Rearrangement and Expression of Immunoglobulin Light Chain Genes Can Precede Heavy Chain Expression during Normal B Cell Development in Mice

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Summary

In mouse mutants incapable of expressing μ chains, $V_\kappa J_\kappa$ joints are detected in the CD43$^+$ B cell progenitors. In agreement with these earlier results, we show by a molecular single cell analysis that 4–7% of CD43$^+$ B cell progenitors in wild-type mice rearrange immunoglobulin (Ig)κ genes before the assembly of a productive $V_H D_H J_H$ joint. Thus, μ chain expression is not a prerequisite to Igκ light chain gene rearrangements in normal development. Overall, ~15% of the total CD43$^+$ B cell progenitor population carry Igκ gene rearrangements in wild-type mice. Together with the results obtained in the mouse mutants, these data fit a model in which CD43$^+$ progenitors rearrange IgH and Igκ loci independently, with a seven times higher frequency in the former. In addition, we show that in B cell progenitors $V_\kappa J_\kappa$ joining rapidly initiates κ chain expression, irrespective of the presence of a μ chain.

Key words: B cell development • bone marrow • immunoglobulin gene rearrangement

During B cell development, genes encoding immunoglobulin V regions are generated by recombination of individual gene segments. Genes encoding Ig heavy chains (IgH genes) are formed by first rearranging a D_H to a J_H segment, followed by a V_H to $D_H J_H$ rearrangement. In the light chain (L) loci, a V_L to J_L recombination event generates an Ig light chain (IgL) gene. If the resulting joints are in a contiguous open reading frame, the rearrangements are referred to as "productive".

In regard to the relative order of $V_H D_H J_H$ and $V_L J_L$ recombination events, two models have been proposed. According to the "ordered" model, expression of a μ heavy chain from a productively rearranged IgH gene induces light chain gene rearrangement. Evidence that formation of $V_H D_H J_H$ complexes usually precedes light chain gene rearrangement comes from the analysis of Abelson murine leukemia virus–transformed pre-B cells in culture (1, 2) and from studies of Ig gene rearrangements in B cell precursor populations isolated ex vivo (3). Furthermore, the expression of a transfected membrane-bound μ chain as well as cross-linking of pre-B cell receptor complexes (consisting of membrane-bound μ chains and the products encoded by the λ5 and V_{preB} genes; reference 4) stimulated the rearrangement of endogenous κ light chain genes in transformed pre-B cell lines (5–7). In addition, an increased number of $V_\kappa J_\kappa$ rearrangements was observed in fetal livers of heavy chain transgenic mice as compared with nontransgenic mice (8).

In contrast, the "stochastic" model of IgH and IgL gene recombination states that μ chain expression and pre-B receptor signaling are not required for IgL gene rearrangement and suggests that IgH and IgL loci rearrange independently of each other (9, 10). This hypothesis is supported by the analysis of Abelson murine leukemia virus–transformed murine pre-B cell lines derived from normal (11) and scid mice (12). In both cases, some cells were shown to rearrange Igκ loci in the absence of a membrane-bound μ heavy chain. In vitro differentiation experiments using normal murine pre-B cell lines have also demonstrated that κ protein could be expressed in the absence of a μ chain (13). Moreover, κ chain expression was detected in the absence of productive $V_H D_H J_H$ rearrangements in immortalized B cell precursors of human fetal bone marrow (14). Examination of transformed embryonic bursal cells showed that during chicken B cell development, IgL genes can also be rearranged before IgH gene rearrangement has been completed (15).

Although in vivo most Igκ rearrangements occur in the pre-B cell compartment into which progenitor cells are driven upon pre-B cell receptor (i.e., μ chain) expression (5, 16), evidence indicates that initially, when gene rearrangements in IgH are set in motion in CD43$^+$ progenitors, $V_\kappa J_\kappa$ rearrangements also occur, albeit at low frequency (17, 18). At this early stage of development, κ rearrangements appear to be independent of μ chain expression and, indeed, any rearrangement in the IgH locus,

as they are also seen in mutant mice unable to either express membrane-bound μ chains (μMT mice; 19) or generate $V_HD_HJ_H$ joints due to a targeted deletion of the J_H elements (20, 18). These data suggest that gene rearrangements in the Igκ locus occur at two stages of development: in early $CD43^+$ progenitors at low frequency and independent of μ chain expression, and later on, at high frequency, in pre-B cells upon pre-B cell receptor expression. However, one might argue that the analysis of the mutant mice could be misleading because in these animals the progenitors do not develop beyond the $CD43^+$ stage and therefore persist in this cellular compartment for a prolonged time, accumulating gene rearrangements that normally would not have occurred. On the other hand, Igκ gene rearrangements seen in $CD43^+$ B cell progenitors of wild-type mice (17) could be derived from cells already expressing μ chains.

We therefore decided to verify the results obtained in the mouse mutants by the analysis of IgH and Igκ rearrangements in individual $CD43^+$ B cell progenitors in wild-type animals. This approach allows us to investigate whether recombination of IgL loci can indeed precede the generation of productive IgH gene rearrangements in the course of B cell development under physiological conditions, and, if so, to evaluate the frequency of these events.

Materials and Methods

Cell Sorting

Single cell suspensions were prepared from bone marrow by flushing femurs with DMEM (containing 5% FCS, 0.1% NaN_3) or from splenic tissues of BALB/c mice (8–12 wk old; Bomholtgaard, Denmark). Cells were treated with Tris-buffered 0.165 M NH_4Cl to eliminate erythrocytes and washed by centrifugation through FCS.

3-83κi mice (21) were used at 8–12 wk of age. Wild-type mice used in the staining shown in Fig. 1 were F1 at the age of 8–12 wk from a 129sv × BALB/c cross.

Cell sorting was performed using a dual laser flow cytometer (FACStar®). Single cells were directly deposited into 0.5-ml microtubes containing 20 μl 1× PCR buffer (GIBCO BRL, 2.5 mM $MgCl_2$) supplemented with 1 μg/ml rRNA from *Escherichia coli* (Boehringer Mannheim), immediately frozen on dry ice, and stored at −80°C. Single cells from the E14 embryonic stem cell line (22) were isolated accordingly as negative controls for the PCR. Depending on the set of the cytometer, up to 20% of the tubes could be empty during a particular sorting procedure.

Isolation of Fraction B, C, and D Cells. Fractions were classified according to Hardy et al. (23). Pooled bone marrow cells from two to six mice were depleted of MAC-1^+ cells (and of IgM^+ cells in the experiment with subsequent κ protein staining) by magnetic cell separation (24) using antibody M1/70.15.11/2 (anti–Mac-1; reference 25) or antibody CD11B (anti–Mac-1; Miltenyi Biotec), and in addition rat anti–mouse IgM (Miltenyi Biotec) antibodies for the experiment with subsequent κ protein staining, coupled to magnetic beads (Miltenyi Biotec). Cells passing through the column in the magnetic field were collected and further stained by a combination of FITC-S7 (anti-CD43; reference 26), PE-BP-1 (anti–BP-1; reference 23), biotin-30F1 (anti–heat-stable antigen; reference 23), and allophycocyanin-RA3-6B2 (anti-CD45R/B220; reference 23) in staining medium, washed, and counterstained by Texas red–avidin (Boehringer Mannheim).

To obtain fraction B cells that expressed κ chains intracellularly, sorted fraction B cells (~10^5 cells) from pooled bone marrow of five mice were fixed in PBS containing 2% formaldehyde for 20 min at room temperature. After washing with PBS, the cells were resuspended in PBS containing 0.1% NaN_3 and 1% BSA, bleached overnight, and then stained with FITC–R33-18 (anti-κ; reference 27) in PBS containing 1% saponin (Sigma Chemical Co.).

The extent of possible contamination of $CD43^+$ by $CD43^-$ B cell precursors (pre-B cells) or by B cells (all bearing productive $V_HD_HJ_H$ joints) can be estimated as not exceeding 10% from the staining data (not shown) for the sortings of fraction B cells. By selecting $κ^+$ cells, one would expect to enrich for contaminating cells, so that the proportion of cells bearing productive $V_HD_HJ_H$ rearrangements would be higher in the κ chain–expressing than in total fraction B cells. Since this is not the case (8 out of 15 $κ^+$ cells compared with 7 out of 11 unselected fraction B cells; see Tables V and VI), a significant contamination of fraction B cells by pre-B or B cells seems excluded. In the sortings of fraction C cells, the staining data do not allow us to rule out the possibility of a contamination by pre-B or B cells that could be >10%. Note, however, that contaminating cells, if present, would appear only among the cells with productive $V_HD_HJ_H$ joints, and would thus lead to an underestimation of the true proportion of cells that form $V_κJ_κ$ joints while lacking productive $V_HD_HJ_H$ rearrangements in early B cell development.

Sorting of κ light chain expressing splenic B cells was done by staining splenocytes with PE-RA3-6B2 (23) and FITC-R33-18 (27).

PCR and Sequence Analysis of Ig Gene Rearrangements

To prepare DNA for amplification, 1 μl of an aqueous solution of proteinase K (10 mg/ml; Boehringer Mannheim) was added, samples were overlaid with paraffin oil, and were incubated for 30 min at 55°C. Subsequently, proteinase K was inactivated for 10 min at 95°C. PCR amplification was carried out in two rounds: the first reaction contained all 5′ primers, JH4E (29), and Jκ5E primers (2.5 pmol each; Table I). Amplification was done over 30 cycles (1 min at 95°C, 1 min at 60°C, and 2.5 min at 72°C). For the second PCR, 1.5-μl aliquots of the first round amplification product were transferred into separate reactions (set up in 96-well microtiter plates; Costar Corp.), each containing a single 5′ primer in combination with either the nested JH4A (amplification of IgH genes; reference 29) or the Jκ5A primer (amplification of Igκ genes) (7 pmol of each primer). 30 cycles were performed (1 min at 95°C, 1 min at 63°C, and 1.5 min at 72°C). All PCRs contained dATP, dCTP, dGTP, dTTP (Pharmacia Biotech) at 200 μM each, PCR buffer (GIBCO BRL), 2.5 mM $MgCl_2$, 5 U of Taq DNA polymerase (GIBCO BRL) in the first round, and 3 U of Taq DNA polymerase in the second round. The final volume of each reaction was 50 μl. Each PCR was followed by a 5–10-min incubation at 72°C. 10 μl of the second-round PCR product was analyzed on agarose gels. Before sequencing, 1.5 μl of second-round product was reamplified for 20 cycles (30 s at 95°C, 1 min at 63°C, and 2 min at 72°C) using appropriate 5′ primers and nested 3′ primers, DNA was isolated from preparative agarose gel using Spin-X columns (Costar Corp.). Cycle sequencing was performed using the Ready Reaction Dye Deoxy Terminator Cycle Sequencing Kit (Applied

Biosystems) following the manufacturer's instructions and an ABI 373A sequencer (Applied Biosystems). Sequencing primers recognize sequences downstream of the respective rearranged Jκ genes (Table I).

The primers used for amplification and sequencing of Ig heavy chain genes have been described by Ehlich et al. (23) and Löffert et al. (30). The VHH primer (30) was not used in the analyses of fraction C. KGI (Table I) was used only in the analyses of fraction B cells irrespective of κ protein staining.

Sequences were analyzed using DNAPLOT at www.genetik.uni-koeln.de/dnaplot/. The database used consists of mouse V gene sequences from an EMBL/GenBank/DDBJ nucleotide sequence database, a Kabat database (31), and the $V_κ$ sequence list compiled by Kofler et al. (32).

Control Experiment to Confirm the Isolation of Single Cells by FACS®

We chose two mutant mouse strains in which a rearranged Ig heavy chain variable region gene was introduced by gene targeting into the heavy chain locus, replacing the J_H elements (T15i mice, reference 28, and B1-8i mice, reference 33, containing a rearranged V_H186.2 gene isolated from the hybridoma B1-8; reference 34). From each of the two mouse strains, which were homozygous for the introduced heavy chain, 4×10^5 κ light chain–positive splenic B cells were isolated by FACS® and subsequently pooled to yield a 1:1 mixture. Of this mixture single cells as well as two cells were sorted directly into microtubes containing PCR buffer. The inserted $V_HD_HJ_H$ complexes of these splenic B cells were amplified in a semi-nested PCR approach analogous to the one described above. The B1-8 (T15i) gene was amplified by the 5′ primer VHA (VHT15) and the 3′ primers JH2E and JH2 (JH1E and JH1) in the first and second rounds of amplification, respectively. The primers used in this experiment have been described elsewhere (30).

Results

PCR Analysis of Single B Cell Precursors. We extended our previously described single cell PCR system for the analysis of IgH genes (29) to simultaneously examine Igκ genes. For this purpose, seven Igκ gene–specific oligonucleotides were included to detect rearranged $V_κJ_κ$ complexes as well as Igκ loci in germline configuration (Table I).

To estimate the efficiency of the amplification of Igκ loci rearrangements, we used splenic, surface Igκ-positive B cells. 197 $V_κJ_κ$ joints were amplified from 210 single B cells (none, one, or two per cell). Assuming that ~30% of all splenic B cells carry two $V_κJ_κ$ complexes (9, 16), this corresponds to a $V_κJ_κ$ rearrangement detection efficiency of ~70%. To determine the detection efficiency of IgH gene joints, $V_HD_HJ_H$ and D_HJ_H gene rearrangements were amplified from 311 B cell precursors of the $CD43^+$ fraction C (reference 23; excluding fraction C′ cells) in the presence of Igκ locus–specific oligonucleotides. Two IgH gene PCR products were obtained from 41% of cells. In the remaining cells, either one (51%) or no (7%) IgH gene PCR products were amplified. Thus, the efficiency of the amplification was sufficient to allow the simultaneous analysis of heavy and light chain loci.

When the interdependence of rearrangements of the various Ig loci is investigated by single cell analysis, it is essential to demonstrate that the amplification products are

Table I. *Igκ Locus–specific Oligonucleotides Used In PCR and Sequencing Reactions*

	Primer	Specificity
A	PCR primers	
	κ light chain genes	
VK1	GCG AAG CTT CCC TGA TCG CTT CAC AGG CAG TGG	
VK2	GCG AAG CTT CCC(AT)GC TCG CTT CAG TGG CAG TGG	
VK3	GCG AAG CTT CCC A(GT)(AC) CAG GTT CAG TGG CAG TGG	
KG	GCG AAG CTT AAG CTT TCG CCT ACC CAC TGC TCT	5′ of $J_κ$1
KG1	ACA GCC AGA CAG TGG AGT ACT ACC ACT GTG	immediately 5′ of $J_κ$1
JK5E	GAT CCA ATC TCT TGG ATG GTG ACC	
JK5A	GGG TCT AGA CAA CTG ATA ATG AGC CCT CTC CAT	
B	Sequencing primers	
	κ light chain genes	
JK1	AGA CAT AGA AGC CAC AGA CAT AG	
JK2	CTT AAC AAG GTT AGA CTT AGT GAA C	
JK4	TTC ACA CAA GTT ACC CAA ACA G	
JK5	GAA CTG ACT TTA ACT CCT AAC ATG	

Sequences are presented from 5′ to 3′. Nucleotides in brackets denote a nucleotide mix at this position. V, KG, and KGI oligonucleotides are 5′ primers, whereas J oligonucleotides are 3′ primers. The $V_κ$ primers, recognizing all $V_κ$ genes listed by Strohal et al. (52) in framework region 3, cross-react and thus cannot be assigned to specific $V_κ$ families. The KG and KGI primers hybridize to a germline region upstream of $J_κ$1. All J primers are homologous to a region downstream of the respective J element. A shows the primers used for amplification, and B shows those used in sequencing reactions.

indeed derived from the same cell, and that the samples do not occasionally contain more than one cell. Therefore, a control experiment similar to the one described by Löffert et al. (30) was performed using two mutant mouse strains in which different heavy chain transgenes were inserted into the heavy chain locus, replacing the J_H elements (T15i mice, reference 28, and B1-8i mice, reference 33). Cell suspension containing equal proportions of Igκ-positive splenic B cells from both strains was prepared. From this, either "one cell" or "two cell" samples were deposited into microtubes using the FACS®. Subsequently, the IgH transgenes were amplified from these cells, using appropriate PCR primers (30).

127 "one cell" samples yielded indeed only one PCR product (Table II). In the case of the "two cell" samples, 50% of the tubes would be expected to contain two cells from the same mouse strain that would not be identified as "two cells" because both have given rise to identical PCR products. Two different PCR products were obtained in 53% of the "two cell" samples (Table II). The rare cases in which no PCR product was obtained (Table II) may be explained by a relatively poor amplification efficiency using this particular primer set, or, alternatively, these tubes may not have contained a cell. These results indicate that the direct deposition of cells by FACS® used in the experiments described below represents a reliable method for obtaining samples containing single cells.

Igκ Gene Rearrangements in Early B Cell Precursors. To investigate whether IgL gene rearrangements in B cell precursors can occur before μ chain expression, we had to look into the compartment of early B cell progenitors, where cells both with and without productively rearranged heavy chain genes are present. To classify different stages of B cell development in the bone marrow, we used the system developed by Hardy et al. (23), which divides $B220^+$, surface Ig^- cells into five cellular fractions according to their differential expression of CD43, heat stable antigen (HSA), and BP-1. For initial studies, we chose fraction C (excluding fraction C′; references 17, 23) of early B cell progenitors in which $V_\kappa J_\kappa$ rearrangements are six to seven times less frequent than in κ^+ splenic B cells (17).

627 fraction C cells were examined. For 14 out of 50 cells bearing $V_\kappa J_\kappa$ rearrangements, the configurations of both IgH alleles were determined (Tables III and VI). Seven cells are potentially able to express μ chains because they harbor functional $V_H D_H J_H$ rearrangements. However, seven other cells contain an Igκ gene rearrangement in the absence of a functional $V_H D_H J_H$ complex. Two of these cells carry nonfunctional $V_H D_H J_H$ rearrangements at both IgH alleles, and four carry a nonproductive $V_H D_H J_H$ rearrangement together with a $D_H J_H$ joint. Two nonproductive $V_H D_H J_H$ joints (in cells 298 and 717) comprise D_H elements rearranged in reading frame 2 (in the nomenclature of Ichihara et al.; reference 35). Thus, these cells could have expressed a truncated heavy chain (Dμ protein; reference 36) before $V_H D_H J_H$ complex formation. The remaining cell harbors a rearranged Igκ allele and contains only $D_H J_H$ complexes (cell 352). The D_H elements in this cell are rearranged in reading frames other than reading frame 2.

It has been suggested that cells incapable of expressing a pre-B cell receptor accumulate in fraction C (29). Thus, at least some of the cells carrying $V_\kappa J_\kappa$ joints observed in fraction C could represent dead-end cells that cannot mature further and may have persisted for a prolonged time in fraction C. Such prolonged persistence may increase the probability to rearrange Igκ genes. Therefore, we decided to also analyze fraction B, the earliest stage at which $V_H D_H J_H$ rearrangements are detected, for the presence of cells containing rearranged κ genes in the absence of productive $V_H D_H J_H$ complexes. According to our previous analysis, $V_\kappa J_\kappa$ rearrangements are 14 times less frequent in this cell population than in κ-positive splenic B cells (17).

To enrich for cells bearing Igκ rearrangements, we isolated cells that stained for κ chains intracellularly. 88 single fraction B cells positive for intracellular κ chains were analyzed. Igκ gene rearrangements were amplified (either one or two per cell) from 47 cells. For 15 of these we were able to determine the configuration of both heavy chain alleles. 8 out of 15 cells bearing $V_\kappa J_\kappa$ rearrangements contained a productive $V_H D_H J_H$ joint. Seven cells were found to harbor either $D_H J_H$ joints on both heavy chain alleles (five cells) or a nonproductive $V_H D_H J_H$ joint on one allele and a $D_H J_H$ joint on the other (two cells) (Tables IV and VI). Reading frame 2, which encodes Dμ protein, appeared on one or both alleles in all five cells that bear only $D_H J_H$ rearrangements and in one of the two cells containing a $D_H J_H$ joint

Table II. *No. of PCR Products Obtained from Control Samples Containing Either "One" or "Two" Cells*

Cells per sample	No. of samples	PCR products			
		B1-8 + T15	B1-8	T15	no product
1	127	0	45	68	14
2	90	48	19	19	4

Either one or two cells of a 1:1 mixture of κ^+ spenic B cells derived from T15i and B1-8i mice were deposited by FACS® into microtubes and their rearranged immunoglobulin genes were amplified by PCR. The numbers of different amplification products are shown for samples containing either one or two cells.

Table III. *Junctional Region Sequences of D_HJ_H, $V_HD_HJ_H$, and $V_\kappa J_\kappa$ Ig Gene Rearrangements in B Cell Progenitors from Fraction C*

No	$V_HD_HJ_H$ and D_HJ_H rearrangements											$V_\kappa J_\kappa$ rearrangements and Igκ germline fragments					
	type	VH	DH	JH	rf	prod	$3'V_H$ or upstream D_H	P,N nucleotides	D_H element	P,N nucleotides	J_H element	V_κ	J_κ	prod	$3'V_\kappa$ or upstream $J_\kappa1$	P,N nucleotides	J_κ element
352	DJ		sp2.10	2	1		TCT ACT ACT GTG		TC TAC TAT AGG TAC	CT	C TTT GAC TAC TGG GGC	4	5	np	TNC CAG CAG TGG NAT TAT CCT CTT ATT ATT C	CC GT	TC ACG TTC GGN
	DJ		sp2.10	3	3		TCT ACT ACT GTG		T CTA CTA TAG GNA CGA C		GG TTT NCT TAC TGG GGC						
298	VDJ	3	fl16.2	3	2	np	ANA TTA CTN TNC	CC TTA A	TC ATT ACN ACG GCN AC	A GGG	GCT TAC TGG GGC	38C	5	p	TGT CTA CAG TAT GAT AAT CN	T CTA CA	G CTC ACG TTC GGT
	DJ		sp2.1 or 2.5	3	3		TCT ACT ACT GTG		T CTA CTA AGG NAA CTA C		AC TAN NCT AAG GAC TAC TGG GGT	2	2	np	TGN CAA GGT ACA CAT TTT CC	CC	G TAC ACG TTC GGA
321	VDJ	5	sp2.1 or 2.3-2.7	2	1	np	TGT GCA AGA	G AGG GGC AAT CG	C TAC TAT GG		C TTT GAC TAC TGG GGC	24	1	np	TGT GCT CAA AAT CTA GAA CTC CCT	C	G TGG ACG TTC GGT
	DJ		fl16.1	3	1		TCT ACT ACT GTG		NT TAT TAC TAC GGT AGT AGC	CCC C	TT GCT TAC TGG GGC	germline fragment					GTACTACCACTGTGGTGGACGTTCGGT
717	VDJ	1	sp2.9	3	2	np	TGT NCA AGA	TT	ATG GTT ACT AC	C CAG TGA	GGC	2	4	np	TGC TGG CAA GGT ACA CAT T	CT	G TTC GGC
	DJ		fl16.1	1	3		TCT ACT ACT GTG		T TTA TTA CTA CGG **TAG** TA		C TGG TAC TTC GAT GTC TGG GGC	germline fragment					GTACTACCACTGTGGTGGACGTTCGGT
718	VDJ	2	sp2.10 or 2.11	3	3	np	TGT TCC AGA	G	A **TAG** GT	CCC	G TTT GCT TAC TGG GGC	4	5	p	TGC CAG CAG TGG AAT TAT CCT		CTC ACG TTC GGT
	DJ		fl16.1	3	3		TCT ACT ACT GTG		T TTA TTA CTA CGG **TAG TAG** CTA C		GG TTT NCT TAC TGG GGC	germline fragment					GTACTACCACTGTGGTGGACGTTCGGT
78	VDJ	2	sp2.9	3	1	np	TGT GCC AG	C	T GAT GGT TAC T	CT	TTT GCT TAC TGG GGC	AI5	2	np	TGT AT	TTC TCC C	CG TTC GGA
	VDJ	5	sp2.11	3	1	np	TGT GCA AGA	CG	CC TAC TAT AGG TAC GAC	G	GG TTT GCT TAC TGG GGC	germline fragment					GTACTACCACTGTGGTGGACGTTCGGT
96	VDJ	1	fl16.1 or 16.2	1	3	np	TGT GCA AGA	C	TCA CT	G GGT G	AC TGG TAC TTC GAT GTC TGG GGC	n.i.	5	p	TGC TTC CAA GGT ACA CAT TTA CC	C GGA CTC	CTC ACG TTC GGT
	VDJ	2	fl16.1 or 16.2	3	3	np	TGT GC	CCC AAG GGG C	AG CT	C GGG CAA CGT AAG	GCT TAC TGG GGC	germline fragment					GTACTACCACTGTGGTGGACGTTCGGT
5	VDJ	5	Q52	3		p	TGT GCA AGA	CAG A	A A CTG GGA C		GG TTT GCT TAC TGG GGC	21	2	p	TGT CAN CCN AAT AAT GAG GAT CC		G TAC ACG TTC GGA
	DJ		fl16.1	2	1		TCT ACT ACT GTG		TT TAT TAC TAC GGT AGT A	CC CCC CTC	TTT GAC TAC TGG GGC	germline fragment					GTACTACCACTGTGGTGGACGTTCGGT
80	VDJ	6	n.i.	3		p	ATT TAT TAC TGC	CCC	TGG		GCT TAC TGG GGC	19	5	np	TGT CAT CAA GAC CTC TCT TC	TC	CTC ACG TTC GGT
	DJ		fl16.1	2	3		TCT ACT ACT GTG		T TTA TTA CTA CGG **TAG TAG** CTA C	GA	TAC TTT GAC TAC TGG GGC						
265	VDJ	2	n.i.	3		p	TGT TCC AGA	G	AGG		GG TTT TCT TAC TGG GGC	2	1	p	TGC TGN CAA GGT ACA CAT TTT CC		G TGG ACG TTC GGN
	DJ		sp2.2	3	1		TCT ACT ACT GTG		TC TAC **TAA** GAT TAC	CAG G	CC TGG TTG GCT TAC TGG GGC	germline fragment					GTACTACCACTGTGGTGGACGTTCGGT
294	VDJ	2	fl16.1	3	1	p	TGT CCC AGA	AAG G	AT TAC TAC GGT AGT AGC	CCC CTT TATGG	T TAC TGG GGC	9B	5	np	TGT CCA CAG TAT GAN GAG TTT C		CTC ACG TTC GGN
	DJ		fl16.1	3	3		TCT ACT ACT GTG		T TTA TTA CTA C	CG G	GG TTT GCT TAC TGG GGC						
499	VDJ	5	sp2.2	4	2	p	TGT GCA AG	G CG	T ATG A	CC C	AC TAT GCT ATG GAC TAC TGG GGT	4	5	p	TGC CAN CAG TGG AGT AGT AAC CC		G CTC ACG TTC GGT
	DJ		fl16.1	2	2		TCT ACT ACT GTG		TTT ATT A	CC TCT	GGC	germline fragment					GTACTACCACTGTGGTGGACGTTCGGT
530	VDJ	1	fl16.1	4	2	p	TGT GCA AGA	GAG	TTT ATT ACT AC	A GA	G GAC TAC TGG GGT	4	5	np	TGC CAT CAG TGG AGT AGT TAC CC	AC CCC CC	C ACG TTC GGT
	DJ		sp2.2	3	3		TCT ACT ACT GTG		T CTA CTA **TGA** TTA CGA C	GG GGG	GCT TAC TGG GGC						
538	VDJ	1	n.i.	4		p	TGT GC	CC	TC TN		G GAC TAC TGG GGT	23	2	np	TGT CAA CNA AGT AAT AGC TGG CCA		AC ACG TTC GGA
	VDJ	9	fl16.1	3	3	np	TGT GCA	G	CTA CGG **TAG TAG** C	CGG	CC TGG TTT GCT TAC TGG GGC						

The two IgH or Igκ alleles of a cell are placed so that each line contains information about one allele. Heavy chain gene sequences are shown in the left part of the table and the corresponding light chain sequences in the right part. (P,N) Nucleotides not encoded in the germline, called either N nucleotides (53, 54) or P nucleotides (55). Sequences of the IgH locus are categorized based on their configuration in the column type as DJ or VDJ. D_H sequences were assigned to published D_H segments (56) if there was homology of at least four nucleotides. Numbers in column rf indicate the D_H element reading frame (35). Reading frame is not identified for DQ52 element, because of its inability to encode for Dμ protein. N.i. indicates that the respective D or V element could not be unambiguously assigned to some gene or gene family. Numbers in JH or J_κ columns indicate the J element used in the respective joint. For $V_HD_HJ_H$ or $V_\kappa J_\kappa$ rearrangements the V gene families used are indicated in the columns VH or V_κ. In the column prod: p, a productive $V_HD_HJ_H$ or $V_\kappa J_\kappa$ joint; np, a nonproductive $V_HD_HJ_H$ or $V_\kappa J_\kappa$ joint. Stop codons are shown in bold.

Table IV. *Sequences of D_HJ_H, $V_HD_HJ_H$, and $V_\kappa J_\kappa$ Junctional Regions Ig Gene Rearrangements in Intracellular κ–expressing B Cell Progenitors from Fraction B*

No	$V_HD_HJ_H$ and D_HJ_H rearrangements											$V_\kappa J_\kappa$ rearrangements and Igκ germline fragments					
	type	VH	DH	JH	rf	prod	$3'V_H$ or upstream D_H	P,N nucleotides	D_H element	P,N nucleotides	J_H element	V_κ	J_κ	prod	$3'$ V_κ or upstream $J_\kappa1$	P,N nucleotides	J_κ element
43	DJ		sp2.1 or 2.5	2	2		TCT ACT ACT GNG		TCT ACT ATG GTA ACT	C	AC TTT GAC TAC TGG GGC	4	4	p	TGC CAG CAG TGG AGT GGT TAC CCA		TTN ACG TTC GGC
	DJ		sp2.10	4	2		TCT ACT ACN GNG		TCT ACT ATA GGT ACG A	GA GGG AG	C TAT GCT ATG GAC TAC TGG GGT						
52	DJ		sp21-25 or 2.10	3	2		TCT ACT ACN GNG		TCT ACT A	AG	TGG TTT GCT TAC TGG GGC	4	5	p	TNC CAG CAG TGG AGT AGT AAC CCA		CTC ACG TTC GGT
	DJ		sp2.8	4	2		TNT ANT ACT GTG		NCT AGT ATG G	GT	TAT NCT ATG GAC TAC TGG GGT						
60	DJ		sp2.9	4	2		TCT ACT ACN GAG		TCT ATG ATG GTN ANT AC	G TAT G	AC TAT GCT ATG GAC TAC TGG GGT	2	2	p	TNC TGG CNA GGT ACA CAT TTT CC	C	TAC ACG TTC GGA
	DJ		sp2.10	3	1		TCT ACT ACT GNN		TC TAC TAT AGG TAC G	GT CC	C TTG GCT TAC TGG GGC						
64	DJ		sp2.8	1	2		TCT ACT ACT GTG		NCT AGT ATG GTN ACT	TAC A	AC TGN TAC TTC NAT GTC TGG GNC	8	1	p	TGT CAT CAA TAC CTC TCC TCG		TGG ACG TTC GGT GGA GGC ACC
	DJ		sp2.10	3	2		TCT ACT ACT GNG		TCT ACT ATA GGT ACG AC	A NN	G TTN GCT TAC TGG GNC	germline fragment					GTACTACCACTGTGGTGGACGTTCGGT
66	DJ		sp2.10	2	2		TCT ACT ACT GTG		TCT ACT ATA GGT ACG A	AG	GAC TAC TGG GGC	1	5	p	TGC TTA CNA GGT ACA CAT CAN NC	C CGA GC	G CTN ACG TTC GGT
	DJ		sp2.1, 25or27	4	1		TCT ACT ACT GTG		TC TNC TAT GGT AA	G A	AT TGC TAT NCT ATG GAC TAC TGG GGT						
62	VDJ	1	sp2.9	3	3	n p	TGT GCA AGA	A GGG GGC	CTA TGA TGG TTA CTA CT		C TGG TTT GCT TAC TGG GGC	4	4	p	TNC CAN CAG TGG AAT TAT CCT CTT	AG	C ACG TTC GGC
	DJ		sp2.10	3	1		TNT ANT ANT NTN		TC TAN TAT AGG NAN GAN	GAG G	GN TTT GCT TAC TGG GGC						
110	VDJ	n.i.	f16.1-2, sp2.1-7 or 2.x	4	1	n p	TGT GN		T NAC NA	TA	CT ATG GAC TAC TGG GGT	n.i.	5	p	TNC TNC CGG GAG AAT NAC TNC	CTN CC	G CTC ACG TTC GGT
	DJ		sp2.1 or 2.5	3	2		TCT ACT ACT GNG		TCT ACT ATG GTA ACN AC	G AGG CC	G TTT GCT TAC TGG GGC						
40	VDJ	2	sp2.1 or 2.3-2.9	3	1	p	TGT GCC AGA	G	AT GG	AGT	C TGG TTT GCT TAC TGG GNC	4	4	p	TNC CAA CAG TGG NGT AGT NAC CCA		TTC ACG TTC GGC
	DJ		sp2.10	4	3		TCT ACT ACT GNG		T CTA CTA TAG GTA CG	A CGG GAA GCA	ATG GAC TAC TGG GGT						
50	VDJ	1	Q52	3		p	TGT GCA AGA		TGG GA	A TTA A	TT GCT TAC TGG GGC	n.i.	5	p	TGN GAN NCT ACC ACN ACT GGC GNN	GTT NAC ATC CC C	CG TTC GGT
	DJ		sp21-25 orsp2.10	3	2		TCT ACT ANN GNG		TNT ACT A	AG	TGG TTT GCT TAC TGG GGC						
57	VDJ	2	sp2.2	3	1	p	TGT GCC AGA	A	AT TAC G	GG C	CC TGG TTT GCT TAC TGG GGC	4	2	p	TGC CAG CAG TGG AGT AGT AAC CCA	CC	C ACG TTC GGA
	DJ		fl16.2	2	1		TCT ACT ACT GTG		TT CAT TAC TAC GGC TAC	CT	C TAC TTN GAC TAC TGG GGC	4	1	p	TGC CAG CAG TAC AGT GGT TAC CCA		TGG ACG TTC GGT
69	VDJ	5	n.i.	2		p	TGT ACA AGA			GGG GGG T	TC TAC TTT GAC TAC TGG GGC	2 3	5	n p	TNT CAA CAG AGT AAC AGC TGG CC		CTC ACG TTC GGT
	DJ		fl16.1	3	1		TCT ACT ACT GTG		TT TAT TAC TAC GGT AGT AGC **TA**	**G** A	CC TGG TTT GCT TAC TGG GGC						
70	VDJ	4	fl16.1-2 or sp2.9	4	1	p	TGT GCA AGG	GTG GGG GTG GG	T TAC TAC	C	AT TAC TAT GCT ATG GAC TAC TGG GGT	4	1	p	TGC CAG CAG TTT ACT AGT TCC CCA		TGG ACG TTC GGT
	DJ		Q52	3			GGA GCA CCA CAG TNN		TAA C	C	C TGG GGC						
113	VDJ	8	n.i.	3		p	TGT NC	CG	AGG	GGG	CC TGG TTT NCT TAC TGG GNC	2 2	4	p	TGT GGA CAG AGT TAC AGC TAT	AT	A TTC ACG TTC GGC
	DJ		sp2.9	3	1		TCT ANT ACN GNG		TC TAN GAT GGT TAC T	CGTTA	GCT TAC TGG GGC						
46	VDJ	2	sp21-25, 29-210	2	2	p	TGT GCC AGA	AAT	TCT A	GG GTG T	AC TAC TTT GAC TAC TGG GGC	8	1	p	TGT CAA CTA CAT TAT AGC ACT CCG		TGG ACG TTC GGT
	VDJ	1	sp2.3-4, sp26or9	4	1	n p	TGT GC	CAC T	AT GGT TAC	CCA AGT ATN	CAN NAC NAN GCN ANG GAC TAC TGG GGT						
87	VDJ	5	Q52	2	2	p	TGT GCN AG	T TCC CAN TCG AGG GGG	CTG GGN C	TG GGG TTT	TTT GAC NAC TGG GGC	RF	1	p	TGT CNA CAG CAT AAT GAA TAC C		GG ACG TTC GGT
	VDJ	1	sp2.7	4	1	n p	TGT NCA AGA	AG GGG GGC C	CC TAC TAT GGT AAC TNC	GGT CGG	GCT ATG GAC TAC TGG GGT	germline fragment					GTACTACCACTGTGGTGGACGTTCGGT

Designations are the same as in Table III.

together with a nonproductive $V_HD_HJ_H$. Only one $V_\kappa J_\kappa$-bearing cell (cell 62, Tables IV and VI) that is unable to produce a (truncated) heavy chain was found in this experiment. It carried a nonproductive $V_HD_HJ_H$ rearrangement (with the D_H element in reading frame 3) on one allele and a D_HJ_H joint in reading frame 1 on the other.

However, in order to maximally enrich for κ chain producers we had isolated only cells that displayed high levels of κ protein. These cells might be already selected for μ chain or Dμ protein expression, considering that the stability of the κ protein could depend upon the presence of a (truncated) heavy chain in the cell. For this reason we decided to look again in cells from fraction B, this time not selected by intracellular staining for κ protein, but randomly selected by PCR for the presence of $V_\kappa J_\kappa$ rearrangements.

373 single cells sorted from fraction B were analyzed. In 32 cells we detected one or two rearrangements at the κ locus. In 11 of these cells we were also able to amplify and sequence rearrangements of both heavy chain alleles. In four cells no productive $V_HD_HJ_H$ joint was present (Tables V and VI). One cell contained two nonproductive $V_HD_HJ_H$ joints (one of which comprises a D_H element in reading frame 2), and three cells carried D_HJ_H rearrangements on both heavy chain alleles. None of these D_HJ_H/D_HJ_H cells harbored D_H elements rearranged in reading frame 2.

Igκ Chain Expression in Early B Cell Precursors. Given the efficiency of $V_\kappa J_\kappa$ joint detection of ~70% and the fact that single cell sorting procedure will leave up to 20% of the tubes empty, the overall frequency of cells bearing $V_\kappa J_\kappa$ rearrangements (either productive or nonproductive) in both fractions B and C is in the range of ~11–16%.

To estimate the frequency of cells that are able to express κ chain at the early stages of B cell development, we stained fraction B cells for intracellular κ protein. We used wild-type mice and the 3-83κi mouse mutant in which a productive $V_\kappa J_\kappa$ gene segment encoding the V_L region of antibody 3-83 (37) was inserted by gene targeting into its natural genomic localization so that its expression is controlled by the endogenous regulatory elements (21). Due to the fact that in wild-type mice two-thirds of the $V_\kappa J_\kappa$ rearrangements are out of frame, 3-83κi mice should show a threefold increase in the number of κ chain–expressing cells in fraction B. The result of this experiment is shown in Fig. 1: ~7% of cells in fraction B in wild-type mice were found to express κ chains, whereas this value was 24% in the 3-83κi mutant, yielding almost exactly the expected 1:3 ratio.

These data are in agreement with the frequency of $V_\kappa J_\kappa$ rearrangements in cells from fraction B estimated by PCR analyses. Together, these results suggest that Igκ gene rearrangement and expression follow each other rapidly.

Discussion

Reliability of the Assay System. A control experiment in which either one or two cells were deflected into each reaction tube (Table II) confirmed that the method to isolate single cells by using the FACS® is highly reliable and that the PCR products obtained from one sample are indeed derived from a single cell. This is further supported by the fact that PCR amplification of one sample never generated more than four products (two from heavy chain loci and two from κ light chain loci; data not shown). There was also no indication for the presence of contaminating DNA molecules in the PCR, because rearranged Ig genes were never amplified from control samples containing embryonic stem cells and the sequences of all rearrangements were different. Therefore, it is unlikely that in the cases where rearranged κ genes were observed in the absence of productive $V_HD_HJ_H$ complexes, the IgH gene rearrangements amplified were derived from a second cell present in the sample or from foreign DNA. The extent of a possible contamination in fractions B and C by $CD43^-$ pre-B or B cells due to inaccurate cell separation during FACS® sorting is discussed in the Materials and Methods section. However, the presence of such contaminating cells (all bearing productive $V_HD_HJ_H$ joints) would result in an underestimation of the percentage of cells bearing $V_\kappa J_\kappa$ joints but no productive $V_HD_HJ_H$ rearrangements in the early fractions of B cell progenitors.

Although most Ig genes present in the germline are recognized by the collection of the primers used, certain combinations of gene rearrangements in a cell could not be detected. In particular, all D_H elements (except the D_HQ52 element) are recognized by the same primer and the primers specific for V_κ genes are highly homologous in structure (Table I). Therefore, most of the D_HJ_H joints using the same J_H genes on both chromosomes or distinct $V_\kappa J_\kappa$ rearrangements with the same J_κ segment could not be resolved. For these reasons the number of cells with D_HJ_H joints at both IgH loci and the number of cells bearing two $V_\kappa J_\kappa$ joints could be underestimated.

Igκ Gene Rearrangements Appear To Be Independent of Heavy Chain Expression in $CD43^+$ B Cell Progenitors. The question of whether expression of a productive $V_HD_HJ_H$ rearrangement is a prerequisite for light chain gene rearrangement during B cell development or whether Igκ gene rearrangement can take place also in the absence of a membrane-bound μ chain has been discussed controversially. The analysis of Ig gene rearrangements of single B cell progenitors isolated ex vivo from wild-type mice addresses this issue directly.

Cells of the earliest B cell progenitor fractions in which $V_\kappa J_\kappa$ rearrangements are detectable, namely, cells of the $CD43^+$ fractions B and C (17), were chosen for analysis. The results obtained are summarized in Table VI. Overall, 18 cells were found to carry Igκ rearrangements in the absence of a productive $V_HD_HJ_H$ joint. However, six of these contained D_HJ_H rearrangements in reading frame 2, and thus were able to express Dμ proteins. Like a μ chain, the Dμ protein could associate with the products of the λ5 and V_{preB} genes to form a pre-B cell receptor–like complex (7). It has been suggested that Dμ protein expression, similar to μ chain expression, provided a stimulatory signal for Igκ gene rearrangements (5, 38, 39). Among the other cells an-

Table V. *Sequences of D_HJ_H, $V_HD_HJ_H$, and $V_\kappa J_\kappa$ Junctional Regions Ig Gene Rearrangements in B Cell Progenitors from Fraction B*

No	$V_HD_HJ_H$ and D_HJ_H rearrangements											$V_\kappa J_\kappa$ rearrangements and Igκ germline fragments					
	type	VH	DH	JH	rf	prod	3'V_H or upstream D_H	P,N nucleotides	$D_H$element	P,N nucleotides	J_H element	V_κ	J_κ	prod	3' V_κ or upstream J_κ1	P,N nucleotides	$J_\kappa$element
S50	DJ		Q52	3			GGA NCA NCA CAG TNC		T AAC T		CC CGG TTT GCT TAC TGG GNC	10	1	p	TGT CAG CAG TAT AGT AAG CTT CC	G G	GG ACG TTC GGT
	DJ		sp2.7	2	3		TCT NCT NCT GTG		N CTA CTA TGG **TAA** C		GG GGC	germline fragment					GTACTACCACTGTGGTGGACGTTCGGT
S190	DJ		sp2.2	2	3		TCT ACT ACT GTG		T CTA CTA **TGA** TTA C		TT GAC TAC TGG GGC	24	1	n p	TGT CAA CAA CTN GTA GAG TAT CC	C	G TGG ACG TTC GGT
	DJ		sp2.9	3	3		TCT ACT ACT GTG		T CTA **TGA** T	TC ACC A	CT TAC TGG GNC	germline fragment					GTACTACCACTGTGGTGGACGTTCGGT
S300	DJ		fl16.1	3	3		TCT ACT ACT GTG		T TNA NNA CNA CGG **NAG TAG** CTA C		GG TTT GCT TAC TGG GGC	11	1	p	NGT CTA CAG CAT AGG TAT CTC CC	C GA	G ACG TTC GGN
	DJ		fl16.1	4	3		TCT ACT ACT GTG		T TTA TTA CTA CGG **TAG TA**	A CTG AGG	TAC TAN GCT ATG GAC TAC TGG GGT	4	5	p	TGC CAN CAG TGG AAT TAT CCT CTT	ATA GG	G CTC ACG NTC GGN
S147	VDJ	5	sp2.9	4	2	n p	TGT GCA AGA	C	ATG ATG GTT ACT AC	C ACG GCG G	AT GCT ATG GAC TAC TGG GGT	KK_5.1	2	p	TGC TCT NCA AGT ACA CAN GTT CC	C ANG	TAC ACG TTC GGA
	VDJ	5	n.i.	3		n p	TGT GCA AGA	C	A C		GG TTT GCT TAC TGG GGC						
S53	VDJ	1	sp2.11	2	1	p	TGT ACA AGA	G	CC TAC TAT AGG TAC	TCA GTC CT	C TAC TTT GAC TAC TGG GNC	24	4	p	TGT ATG CAA CAT CTA GAA TAT CC		A TTC ACG TTC GGC
	DJ		n.i.	4			TCT ACT ACT GTG		TC T	T	C TAC TGG GGT						
S127	VDJ	3	fl16.1	2	1	p	TGT GCA AGA	CGG AGG GAA GGC TC	T TAC TAC GGT AGT		TTT GAC TAC TGG GGC	23	1	n p	TGT CAA CAG AGT AAC AGC TGG CCT	CA	TGG ACG TTC GGT
	DJ		sp2.1 or 2.5	3	3		TCT ACT ACT GNG		T CTA CTA TGG **TAA** CT	C CTT	TTN GCT TAC TGG GGC						
S196	VDJ	1	fl16.1 or 16.2	4	3	p	TGT	ANC AAA AAA AGG A	AC GG	A GAT G	AT GGT ATG GAC TAC TGG GGT	10	1	p	NGT CAG CAA TTT AGT A	AC CTT CC	G TGG ACG TTC GGT
	DJ		sp2.2	3	3		TCT ACT ACT GNG		T CTA CTA **TGA** TT	C CC	G TTT GCT TAC TGG GGC	germline fragment					GTACTACCACTGTGGTGGACGTTCGGT
S44	VDJ	4	n.i.	1		p	TGT GCA CGA	CGA GGN GCN GGN C	TC		TGG TAC TTC GAN GTC TGG GGC	n.i.	1	n p	TGT TCT ACA GCG TTC TGC GTT TCC T	CT	G ACG TTC GGT
	VDJ	n.i	sp2.1-7, 2.10-11 or 2.x	4	1	n p	TGT CGG GTA	T AGA GGA AAA ATC C	AC TAT	TGT	GNT TAC TAT GCT ATG GAC TAC TGG GGT						
S219	VDJ	6	n.i.	3		p	TGT ACA	TGT TTT GAT GGC CCC	TTC		TTT GCT TAC TGG GGC	8	4	p	TGC AAG CAA TCT TAT AAT CTA		TTC ACG TTC GGC
	VDJ	n.i.	fl16.1-2 or sp2.9	4	1	n p	T GT G	A	T TAC TA		T ACT ATG GAC TAC TGG GNT	germline fragment					GTACTACCACTGTGGTGGACGTTCGGT
S258	VDJ	1	Q52	2	2	p	TGT GCA AGA	TCC AA	A CTG GGA C	GA GGG ACT	TTT GAC TAC TGG GGC	4	2	p	TNC CAG CAG NGT CAT AGT TAC CC	G	CTC ACG TTC GGT
	VDJ	6	sp2.1, 5 or 7	2	3	n p	TGT AC	A TGG AGC	CTA CTA TGG **TAA** C	CA C	TT GAC TAC TGG GGC						
S270	VDJ	11	sp2.1, 5, 7 or 8	1	1	p	TGT ATG AGA		TAT GGT AA		C TAC TGG TAC CTC GAT GTC TGG GGC	9B	2	p	TGT CTA CAG CAT GGT GAG AGC NC	G	TAC ACG TTC GGA
	VDJ	10	Q52	3		p	TGT	T	TA ACT GGG AC	G	TGG TTN GCT TAC TGG GGC	germline fragment					GTACTACCACTGTGGTGGACGTTCGGT

Designations are the same as in Table III.

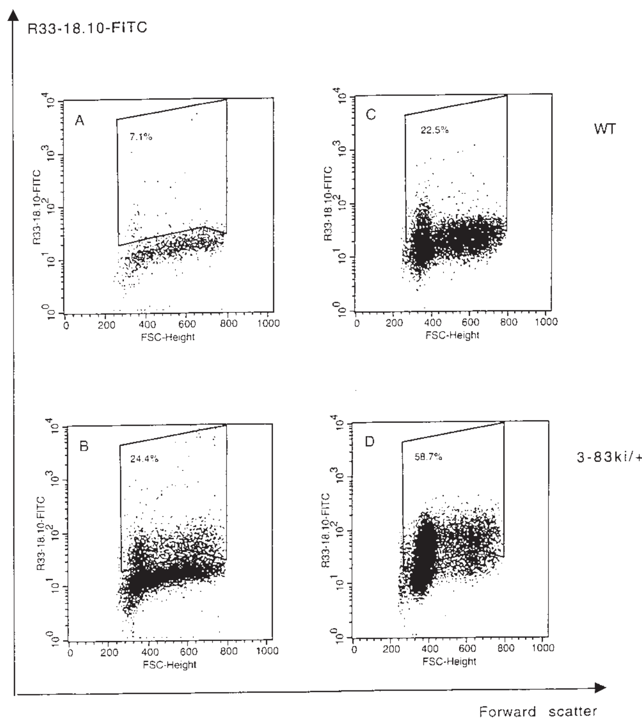

Figure 1. Staining of bone marrow B cell precursors for intracellular Igκ expression. Fraction B cells from wild-type (A) and 3-83κi/+ mice (B). Fraction D cells from wild-type (C) and 3-83κi/+ mice (D). Anti-Igκ chain antibody is shown on the y-axis, and the forward scatter of the cells is shown on the x-axis. Fraction D cells were used to gate κ^+ cells. Numbers indicate the percentage of cells in the window.

alyzed, three (cells 298, 717, and s147; Tables III, V, and VI) had nonproductive $V_HD_HJ_H$ joints in which D_H elements were rearranged in reading frame 2, and thus could have expressed a Dμ protein earlier. For these cells, as well as for the ones containing Igκ rearrangements and productive $V_HD_HJ_H$ joints, we can neither deduce the order of rearrangements at heavy and light chain loci nor state their interdependence. However, the remaining nine cells have either nonproductive $V_HD_HJ_H$ rearrangements (with the D_H elements in reading frames 1 or 3) and/or D_HJ_H joints in reading frames 1 or 3 (Table VI) and are thus unable to express μ or Dμ chains.

We cannot rule out the possibility that some of the heavy chain gene joints detected were formed by secondary rearrangement events; specifically, previously productive $V_HD_HJ_H$ rearrangements could have been rendered nonproductive by V_H gene replacement (40–42), and D_HJ_H joints could also have been substituted by recombining upstream D and downstream J elements with possible changes to the reading frame (5, 40, 42, 43). However, it has been implied that expression of the recombination activating genes RAG1 and RAG2 is downregulated upon pre-B cell receptor expression, suggesting that recombination of heavy chain genes is terminated once a μ chain is expressed (44). Furthermore, Dμ protein expression has also been suggested to prevent further IgH gene rearrangements (20, 30). In line with this idea, recent data have shown that Dμ protein transgene expression leads to a partial block in V_H to D_HJ_H rearrangements (39). For these reasons, it is unlikely that a major fraction of cells carrying $V_\kappa J_\kappa$ joints but no D_HJ_H rearrangement using reading frame 2 or no productive $V_HD_HJ_H$ rearrangement had assembled their IgL genes while expressing Dμ or μ chains, respectively, and altered their IgH gene complexes during subsequent rearrangements.

The data presented here are consistent with the earlier detection of $V_\kappa J_\kappa$ joints in B cell progenitors of mouse mutants unable to express μ chains (17–19) and support the view that Ig gene rearrangements in $CD43^+$ B cell progenitors of the mouse follow the "stochastic" model.

If rearrangements of IgH and IgL loci indeed occur independently in $CD43^+$ B cell progenitors, productive and nonproductive $V_HD_HJ_H$ joints should distribute randomly in cells bearing $V_\kappa J_\kappa$ rearrangements. Although this is true insofar as the ratio of productive to nonproductive joints is similar in κ chain$^+$ $V_\kappa J_\kappa$ rearrangement–containing and in total $CD43^+$ cells (∼50%; Table VI and reference 29), it is also obvious that, overall, the $CD43^+$ progenitor population is selected for productive $V_HD_HJ_H$ joints, as their fre-

Table VI. *Classification of B Cell Progenitors Carrying $V_\kappa J_\kappa$ Rearrangements by the Configuration of Their IgH Loci*

Fraction	DJ/DJ	VDJ⁻/DJ	VDJ⁻/VDJ⁻	VDJ⁺/DJ	VDJ⁺/VDJ⁻
C	1	4	2	6	1
	(352)	(**298**, 321, 717, **718**)	(78, **96**)	(**5**, 80, **265**, 294, **499**, 530)	(538)
κ chain⁺ B	5	2	0	6	2
	(**43**, **52**, **60**, **64**, **66**)	(**62**, **110**)		(**40**, **50**, **57**, 69, **70**, **113**)	(**46**, **87**)
B	3	0	1	3	3
	(**s50**, s190, **s300**)		(**s147**)	(**s53**, s127, s196)	(s44, **s219**, **s258**)

B cell progenitors of fractions B and C that carry $V_\kappa J_\kappa$ joints (Tables III–V) are classified into five groups according to the rearrangements of the two IgH alleles. The number of cells in each group is indicated. Numbers in parentheses denote the designations of the cells as given in Tables III–V. VDJ⁻ and VDJ⁺ represent nonproductive and productive $V_HD_HJ_H$ rearrangements, respectively. Cells with productive $V_\kappa J_\kappa$ rearrangements are shown in bold, and cells with a D_HJ_H joint in reading frame 2 that can encode a Dμ protein are underlined.

quency would be only 24% in a random distribution (considering that one-third of the joints are in-frame and that ~80% of the D elements in reading frame 3 contain stop codons). An over-representation of productive versus nonproductive $V_HD_HJ_H$ joints in these early progenitors has been repeatedly observed in other experiments: 0.6 (reference 45), 0.6 (reference 30), and 0.8 (reference 46). How can this selection be explained? Two possibilities can be considered: either the bias is introduced by the expansion of pre-B cell receptor–expressing (and therefore μ^+) $CD43^+$ progenitors that have downregulated RAG-1 and -2 expression (44, 47), or the CD43-expressing progenitors that we have analyzed contain a subset of classical pre-B cells in which RAG-1 and -2 are re-induced to mediate gene rearrangement in IgL loci, but surface CD43 expression is (still) retained. The existence of such cells could explain the finding of Pelanda et al. (21) that in surface (s)Ig^-, $CD43^+$, HSA^+ B cell precursors, the frequency of cells expressing κ light chains intracellularly is reduced approximately fourfold in the absence of the λ5 gene product. However, it is also possible that in the absence of λ5, κ and μ chain–expressing progenitors transit more rapidly into the compartment of $sIgM^+$ B cells than in the wild-type. That $CD43^+$ B cell precursors are in principle able to express sIg has been shown in mice containing productively rearranged heavy and light chain genes targeted into the corresponding Ig loci (Lam, K.-P., personal communication).

Given those complexities, we cannot exclude that some of the $V_\kappa J_\kappa$ rearrangements that we have found in the $CD43^+$ B cell progenitors were induced upon pre-B cell receptor expression, although we consider this unlikely. However, the finding that about half of the $CD43^+$ cells bearing $V_\kappa J_\kappa$ joints have yet to undergo IgH gene rearrangements for μ chain expression supports the concept originally developed from the analyses of mutant mice unable to express IgH chains (17), namely that in $CD43^+$ B cell progenitors, rearrangements of heavy and light chain loci are initiated "stochastically", with an approximately seven times higher frequency of rearrangements at the IgH than at the Igκ loci (see below).

The order of the rearrangements at the IgH and IgL loci determines the subsequent developmental route of the cell. If a μ chain is assembled first, a pre-B cell receptor will be expressed. The pre-B cell receptor gives a proliferative signal and directs the development of the cell to the $CD43^-$ pre-B cell compartment, where most IgL chain genes are rearranged (16, 48). However, if an IgL chain is expressed before or simultaneously with a μ chain, the cell is no longer dependent on the pre-B cell receptor to enter the B cell pool: as shown by Pelanda et al. (21), at least some conventional κ chains can substitute for the surrogate light chain and promote the development of progenitor B cells. Since we do not see any obvious bias towards some particular V_κ gene families among the κ chain sequences derived from $CD43^+$ B cell progenitors, it seems that a large repertoire of $V_\kappa J_\kappa$ joints can be generated in this compartment.

The existence of a pre-B cell receptor–independent developmental pathway that may be evolutionary more ancient than the pre-B cell receptor–driven pathway (17) may allow the generation of B cells whose μ chains are incapable of pairing with the surrogate light chain and thus are bound to die unless rescued by a conventional IgL chain. For example, a fraction of V_H81X-bearing heavy chains does not associate with the surrogate light chain (49), and thus these V_H81X-expressing B cells must be generated via the pre-B cell receptor–independent pathway. ten Boekel et al. (49) found that ~50% of heavy chains of early B cell progenitors using V_H elements of the V_HQ52 or V_HJ558 families are unable to pair with the surrogate light chain. IgH chain–independent recombination of IgL chain genes thus might add antigen receptor specificities to the B cell repertoire that would not arise via the pre-B cell receptor–driven pathway.

Frequency of Igκ Gene Rearrangement and Expression in $CD43^+$ B Cell Progenitors. We found 50 out of 627 fraction C cells and 32 out of 373 fraction B cells harboring $V_\kappa J_\kappa$ rearrangements. (We disregard the data obtained from sorted κ chain expressing cells from fraction B, because this cell population was selected for high levels of κ chain expression; see Results.) Taking into account the detection efficiency of the assay (70%) and the proportions of cells bearing $V_\kappa J_\kappa$ joints in the absence of productive $V_HD_HJ_H$ rearrangements (7 out of 14 in fraction C and 4 out of 11 in fraction B; Table VI), we estimate that 4–7% of cells in fractions B and C carry $V_\kappa J_\kappa$ joints in the absence of a productive $V_HD_HJ_H$ joint, and a similar proportion of cells contains both $V_\kappa J_\kappa$ rearrangement(s) and a productive V_H-D_HJ_H joint. Overall, the frequency of the cells carrying Igκ gene rearrangements is ~15% of the total $CD43^+$ B cell progenitor population in wild-type mice. This value correlates well with B cell production observed in λ5-deficient animals, which is reduced by ~95% (17, 50) and is dependent on the generation of Ig light chains in the absence of pre-B cell receptor function. To obtain 5% of B cells generated in wild-type mice, Igκ genes must be rearranged in 15% of the B cell progenitors, assuming that one-third of the joints are in-frame and that the B cell receptor induces a similar extent of proliferation in the progenitor compartment as does the pre-B cell receptor.

The results reported here are in a good agreement with previous data based on quantitative PCR analyses, in which $V_\kappa J_\kappa$ rearrangements represented ~7 and 15% in fractions B and C, respectively, taking the level of $V_\kappa J_\kappa$ rearrangements in splenic B cells as 100% (17). Our results do not contradict the experiments of ten Boekel et al. (16), who did not detect $V_\kappa J_\kappa$ rearrangements among 24 cells of early progenitor B cell phenotype (c-kit^+, $CD25^-$, $B220^+$). Since this population includes fractions A, B, and C (according to Hardy's classification, reference 23), and no $V_\kappa J_\kappa$ rearrangements are detectable in fraction A (17), the frequency of cells bearing $V_\kappa J_\kappa$ joints in the population analyzed by ten Boekel and colleagues is expected to be lower than 1 in 24 in these cells.

Immunoglobulin gene transcription and rearrangements are coordinately regulated during B cell development (for

review see reference 51). It has been suggested that transcription of unrearranged genes is required for the initiation of the V(D)J joining process. We have observed that in 3-83κi mice carrying a productively rearranged $V_\kappa J_\kappa$ joint in the germline there are approximately three times more cells expressing κ light chains in fraction B compared with the wild-type situation (Fig. 1). This difference is quantitatively accounted for by the fact that two-thirds of the newly formed rearrangements in the wild-type cells are nonproductive. Therefore, this result suggests that the "opening" of the Igκ locus for transcription and for recombination occurs simultaneously and may thus be controlled by the same factor(s). Moreover, this result shows that at this early developmental stage wild-type cells rearranging Igκ genes express the recombinatorial products at the protein level.

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