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The status of Ig loci rearrangements in single cells from different stages of B cell development

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Abstract

Differential expression of c-kit, CD25 (TAC), surrogate L chain and cytoplasmic μH chain, and surface expression of IgM and IgD allows the separation of B220 ($CD45^+$) B cell subpopulations. PCR analyses with DNA of single cells developed by others and by us have been used to monitor the conformation of the Ig H and L chain gene loci in these different B lineage subpopulations. The results of these analyses indicate that $B220^+/c\text{-}kit^+/CD25^-$ cells are the precursors of large $B220^+/CD25^+/sIgM^-$ which, in turn, are the precursors of small $B220^+/CD25^+/sIgM^-$ cells. The majority of $B220^+/c\text{-}kit^+/CD25^-$ cells are D_HJ_H-rearranged, with L chain loci in germline configuration and are thus pre-B I cells. More than 90% of all large $B220^+/CD25^+/sIgM^-$ cells have at least one H chain locus $V_HD_HJ_H$ rearranged; half of them have also the second locus $V_HD_HJ_H$ rearranged and are thus large pre-B II cells. Rearrangements of at least one allele of the κL chain loci become detectable in 65% of the small $B220^+/CD25^+/sIgM^-$ cells, 67% of the immature B and >75% of the mature B cells. The ratio of κL to λL gene rearrangements in all three subpopulations is ~10:1, indicating that the κL/λL ratio is established as soon as rearrangements are made.

Introduction

Bone marrow is the major site of B lymphopoiesis during adult life (1–5). Development from early B-lymphocyte-lineage-committed progenitors (pro-B) to precursor (pre-B) and immature B cells in mouse bone marrow and to mature, antigen-sensitive B cells in peripheral lymphoid organs can be dissected into different stages. These cellular stages are characterized by the differential expression of surface-located and intra-cellular markers, and by the differential capacities of cells to proliferate '*in vitro*' (Fig. 1) (2,5–8). Hardy and his colleagues (6) have used the differential expression of CD43, heat stable antigen (HSA) and BP-1 to separate precursor B cell subpopulations while our laboratory has employed the analysis of cell size and the expression of c-kit, CD25 (TAC) and surrogate L (SL) chain to do so (7).

Progression along the pathways of B lymphocyte differentiation is also marked by successive rearrangements of the Ig gene loci. They begin with D_H to J_H rearrangements in the H chain gene loci where they appear to involve both alleles within a precursor B cell (9). These so called pro/pre-B I cells express c-kit, but not CD25, and are large, cycling cells when prepared '*ex vivo*' (7).

D_HJ_H rearrangements are followed by V_H to D_HJ_H rearrangements. Cells with productively $V_HD_HJ_H$-rearranged Ig H chain alleles, i.e. those capable of expressing a μH chain, appear positively selected over non-productively $V_HD_HJ_H$-rearranged cells (7). This occurs through proliferative expansion of the μH chain-expressing cells, in all likelihood initiated by the signaling through the μH chain/SL chain pre-B receptor (7). Hence, we expect these cells, again, to be large cycling cells. Large, cytoplasmic μH chain expressing cells in bone marrow are $c\text{-}kit^-/CD25^+$. About 25% of them express the μH/SL pre-B cell receptor which can appear on the surface when the cells are cultured '*in vitro*' (7,10).

The next stage of development is thought to be small, resting $c\text{-}kit^-/CD25^+$ cells expressing cytoplasmic μH chain, but not yet L chains, which are no longer in cell cycle.

L chain expression then leads to deposition of IgM on the surface of so-called immature B cells. These immature B cells are thought to be the precursors of mature, antigen-sensitive, mitogen-reactive $sIgM^+/sIgD^+$ B cells, which are found in part in bone marrow, but are most abundant in peripheral lymphoid organs.

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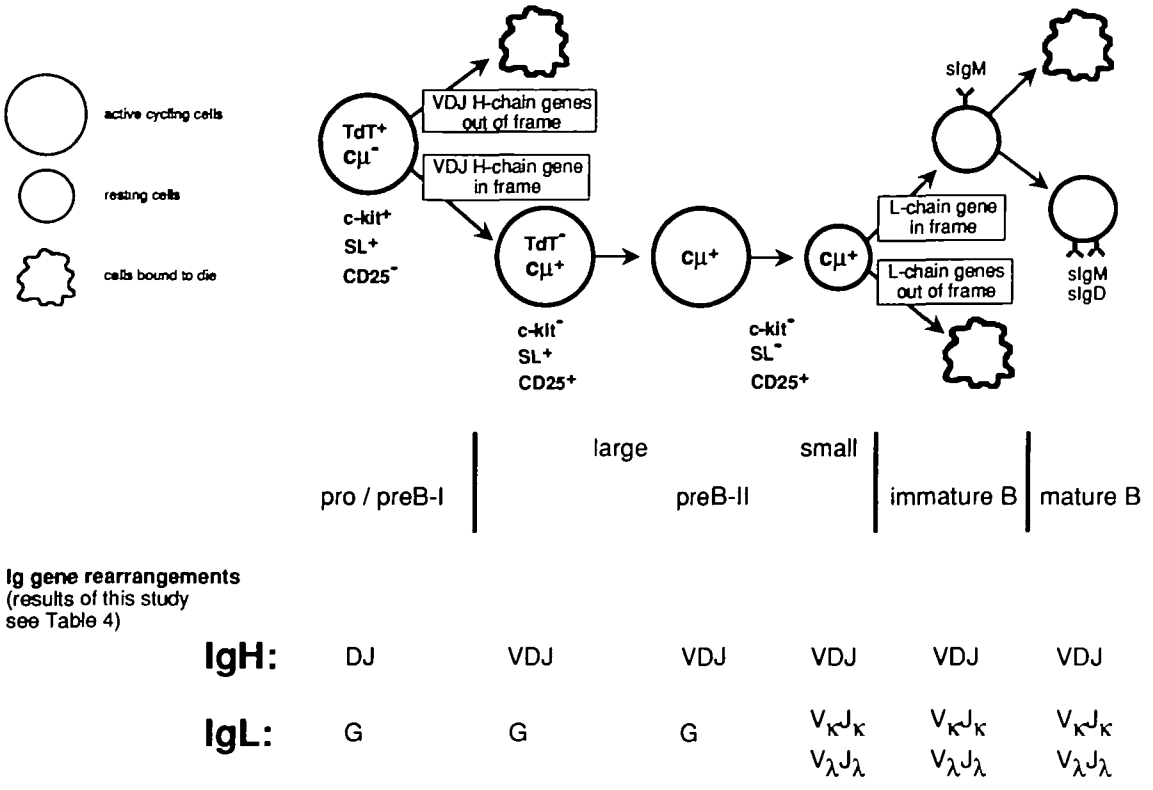

Fig. 1. B lymphopoiesis in mouse bone marrow, as analyzed by differential marker expression and status of IgH and L chain gene rearrangements.

An assay has been developed by Ehlich *et al.* (5) that allows the characterization of Ig H chain gene rearrangements in single cells. This assay has been previously used to monitor the rearrangement status of the Ig gene loci of B lineage precursors in mouse bone marrow characterized, and thus separable, by the differential expression of CD43, HSA and BP-1 (5). We use this assay in the present study to determine the status of Ig H and L chain gene rearrangements in B lineage precursor cells of mouse bone marrow, as characterized and thus separable by their differences in cell size, and c-kit, CD25, μH/SL pre-B cell receptor IgM and IgM/IgD B cell receptor expression. The results define the cells in mouse bone marrow in which the majority of either D_H–J_H, V_H–D_HJ_H or V_L–J_L rearrangements have taken place. The results also allow a comparison with Hardy's bone marrow cell subpopulations defined by CD43, HSA and BP-1 expression, and their status of Ig gene rearrangements (6). It appears that separation of B lineage committed cells differentially expressing c-kit, CD25 and SL chain allows a clear distinction and thus purification of precursors before and after productive $V_HD_HJ_H$ rearrangements and after V_LJ_L rearrangements.

Methods

Mice

(C57BL/6×DBA/2) F_1 (BDF_1) mice, 6–12 weeks of age, were obtained from the Institut für Biologische-Medizinische Forschung AG (Füllinsdorf, Switzerland).

FACS staining, sort and DNA preparation

Cells for FACS sorting were prepared from bone marrow and spleen as described (7). Bone marrow cells were stained with the FITC-conjugated mAb RA3 6B2 (anti-CD45R, B220) (PharMingen, San Diego, CA) and double stained with biotin-conjugated mAb ACK-4 (anti-c-kit) (11), LS156 (anti-λ5/μH) (10), 7D4 (anti-CD25, TAC), M41 (anti-μH) (12) or 1.19 (anti-δ) (13). Binding of biotin-conjugated mAb was visualized using streptavidin–phycoerythrin (Southern Biotechnology Associates, Birmingham, AL)

Single cells were sorted using the FACStar Plus equipped with an automatic cell deposition unit (Becton Dickinson, Mountain View, CA). Single cells were directly sorted into 96-well PC plates type H (Costar, Cambridge, MA) containing 3 μl of 10 times concentrated PCR buffer, 7 μl H_2O and 10 ng tRNA. To prepare DNA, samples were overlaid with PCR oil (Fluka, Buchs, Switzerland) and 2 μl of proteinase K (5 mg/ml; Boehringer, Mannheim, Germany) was added. The samples were digested for 1 h at 55°C and proteinase K was subsequently inactivated for 10 min at 95°C. Plates were then stored at –70°C until use for DNA amplification.

PCR analysis of Ig H and L gene rearrangements

PCR amplification was carried out in two rounds using a Hypaid Omnigene PCR machine (Hybaid Ltd, Middlesex, UK). The first round was done over 28 cycles and contained all 5′ and 3′ primers listed in Table 1 as first round primers. The PCR amplification conditions were as described by Ehlich

Table 1. Oligonucleotides used for amplification of rearranged Ig H and L genes

Sequence	Specificity	Ref.	PCR round usage	
			First	Second
5′ primers				
GCGAAGCTTA(AG)GCCTGGG(AG)CTTCAGTGAAG	V_HJ588	(5)	+	+
GCGAAGCTTCTCACAGAGCCTGTCCATCAC	V_HQ52	(5)	+	+
GCGAAGCTTTCTCAG(AT)CTCTGTC(CT)CTCACC	V_H36–60	(5)	+	+
GCGAAGCTTCTCGAGTCTGGAGGTGGCCTG	V_HX24	(5)	+	+
GCGAAGCTTGTGGAGTCTGGGGGAGGCTTA	V_H7183	(5)	+	+
GCGAAGCTT(AT)CTGGAGGAGGCTTGGTGCAA	V_HJ606, V_HS107, V_HX24	(5)	+	+
GCGAAGCTTGGAGAGACAGTCAAGATCTCC	V_HGAM3	(5)	+	+
CACAGAGAATTCTCCATAGTTGATAGCTCAG	D_HQ52		+	−
GCCTCAGAATTCCTGTGGTCTCTGACTGGT	D_HQ52		−	+
ACAAGCTTCAAAGCACAATGCCTGGCT	D_HFL16, D_HSP2	(2)	+	−
ACGTCGACTTTTGT(GC)AAGGGATCTACTACTGT	D_HFL16, D_HSP2	(5)	−	+
GGAGAGAGTTTTAGTATAGGAACACAGAGGC	5′ of J_H1		+	+
GGCTGCAG(CG)TTCAGTGGCAGTGG(AG)TC(AT)GG(AG)AC	V_κ	(15)	+	+
GCTACCCACTGCTCTGTTCCTCTTCAGTG	5′ of $J_\kappa1$		+	+
GCCATTTCCCAGGCTGTTGTGACTCAGG	V_λ		+	−
AATCTGCACTCACCACATCACCTGGTG	V_λ		−	+
3′ primers				
AGGCTCTGAGATCCCTAGACAG	3′ of J_H4	(5)	+	−
GGGTCTAGACTCTCAGCCGGCTCCCTCAGGG	3′ of J_H4	(5)	−	+
CAAAACCCTCCCTAGGTAGACAATTATCCCTC	3′ of $J_\kappa2$		+	−
CCTCTCAACTAAAGCCTCTTTTTGCCCCTAATC	3′ of $J_\kappa5$		+	−
ACTCACCTAGGACAGTCAGCTTGGTTCC	J_λ		+	+
GGACAGATCTCCCTCCTTAACACCTGATCTG	3′ of $J_\kappa2$		−	+
CATAACTGACTGTGCTGGCTGGGTTGG	RS		+	−
CTGCCCACACGACTCCTTCAGGCAGACG	RS		−	+
ATGCGACGTCAACTGATAATGAGCCCTCTCC	3′ of $J_\kappa5$		−	+

et al. (5). For the second PCR round, 1 µl of the first PCR amplification was reamplified with one specific 5′ primer and a specific 3′ primer (Table 1, second round PCR primers). The second round was done over 35 cycles (20 s at 95°C, 1 min at 65°C, 2 min at 72°C). All PCR contained dATP, dCTP, dTTP and dGTP (Pharmacia, Uppsala, Sweden) at 200 µM each and 5 U Taq DNA polymerase (Roche, Basel, Switzerland) in PCR buffer (0.05 mM 2-mercaptoethanol, 50 mM KCl, 10 mM Tris–HCl, pH 8.3, 1.5 mM $MgCl_2$ and 0.001% gelatine). The first round PCR reaction was performed in 30 µl, the second in 20µl. Then, 10 µl of the second round PCR product was analyzed on agarose gels stained with ethidium bromide. The lengths of the different PCR products are summarized in Table 2.

Table 2. Approximate PCR product lengths

Rearrangements	Product lengths (bp)
Germline Ig H	1500
D_HJ_H (SP2/FL16)	1460 (J_H1), 1150 (J_H2), 730 (J_H3) and 200 (J_H4)
$D_HQ_{52}J_H$	1500 (J_H1), 1190 (J_H2), 770 (J_H3) and 240 (J_H4)
$V_HD_HJ_H$	1720 (J_H1), 1410 (J_H2), 990 (J_H3) and 460 (J_H4)
Germline Ig κ	600
$V_\kappa J_\kappa1$ or 2	650 ($J_\kappa1$) and 280 ($J_\kappa2$)
$V_\kappa J_\kappa4$ or 5	600 ($J_\kappa4$) and 260 ($J_\kappa5$)
RS_κ	320
V_λ J_λ	318

Results and discussion

PCR assay for the analyses of Ig gene rearrangements in single cells

We have used assays for H chain gene rearrangements developed by Ehlich *et al.* (5) and by Haasner *et al.* (14), and assays for κL chain gene rearrangements developed by Schlissel and Baltimore (15), and have modified and extended these assays, particularly to include λL chain gene rearrangements, as described in Methods. At the single cell level, these assays are based on PCR amplification of gene segments in two steps. In the first, both rearranged Ig loci of a cell were amplified simultaneously using a mixture of 13 5′ primers, homologous to V_H, V_L and D_H genes, and upstream of the J_H1 and $J_{L\kappa}1$ segment in combination with five 3′ primers binding downstream of J_H4, $J_{L\kappa}2$, $J_{L\kappa}5$, $J_{L\lambda}$ and RS_κ. The primers are listed in Table 1. In the second PCR round, the products of the first PCR were analyzed in separate reactions. For VDJ and DJ PCR, each reaction contained respectively a single V_H and D_H primer together with a nested 3′ J_H4 primer. The unrearranged state of an Ig H locus was determined by the reaction containing a primer recognizing 5′ of the J_H1 segment and a nested 3′ J_H4 primer. PCR assays for L_κ chain rearrangements used a V_κ consensus primer located at the 5′ end of framework 3, as well as two primers in the J_κ locus; $J_\kappa1$–2 and $J_\kappa4$–5, located downstream of $J_\kappa2$ and $J_\kappa5$ respectively. In the second PCR step, a nested primer (upstream) of primer $J_\kappa1$–2 and $J_\kappa4$–5 was used. We used two different J_κ primers in the first PCR, because amplification

of V–J_κ1 and V–J_κ2 rearrangements by using the J_κ4–5 primer only resulted in a low frequency of J_κ1 and J_κ2 rearrangement (results not shown). The λ PCR assay utilized a V_λ consensus primer and a universal J_λ primer in the first PCR round and a nested V_λ primer together with the universal J_λ primer in the second PCR round. It has been described that expression involving J_λ1 segments occurs 10 times more frequently than J_λ2 and 3 whereas expression of J_λ4 was undetectable (16). Therefore we used a J_λ primer which binds to J_λ1, rather than J_λ2, 3 and 4 genes. Thus, we cannot exclude that some λ rearrangements are not detectable in these assays.

Ig H and L chain gene rearrangements in B cell subpopulations

A total of 24 single B220$^+$/c-kit$^+$ cells, 48 single large B220$^+$/μH/SL$^+$ cells, 48 single large B220$^+$/CD25$^+$/sIgM$^-$ cells, 48 single small B220$^+$/CD25$^+$/sIgM$^-$ cells, 48 single immature B cells and 44 single mature B cells were sorted as described in Methods, and analyzed for the status of their two Ig H, κL and λL chain alleles. A representative picture of V_HJ558–D_HJ_H rearrangements and $V_\kappa J_\kappa$1 or 2 rearrangments found in single immature B cells is shown in Fig. 2.

H chain alleles. In 42% of the cells, both Ig H alleles were detected and in 36% of the cells only a single PCR product was observed. No amplification was obtained in 22% of the tested cells. However, it should be noted that cells containing certain combinations of rearrangements in Ig H loci might be under-represented Due to the recognition of D_{FL} and D_{SP} segments by the same primer, the present assay does not resolve two DJ_H joints involving D_H elements of these two families rearranged to the same J_H genes on both chromosomes, because these rearrangements will appear as a single PCR band. Moreover, two V_HDJ_H joints involving V_H genes of the same family rearranged to the same J_H gene on both chromosomes cannot be discriminated on the gel. Furthermore, the $D_{SP/FL}$ primer does not bind to the FL16.2 segment Hence, ~7% of the DJ_H rearrangements will not be scored, assuming that the usage of the D segments in rearrangements is random, as suggested earlier (17).

The results of the analyses of the H chain alleles in single cells of different stages of B cell development are given in Tables 3 and 4. They show that the majority of all H chain alleles in B220$^+$/c-kit$^+$ cells are D_HJ_H-, but not yet $V_HD_HJ_H$-rearranged and are thus pre-B I cells. This is in agreement with previous analyses from our laboratory either on B220$^+$/c-kit$^+$ cell populations obtained '*ex vivo*' (8,14) or detected in lines and clones of B220$^+$/c-kit$^+$ cells proliferating on stromal cells in the presence of IL7 (8,14).

$V_HD_HJ_H$-rearranged alleles are detected at equally high frequencies in B220$^+$/μH/SL$^+$, large and small B220$^+$/CD25$^+$/sIgM$^-$, immature B and mature B cells. More than 90% of the PCR positive cells of these showed at least one H chain allele in the $V_HD_HJ_H$-rearranged configuration. This is in agreement with the observation that >90% of all large and small B220$^+$/CD25$^+$/sIgM$^-$ cells express μH chains in their cytoplasm (7).

Among the cells in which both alleles were detectable, ~50% had both alleles $V_HD_HJ_H$ rearranged while the other 50% was $V_HD_HJ_H/D_HJ_H$ rearranged. Again, this was true for

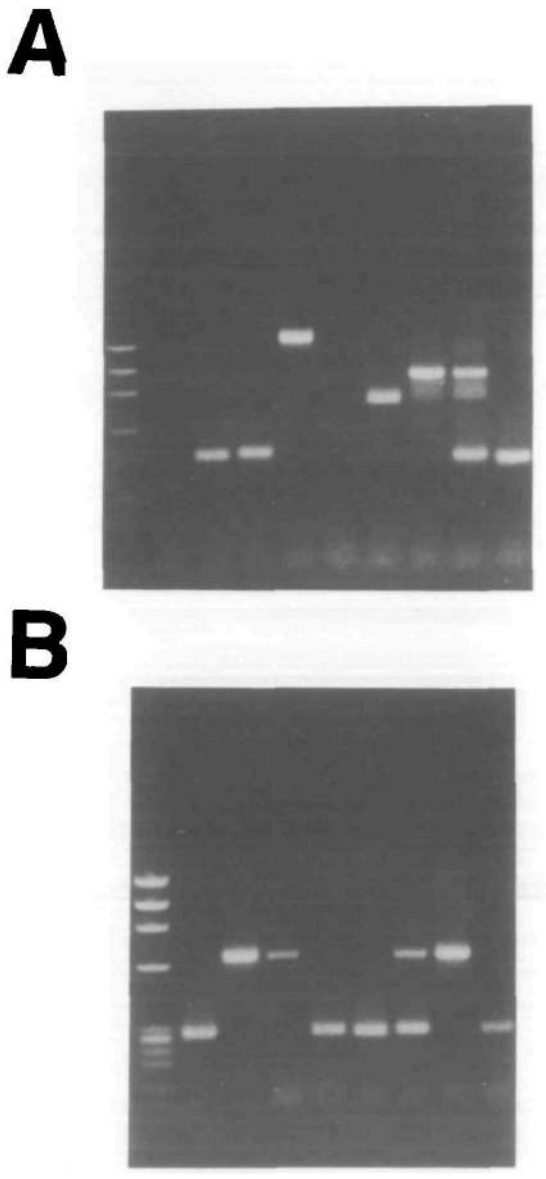

Fig. 2. A representative example of V_HJ558–D_HJ_H rearrangments (A) and $V_\kappa J_\kappa$1 or 2 rearrangements (B) as found in eight single immature B cells (lanes 2–9) Molecular weight markers are shown in lane 1.

B220$^+$/μH/SL$^+$ large and small B220$^+$/CD25$^+$/sIgM$^-$, immature B and mature B cells (Table 3). These results agree with and extend the observation that 40% of the peripheral B cells have both H chain alleles in a $V_HD_HJ_H$-rearranged configuration (18). Future sequencing of all alleles should answer the question of which alleles are, or are not, productively $V_HD_HJ_H$ rearranged, and quantitate the reading frame distribution amongst D_HJ_H rearranged H chain alleles (5,14,19). They will also allow an investigation of the usage of V gene segments in the H chain alleles of different B cell subpopulations during development.

L chain alleles. Approximately 40% of V_κ genes rearrange by inversion (20), thus retaining previous rearrangements on the same chromosome. In addition, any excised DNA containing

Table 3. Configuration of Ig H loci in pro/pre-B I, pre-B II, immature and mature B cells

	B cell subpopulations[a]					
	c-kit$^+$/CD25$^+$	c-kit$^+$/CD25$^+$/SL/μH$^+$ large	c-kit$^-$/CD25$^+$ /sIgM$^-$ large	c-kit$^-$/CD25$^+$ /sIgM$^-$ small	IgM$^+$B	IgM$^+$/IgD$^+$ B
Total no. of cells tested	24	48	48	48	48	44
No. of cells with[b]						
VDJ/VDJ	1	12	7	12	6	5
VDJ/DJ	2	13	9	12	11	7
DJ/DJ	8	2	0	0	0	0
GL/VDJ	0	2	2	0	0	0
VDJ/-	0	8	3	12	18	18
DJ/-	8	2	11	5	3	2
-/-	5	9	16	7	10	12

[a]All subpopulations are B220 (CD45R)$^+$
[b]-: one or two alleles not detected.

Table 4. The rearrangement status of Ig H and L chain alleles of single cells at different stages of development

	B cell subpopulations[a]						
	c-kit$^+$/CD25$^-$	c-kit$^-$/CD25$^+$/SL/μH$^+$ large	c-kit$^-$/CD25$^+$ /sIgM$^-$ large	c-kit$^-$/CD25$^+$/sIgM$^-$ small	IgM$^+$B	IgM$^+$/IgD$^+$ B	IgM$^+$IgD$^+$λL$^+$B
No of cells analyzed	24	48	48	48	48	44	36
Configuration of Ig H chain alleles							
germline	0	2	2	0	0	0	ND[b]
D_HJ_H	26	19	20	17	14	9	ND
$V_HD_HJ_H$	4	46	28	48	41	35	ND
efficiency of detection	63%	70%	52%	68%	57%	50%	-
Configuration of Ig L chain alleles							
germline κL	ND	ND	ND	20	26	23	2
$V_\kappa J_\kappa$	0	1	1	47	50	47	36
$V_\lambda J_\lambda$	0	0	0	6	4	6	36
RS	ND	ND	ND	ND	ND	ND	22

[a]All subpopulations are B220 (CD45R)$^+$
[b]ND: not done.

the 'germline fragment' ($J_\kappa1$–$J_\kappa2$ region) or primary $V_\kappa J_\kappa$ rearrangements may still be present in the cell and thus be available for PCR amplification, since we employed an oligonucleotide ($J_\kappa1$–2) priming inside the J_κ cluster. When an inverted V_κ gene or a non-inverted V_κ gene joins to $J_\kappa4$ or 5, the germline fragment is retained in the same chromosome in the former case and is deleted in the latter. Hence, when a secondary (inversional) rearrangement has occurred, more than two PCR bands can be found. The numbers of alleles given in Table 4 as the results of the analyses of the κL chain alleles in different B cell subpopulations make the assumption that only one cell with maximally two κL chain alleles was analyzed in each assay. Hence, in cases where more than two PCR products of the κL chain locus were detectable (in fact in 12 of a total of 164 cells with detectable κL chain loci), they were counted as two alleles.

Of the 24 B220$^+$/c-kit$^+$ cells, 48 B220$^+$/μH/SL$^+$ cells and 48 large B220$^+$/CD25$^+$/sIgM$^-$ cells, only two $V_\kappa J_\kappa$-rearranged alleles were detectable (Table 4). This shows that the vast majority of precursor B cells rearrange the D_H–J_H and V_H–D_HJ_H segment on the H chain loci before they rearrange the L chain loci. It makes the possible alternate pathway of B cell development improbable, or at least infrequent (21). While the large μH/SL$^+$ and μH/SL$^-$ pre-B II cells are both candidates for precursor of small pre-B II cells, it remains to be investigated whether both of them, or only one of them, play(s) this role in B cell differentiation.

Rearrangements of at least one allele of the κL chain gene locus became detectable in 65% of the small B220$^+$/CD25$^+$/sIgM$^-$ cells, in 67% of the immature B and in >75% of the mature B cells (data for single cells not shown, but summarized as numbers of alleles in Table 4). This indicates that κL chain gene rearrangements are induced when small resting B220$^+$/CD25$^+$/sIgM$^-$ cells develop and suggests that large B220$^+$/CD25$^+$/sIgM$^-$ cells are the precursors of their small counterparts. Collectively, these results define c-kit$^+$ CD25$^-$ cells as mostly D_HJ_H-rearranged pre-B I cells and which are the precursors of large c-kit$^-$/CD25$^+$/μH/SL$^{+/-}$ $V_HD_HJ_H$-rearranged preB II cells, which are the precursors of the small c-kit$^-$/CD25$^+$/μH/SL$^-$ $V_HD_HJ_H/V_LJ_L$-rearranged pre-B II cells, which, in turn, are the precursors of the sIgM$^+$ immature B cells.

Rearrangements in κL versus λL gene loci

The majority of the L chain rearrangements are found in the κ loci. The ratio of rearrangements in the κL versus λL chain is 10:1 (Table 3). This ratio is stable throughout the different B lineage populations with increasing maturity, i.e. from small $B220^+/CD25^+/sIgM^-$ to immature B to mature B cells. The majority of $V_\kappa J_\kappa$-rearranged small pre-B II, immature B and mature B cells do not have λL chain gene rearrangements. However, 12 of the 16 cells with λL gene rearrangements have $V_\kappa J_\kappa$ rearrangements using the most 3′ $J_\kappa 5$ segment.

Furthermore, mature λL chain-producing B cells from spleen were analyzed for the configurations of their L chain alleles (Table 4, last column). A rearranged λL locus was detected in all λ-producing B cells Although we have not yet ruled out by sequencing that all 36 cells analyzed (Table 4) produced λ_1L chain, we take this as a strong indication that the λ PCR assay is very efficient and can probably detect rearrangements in all the λL chain loci. Moreover, all λL-producing cells showed rearrangements in their κL chain loci Twenty-nine of the 36 cells analyzed, again, had $V_\kappa J_\kappa$ rearrangements using $J_\kappa 5$.

These findings are consistent with previous reports (22,23). However, λL chain gene-rearranged B cells in which no κL chain gene rearrangements were detectable have been described (24). While this might imply that some λL chain-expressing cells have their κL chain loci in germline configuration, the more frequently observed $V_\lambda J_\lambda$-rearranged pre-B II cells (five of six), immature B cells (two of four) or mature B cells (seven of eight), is one in which κL chain gene loci are rearranged to downstream J_κ segments or even deleted via RS. In fact, deletion was observed in 22 of the 36 λL $chain^+sIg^+$ B cells (Table 4, last column). In addition, due to the complexity of the κL chain gene locus introduced by opposite polarities of V_κ segments, it is not even certain that the λL chain-rearranged cells, in which no κL chain rearrangements were observed, have, in fact, these κL chain loci in germline configuration.

All these results suggest that the κL/λL chain ratio of the mature peripheral B cell pool, which also is of the order of 10:1, is established as soon as L chain gene rearrangements are induced, i.e. in the pre-B II cells. It will require the analysis of a much larger number of cells from the different B lineage compartments in bone marrow and the sequencing of the rearranged Ig L chain loci to determine whether this ratio is exactly the same in all stages of B cell development with rearranged L chain loci or whether selective forces on a small number of the total cells influence this ratio with increasing maturity. It is also too early to conclude from our data that all λL chain rearrangements obligatorily follow κL chain rearrangements, although the majority of them appear to do so.

Finally, since small pre-B II cells do not deposit IgM on the surface and since L chains so far have not been detectable in the cytoplasm by immunofluorescence with specific antibodies (while μH chains are easily detectable), it might well be that κL chain gene rearrangements and the expression of the rearranged L chain loci as proteins are separately regulated. One possible example of such a cell with rearranged κL chain loci but without expression of the rearranged gene locus is the 70Z/3 preB lymphoma (25).

A comparision of the B-lineage-committed, CD45RA ($B220^+$) subpopulations of bone marrow characterized by different markers in different laboratories

Hardy's (6) and Rajewsky's (5) laboratories have used the differential expression of CD43, HSA and BP-1 to separate CD45RA ($B220^+$) B-lineage-committed precursors into fractions B, C, C′ and D, and into the immature and mature, $sIgM^+/sIgD^-$ and $sIgM^+/sIgD^+$ populations E and F. Analyses of the status of the Ig gene loci in these fractions have shown that fraction B is enriched for D_HJ_H-rearranged H chain loci which have not yet undergone $V_HD_HJ_H$ rearrangements. Fraction B is therefore likely to be largely the same as our pro/pre-B I population (Fig. 1). Fraction C was found to be enriched for cells with two non-productively $V_HD_HJ_H$-rearranged Ig H alleles. We have hypothesized (see Fig. 1) but never detected these cells in our assays.

Ehlich *et al.* (5) have suggested that fractions C′ and D might have similar IgH rearrangements. They therefore only analyzed fraction D and found all cells to contain one productively $V_HD_HJ_H$-rearranged IgH allele. Fractions C′ and D could therefore be, at least in part, the same as the large, $\mu H/SL^+$ and $\mu H/SL^-$ pre-B II cells, and the small pre-B II cells In our analyses, 60% of the large pre-B II cells and 30% of the small pre-B II cells express BP-1 (7), indicating that the comparison of fractions C′ and D with pre-B II cells is complicated. Also, fractions C′ and D include to a good part the large, cycling cells that should contain the large, $\mu H/SL^+$ and $\mu H/SL^-$ pre-B II cells. Our scheme of separation on the basis of large, cycling and small, resting cells, as well as the patterns of expression of c-kit, CD25 and SL and the analysis of the rearrangement status of L chain gene loci appears to allow a better resolution of crucial steps in the development of precursor B cells, especially since L chain gene rearrangements are almost totally absent in large pre-B II cells and appear in full when they become resting, small pre-B II cells. With all the analyses on marker expression and status of Ig gene loci rearrangements, we should now be in the position to simplify the nomenclature of precursor B cells in bone marrow and to propose protocols for the analysis of precursor B cell compartments that might vary in their contents due to genetic or environmental influences. More importantly, we are now in a position to clearly separate cells with defined stages of Ig gene rearrangements according to their cellular program of differentiation, i.e. marker expression. This will facilitate the generation of differential cDNA libraries in the search for genes and molecules which control these B cell developmental steps.

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Abbreviations

HSA	heat stable antigen
RS	recombination signal sequence (up- and downstream of Cκ)
SL	surrogate light chain

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