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 \( \mu \) chains, \( V_{\mu}J_{\mu} \) 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)\( \kappa \) genes before the assembly of a productive \( V_{\mu}D_{\mu}J_{\mu} \) joint. Thus, \( \mu \) chain expression is not a prerequisite to Ig\( \kappa \) light chain gene rearrangements in normal development. Overall, ~15% of the total CD43\(^+ \) B cell progenitor population carry Ig\( \kappa \) 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 Ig\( \kappa \) loci independently, with a seven times higher frequency in the former. In addition, we show that in B cell progenitors \( V_{\mu}J_{\mu} \) joining rapidly initiates \( \kappa \) chain expression, irrespective of the presence of a \( \mu \) 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\( _{\mu}J_{\mu} \) recombination events, two models have been proposed. According to the "ordered" model, expression of a \( \mu \) 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 \( \mu \) chain as well as cross-linking of pre-B cell receptor complexes (consisting of membrane-bound \( \mu \) chains and the products encoded by the \( \lambda 5 \) and V\( _{\mu \text{preB}} \) genes; reference 4) stimulate the rearrangement of endogenous \( \kappa \) light chain genes in transformed pre-B cell lines (5–7). In addition, an increased number of V\( _{\mu}J_{\mu} \) 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 \( \mu \) 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 Ig\( \kappa \) loci in the absence of a membrane-bound \( \mu \) heavy chain. In vitro differentiation experiments using normal murine pre-B cell lines have also demonstrated that \( \kappa \) protein could be expressed in the absence of a \( \mu \) chain (13). Moreover, \( \kappa \) chain expression was detected in the absence of productive V\( _{\mu}D_{\mu}J_{\mu} \) 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 Ig\( \kappa \) rearrangements occur in the pre-B cell compartment into which progenitor cells are driven upon pre-B cell receptor (i.e., \( \mu \) chain) expression (5, 16), evidence indicates that initially, when gene rearrangements in IgH are set in motion in CD43\(^+ \) progenitors, V\( _{\mu}J_{\mu} \) rearrangements also occur, albeit at low frequency (17, 18). At this early stage of development, \( \kappa \) rearrangements appear to be independent of \( \mu \) chain expression and, indeed, any rearrangement in the IgH locus,

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as they are also seen in mutant mice unable to either express membrane-bound μ chains (μMT mice; 19) or generate V_μ D_JH joints due to a targeted deletion of the J_μ 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 mice 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 Igκ 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 NaH_2C_1 to eliminate erythrocytes and washed by centrifugation through FCS. 3-83ki 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. Single cells were directly deposited into 0.5-ml microtubes or into 1.5 ml of an aqueous solution containing 0.1% saponin (Sigma Chemical Co., St. Louis, MO) and 1% BSA, bleached overnight, and then stained with FITC–R33-18 mAb. In separate experiments (not shown) for the sortings of fraction B cells, FITC–R33-18 mAb was applied to separate fractions of the same cell sortings.

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, 5 U of Taq DNA polymerase (GIBCO BRL) in the first reaction, and 3 U of Taq DNA polymerase in the second reaction, 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 primer in combination with either the nested JH4A primer or the JH4E primer (amplification of Igκ 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 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 primer (amplification of Igκ 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 60°C, and 1.5 min at 72°C). All PCRs contained dATP, dCTP, dGTP, dTTP (Pharmacia Biotech) at 200 μM each, and Taq DNA polymerase at 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 60°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).
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 Vκ sequence list compiled by Koffler 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 Jκ elements (T15i) mice, reference 28, and B1-8i mice, reference 33, containing a rearranged Vκ186.2 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 Vκ186.2 gene was amplified by the Jκ1 primer (reference 30; excluding fraction C) and subsequently sequenced. The Vκ186.2 gene was amplified from 210 single B cells (none, one, or two per cell). Assuming that ~30% of all splenic B cells carry two Vκ186.2 complexes (9, 16), this corresponds to a Vκ186.2 rearrangement detection efficiency of ~70%. To determine the detection efficiency of Vκ186.2 gene complexes, we 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 Igκ gene PCR products were obtained from 41% of cells. In the remaining cells, either one (51%) or no (7%) Igκ 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 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 Vκ-Jκ 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 Vκ-Jκ complexes were amplified from 210 single B cells (none, one, or two per cell). Assuming that ~30% of all splenic B cells carry two Vκ-Jκ complexes (9, 16), this corresponds to a Vκ-Jκ rearrangement detection efficiency of ~70%. To determine the detection efficiency of Igκ gene joints, Vκ-Dκ-Jκ complexes 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 Igκ gene PCR products were obtained from 41% of cells. In the remaining cells, either one (51%) or no (7%) Igκ 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 described elsewhere (30).

| 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 CAC TGG CAG TGG |
| VK3 | GCG AAG CTT CCC AG(T) AC CAG GTT CAG TGG CAG TGG |
| KG | GCG AAG CTT AAG CCT TCG CCT ACC CAC TGG CTC |
| KG1 | ACA GCC AGA CAG TGG AGT ACT ACC ACT GTG |
| JK5E | GAT CCA ATC TCT TGG ATG GTG ACC |
| JK5A | GGG TCT AGA CAA CTG ATG CTG 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 AGA 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 Vκ primers, recognizing all Vκ genes listed by Strohal et al. (52) in framework region 3, cross-react and thus cannot be assigned to specific Vκ families. The KG and KGI primers hybridize to a germline region upstream of Jκ1. All Jκ primers are homologous to a region downstream of the respective Jκ 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 JH elements (T15i mice, reference 28, and B1-8i mice, reference 33). Cell suspension containing equal proportions of Igk-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.

Igk 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 IgM 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 VJk 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 VJk 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 VH-DHJH rearrangements. However, seven other cells contain an Igk gene rearrangement in the absence of a functional VH-DHJH complex. Two of these cells carry nonfunctional VH-DHJH rearrangements at both IgH alleles, and four carry a nonproductive VH-DHJH rearrangement together with a DHJH joint. Two nonproductive VH-DHJH joints (in cells 298 and 717) comprise DH 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 VH-DHJH complex formation. The remaining cell harbors a rearranged Igk allele and contains only DHJH complexes (cell 352). The DH 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 VHJk 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 Igk genes. Therefore, we decided to analyze fraction B, the earliest stage at which VH-DHJH rearrangements are detected, for the presence of cells containing rearranged κ genes in the absence of productive VH-DHJH complexes. According to our previous analysis, VHJk rearrangements are 14 times less frequent in this cell population than in κ+ positive splenic B cells (17).

To enrich for cells bearing Igk rearrangements, we isolated cells that stained for κ chains intracellularly. 88 single fraction B cells positive for intracellular κ chains were analyzed. Igk 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 VHJk rearrangements contained a productive VH-DHJH joint. Seven cells were found to harbor either DHJH joints on both heavy chain alleles (five cells) or a nonproductive VH-DHJH joint on one allele and a DHJH 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 DHJH rearrangements and in one of the two cells containing a DHJH joint.

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

| Cells per sample | No. of samples | 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+ splenic 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.

78 κ Gene Rearrangements and Expression before μ Chain Expression
### Table III. Junctional Region Sequences of D_{k}J_{k}, V_{k}D_{k}J_{k}, and V_{k}J_{k} Ig Gene Rearrangements in B Cell Progenitors from Fraction C

| N  | V_{k}D_{k}J_{k} and D_{k}J_{k} rearrangements | V_{k}J_{k} rearrangements and IgC germine fragments |
|----|---------------------------------------------|-------------------------------------------------|
| No | N  | J  | H  | p  | \(X_{k}\) of sequence \(D_{k}\) | \(N_{k}\) nucleotides | \(P_{k}\) nucleotides | \(I_{k}\) element | \(J_{k}\) element | \(V_{k}\) | \(J_{k}\) | \(P_{k}\) nucleotides | \(I_{k}\) element | \(\lambda^\prime\) element |
|----|----|----|----|----|----------------|------------------|------------------|----------------|----------------|----------|----------|------------------|----------------|----------------|
| 352 | DJ 1 | sp2.10 | 2 | 1 | TCT ACT ACT GCRG | TAC TAT ACC GAC CC | C | C TCT AGC TAC TGC GGC | GC TCT AGC TAC TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC |
| 298 | DJ 3 | sp2.10 | 3 | 3 | TCT ACT ACT GCRG | TCT ACT TAC GAC CC | C | C TCT AGC TAC TGC GGC | GC TCT AGC TAC TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC |
| 321 | DJ 3 | sp2.10 | 2 | 1 | TCT ACT ACT GCRG | TCT ACT TAC GAC CC | C | C TCT AGC TAC TGC GGC | GC TCT AGC TAC TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC |
| 317 | DJ 1 | sp2.10 | 3 | 3 | TCT ACT ACT GCRG | TCT ACT TAC GAC CC | C | C TCT AGC TAC TGC GGC | GC TCT AGC TAC TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC |
| 318 | DJ 2 | sp2.10 | 3 | 3 | TCT ACT ACT GCRG | TCT ACT TAC GAC CC | C | C TCT AGC TAC TGC GGC | GC TCT AGC TAC TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC |
| 38 | DJ 2 | sp2.10 | 3 | 3 | TCT ACT ACT GCRG | TCT ACT TAC GAC CC | C | C TCT AGC TAC TGC GGC | GC TCT AGC TAC TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC |
| 36 | DJ 2 | sp2.10 | 3 | 3 | TCT ACT ACT GCRG | TCT ACT TAC GAC CC | C | C TCT AGC TAC TGC GGC | GC TCT AGC TAC TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC |
| 35 | DJ 2 | sp2.10 | 3 | 3 | TCT ACT ACT GCRG | TCT ACT TAC GAC CC | C | C TCT AGC TAC TGC GGC | GC TCT AGC TAC TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC |
| 34 | DJ 2 | sp2.10 | 3 | 3 | TCT ACT ACT GCRG | TCT ACT TAC GAC CC | C | C TCT AGC TAC TGC GGC | GC TCT AGC TAC TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC |
| 33 | DJ 2 | sp2.10 | 3 | 3 | TCT ACT ACT GCRG | TCT ACT TAC GAC CC | C | C TCT AGC TAC TGC GGC | GC TCT AGC TAC TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC |
| 32 | DJ 2 | sp2.10 | 3 | 3 | TCT ACT ACT GCRG | TCT ACT TAC GAC CC | C | C TCT AGC TAC TGC GGC | GC TCT AGC TAC TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC |
| 31 | DJ 2 | sp2.10 | 3 | 3 | TCT ACT ACT GCRG | TCT ACT TAC GAC CC | C | C TCT AGC TAC TGC GGC | GC TCT AGC TAC TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC |
| 30 | DJ 2 | sp2.10 | 3 | 3 | TCT ACT ACT GCRG | TCT ACT TAC GAC CC | C | C TCT AGC TAC TGC GGC | GC TCT AGC TAC TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC |
| 29 | DJ 2 | sp2.10 | 3 | 3 | TCT ACT ACT GCRG | TCT ACT TAC GAC CC | C | C TCT AGC TAC TGC GGC | GC TCT AGC TAC TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC |
| 28 | DJ 2 | sp2.10 | 3 | 3 | TCT ACT ACT GCRG | TCT ACT TAC GAC CC | C | C TCT AGC TAC TGC GGC | GC TCT AGC TAC TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC |
| 27 | DJ 2 | sp2.10 | 3 | 3 | TCT ACT ACT GCRG | TCT ACT TAC GAC CC | C | C TCT AGC TAC TGC GGC | GC TCT AGC TAC TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC |
| 26 | DJ 2 | sp2.10 | 3 | 3 | TCT ACT ACT GCRG | TCT ACT TAC GAC CC | C | C TCT AGC TAC TGC GGC | GC TCT AGC TAC TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC |
| 25 | DJ 2 | sp2.10 | 3 | 3 | TCT ACT ACT GCRG | TCT ACT TAC GAC CC | C | C TCT AGC TAC TGC GGC | GC TCT AGC TAC TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC | TGG TCT ACT TGC GGC |

The two IgH or Igk 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 germine, called either N nucleotides (53, 54) or P nucleotides (55). Sequences of the light locus are categorized based on their configuration in the column types (D) or (VJ). D sequences were assigned to published DH segments (56) if there was homology of at least four nucleotides. Numbers in column rf indicate the DH element reading frame (35). Reading frame is not identified for DQ52 element, because of its inability to encode for Dm 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 Jk columns indicate the J element used in the respective joint. For V_{k}D_{k}J_{k} or V_{k}J_{k} rearrangements the V gene families used are indicated in the columns VH or Va. In the column prod: p, a productive V_{k}D_{k}J_{k} or V_{k}J_{k} joint; np, a nonproductive V_{k}D_{k}J_{k} or V_{k}J_{k} joint. Stop codons are shown in bold.
### Table IV. Sequences of $D_{\gamma LH}$, $V_{\gamma LH}$, and $V_{\delta JH}$ Junctional Regions Ig Gene Rearrangements in Intracellular $k$-expressing B Cell Progenitors from Fraction B

| No | type | VH | DH | JJ | re | $V_{\gamma LH}$  | $V_{\delta LH}$  | $D_{\gamma LH}$  | $\gamma JH$  | $\delta JH$  | $\gamma Jk$  | $\delta Jk$  | $\gamma Jk$  | $\delta Jk$  | junctional | germ line fragment |
|----|------|----|----|----|----|-----------------|-----------------|-----------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|------------------|
| 43 | DJ   | sp2 | 2  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 44 | DJ   | sp2.10 | 2  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 51 | DJ   | sp4.5 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 52 | DJ   | sp2.8 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 53 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 60 | DJ   | sp2.5 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 61 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 62 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 63 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 64 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 65 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 66 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 67 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 68 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 69 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 70 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 71 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 72 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 73 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 74 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 75 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 76 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 77 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 78 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 79 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 80 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 81 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 82 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 83 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 84 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 85 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 86 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |
| 87 | DJ   | sp2.10 | 3  | 2  |    | TCA ACT AGT GTA GCT C | 4 | 4 | p | ECC CAGG AGG A ATG GAT GAT TAC CUA | TEN AUG P TC GGC |

Designations are the same as in Table III.
together with a nonproductive V<sub>k</sub>D<sub>j</sub><sub>k</sub>. Only one V<sub>k</sub>J<sub>k</sub>-bearing cell (cell 62, Table IV and VI) that is unable to produce a (truncated) heavy chain was found in this experiment. It carried a nonproductive V<sub>k</sub>D<sub>j</sub><sub>k</sub> rearrangement (with the D<sub>k</sub> element in reading frame 1) on one allele and a D<sub>j</sub><sub>k</sub> joint in reading frame 2 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 D<sub>μ</sub> 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<sub>k</sub>J<sub>k</sub> 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<sub>k</sub>D<sub>j</sub><sub>k</sub> joint was present (Tables V and VI). One cell contained two nonproductive V<sub>k</sub>D<sub>j</sub><sub>k</sub> joints (one of which comprises a D<sub>k</sub> element in reading frame 2), and three cells carried D<sub>k</sub> rearrangements on both heavy chain alleles. None of these D<sub>k</sub><sub>j</sub><sub>k</sub>/D<sub>k</sub><sub>j</sub><sub>k</sub> cells harbored D<sub>k</sub> elements rearranged in reading frame 2.

Ig<sub>k</sub> chain expression in early B cell precursors. Given the efficiency of V<sub>k</sub>J<sub>k</sub> 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<sub>k</sub>J<sub>k</sub> 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 3-83<sup>ki</sup> mouse mutant in which a productive V<sub>k</sub> gene segment encoding the V<sub>k</sub> 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<sub>k</sub><sub>j</sub> rearrangements are out of frame, 3-83<sup>ki</sup> 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 3-83<sup>ki</sup> mutant, yielding almost exactly the expected 1:3 ratio.

These data are in agreement with the frequency of V<sub>k</sub>J<sub>k</sub> rearrangements in cells from fraction B estimated by PCR analyses. Together, these results suggest that Ig<sub>k</sub> 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<sub>k</sub>D<sub>j</sub><sub>k</sub> 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<sup>−</sup> 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<sub>k</sub>D<sub>j</sub><sub>k</sub> joints) would result in an underestimation of the percentage of cells bearing V<sub>k</sub>J<sub>k</sub> joints but no productive V<sub>k</sub>D<sub>j</sub><sub>k</sub> 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<sub>k</sub> elements (except the D<sub>k</sub>Q52 element) are recognized by the same primer and the primers specific for V<sub>k</sub> genes are highly homologous in structure (Table I). Therefore, most of the D<sub>k</sub><sub>j</sub><sub>k</sub> joints using the same J<sub>k</sub> genes on both chromosomes or distinct V<sub>k</sub><sub>j</sub><sub>k</sub> rearrangements with the same J<sub>k</sub> segment could not be resolved. For these reasons the number of cells with D<sub>k</sub><sub>j</sub><sub>k</sub> joints at both IgH loci and the number of cells bearing two V<sub>k</sub><sub>j</sub><sub>k</sub> joints could be underestimated.

Ig<sub>k</sub> Gene Rearrangements Appear To Be Independent of Heavy Chain Expression in CD43<sup>−</sup> Pre-B Cell Progenitors. The question of whether expression of a productive V<sub>k</sub>D<sub>j</sub><sub>k</sub> rearrangement is a prerequisite for light chain gene rearrangement during B cell development or whether Ig<sub>k</sub> gene rearrangement can take place also in the absence of a membrane-bound μ chain has been discussed controversially. The analysis of Ig<sub>k</sub> 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<sub>k</sub><sub>j</sub><sub>k</sub> rearrangements are detectable, namely, cells of the CD43<sup>+</sup> fractions B and C (17), were chosen for analysis. The results obtained are summarized in Table VI. Overall, 18 cells were found to carry Ig<sub>k</sub> rearrangements in the absence of a productive V<sub>k</sub>D<sub>j</sub><sub>k</sub> joint. However, six of these contained D<sub>k</sub><sub>j</sub><sub>k</sub> rearrangements in reading frame 2, and thus were able to express D<sub>k</sub> proteins. Like a μ chain, the D<sub>k</sub> protein could associate with the products of the S and V<sub>preB</sub> genes to form a pre-B cell receptor-like complex (7). It has been suggested that D<sub>k</sub> protein expression, similar to μ chain expression, provided a stimulatory signal for Ig<sub>k</sub> gene rearrangements (5, 38, 39). Among the other cells an-
### Table V.
Sequences of \(D_{i}J_{k}\), \(V_{i}D_{i}J_{k}\), and \(V_{i}J_{k}\) Functional Regions Ig Gene Rearrangements in B Cell Progenitors from Fraction B

| No | tpt | VH | DH | JH | rf | prod | \(3'_{V_{k}}\) or upstream \(D_{i}\) | PN nucleotides | 5' element | PN nucleotides | \(3'_{J_{k}}\) element | \(\lambda_{k}\) element | \(V_{i}D_{i}J_{k}\) rearrangements and Ig germine fragments |
|----|-----|----|----|----|----|------|-----------------|----------------|-------------|----------------|-----------------|----------------|--------------------------------------------------|
| 889 | DJ | sp2.7 | 2 | 2 | 3 | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG |
| 890 | DJ | sp2.7 | 3 | 2 | 2 | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG |
| 891 | DJ | sp2.7 | 3 | 2 | 2 | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG |
| 892 | DJ | sp2.7 | 3 | 2 | 2 | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG |
| 893 | DJ | sp2.7 | 3 | 2 | 2 | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG |
| 894 | DJ | sp2.7 | 3 | 2 | 2 | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG |
| 895 | DJ | sp2.7 | 3 | 2 | 2 | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG |
| 896 | DJ | sp2.7 | 3 | 2 | 2 | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG |
| 897 | DJ | sp2.7 | 3 | 2 | 2 | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG |
| 898 | DJ | sp2.7 | 3 | 2 | 2 | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG |
| 899 | DJ | sp2.7 | 3 | 2 | 2 | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG | TCTCTACTGCTG |

Designations are the same as in Table III.
Fraction D cells from wild-type (C) and 3-83k Ig cells is shown on the x-axis. Fraction D cells were used to gate as for the ones containing Ig have expressed a D elements were rearranged in reading frame 2, and thus could have been substituted by recombinizing 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 Vh to DμJH rearrangements (39). For these reasons, it is unlikely that a major fraction of cells carrying VJμ joints but no DμJH rearrangement using reading frame 2 or no productive VhDμJH 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 VJμ 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 VhDμJH joints should distribute randomly in cells bearing VJμ rearrangements. Although this is true insofar as the ratio of productive to nonproductive joints is similar in κ chain+ VJμ 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 VhDμJH joints, as their fre-

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Staining of bone marrow B cell precursors for intracellular Igκ expression. Fraction B cells from wild-type (A) and 3-83k/+ mice (B). Fraction D cells from wild-type (C) and 3-83k/+ 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.

| Fraction | DJ/DJ | VDJ−/VDJ− | VDJ+/VDJ− | 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     | 1         | 0         | 3         | 3         |
|          | (s50, s190, s300) | (s147) | (s53, s127, s196) | (s44, s219, s258) |

| Table VI. | Classifcation of B Cell Progenitors C aning Vjμ R rearrangements by the C onfiguration of their IgH Loci |
|-----------|--------------------------------------------------|
| Fraction | DJ/DJ | VDJ−/VDJ− | 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     | 1         | 0         | 3         | 3         |
|          | (s50, s190, s300) | (s147) | (s53, s127, s196) | (s44, s219, s258) |

B cell progenitors of fractions B and C that carry Vjμ joints (Tables III–V) are classified into five groups according to the rearrangements of the two Ig light 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 VhDμJH rearrangements, respectively. Cells with productive Vjμ rearrangements are shown in bold, and cells with a DμJH joint in reading frame 2 that can encode a Dμ protein are underlined.
frequency 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 non-productive V<sub>H</sub>D<sub>l</sub> 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<sup>+</sup>) CD43<sup>+</sup> progenitors that have downregulated RAG-1 and -2 expression (44, 47), or the CD43<sup>+</sup>-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<sup>+</sup>, CD43<sup>+</sup>, HSA<sup>+</sup> 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<sup>+</sup> B cells than in the wild-type. That CD43<sup>+</sup> 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<sub>J<sub>k</sub></sub> rearrangements that we have found in the CD43<sup>+</sup> 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<sup>+</sup> cells bearing V<sub>J<sub>k</sub></sub> 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<sup>+</sup> 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 Ig<sub>k</sub> 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<sup>+</sup> 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<sub>κ</sub> gene families among the κ chain sequences derived from CD43<sup>+</sup> B cell progenitors, it seems that a large repertoire of V<sub>J<sub>k</sub></sub> 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<sub>H</sub>81X-bearing heavy chains does not associate with the surrogate light chain (49), and thus these V<sub>H</sub>81X-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<sub>κ</sub> elements of the V<sub>κ</sub>Q52 or V<sub>κ</sub>J558 families are unable to pair with the surrogate light chain. IgL 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 Ig<sub>k</sub> Gene Rearrangement and Expression in CD43<sup>+</sup> B Cell Progenitors. We found 50 out of 627 fraction C cells and 32 out of 373 fraction B cells harboring V<sub>J<sub>k</sub></sub> 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<sub>J<sub>k</sub></sub> joints in the absence of productive V<sub>H</sub>D<sub>l</sub> 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<sub>J<sub>k</sub></sub> joints in the absence of a productive V<sub>H</sub>D<sub>l</sub> joint, and a similar proportion of cells contains both V<sub>J<sub>k</sub></sub> rearrangement(s) and a productive V<sub>H</sub>D<sub>l</sub> joint. Overall, the frequency of the cells carrying Ig<sub>k</sub> gene rearrangements is ~15% of the total CD43<sup>+</sup> 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 a pre-B cell receptor function. To obtain 5% of B cells generated in wild-type mice, Ig<sub>k</sub> 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<sub>J<sub>k</sub></sub> rearrangements represented ~7 and 15% in fractions B and C, respectively, taking the level of V<sub>J<sub>k</sub></sub> 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<sub>J<sub>k</sub></sub> rearrangements among 24 cells of early progenitor B cell phenotype (c-kit<sup>+</sup>, CD25<sup>+</sup>, B220<sup>-</sup>). Since this population includes fractions A, B, and C (according to Hardy’s classification, reference 23), and no V<sub>J<sub>k</sub></sub> rearrangements are detectable in fraction A (17), the frequency of cells bearing V<sub>J<sub>k</sub></sub> 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 unarranged genes is required for the initiation of the V(D)J joining process. We have observed that in 3-83i mice carrying a productively rearranged V_{mu} joining site in the germline there are approximately three times more cells expressing \( \kappa \) 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 Igk 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 Igk genes express the recombinatorial products at the protein level.

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