. Each cycle consisted of 1.5 min at 94°C, 1 min at 72°C and 1 min at 74°C. The reaction buffer contained 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 3 mM $MgCl_2$, 400 μM of each dNTP, 0.01% gelatin, 50 pmol of each primer, and 5 U *Taq* polymerase (BRL). The primers used are as follows: $V\lambda_{1,2}$, AGAAGCTTGTGACTCAG-GAATCTGCA; $J\lambda_1$, CAGGATCCTAGGACAGTCAGTTTGGT and $J\lambda_{2,3}$, CAGGATCCTAGGACAGTGACCTTG.

PCR products (rearranged $V\lambda_1$–$J\lambda_1$, $V\lambda_1$–$J\lambda_3$, $V\lambda_2$–$J\lambda_2$ joints) were cloned into the PTZ19R vector (Pharmacia). Bacterial colonies containing Vλ–Jλ joints were randomly picked and the Vλ–Jλ joints were sequenced by direct plasmid sequencing using the Sequenase Kit (USB).

Since Vλ–Jλ joints exhibit only limited diversity and, in particular, since most of the productive joints are identical for each of the Vλ and Jλ combinations, it cannot be formally proven that what we isolate from the amplified material is representative of the joints carried in the original cell population. However, we are confident that this is largely the case for the following reasons. (i) The Vλ–Jλ joints were amplified by primers which should exactly match each of the target sequences, and the amplified sequences differ from each other in a few base pairs at most. In extensive work in the past in which Ig heavy chain rearrangements (D_HH_H and $V_HD_HJ_H$ joints) were amplified which exhibit much more diversity in terms of the genetic elements involved and because of N region addition, there was no indication of a preferential amplification of certain sequences (see Gu *et al.*, 1990, 1991a,b). (ii) The distribution of productive versus non-productive joints is consistent between the experimental groups: in λ$^+$ B cells, most joints were productive, while in κ$^+$ B cells joints were almost exclusively non-productive; from the total B cell population equal numbers of non-productive joints were isolated (Table IV). (iii) Among the non-productive joints, one single $V\lambda_1$–$J\lambda_1$ rearrangement was repeatedly (13 times) isolated within and between experiments, whereas all others are unique (except a $V\lambda_2$–$J\lambda_2$ joint which was found twice). This rearrangement was the only one exhibiting a 1 bp homology between Vλ and Jλ at the breakpoint of recombination. There is evidence that such homologies promote the joining process (Gu *et al.*, 1990, 1991a). However, even if

one eliminates these repeats from the sequence collection, the picture does not fundamentally change.

Acknowledgements

We would like to thank Dr M.Reth, Freiburg, for giving us the RS probe and him and F.Alt for a critical review of this manuscript as well as discussion. We are also grateful to J.Chen and F.Alt for letting us see their data before publication. Finally, we thank S.Schaal and C.Göttlinger for technical help, R.Torres, W.Müller, H.Gu and R.Kühn for advice and discussion, U.Ringeisen for the graphical work, and E.Siegmund and G.Schmall for typing the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft through SFB 243, the Fazit Foundation and by the Human Frontier Science Programme.

References

Alt,F.W., Enea,V., Bothwell,A.L.M. and Baltimore,D. (1980) *Cell*, **21**, 1–12.

Alt,F.W., Blackwell,T.K., DePinho,R.A., Reth,M.G. and Yancopoulos,G.D. (1986) *Immunol. Rev.*, **89**, 5–30.

Berg,J., McDowell,M., Jäck,H.-M. and Wabl,M. (1990) *Dev. Immunol.*, **1**, 53–57.

Blackwell,K., Malynn,B., Pollock,R., Ferrier,P., Covey,L., Fulop,G., Phillips,R., Yancopoulos,G. and Alt,F. (1989) *EMBO J.*, **8**, 735–742.

Blomberg,B., Traunecker,A., Gisen,H. and Tonegawa,S. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 3765–3769.

Chen,J., Trounstine,M., Kurahara,C., Young,F., Kuo,C.C., Xu,Y., Loring,J.F., Alt,F.W. and Huszar,D. (1993) *EMBO J.*, **12**, 821–830.

Claverie,J.-M. and Langman,R. (1984) *Trends Biochem. Sci.*, **9**, 293–296.

Cockerill,P.N. and Garrard,W.T. (1986) *Cell*, **44**, 273–282.

Coffman,R.L. (1982) *Immunol. Rev.*, **69**, 5–23.

Cohn,M. and Langman,R.E. (1990) *Immunol. Rev.*, **115**, 7–147.

Coleclough,C. (1990) *Immunol. Rev.*, **115**, 173–181.

Coleclough,C., Perry,R.P., Karjalainen,K. and Weigert,M. (1981) *Nature*, **290**, 372–378.

Cory,S., Typler,B.M. and Adams,J.M. (1981) *J. Mol. Appl. Genet.*, **1**, 103–116.

Davidson,I., Xiao,J.H., Rosales,R., Staub,A. and Chambon,P. (1988) *Cell*, **54**, 931–942.

Dildrop,R., Gause,A., Müller,W. and Rajewsky,K. (1987) *Eur. J. Immunol.*, **17**, 731–734.

Durdik,J., Moore,M.W. and Selsing,E. (1984) *Nature*, **307**, 749–752.

Feddersen,R.M., Martin,D.J. and Van Ness,B.G. (1990) *J. Immunol.*, **144**, 1088–1093.

Felsher,D.W., Ando,D.T. and Braun,J. (1991) *Int. Immunol.*, **3**, 711–718.

Förster,I., Vieira,P. and Rajewsky,K. (1989) *Int. Immunol.*, **1**, 321–331.

Fromental,C., Kanno,M., Nomiyama,H. and Chambon,P. (1988) *Cell*, **54**, 943–963.

Golding,H., Tittle,T.V., Foiles,P.G. and Rittenberg,M.B. (1980) *Fed. Proc.*, **39**, 4145.

Gollahon,K.A., Hagman,J., Brinster,R.L. and Storb,U. (1988) *J. Immunol.*, **141**, 2771–2780.

Grützmann,R. (1981) Vergleichende Idiotypische Analyse von Rezeptoren mit Spezifität für Histokompatibilitätsantigene. Ph.D. Thesis, University of Cologne.

Gu,H., Förster,I. and Rajewsky,K. (1990) *EMBO J.*, **9**, 2133–2140.

Gu,H., Kitamura,D. and Rajewsky,K. (1991a) *Cell*, **65**, 47–54.

Gu,H., Tarlinton,D., Müller,W., Rajewsky,K. and Förster,I. (1991b) *J. Exp. Med.*, **173**, 1357–1371.

Harada,K. and Yamagishi,H. (1991) *J. Exp. Med.*, **173**, 409–415.

Hardy,R.R., Dangl,J.L., Hayakawa,K., Jager,G., Herzenberg,L.A. and Herzengerg,L.A. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 1438–1442.

Hardy,R.R., Carmack,C.E., Shinton,S.A., Kemp,J.D. and Hayakawa,K. (1991) *J. Exp. Med.*, **173**, 1213–1225.

Haughton,G., Lanier,L.L. and Babcock,G.F. (1978) *Nature*, **275**, 154–157.

Hieter,P.A., Korsmeyer,S.J., Waldmann,T.A. and Leder,P. (1981) *Nature*, **290**, 368–372.

Holmberg,D., Lundkvist,I., Forni,L., Ivars,F. and Coutinho,A. (1985) *Mol. Cell. Immunol.*, **2**, 51–56.

Hood,L., Gray,W.R., Sanders,B.G. and Dreyer,W.J. (1967) *Cold Spring Harbor Symp. Quant. Biol.*, **32**, 133–146.

Huber,C., Klobeck,H.-G. and Zachau,H.G. (1992) *Eur. J. Immunol.*, **22**, 1561–1565.

Kessler,S., Kim,K.J. and Scher,I. (1981) *J. Immunol.*, **127**, 1674–1678.

Kitamura,D. and Rajewsky,K. (1992) *Nature*, **356**, 154–156.

Klobeck,H.G. and Zachau,H.G. (1986) *Nucleic Acids Res.*, **14**, 4591–4603.

Koller,B.H. and Smithies,O. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 8932–8935.

Kubagawa,H., Cooper,M.D., Carroll,A.J. and Burrows,P.D. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 2356–2360.

Ledermann,B. and Burki,K. (1991) *Exp. Cell Res.*, **197**, 254–258.

Le Jeune,J.M., Briles,D.E., Lawton,A.R. and Kearny,J.F. (1981) *J. Immunol.*, **129**, 673–677.

Leo,O., Foo,M., Sachs,D.H., Samelson,L.E. and Bluestone,J.A. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 1374–1378.

Lewis,S., Rosenberg,N., Alt,F. and Baltimore,D. (1982) *Cell*, **30**, 807–816.

Mansour,S.L., Thomas,K.R. and Capecchi,M.R. (1988) *Nature*, **336**, 348–352.

McGuire,K.L. and Vitetta,E.S. (1981) *J. Immunol.*, **127**, 1670–1673.

Meyer,K.B. and Neuberger,M.S. (1989) *EMBO J.*, **8**, 1959–1964.

Müller,B. and Reth,M. (1988) *J. Exp. Med.*, **168**, 2131–2137.

Nadel,B., Cazenave,P.-A. and Sanchez,P. (1990) *EMBO J.*, **9**, 435–440.

Nishikawa,S.-I., Kina,T., Gyotoku,J.-I. and Katsura,Y. (1984) *J. Exp. Med.*, **159**, 617–622.

O'Gorman,S., Fox,D.T. and Wahl,G.M. (1991) *Science*, **251**, 1351–1355.

Osmond,D.G. (1991) *Curr. Opin. Immunol.*, **3**, 179–185.

Persiani,D., Durdik,J. and Selsing,E. (1987) *J. Exp. Med.*, **165**, 1655–1674.

Primi,D., Levi-Strauss,M. and Cazenave,P.-A. (1986) *Eur. J. Immunol.*, **16**, 53–59.

Ramsden,D.A. and Wu,G.E. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 10721–10725.

Rolink,A., Streb,M. and Melchers,F. (1991) *Eur. J. Immunol.*, **21**, 2895–2898.

Sanchez,P. and Cazenave,P.-A. (1987) *J. Exp. Med.*, **166**, 265–270.

Sanchez,P., Marche,P.N., Le Guern,C. and Cazenave,P.-A. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 9185–9188.

Sauter,H. and Paige,C.J. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 4989–4993.

Schlissel,M.S. and Baltimore,D. (1989) *Cell*, **58**, 1001–1007.

Siminovitch,K.A., Bakhshi,A., Goldman,P. and Korsmeyer,S.J. (1985) *Nature*, **316**, 260–261.

Siminovitch,K.A., Moore,M.W., Durdik,I. and Selsing,E. (1987) *Nucleic Acids Res.*, **15**, 2699–2705.

Takemori,T. and Rajewsky,K. (1981) *Eur. J. Immunol.*, **11**, 618–625.

Thomas,K.R. and Capecchi,M.R. (1987) *Cell*, **51**, 503–512.

Tonegawa,S. (1983) *Nature*, **302**, 575–581.

Received on October 12, 1992; revised on November 30, 1992