International Immunology, Vol 6, No 1, pp 21 - 30

Influence of surrogate L chain on D_HJ_H-reading frame 2 suppression in mouse precursor B cells

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Key words: D_HJ_H rearrangement, reading frame, surrogate L chain

Abstract

D_HJ_H rearrangements start in progenitor and precursor B cells and occur in three reading frames (rf). A strong bias for rf I has been noticed in murine and chicken antibodies, while the representation of rf II has been found suppressed both in peripheral as well as in precursor B cells. H chain gene loci D_HJ_H rearranged in rf II are potentially capable of expressing a truncated $D_HJ_HC_\mu$ protein on the cell surface. Mice incapable of expressing this protein on the surface have previously been shown to have all reading frames represented in near equal frequency, suggesting that membrane-bound $D_HJ_HC_\mu$ protein is involved in the suppression of rf II. In this paper we show that suppression of rf II is not yet established in c-*kit*$^+$ CD43$^+$ IL-7/stromal cell-reactive pre-B I cells of fetal liver at day 15 of gestation, but becomes established when such precursor cell populations are expanded *in vitro* on stromal cells in the presence of IL-7. H chain gene loci using the D_{Q52} segment for rearrangements (which contains a stop codon in rf II, thus being unable to make $D_HJ_HC_\mu$ protein) do not show rf II suppression under these conditions. The same type of fetal liver-derived pre B-I cells from λ5 deficient mice also do not show rf II suppression after *in vitro* expansion. Bone marrow-derived pre B-I cells from normal mice assayed *ex vivo* and expanded *in vivo* show rf II suppression, while the corresponding pre-B I cells from λ5T mice do not. Collectively these experiments suggest that surrogate L chain is involved in rf II suppression. This may happen by inhibition of proliferation of pre-B cells expressing a complex of $D_HJ_HC_\mu$ protein and surrogate L chain.

Introduction

The Ig heavy (H) chain gene locus on chromosome 12 of the mouse consists of probably >100 V_H gene segments, 15 D_H gene segments, and four J_H gene segments (1 - 3). Of the 15 D_H segments, two are D_{FL} segments ($D_{FL16.1}$ and $D_{FL16.2}$), 12 are D_{SP} segments ($D_{SP2.1}$ - $DSP_{SP2.11}$ plus $D_{SP2.X}$), and one is a D_{Q52} segment.

During differentiation of progenitors (pro-B cells) to precursors of the B lineage pathway (pre-B I cells; for nomenclature see 4), D_H segments are first rearranged to J_H segments (5). The intervening sequences between the rearranged D_H and J_H are frequently deleted as circular DNA. As long as D_H segments at the 5′ side and J_H segements at the 3′ side remain in the rearranged H chain gene locus, secondary rearrangements are possible. Such secondary D_HJ_H rearrangements were shown by studies of circular excision products in which pre-existing D_HJ_H joints were found (6). Secondary D_HJ_H rearrangements have also been implicated from analyses of D_HJ_H joints in clones of pro- and pre-B I cells of fetal liver and bone marrow early in development (7).

D_HJ_H rearrangements can occur in three reading frames (rf) (8,9). The D_H segments carry promoter-like elements upstream of the coding sequences and an ATG start codon (at position - 63 for all D_{SP} segments, - 108 for D_{FL} segments, and - 120 for the D_{Q52} segment) which is 'in frame' with J_H when the D_HJ_H rearragement occurred in rf II. The protein product of such a rf II rearranged locus, a truncated H chain consisting of D_H, J_H, and C_μ, has been detected in one cell line (10). Since the D_{Q52} segment carries a stop codon 48 nucleotides after the start, only D_{SP}- and D_{FL}-, but not D_{Q52}-containing D_HJ_H rearranged H chain loci can express the rf II rearranged locus as a truncated μH chain.

Pre-B cells express the proteins λ5 and $V_{pre\text{-}B}$, which can be found on the surface as a surrogate light (L) chain (11,12), reviewed in (13). One Abelson virus-transformed cell line has been found to express the truncated $D_HJ_HC_\mu$ protein together with surrogate L chain on the surface (14).

D_HJ_H joints in rf I are over-represented. Short stretches of sequence homology in the D_H and J_H segments might help to

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Transmitting editor: O Mäkelä

Received 7 September 1993, *accepted* 22 September 1993

align the recombining DNA strands and this is likely to contribute to the rf I over-representation (3,8,9,15–17). When D–J recombination reactions are measured with extrachromosomal substrates transfected into murine pre-B cell lines *in vitro*, i.e. when D_HJ_H rearrangements can occur without cellular selection, a rearrangement using a four nucleotide overlap (as in D_HJ_H rf I) occurs at 55% (18). A similar preference for rf I rearrangements is observed in pre-B cells from fetal liver which do not express high levels of the enzyme terminal deoxynucleotidyl transferase (TdT) (19,20), and, therefore, do most often not insert non-templated (N) nucleotides into the D_HJ_H joints (7,9,15,16,21,22). The insertion of N nucleotides appears to correlate with the level of TdT expression (23,24) which is high in bone marrow-derived pre-B cells. N-region insertion reduces the preference for overlap-mediated joints (18). A preference for rf I has also been found in chicken (25).

A different mechanism, apparently independent of TdT expression, is the insertion of so called P nucleotides, which consist of short palindromic (P) sequences specific to the joining ends of the gene segments (26). P nucleotides are present at the coding joint when exonuclease activity is low (16) and have been frequently observed in sequences derived from fetal liver (9,15,16,22).

While the preference for D_HJ_H joints in rf I appears to be the consequence of a molecular selection of DNA strands during rearrangement, the suppression of rf II is thought to arise through cellular counterselection against those pre-B cells which expess the truncated $D_HJ_HC_\mu$ protein on their surface (4,27,28). Further evidence comes from μMT mice which can neither express $D_HJ_HC_\mu$ protein nor μH chains on their surface due to disruption of the transmembrane portion-encoding exon. In the non-functional, targeted alleles of splenic B cells of heterozygous μMT mice, rf II D_HJ_H joints are normally represented and not suppressed (27).

The surrogate L chain can form a disulfide-bonded complex with $D_HJ_HC_\mu$ protein as well as with μH chains (14, 29). Membrane deposition on pre-B cells of $D_HJ_HC_\mu$ protein and μH chains may well be dependent on this association with surrogate L chain, just as membrane deposition of IgM on mature B cells is dependent on the association of μH chains with normal L chains. If this were so, then pre-B cells of mice with a defective λ5 gene (λ5T mice; (30)) should be unable to deposit $D_HJ_HC_\mu$ proteins in membranes, which would also abolish the suppression of pre-B cells with H chain loci D_HJ_H rearranged in rf II.

In this paper we analyze the structures of D_HJ_H joints in pre-B cells obtained from fetal liver and bone marrow of normal as well as λ5T mice to determine their rf distribution. B220$^-$ c-*kit*^{low} pro-B cells and B220$^+$ c-*kit*$^+$ CD43$^+$ pre-B I cells are clonable *ex vivo* with high efficiency and proliferate for long periods of time *in vitro* on stromal cells in the presence of IL-7 (7,31). Although a defective λ5 gene leads to the depletion of later stages of B cell development (pre-B II cells, immature B cells) and to a delayed appearance of sIg$^+$ cells in the periphery (30), the defect does not impair the generation of normal numbers of pro-B and pre-B I cells (32). In this paper we, therefore, also analyze the structures of D_HJ_H joints of pre-B I cell lines and clones obtained from fetal liver and bone marrow of normal and λ5T mice. All D_{SP} segments and one of the two D_{FL} segments (i.e. $D_{FL16.1}$), but not $D_{FL16.2}$ and D_{Q52} are detected by a polymerase chain reaction (PCR) with an oligonucleotide primer which hybridizes in the 5′ regions of all these D segments. A second oligonucleotide primer is employed to detect D_{Q52}–J_H joints.

Collectively our results present evidence for the involvement of surrogate L chain in the selection of rfs of D_HJ_H joints in pre-B cells, which appears to function by a suppression of proliferation of pre-B I cells with rf II D_HJ_H rearranged H chain gene loci.

Methods

Mice

(C57BL/6 (×) DBA/2)F_1 (BDF_1) mice were obtained from Institut für Biologisch-Medizinische Forschung AG (*Füllinsdorf, Switzerland*). Homozygous λ5T mice (30) were bred at the Institute's animal facilities. Cells for FACS sorting and pre-B cell culture were prepared from different lymphopoietic organs at different developmental stages (fetal liver, newborn and adult liver, spleen, and bone marrow) as described (7).

FACS staining and sort

Cell staining procedures and antibodies employed in the staining reaction have been described previously (7,31). Cells expressing B220, c-*kit*, S7(CD43), BP1, or combinations thereof were sorted using the FACS-Star Plus (Beckton-Dickinson, Mountain View, CA).

Cell culture

Cell suspensions were plated under limiting dilution conditions on a feeder layer of 3300 rad irradiated stromal cells (PA-6 cells, (37)) in serum free medium containing murine rIL-7 at a concentration of 100–200 U/ml. The culture conditions for the growth of these non-transformed pre-B cells have been described previously (31). Pre-B cell clones were expanded to $\sim 5 \times 10^6$ cells before they were harvested for the preparation of DNA.

DNA Preparation

Cells (5×10^5) were harvested, washed in PBS, and lysed by boiling for 5′ in 500 μl PBS. Proteinase K (Boehringer, Mannheim, Germany; 20 μg/ml final) was added, the lysate digested at 55°C for 3 h and boiled again to destroy Proteinase K activity.

The solution was extracted once with phenol:chloroform:isoamylalcohol (25:24:1) and once with chloroform:isoamylalcohol (24:1). DNA was precipitated with 1 volume isopropanol/0.1 volume NaAcetate, pH 5.2, centrifuged, the pellet washed with 1 volume 70% Ethanol, and air dried. The DNA pellet was dissolved in 500 μl 10 mM Tris, pH 8.3. Aliquots of 5 μl of this preparation (containing the DNA of 5000 cells) were subsequently used for PCR amplification.

PCR Amplification and Cloning

The PCR conditions and oligonucleotide primers to amplify D_H–J_H rearrangements have been previously described by Gu *et al.* (27). The upstream primer binds 5′ of all D_{SP} segments and $D_{FL16.1}$. The downstream primer binds 3′ of J_H4, thereby generating PCR products of different lengths according to the J_H segment used for rearrangement (7). A Perkin-Elmer Cetus DNA Thermal Cycler and Cetus Taq Polymerase (Perkin-Elmer Cetus, Norwalk, CT) were used for PCR amplification. D_H–J_H1 rearrangements generate products of 1700 bp, D_H–J_H2 1450 bp, D_H–J_H3 1100 bp, and D_H–J_H4 600 bp respectively.

A different 5′ primer was used to amplify D_{Q52} rearrangements in conjunction with the 3′ J_H primer. The primer sequence is 5′-GCC TCA GAA TTC CTG TGG TCT CTG ACT GGT-3′; it contains an *Eco*RI cloning site. PCR product lengths are 2200 bp for

germline, 1500 bp for $D_Q - J_H1$, 1170 bp for $D_Q - J_H2$, 790 bp for $D_Q - J_H3$, and 230 bp for $D_Q - J_H4$ respectively.

The PCR products and the M13mp19 vector (New England Biolabs, Beverly, MA) were digested with two different restriction enzymes (New England Biolabs) according to the enzyme manufacturer's conditions (forced cloning), and size fractionated on a low melting point agarose gel (Ultra Pure LMP Agarose; BRL, Bethesda, MD). Bands of interest and the linearized vector were cut out under UV illumination, the agarose remelted, ligation reagents and T4 DNA Ligase (Pharmacia, Uppsala, Sweden) mixed into the agarose, and ligation carried out directly (39). Ligated vector was heat shock transfected into competent *Escherichia coli* JM 105 bacteria and plated out on YT plates. Recombinant plaques were identified by plaque hybridization with a radioactive J_H4 oligonucleotide probe (AGGAACCTCAGTCACCGGATCCGT) (all procedures according to (39)). Single-stranded M13 DNA was sequenced using a Sequenase 2.0 sequencing kit (United States Biochemicals, Cleveland, OH) and an IBI sequencing gel apparatus (Eastman Kodak, Rochester, NY).

Results

Structures of D_HJ_H joints in FACS-sorted pre-B cells from fetal liver and bone marrow of normal and λ5T mice

Cell suspensions from fetal liver or bone marrow of normal BDF_1 and surrogate L chain deficient λ5T mice were sorted for $B220^+$ c-*kit*$^+$ or $B220^+$ $CD43^+$ pre-B cells as described (7), and DNA was prepared from these cells for D_HJ_H joint sequencing. Table 1 shows 36 sequences of normal fetal liver cells (day 15, $B220^+$ c-*kit*$^+$), 25 sequences of λ5T fetal liver cells (day 13, $B220^+$ c-*kit*$^+$), 58 sequences of normal bone marrow cells (3 weeks of age, $B220^+$ c-*kit*$^+$ or $S7^+$ $BP1^+$) and 31 sequences of λ5T bone marrow (14 weeks of age, $B220^+$ c-*kit*$^+$). Pre-B cells from fetal liver of normal (day 15) as well as λ5T (day 13) mice only rarely displayed N or P nucleotides (Table 1a and b), whereas bone marrow-derived pre-B cells (normal: w3, λ5T w14) displayed high junctional diversity due to added nucleotides (Table 1c and d). Table 3 summarizes the sequencing data and was used to generate Fig. 1(a and b).

In the fetal liver of both normal and λ5T mice, most D_HJ_H joints were in rf I (60 – 67%) (Fig. 1a, 1 + 2). This is presumed to be the consequence of a preferred alignment of short stretches of sequence homology in the D_H and J_H segments. In these cells, D_HJ_H joints in rf II and rf III were present in approximately equal amounts (18 – 23%). This distribution of frequencies of rf I versus rf II versus rf III D_HJ_H joints closely resembles the distribution found in a system where recombinants of the D_HJ_H type were generated on extrachromosomal substrates transfected into murine pre-B cells of fetal liver origin *in vitro*, i.e. without selection *in vivo* (18). In addition, the results in Fig. 1(a) also show that rf II is not suppressed in fetal liver pre-B cells *ex vivo*. It suggests that no selection of H chain alleles and/or cells carrying those alleles has taken place at that early stage of B cell development.

In bone marrow of normal and of λ5T mice, rf I and rf III D_HJ_H joints are present at similar frequencies, i.e. rf I is not over-represented, and most of these joints carry N region sequences inserted by TdT. Hence this result agrees with Gerstein and Lieber's findings (18) with extrachromosomal rearrangement substrates that the constraint to align short homologous sequences of D_H and J_H is diminished by TdT and N-region insertion. Although $D_{Q52} - J_H$ joints are not analyzed here, we expect that they show a similar pattern of rf usage (see Note added in proof).

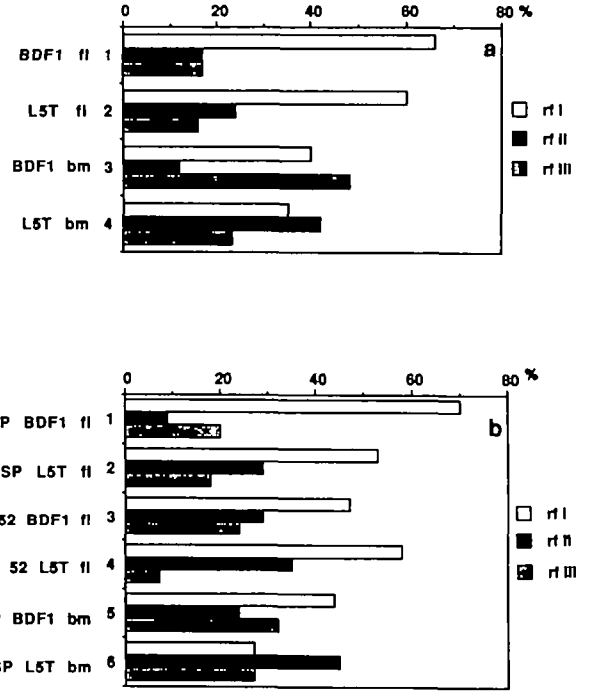

Fig. 1. Presence or absence of rf II suppression in different populations of pre-B cells from normal and λ5T mice. The figure shows the rf usage in FACS-sorted pre-B cells (a) and cultured pre-B cells (b). Values are given as percent of total sequences analyzed. The numbers of total sequences are given in Table 2. Panel (a) includes D_HJ_H rearrangements using $D_{FL16.1}$ or all D_{SP} segments in cells of BDF1 fetal liver (1), λ5T fetal liver (2), BDF_1 bone marrow (3), and λ5T bone marrow (4). Panel (b) includes D_HJ_H rearrangements using $D_{FL16\ 1}$ or all D_{SP} segments in cultured cells obtained from BDF_1 fetal liver (1), λ5T fetal liver (2), BDF_1 bone marrow (5), and λ5T bone marrow (6), and D_HJ_H rearrangements using the DQ52 segment in cultured cells obtained from BDF_1 fetal liver (3) and λ5T fetal liver (4). Details for sequencing of D_HJ_H joints are given in Methods.

Most important for the possible role of surrogate L chain in the rf selection process is the finding that rf II is represented in bone marrow cells of λ5T mice in frequencies nearly equal to those for rf I and rf III, while it is suppressed in bone marrow cells of normal mice ($P = 0.003$) (Fig. 1a, 3 + 4). These results indicate that the inability of pre B cells to express the surrogate L chain $V_{pre\text{-}B}/λ5$ abolishes the mechanism(s) which suppresses rf II representation in D_HJ_H joints.

Structures of D_HJ_H joints in pre-B cell lines and clones proliferating in vitro

Pre-B cells from fetal liver and bone marrow of normal and λ5T mice were cloned by limiting dilution and expanded by proliferation on stromal cells in the presence of IL-7 to $\sim 10^6$ cells (i.e. for at least 20 divisions) as described (31). DNA was prepared and D_HJ_H joints sequenced. Again, D_HJ_H joints of fetal liver-derived lines and clones only rarely contained P or N nucleotides while those of bone marrow frequently did (Table 2a – d). The rf I D_HJ_H joints were found to be strongly over-represented in all lines and clones except in $D_{FL}/D_{SP} - J_H$ joints of bone marrow from normal and λ5T mice (Fig. 1b, 5 + 6). While $D_{Q52} - J_H$ joints in rf III were unexpectedly

Table1 a-b: D-J sequences (DFL/DSP) of BDF1 and L5T/L5T sorted fetal liver preB cells

1a) sorted BDF1 fetal liver preB cells day 15 (B220+/c-kit+ markers)

name, origin	D-segment	N/P-sequence	J-segment	D	J	reading frame	N/P-seq
A21	CC TAC TAT AGT TAC TAT AGT TAC		GAC TAC TTT GAC TAC TGG	SP2.11+X	2	I+I	-
A22	CC TAC TAT AGT AAC TAC		TTT GAC TAC TGG	SP2.X	2	I	-
A24	GAT GGT TAC TA		T GCT ATG GAC TAC TGG	SP2.9	4	I	-
A31	TC TAC TAT GGT AAC T		TT GCT TAC TGG	SP2.1	3	I	-
A32	TC TAT GAT GGT T		TT GCT TAC TGG	SP2.9	3	I	-
A33	TC TAC TAT GGT AAC T		GG TTT GCT TAC TGG	SP2.1	3	I	-
A34	CCT ACT ATA GTA AC		C TGG TTT GCT TAC TGG	SP2.X	3	I	-
A41	CC TAC TAT AGT TAC G		CT ATG GAC TAC TGG	SP2.11	4	I	-
A42	CC TAC TAT AGT TAC GAC		TAT GCT ATG GAC TAC TGG	SP2.11	4	I	-
A43	CC TAC TAT AGT AAC TAC		TAT GCT ATG GAC TAC TGG	SP2.X	4	I	-
B11	TC TAT GAT GGT TAC		TTT GAC TAC TGG	SP2.9	2	I	-
B12	CCT ACT ATA		TGG TAC TTC GAT GTC TGG	SP2.11/X	1	I	-
B14	TC TAT GAT GGT TAC TAC		TAC TAT GCT ATG GAC TAC TGG	SP2.9	4	I	-
B21	TC TAC TAT GGT AAC TAC		TGG	SP2.5	2	I	-
B22	TC TAC TAT GAT TAC GA		T TAC TAT GCT ATG GAC TAC TGG	SP2.2	4	I	-
B23	CC TAC TAT AGT AAC TAC		GCC TGG TTT GCT TAC TGG	SP2.X	3	I	-
B24	TC TAC TAT GGT AAC TAC		TAC TTT GAC TAC TGG	SP2.5	2	I	-
B31	TC TAC TAT GAT TAC G		CT TAC TGG	SP2.2	3	I	-
B32	TCT ATG ATG GTT ACT AC	G	TGG TTT GCT TAC TGG	SP2.9	3	I	P+-
B33	TC TAT GAT GGT TAC TAC		GCC TGG TTT GCT TAC TGG	SP2.9	3	I	-
B41	T CTA CTA TGG TAA CTA C	G	T TAC TAT GCT ATG GAC TAC TGG	SP2.5	4	I	P+
C11	T CTA CTA TGG TAA CTA		TTT GCT TAC TGG	SP2.5	3	I	-
C12	T CTA TGA TGG TTA CTA C	G	C TAC TGG	SP2.9	2	I	P+-
C13	TC TAC TAT GGT AAC TA		T GCT ATG GAC TAC TGG	SP2.1	4	I	-
C21	CC TAC TAT AGT AAC TAC		TAC TTT GAC TAC TGG	SP2.X	2	I	-
C22	C CTA CTA TAG TAA CTA C		TT GCT TAC TGG	SP2.X	3	I	-
C23	T CTA TGA TGG TTA CTA C	GT AC	C TAC TTT GAC TAC TGG	SP2.9	2	I	P+
C24	TC TAC TA		C TGG	SP2.5	2	I	-
C31	TCT ATG ATG GTT ACT AC		C TGG TTT GCT TAC TGG	SP2.9	3	I	-
C32	TCT ACT ATG ATT ACG		GCC TGG TTT GCT TAC TGG	SP2.2	3	I	-
C33	TCT ACT ATG GT		C TGG TTT GCT TAC TGG	SP2.5	3	I	-
C34	CC TAC TAT AGT AAC T		TT GCT TAC TGG	SP2.X	3	I	-
C41	TC TAC TAT GGT TAT GAC		GCT ATG GAC TAC TGG	SP2.3/4	4	I	-
C42	T CTA TGA TGG TTA C		AT TAC TAT GCT ATG GAC TAC TGG	SP2.9	4	I	-
C43	TC TAC TAT GGT AAC TAC		TAC TAT GCT ATG GAC TAC TGG	SP2.1	4	I	-
C44	TC TAC TAT GGT AAC TAC		TAT GCT ATG GAC TAC TGG	SP2.5	4	I	-

1b) sorted L5T/L5T fetal liver preB cells day 13 (B220+/c-kit+ markers)

name, origin	D-segment	N/P-sequence	J-segment	D	J	reading frame	N/P-seq
D11	TC TAC TAT GGT AAC TAC		TGG TAC TTC GAT GTC TGG	SP2.5	1	I	-
D21	CC TAC TAT AGT AAC TAC		TTT GAC TAC TGG	SP2.X	2	I	-
D22	T CTA CTA TGG TAA CTA C		AC TAC TGG	SP2.5	2	I	-
D23	TC TAT GAT GGT TAC T		TT GAC TAC TGG	SP2.9	2	I	-
D24	TCT ACT ATG ATT ACG AC	G T	AC TAC TTT GAC TAC TGG	SP2.2	2	I	P+
D32	TCT ACT ATG GTA ACT		TTT GCT TAC TGG	SP2.5	3	I	-
D33	TC TAC TAT GGT AAC TAC		GCC TGG TTT GCT TAC TGG	SP2.1	3	I	-
D41	CC TAC TAT AGT AAC TA		T GCT ATG GAC TAC TGG	SP2.X	4	I	-
D42	TC TAC TAT GGT AAC TAC		TAT GCT ATG GAC TAC TGG	SP2.5	4	I	-
E11	TCT ATG ATG GTT ACT AC	G A	AC TAC TGG	SP2.9	2	I	P+
E23	TC TAC TAT GGT TAC		TTT GAC TAC TGG	SP2.3/4	2	I	-
E24	TC TAC TAT GAT TAC		TTT GAC TAC TGG	SP2.2	2	I	-
E31	TC TAC TAT GAT TA		T GCT TAC TGG	SP2.2	3	I	-
E33	TC TAC TAT GGT AAC TA		T GCT ATG GAC TAC TGG	SP2.1	4	I	-
E34	TC TAT GAT GGT TAC T		TT GCT TAC TGG	SP2.9	3	I	-
E41	TCT ACT ATG GTA ACT	T	AT TAC TAT GCT ATG GAC TAC TGG	SP2.5	4	I	P+-
E43	TC TAC TAT GAT TAC GAC		TAT GCT ATG GAC TAC TGG	SP2.2	4	I	-
F11	T CTA CTA TGG TAA		TAC TAT GCT ATG GAC TAC TGG	SP2.5	4	I	-
F12	T CTA CTA TGA TTA CGA C	G	T GCT ATG GAC TAC TGG	SP2.2	4	I	P+-
F21	TCT ATG ATG GTT AC		C TGG TTT GCT TAC TGG	SP2.9	3	I	-
F22	T CTA CTA TGG		TTT GAC TAC TGG	SP2.5	2	I	-
F32	TC TAC TAT GAT TAC		TAC TGG	SP2.2	3	I	-
F33	TC TAC TAT GAT TAC G		CC TGG TTT GCT TAC TGG	SP2.2	3	I	-
F42	TCT ACT ATG GTT ACG AC		T TAC TAT GCT ATG GAC TAC TGG	SP2.3/4	4	I	-
F43	TC TAC TAT GAT TAC GAC		TAT GCT ATG GAC TAC TGG	SP2.2	4	I	-

Table 1 c-d: D-J sequences (DFL/DSP) of BDF1 and L5T/L5T sorted bone marrow preB cells

1c) sorted BDF1 bone marrow preB cells (B220+/c-kit+ markers or S7+/BP1+ markers)

name, origin		D-segment	N/P-sequence	J-segment	D	J	reading frame	N/P-seq
o1	k2	TTT ATT ACT ACG ATG GTA GCT A	GA	GAT TAC TAT GCT ATG GAC TAC TGG	FL16.1	4	I	N+
k2	k2	TC TAC TAT GGT AAC TAC	GGA T	AT TAC TAT GCT ATG GAC TAC TGG	SP2.1/5	4	I	N+
k2	k5	TCT ACT GTG GTA A	GG	GAT TAC TAT GCT ATG GAC TAC TGG	SP2.1	4	I	P+
k3	k5	TC TAT GAT GGT I	GA T	CT ATG GAC TAC TGG	SP2.9	4	I	P+
k4	k5	T CTA CTA TGG TAA CTA C	C	C TGG TAC TTC GAT GTC TGG	SP2.1	1	I	P+
k5	k5	T CTA CTA	CGG	GAC TAC TGG	SP2.3/4	2	I	N+
k5	k5	T CTA CTA TGG TAA CTA C	GT A	TT GAC TAC TGG	SP2.1	2	I	N+
k4	k5	T CTA TGA TGG TTA CTA C	GA GG	C TAC TGG	SP2.9	2	I	N+
k2	k5	TC TAC TAT GA	C CCC	GCC TGG TTT GCT TAC TGG	SP2.2	3	I	N+
k3	k5	CCT ACT ATA GTA ACT AC	G AAA G	GG TTT GCT TAC TGG	SP2.X	3	I	N+
k1	k5	TC TAC TAT GG	G AG	T TAC TAT GCT ATG GAC TAC TGG	SP2.3/4	4	I	N+
k3	k5	CC TAC TAT AGT TAC TA	T AGT TGA	GCT ATG GAC TAC TGG	SP2.X	4	I	N+
k3	k5	TC TAC TAT GGT AAC TAC	G	AC TAT GCT ATG GAC TAC TGG	SP2.1	4	I	P+
k4	k5	T CTA CTA TGG TAA GT	G AGG	GCT ATG GAC TAC TGG	SP2.1	4	I	N+
l1	k5	T CTA CTA TGG	GCT	TTT GCT TAC TGG	SP2.5	3	I	N+
l3	k5	TC TAC TAT GAT TAC GAC	G	GG TTT GCT TAC TGG	SP2.2	3	I	P+
g1	k5	TC TAC TAT GGT AAC TAC	GCG	TAT GCT ATG GAC TAC TGG	SP2.1	4	I	N+
k5	k5	CC TAC TAT AGT TAC TAT AGG TAC GAC GTA A	GA G	AT GCT ATG GAC TAC TGG	SP2.X+3+Q52	4	I	N+
k4	k5	TCT ACT ATG GT	C	GAT TAC TAT GCT ATG GAC TAC TGG	SP2.5	4	I	P+
k2	k5	C CTA CTA TAG TTA CTA TAG GTA CGA C	GG T	AC TAC TTT GAC TAC TGG	SP2.X+2.11	2	I	P+
k3	k5	TC TAC TAT GAT TAC GAC	GAG GG	C TAC TGG	SP2.2	2	I	N+
l1	k5	CC TAC TAT GGT T		TT GCT TAC TGG	SP2.6	3	I	-
k2	k5	C CTA CTA TAG TAA CTA C	AG GAG	TGG TTT GCT TAC TGG	SP2.X	3	I	N+
k1	k5	C CTA CTA TAG TAA CTA C	GT TC	C TAT GCT ATG GAC TAC TGG	SP2.X	4	I	N+
k2	k5	T CTA CTA TGG TAA CT	T TCG T	CT ATG GAC TAC TGG	SP2.1	4	I	N+
l1	k5	T CTA CTA TGG TAA CT	T A	AC TGG TAC TTC GAT GTC TGG	SP2.5	1	I	P+
k2	k5	T CTA CTA TGA TTA CGA C	GG GGG	TGG TAC TTC GAT GTC TGG	SP2.2	1	I	N+
l3	k5	CC TAC TAT AGT AAC TAC	GAG GGA	TAC TGG TAC TTC GAT GTC TGG	SP2.X	1	I	N+
k4	k5	T CTA CTA TGG TAA CTA C	GA GGG	TGG TAC TTC GAT GTC TGG	SP2.1	1	I	N+
a1	k5	T CTA CTA TGG TAA AAC C GT AAC	GAG G	TT GAC TAC TGG	SP2.1+Q52	2	I	P+
k3	k5	T CT	G CT	GG	SP2.5	2	I	N+
k4	k5	CCT ACT ATA GTA ACC AC	A CC	T GAC TAC TGG	SP2.X	2	I	P+
a1	k5	CC TAC TAT AGT AAC T		GG TTT GCT TAC TGG	SP2.X	3	I	-
a2	k5	TCT ACT ATG GTA AC	C CC	G TTT GCT TAC TGG	SP2.5	3	I	N+
a3	k5	C CTA CTA TAG	CCC GT	C TGG	SP2.X/2.11	3	I	N+
a4	k5	C CTA CTA TAG TTA CTA	TA	T GCT TAC TGG	SP2.X	3	I	P+
o1	k5	C CTA CTA TAG TAA CTA C	GA	GAC TAC TGG	SP2.X	4	I	P+
g3	k5	CC TAC TAT AGT AAC TAC	GGG AGG	TAT GCT ATG GAC TAC TGG	SP2.X	4	I	N+
g4	k5	C CTA CTA TAG TAA CTA C	GT GGG GTT TTT	TAC TAT GCT ATG GAC TAC TGG	SP2.X	4	I	N+
g1	k5	T CTA CTA TGG TAA C	CC TGG G	TT GCT TAC TGG	SP2.1	3	I	N+
k2	k5	T CTA TGA TGG TTA C	CC CC	C GAT GTC TGG	SP2.9	1	I	N+
l1	k5	TC TAC TAT GGT AAC	CCT T	AC TAC TTT GAC TAC TGG	SP2.5	2	I	P+
k2	k5	C CTA CTA TAG TTA CTA TAG TTA CG		T GAC TAC TGG	SP2.X+2.6	2	I	-
k3	k5	T CTA CTA TGA CT	T	TAT GCT ATG GAC TAC TGG	SP2.2	4	I	P+
k4	k5	TC TAC TAT GAT TAC	TTG T	AC TAC TTT GAC TAC TGG	SP2.2	2	I	P+
a1	k5	TC TAC TAT GGT AAC T		CC TGG TTT GCT TAC TGG	SP2.1	3	I	-
a2	k5	CC TAC TAT AGT TAC GAC	GAG G	TT GCT TAC TGG	SP2.8	3	I	N+

Table 1 (cont.)

s3	ws3	TC TAC TAT GGT AAC TAC	CTC TTT G	GG	SP2.1	3	I	N+
s4	ws3	TCT ATG ATG GTT ACT AC	C TC	G TTT GCT TAC TGG	SP2.9	3	II	P+
t1	ws3	T CTA TGA TGG TTA CTA C	C	C TAT GCT ATG GAC TAC TGG	SP2.9	4	III	P+
t2	ws3	TC TAC TAT GAT TAC	TC	C TAT GCT ATG GAC TAC TGG	SP2.2	4	I	P+
t3	ws3	T CTA CTA TGG TAA CTA C	GC CC	T TAC TAT GCT ATG GAC TAC TGG	SP2.1	4	III	N+
t4	ws3	T CTA CTA TGA	CTT	TAT GCT ATG GAC TAC TGG	SP2.2	4	II	P+
u1	ws32	T CTA C	CC TTT G	AT GCT ATG GAC TAC TGG	SP2.2/3/4	4	II/+	N+
u1	ws32	TC TAC TAT GGT AAC T	CC C	AT TAC TAT GCT ATG GAC TAC TGG	SP2.1/5	4	I	N+
u4	ws32	TC TAC TAT GGT	G	AC TAT GCT ATG GAC TAC TGG	SP2.3/4	4	I	N+?
w1	ws32	C CTA CTA TAG T		AT GCT ATG GAC TAC TGG	SP2.X	4	III	-
w3	ws32	CC TAC TAT AGT AAC	CAC C	CT ATG GAC TAC TGG	SP2.X	4	I	N+

1d) sorted L5T/L5T bone marrow preB cells week 14 (B220+/c-kit+ markers)

name, origin	D-segment	N/P-sequence	J-segment	D	J	reading frame	N/P-seq
G11	TCT ACT ATG ATT ACG AC	G GGG	GCT ATG GAC TAC TGG	SP2.2	4	II	+
G12	T CTA TGA TGG TTA	TC	C GAT GTC TGG	SP2.9	1	III	+
G13	TC TAC GAT GGT TAC	TGG	GCT ATG GAC TAC TGG	SP2.1	4	I	+
G14	TCT ACT ATG ATT ACG AC	G GAG	TTT GCT TAC TGG	SP2.2	3	II	+
G21	TCT ACT ATG GTT ACG AC	G GTG A	AC TTT GAC TAC TGG	SP2.3/4	2	II	+
G24	TCT ACT ATG ATT ACG AC	G GGG	GCT ATG GAC TAC TGG	SP2.2	4	II	+
G31	TC TAC TAT GGT AAC TAC	ACC	TTT GCT TAC TGG	SP2.5	3	I	+
G32	TCT ATG ATG GTT ACT AC	G TCG	GCT TAC TGG	SP2.9	3	II	+
G33	TCT ACT ATG ATT AC	C CCT	TTT GCT TAC TGG	SP2.2	3	II	+
G41	TC TAC TAT GAT TAC GAC	AAG	GCT ATG GAC TAC TGG	SP2.2	4	I	+
G42	CC TAC TAT AGT AAC TAC	GGG GG	T GCT ATG GAC TAC TGG	SP2.X	4	I	+
G43	TC TAT GAT GGT TAC	CTT TT	T TAC TAT GCT ATG GAC TAC TGG	SP2.9	4	I	+
G44	TC TAT GAT GGT TAC TAC		TAT GCT ATG GAC TAC TGG	SP2.9	4	I	-
H11	TCT ACT ATG GTA A	GG GG	C TAT GCT ATG GAC TAC TGG	SP2.5	4	II	+
H12	TCT ACT ATG GTA ACT AC	G TCG	GCC TGG TTT GCT TAC TGG	SP2.5	3	II	+
H21	T CTA TGA TGG T	AG G	GG TTT GCT TAC TGG	SP2.9	3	III	+
H31	T CTA TGA TGG TTA C	CA AGC CTA CGA	GCT TAC TGG	SP2.9	3	III	+
H32	C CTA CTA TAG TAA CT	T CCG A	TT GCT TAC TGG	SP2.X	3	III	+
H34	CCT ACT ATA GTA AC	C CCT	TTT CCT TAC TGG	SP2.X	3	II	+
H42	T CTA CTA TGG TT	G GGG	GCT ATG GAC TAC TGG	SP2.3/4	4	III	+
H43	TCT ACT ATG G	GG GG	C TAT GCT ATG GAC TAC TGG	SP2.5	4	II	+
H44	CCT ACT ATA GTA ACT AC	T A	AT GCT ATG GAC TAC TGG	SP2.X	4	II	+
J11	TCT ATG ATG GTT ACT AC	G	TAC TAT GCT ATG GAC TAC TGG	SP2.9	4	II	P+
J13	TC TAT GAT GGT TAC	CCT	TTT GCT TAC TGG	SP2.9	3	I	+
J21	TCT ACT ATG GTA ACT	TCT	TAT GCT ATG GAC TAC TGG	SP2.5	4	II	+
J22	TC TAC TAT GAT TAC GAC	CCC TTT T	AC TAC TTT GAC TAC TGG	SP2.2	2	I	+
J31	CC TAC TAT AGT A	CG CCT	TTT GCT TAC TGG	SP2.X	3	I	+
J41	TC TAC TAT GGT TA	G AGA	TAC TAT GCT ATG GAC TAC TGG	SP2.3/4	4	I	-
J42	T CTA TGA TGG TTA	AAG GG	T GCT ATG GAC TAC TGG	SP2.9	4	III	+
J43	CC TAC	C	CT ATG GAC TAC TGG	SP2.8/7/11/X	4	I	P+
J44	T CTA TGA TGG TTA CTA C	AG TTT T	CT ATG GAC TAC TGG	SP2.9	4	III	+

Table 2 a-b: D-J sequences (DFL/DSP) of BDF1 and L5T/L5T fetal liver preB cell cultures

2a) cultured BDF1 fetal liver preB cells

name, origin	D-segment	N/P-sequence	J-segment	D	J	reading frame	N/P-seq
A3 clone 1	TC TAC TAT GGT TAC GAC		TGG TAC TTC GAT GTC TGG	SP 2.3/4	1	I	-
B1 clone 5	TT TAT TAC TAC GGT AGT AGC TAC		TAC TTT GAC TAC TGG	FL 16.1	2	I	-
B2 clone 5	TTT ATT ACT GCG GTA GTA GCT		GAC TAC TGG	FL 16.1	2	II	-
C1 clone 8	TT TAT TAC TAC GGT AGT AGC T		TT GAC TAC TGG	FL'16.1	2	I	-
E1 clone 18	T TTA TTA CTA CGG TAG TA		C TTT GAC TAC TGG	FL 16.1	2	III	-
a2.1 PAL.1	TAT TAC TAC GGT AGT AGC TAC		TAC TTT GAC TAC TGG	FL 16.1	2	I	-
b2.1 fl d 12	CC TAC TAT GGT AAC TAC		TAC TTT GAC TAC TGG	SP 2.7	2	I	-
b2 fl d 12	TCT ACT ATG GTA ACT AC		T GCT ATG GAC TAC TGG	SP 2.1/5	4	II	-
b2.3 fl d 12	TC TAC TAT GGT AAC		TAC TGG	SP 2.1/5	2	I	-
c1 fl d 12	TC TAC TAT GGT TAC GAC		GCT ATG GAC TAC TGG	SP 2.3/4	4	I	-
c2 fl d 12	TC TAC TAT GGT AAC TA		T TAC TAT GCT ATG GAC TAC TGG	SP 2.1/5	4	I	-
c2.1 fl d 12	CC TAC TA		C TTT GAC TAC TGG	SP 2.6/7	2	I	-
c2.2 fl d 12	TC TAC TAT GGT AAC TAC		TTT GAC TAC TGG	SP 2.1/5	2	I	-
c3 fl d 12	T CTA CTA TGG		TAT GCT ATG GAC TAC TGG	SP 2.3/4	4	III	-
d1 fl d 12	TC TAT TAT GGT TAC		TAC TAT GCT ATG GAC TAC TGG	SP 2.3/4	4	I	-
d2 fl d 12	T CTA TCA TGG TTA C		AT TAC AGT GCT ATG GAC TAC TGG	SP 2.3/4	4	III	-
d2.1 fl d 12	TC TAC TAT		GGC CAA GGC	SP 2.3/4	2	I	-
d2.2 fl d 12	CC TAC TAT AGT TAC GAC		TAC TGG GAC TAC TGG	SP 2.6	2	I	2.DJ
d2.3 fl d 12	T CTA CTA TGG		TTT GAC TAC TGG	SP 2.3/4	2	III	-
d3 fl d 12	TC TAC TAT GGT A		AC TAT GCT ATG GAC TAC TGG	SP 2.3/4	4	I	-
H1 fl d 13	CC TAC TAT AGT AAC TA		T TAC TAT GCT ATG GAC TAC TGG	SP 2.X	4	I	-
H3 fl d 13	CC TAC TAT AGT AAC TA		T TAC TAT GCT ATG GAC TAC TGG	SP 2.X	4	I	-
m1 F13L7.4 (d13)	T CTA CTA TGG		GCT TGG TTT GCT TAC TGG	SP 2.3/4	3	III	-
f 1.1 fl d 14	TC TAC TAT GGT AAC TAC		TGG TAC TTC GAT GTC TGG	SP 2.1/5	1	I	-
7.1 fl d 14	TC TAC TAT GGT AAC TAC		GCT ATG GAC TAC TGG	SP 2.1/5	4	I	-
7.2 fl d 14	CC TAC TAT GGT AAC TA		T TAC TAT GCT ATG GAC TAC TGG	SP 2.7	4	I	-
e1 fl d 15	T CTA CTA TGG TAA CTA		TAC TAT GCT ATG GAC TAC TGG	SP 2.1/5	4	III	-
f1 fl d 15	CC TAC TAT GGT AAC TAC		TAT GCT ATG GAC TAC TGG	SP 2.7	4	I	-
g1 fl d 15	TCT ACT ATG GTA ACT AC		T TAC TAT GCT ATG GAC TAC TGG	SP 2.1/5	4	II	-
g2 fl d 15	T CTA CTA TGG TTA		TAC TAT GCT ATG GAC TAC TGG	SP 2.3/4	4	III (+)	-
g2.2 fl d 15	CC TAC TAT CGT A		AC TAC TGG	SP 2.7	2	I	-
J1 fl d 15	C CTA CTA TAG TA		T GCT ATG GAC TAC TGG	SP 2.X	4	III	-
J3 fl d 15	TC TAC TAT AGT AAC		GCT ATG GAC TAC TGG	SP 2.1/5	4	I	-
h2.2 fl d 16	CC TAC TAT AGT AAC		GAC TAC TGG	SP 2.X	2	I	-
a1 fl d 16	C CTA CTA TGG		TAT GCT ATG GAC TAC TGG	SP 2.7	4	III	-
j1 fl d 16	TT TAT TAC TAC GGT AGT AGC TA		T GAC TAC TGG	FL 16.1	2	I	-
k2.1 fl d 16	C CTA CTA TGG		TAC TGG	SP 2.6/7	2	III	-
B1 18L.1	TC TAT GAT GGT TAC		TGG TTT GCT TAC TGG	SP2.9	3	I	-
B2 18L.2	CC TAC TAT AGT AAC TA		T TAC TAT GCT ATG GAC TAC TGG	SP2.X	4	I	-
B4 18L.2	TCT ACT ATG GTA ACT AC		G TTT GCT TAC TGG	SP2.1/5	3	II	-
C1 18L5.2	TC TAC TAT GGT AAC TAC		TAT GCT ATG GAC TAC TGG	SP2.1/5	4	I	-
D1 19.1	TC TAC TAT GGT AAC T		TT GAC TAC TGG	SP2.1/5	2	I	-
E1 19.2	TC TAC TAT GGT AAC TAC	GTG GG	C TAC TGG	SP2.1/5	2	I	P/N+
E2 19.2	CCT ACT ATA GT T ACT ATA GTT ACG AC G TAG ACC		TAC TGG	SP2.X+2.11	2	II	P+,D-D
E3 19.2	TC TAC TAT GG	C G	AC TTT GAC TAC TGG	SP2.3/4	2	I	P+
E4 19.2	CC TAC TAT AGT AAC TAC		GAC TAC TGG	SP2.X	2	I	-
F1 F19L2	TC TAC TAT GAT TAC G		CT TAC TGG	SP2.2	3	I	-
G1 19.3	TC TAC TAT GGT AAC TAC	GTG G	GG	SP2.1/5	2	I	P+
H1 F19L4		AC CG	G TTT GCT TAC TGG	SP7	3	T	P+
H2 F19L4	TC TAC TAT GGT AAC TAC	TTT	TTT GCT TAC TGG	SP2.1/5	3	I	P+
J1 F19L5	T CTA CTA TGG TT		T GCT TAC TGG	SP2.3/4	3	III(+)	-
J2 F19L5	CC TAC TAT AGT AAC TAC		TAC TTT GAC TAC TGG	SP2.X	2	I	-
J4 F19L5	TC TAC TAT GGT TAC GAC		TGG TAC TTC GAT GTC TGG	SP2.3/4	1	I	-
K1 F19L6	TT TAT TAC TAC GGT AGT AG		G TAC TTC GAT GTC TGG	FL16.1	1	I	-

2b) cultured L5T/L5T fetal liver preB cells

name, origin	D-segment	N/P-sequence	J-segment	D	J	reading frame	N/P-seq
A1 L5.12.1	TCT ATG ATG GTT ACT AC		C TGG TTT GCT TAC TGG	SP2.9	3	II	-
A2 L5.12.1	TC TAC TAT TGG T	TA CTG ACT	TGG TTT GCT TAC TGG	SP2.10	3	I	+
A3 L5.12.1	TCT ACT ATG GTA ACT AC	G T	AT TAC TAT GCT ATG GAC TAC TGG	SP2.1/5	4	II	+
a1 L5.12.1	T CTA CTA TGG TT		T GCT TAC TGG	SP2.3/4	3	III(+)	-
a2 L5.12.1	CC TAC TAT AGT AAC TAC		GCT ATG GAC TAC TGG	SP2.X	4	I	-
a3 L5.12.1	TC TAC TAT GGT AAC TAC		TTT GAC TAC TGG	SP2.1/5	2	I	-
a4 L5.12.1	TCT ATG ATG GTT ACG		TAT GCT ATG GAC TAC TGG	SP2.3/4	4	II	-
B1 L5.12.2	OC TAC TAT AGT AAC T		GG TTT GCT TAC TGG	SP2.X	3	I	-
B2 L5.12.2	TC TAC TAT GGT AAC TA		T GCT ATG GAC TAC TGG	SP2.1/5	4	I	-

Table 2 (cont.)

B3	L5.12.2	TC TAC TAT GAT TAC GAC	T	AC TAC TTT GAC TAC TGG	SP2.2	2	I	+
B4	L5.12.2	T CTA CTA TGG TA		C TTC GAT GTC TGG	SP2.1/5	1	III(+)	-
b1	L5.12.2	CC TAC T		TT GAC TAC TGG	SP2.6/7	2	I	-
b2	L5.12.2	TCT ACT ATG ATT TCG		GCC TGG TTT GCT TAC TGG	SP2.2	3	II	-
C1	L5.12.3	TC TAT GAT GGT TAC TAC		TAC TTT GAC TAC TGG	SP2.9	2	I	-
C2	L5.12.3	TC TAC TAT GGT AAC TAC		TGG TAC TTC GAT GTC TGG	SP2.1/5	1	I	-
C3	L5.12.3	TC TAC TAT GAT TA		T GCT ATG GAC TAC TGG	SP2.2	4	I	-
C4	L5.12.3	TCT ACT ATG ATT ACG		TTT GCT TAC TGG	SP2.2	3	II	-
c1	L5.12.3	TCT		TGG	SP2.1/5	2	II	-
c3	L5.12.3	C CTA CTA TAG TAA CTA C	GT	GAC TAC TGG	SP2.X	2	III	P+
D2	L5.12.4	T CTA CTA TGG TAA CT		T GAC TAC TGG	SP2.1/5	2	III	-
D3	L5.12.4	TT TAT TAC TAC GGT AGT AGC TAC		TGG TAC TTC GAT GTC TGG	FL16.1	1	I	-
d1	L5.12.4	TC TAC TAT GGT AAC TAC		TAT GCT ATG GAC TAC TGG	SP2.1/5	4	I	-
e1	L5.12.5	TC TAT GAT GGT TAC TAC		TAC TTT GAC TAC TGG	SP2.3/4	2	I	-
e3	L5.12.5	CC TAC TAT AGT AAC TAC		TTT GAC TAC TGG	SP2.X	2	I	-
e4	L5.12.5	TC TAC TAT GGT AAC TAC		TAT GCT ATG GAC TAC TGG	SP2.1/5	4	I	-
F1	L5.12.7	T CTA TGA TGG TTA CT		T GCT TAC TGG	SP2.9	3	III	-
F2	L5.12.7	CC TAC TAT AGT AAC TAC		TAC TTT GAC TAC TGG	SP2.X	2	I	-
F4	L5.12.7	CC TAC TAT AGT AAC T	C	C TAT GCT ATG GAC TAC TGG	SP2.X	4	I	+
f2	L5.12.7	TCT ACT ATG GTA ACT		GCT TAC TGG	SP2.1/5	3	II	-
f3	L5.12.7	TCT ACT ATG ATT ACG A	GG	GCT ATG GAC TAC TGG	SP2.2	4	II	P+
G1	L5.12.8	TC TAC TAT GAT TAC GAC		TAT GCT ATG GAC TAC TGG	SP2.2	4	I	-
g1	L5.12.8	C CTA CTA TAG TAA CTA C	GT	TAC TAT GCT ATG GAC TAC TGG	SP2.X	4	III	P+
g2	L5.12.8	TCT ACT ATG ATT ACG AC	G TGA T	AT TAC TAT GCT ATG GAC TAC TGG	SP2.2	4	I/stop	P+
g3	L5.12.8	T TTA TTA CTA CGG TAG TAG CTA C	GG	GCT ATG GAC TAC TGG	FL16.1	4	III	P+
H1	L5.12.9	TC TAC TAT GAT TAC GAC		GCT ATG GAC TAC TGG	SP2.2	4	I	-
H2	L5.12.9	TCT ATG ATG GTT AC		C TGG TTT GCT TAC TGG	SP2.9	3	II	-
h1	L12.5.9	TCT ATG ATG GTT ACT AC		C TGG TTT GCT TAC TGG	SP2.3	3	II	-
h2	L5.12.9	TC TAC TAT GAT TAC	CCT	TTT GAC TAC TGG	SP2.2	2	I	P+
M4	L5.12.9	TC TAC TAT GAT TAC GAC	C	TT GAC TAC TGG	SP2.2	2	I	P+

Table 2 c-d: D-J sequences (DFL/DSP) of BDF1 and L5T/L5T bone marrow preB cell cultures

2c) cultured BDF1 newborn blood, liver, spleen, bone marrow and "adult" bone marrow preB cells

name, origin		D-segment	N/P-sequence	J-segment	D	J	reading frame	N/P-seq
8.1	blood d 7	TT TAT TAC TAC GGT AGT AGC TAC	GGG GG	T TAC TAT GCT ATG GAC TAC TGG	FL 16.1	4	I	+
12.1	liver d 7	TTT ATT ACT ACG G	C	C TAT GCT ATG GAC TAC TGG	FL 16.1	4	II	+
17.1	liver d 21	TC TAT GAT GGT TAC	T	CT ATG GAC TAC TGG	SP 2.3/4	4	I	+
17.2	liver d 21	TCT ACT ATG GTT	CCC TC	T TAC TAT GCT ATG GAC TAC TGG	SP 2.3/4	4	II	+
17.4	liver d 21	TC TAC TAT GAT	CCA CTC C	AT TAC TAT GCT ATG GAC TAC TGG	SP 2.2	4	I	+
18.1	liver d 28	TCT ATG ATG GTT AC	T ACG TGA	GCT ATG GAC TAC TGG	SP 2.3/4	4	III	+
18.2	liver d 28	T CTA	GCA T	AT TAC TAT GCT ATG GAC TAC TGG	SP 2.2	4	III	+
13.1	spleen d 7	C CTA CTA TAG TAA	AGG GG	C TAT GCT ATG GAC TAC TGG	FL 16.1	4	III	+
14.1	spleen d 21	T CTA C	CC TTT G	AT GCT ATG GAC TAC TGG	SP 2.2	4	III (+)	+
15.2	spleen d 21	TCT ACT ATG ATT ACG		TAT GCT ATG GAC TAC TGG	SP 2.2	4	II	-
a1	Bm2L1 (w2)	TC TAT GAT GGT TAC		TAC TAT GCT ATG GAC TAC TGG	SP 2.3/4	4	I	-
c3	Bm2L4 (w2)	C CTA CTA TAG TAA CTA C	TA GG	C TAC TGG	SP 2.X	2	III	+
e1	Bm2L4 (w2)	CC TAC TAT CG	A GTC CAT TGC CAG AGC CTA A TT GCT TAC TG		SP 2.7	3	I	+
d1	Bm2L5 (w2)	TCT ACT ATG ATT ACG AC	G GAC GAC GGA C	AC TAC TGG	SP 2.2	2	II	+
f1	Bm2L10 (w2)	TC TAC TAT GGT AAC T	CC	TTT GCT TAC TGG	SP 2.1/5	3	I	+
g1	Bm2L12 (w2)	CC TAC TAT AGT AAC TAC	C	GG TTT GCT TAC TGG	SP 2.X	3	I	+
l1.1	bm w 3	CCT ACT ATC GTA ACT AC	T GGA	TTT GAC TAC TGG	SP 2.7	2	II	+
l1.4	bm w 3	T TTA TTA CTA CGG TAG TAG CT	C CGT TG	T GTC TGG	FL 16.1	1	III	+
l2.1	bm w 3	CC TAC TAT CGT	GTG G	AC TTT GAC TAC TGG	SP 2.7	2	I	+
10.1	bm w 3	TCT ACT ATG GTT AC	T CT	C TAT GCT ATG GAC TAC TGG	SP 2.3/4	4	II	+
10.3	bm w 3	TCT ACT ATG ATT A	G	C TAT GCT ATG GAC TAC TGG	SP 2.2	4	II	+
10.4	bm w 3	A TGA TGG TTA C	TA CCC CCC	TAT GCT ATG GCC TAC TGG	SP 2.3/4	4	III	+
2.2	bm w 4	CC TAC TAT AGT TAC TAT AGT GAC GAC		TGG TTT GCT TAC TGG	SP 2.6	3	I	D-D
6.1	bm w 4	TC TAC TAT GAT TAC GAC	GTG GGG	GCT ATG GAC TAC TGG	SP 2.2	4	I	+
h1	Bm5L2 (w5)	CC TAC TAT AGT AAC TAC		TGG TTT GCT TAC TGG	SP 2.X	3	I	-
h3	Bm5L2 (w5)	TTT ATT ACT ACG GTA	CCG AGA G	GG TTT GCT TAC TGG	FL16.1	3	III	+
j1	Bm5L4 (w5)	T CTA CTA TGG TAA CTA C	TT TTC	GCC TGG TTT GCT TAC TGG	SP 2.5	3	III	+
l1	Bm5L6 (w5)	C CTA CTA TAG TTA CGA		TGG TTT GCT TAC TGG	SP 2.6	3	III	-
d1	Bm5L8 (w5)	TC TAC TAT GGT AAC T	T	C TAT GCT ATG GAC TAC TGG	SP 2.1/5	4	I	+
m1	Bm5L9 (w5)	TC TAC TAT CGT AAC TAC	AAT TTC	GCC TGG TTT GCT TAC TGG	SP 2.1/5	3	I	+
n2	BDB14.2	CC TAC TAT AGT TAC TAT AGT TAC GAC	GTA GGA G	AT TAC TAT GCT ATG GAC	SP 2.6	4	I	+ D-D
11.1	bm w 15	CC TAC TAT AGT TAC TAT AGT TAC GAC	CTT CA	G TTT GCT TAC TGG	SP 2.6	3	I	+ D-D
L1	bm w 15	CC TAC TAT AGT AAC TA	T	T TAC TAT GCT ATG GAC TAC TGG	SP 2.X	4	I	-
m1	bm w 15	T CTA CTA TGG TAA CTA C	TC GG	C TTT GAC TAC TGG	SP 2.1/5	2	III	+
m2	bm w 15	C CTA CTA TCG TAG	GGG GGA	TTT GAC TAC TGG	SP 2.7	2	III	+

2d) cultured L5T/L5T bone marrow preB cells

name, origin		D-segment	N/P-sequence	J-segment	D	J	reading frame	N/P-seq
A1	PL5–3	CC TAC TAT AGT AAC TAC		GCT ATG GAC TAC TGG	SP2.X	4	I	-
A4	PL5–3	TTT ATT ACT ACG GT		T TAC TAT GCT ATG GAC TAC TGG	FL16.1	4	II	-
B1	PL5–20	TCT ACT ATG GTA ACT AC	G TAG AAG	GCT TAC TGG	SP2.1/5	3	II/Stop	+
B2	PL5–20	TTT ATT ACT ACG GT		C TGG TTT GCT TAC TGG	FL16.1	3	II	-
B3	PL5–20	CTA TAG GTA CGA C	T	T GCC TGG TTT GCT TAC TGG	SP2.11	3	III	+
C1	L5–3.2	C CTA CTA TAG	AGG	GCT TAC TGG	SP2.11	3	III	+
D1	L5–3.4	CT ACT ATA	AAC GTC CC	C TGG TAC TTC GAT GTC TGG	SP2.10	1	II	+
E5	L5–3.7	CC TAC TAT AGT AAC TA		T TAC TAT GCT ATG GAC TAC TGG	SP2.X	4	I	-
E6	L5–3.7	TT TAT TAC TAC GGT AGT AGC TAC		GAT TAC TAT GCT ATG GAC TAC TGG	FL16.1	4	I	-
F1	L5–3.10	TCT ACT ATG GTA AC	G GGG GT	C TTT GAC TAC TGG	SP2.1/5	2	II	+
G1	L5–20.2	C CTA CTA TGG	GAG AGG T	AC TAC TTT GAC TAC TGG	SP2.6/7/8	2	III(+)	+

Table 2 e-f: D-J sequences (DQ52) of BDF1 and L5T/L5T fetal liver preB cell cultures

2e) cultured BDF1 fetal liver preB cells (DQ52)

name, origin		D-segment	N/P-sequence	J-segment	D	J	reading frame	N/P-seq
A1	F12.2	CT AAC TGG GAC		TAC TGG	DQ, O+	2	I	-
A2	F12.2	CTA ACT GGG A	GA	TAC TTT GAC TAC TGG	DQ, O-	2	II	P+
a1	F12.2	C TAA CTG GG		C TAT GCT ATG GAC TAC TGG	DQ, O-	4	III	-
a2	F12.2	CT AAC TGG G		CT ATG GAC TAC TGG	DQ, O+	4	I	-
a3	F12.2	CT AAC T		AC TGG	DQ, O+	4	I	-
a4	F12.2	CT AAC TGG GAC		TAT GCT ATG GAC TAC TGG	DQ, O+	4	I	-
B1	F13L2	CTA ACT GGG ACC		TGG TTT GCT TAC TGG	DQ, O+	3	II	-
B2	F13L2	C TAA CTG GGA C	G	T GAC TAC TGG	DQ, O-	2	III	P+
B3	F13L2	CTA ACT GGG A		GG TTT GCT TAC TGG	DQ, O-	3	II	-
B4	F13L2	CTA ACT GGG AC		C TGG TTT GCT TAC TGG	DQ, O-	3	II	-
b1	F13L1	C TAA CTG GGA C	GG GG	C TAT GCT ATG GAC TAC TGG	DQ, O-	4	III	N/P+
b2	F13L1	C TAA CT	T T	AT TAC TAT GCT ATG GAC TAC TGG	DQ, O-	4	III	P+
c1	F13L2	CT AAC TGG GAC		TAT GCT ATG GAC TAC TGG	DQ, O+	4	I	-
c2	F13L2	C TAA CTG GG	C T	AT TAC TAT GCT ATG GAC TAC TGG	DQ, O-	4	III	P+
c3	F13L2	C TAA CTG GGA C	AT T	AT TAC TAT GCT ATG GAC TAC TGG	DQ, O-	4	III	P+
c4	F13L2	GT AAC	CT	C TAT GCT ATG GAC TAC TGG	DQ, O-	4	I	P+
C1	F14L1	CT AAC TGG G	GG	GCT TAC TGG	DQ, O-	3	I	+
C2	F14L1	CT AAC TGG GAC		TAC TTT GAC TAC TGG	DQ, O+	2	I	-
C3	F14L1	CT AAC TGG		TAC TTC GAT GTC TGG	DQ, O+	1	I	-
C4	F14L1	CT AAC TGG GAC		TGG TTT GCT TAC TGG	DQ, O+	3	I	-
d1	F14L1	CT AAC TGG GAC	GGG G	AT GCT ATG GAC TAC TGG	DQ, O-	4	I	+
D2	F15.2	A A	GG	TAC TTT GAC TAC TGG	DQ, O-	2	?	+
D3	F15.2	C TAA CTG G		AT TAC TAT GCT ATG GAC TAC TGG	DQ, O+	4	III	-
e1	F15.2	CT AAC TGG GAC		GCT ATG GAC TAC TGG	DQ, O-	4	I	-
e2	F15.2	CTA ACT GGG AC	C T	AT TAC TAT GCT ATG GAC TAC TGG	DQ, O-	4	II	P+
M1	F15.3	CT AAC TGG GAC		TTT GAC TAC TGG	DQ, O+	2	I	-

Table 2 (cont.)

E1	F16L1	CTA ACT GGG AC	G	GAC TAC TGG	DQ, O-	2	II	+
E3	F16L1	CT AAC TGG G		GG TAC TTC GAT GTC TGG	DQ, O-	1	I	-
E4	F16L1	CT AAC TGG GAC		TAC TGG	DQ, O+	2	I	-
f1	F16L1	CTA ACT G	AC C	AT TAC TAT GCT ATG GAC TAC TGG	DQ, O-	4	II	P+
f2	F16L1	CTA ACT GGG A		AC TAT GCT ATG GAC TAC TGG	DQ, O-	4	II	-
f3	F16L1	CTA ACT GGG AC	T AGG AGG	GCT ATG GAC TAC TGG	DQ, O-	4	II	+
F1	16L2	CT AAC TGG GAC		TAC TGG	DQ, O+	2	I	-
F4	16L2	CT AAC TGG G	GG	TTT GAC TAC TGG	DQ, O-	2	I	+
g1	16L2	CT AAC TGG GAC		TAT GCT ATG GAC TAC TGG	DQ, O+	4	I	-
g3	16L2	C TAA CTG G		AC TAC TGG	DQ, O+	4	III	-
g4	16L2	CTA ACT GGG AC		T TAC TAT GCT ATG GAC TAC TGG	DQ, O-	4	II	-
h1	16L2	C TAA CTG GGA C	GT ACT G	AC TAC TTT GAC TAC TGG	DQ, O-	2	III	P+
i2	16L2	CT AAC TGG	T	AC TAC TTT GAC TAC TGG	DQ, O-	2	I	P+
i3	16L2	C TAA CTG GGA		GAC TAC TTT GAC TAC TGG	DQ, O-	2	III	-
k4	16L2	CT AAC TGG GAC		TTT GAC TAC TGG	DQ, O+	2	I	-
G1	16L3	CT AAC TGG GAC		TAC TTT GAC TAC TGG	DQ, O+	2	I	-
H1	F16L4	CTA ACT GGG	G	AC TGG TAC TTC GAT GTC TGG	DQ, O-	1	II	+
H4	F16L4	CT AAC T	T	C TAC TGG TAC TTC GAT GTC TGG	DQ, O-	1	I	+
I1	F16L4	CT AAC TGG GAC	GAA	GCT ATG GAC TAC TGG	DQ, O-	4	I	+
I3	F16L4	C TAA CTG GGA C	GG GA	G GAC TAC TGG	DQ, O-	4	III	+
v1	F16L4	CTA ACT GGG ACC		GAC TAC TGG	DQ, O-	2	II	-
v2	F16L4	CTA ACT GGG A	AA GG	T GAC TAC TGG	DQ, O-	2	II	+
h1	16.2	CTA ACT GGG AC	G GGG	GCT ATG GAC TAC TGG	DQ, O-	4	II	+
M4	16.2	C TAA CTG G		AC TAC TGG	DQ, O+	4	III	-
J1	16.2	CT AAC TGG		TGG TAC TTC GAT GTC TGG	DQ, O+	1	I	-
J3	16.2	CTA ACT GGG		TGG TAC TTC GAT GTC TGG	DQ, O-	1	II	-
s1	16.2	CT AAC T		AC TAC TTT GAC TAC TGG	DQ, O-	2	I	-
s2	16.2	C TAA CTG GGA C	GG GG	T GAC TAC TGG	DQ, O-	2	III	+
s3	16.2	CTA ACT GGG		TAC TTT GAC TAC TGG	DQ, O-	2	II	-
s4	16.2	CT AAC T		AC TAC TTT GAC TAC TGG	DQ, O-	2	I	-

2f) cultured L5T/L5T fetal liver preB cells (DQ52)

name, origin	D-segment	N/P-sequence	J-segment	D	J	reading frame	N/P-seq
P1	L5.12.1	CT AAC TGG GAC	TTT GAC TAC TGG	DQ, O+	2	I	-
k1	L5.12.1	CT AAC TGG GAC	GCT ATG GAC TAC TGG	DQ, O-	4	I	-
k3	L5.12.1	CT AAC TGG GAC	TAC TAT GCT ATG GAC TAC TGG	DQ, O-	4	I	-
K4	L5.12.1	CTA ACT GGG A	GG GG	C TAT GCT ATG GAC TAC TGG	DQ, O-	4	II	+
K2	L5.12.2	CTA AAC TGG G	G	C TAC TGG	DQ, O-	2	II	+
K4	L5.12.2	CT AAC TGG G	TG	GAC TAC TGG	DQ, O-	2	I	+
f1	L5.12.2	CT AAC TGG GA		T GCT ATG GAC TAC TGG	DQ, O+	4	I	-
f2	L5.12.2	CT AAC TGG	AT	C TAT GCT ATG GAC TAC TGG	DQ, O-	4	I	P+
f4	L5.12.2	CTA ACT GGG A	T	T TAC TAT GCT ATG GAC TAC TGG	DQ, O-	4	II	P+
L1	L5.12.3	CT AAC TGG GAC	TGG TAC TTC GAT GTC TGG	DQ, O+	1	I	-
L2	L5.12.3	CTA ACT GGG	T	AC TAC TTT GAC TAC TGG	DQ, O-	2	II	P+
L3	L5.12.3	CTA ACT GGG		TAC TTC GAT GTC TGG	DQ, O+	1	II	-
m1	L5.12.3	CT AA		T TAC TAT GCT ATG GAC TAC TGG	DQ, O+	4	I	-
m2	L5.12.3	CTA ACT GGG A		TG GAC TAC TGG	DQ, O+	4	II	-
m3	L5.12.3	CT AAC TGG GAC		GCT ATG GAC TAC TGG	DQ, O-	4	I	-
m4	L5.12.3	CTA ACT G	C	C TAT GCT ATG GAC TAC TGG	DQ, O-	4	II	P+
w1	L5.12.3	CTA ACT GGG	GGG	TTT GAC TAC TGG	DQ, O-	2	II	+
w2	L5.12.3	CT AAC TGG GAC		TTT GAC TAC TGG	DQ, O+	2	I	-
w3	L5.12.3	CT AAC T		AC TGG	DQ, O+	2	I	-
M1	L5.12.4	CT AAC TGG GAC	TGG TAC TTC GAT GTC TGG	DQ, O+	1	I	-
M2	L5.12.4	CTA ACT GGG	CCT	TAC TGG TAC TTC GAT GTC TGG	DQ, O-	1	II	P+
n1	L5.12.4	CTA ACT GGG AC	G GGG	GCT ATG GAC TAC TGG	DQ, O-	4	II	+
n2	L5.12.4	CT AAC TGG G	CC CC	T TAC TAT GCT ATG GAC TAC TGG	DQ, O-	4	I	+
n4	L5.12.4	C TAA CTG GGA C	GT TG	T GCT ATG GAC TAC TGG	DQ, O-	4	III	P+
N1	L5.12.5	CT AAC TGG GAC	TAC TTT GAC TAC TGG	DQ, O+	2	I	-
N2	L5.12.5	C TAA CTG	T	AC TAC TTT GAC TAC TGG	DQ, O-	2	III	P+
o1	L5.12.5	CT AAC T	AC TAT GCT ATG GAC TAC TGG	DQ, O+	4	I	-
o3	L5.12.5	CTA ACT GGG AC	T GCT ATG GAC TAC TGG	DQ, O-	4	II	-
O1	L5.12.7	CT GAC TGG GAC	A	TT GAC TAC TGG	DQ, O+	2	I	P+
O2	L5.12.7	CTA ACT G	AC AT	C TTT GAC TAC TGG	DQ, O-	2	II	P+
O3	L5.12.7	CTA ACT GGG AC		C TAC TGG	DQ, O-	2	II	-
O4	L5.12.7	CT AAC TGG G	GC T	AC TAC TTT GAC TAC TGG	DQ, O-	2	I	+
p1	L5.12.7	CT AAC TGG G	C	T TAC TAT GCT ATG GAC TAC TGG	DQ, O-	4	I	P+
p2	L5.12.7	CT AAC T		AT GCT ATG GAC TAC TGG	DQ, O+	4	I	-
p3	L5.12.7	CTA ACT GGG AC		C TAT GCT ATG GAC TAC TGG	DQ, O-	4	II	-
P2	L5.12.8	CTA AC	C	GAC TAC TGG	DQ, O-	2	II	P+
r1	L5.12.8	CT AAC T		AT GCT ATG GAC TAC TGG	DQ, O+	4	I	-
R1	L5.12.9	CT AAC TGG GAC		TTT GAC TAC TGG	DQ, O+	2	I	-
R2	L5.12.9	CT AAC TGG GAC		TGG TAC TTC GAT GTC TGG	DQ, O+	1	I	-
R4	L5.12.9	CT AAC TGG GAC		TAC TTT GAC TAC TGG	DQ, O+	2	I	-
s1	L5.12.9	CT AAC TGG GAC		TAC TAT GCT ATG GAC TAC TGG	DQ, O-	4	I	-
s2	L5.12.9	CT AAC T		AC TAT GCT ATG GAC TAC TGG	DQ, O+	4	I	-
s4	L5.12.9	C TA		C TAT GCT ATG GAC TAC TGG	DQ, O+	4	III(+)	-

Tables 1 and 2

DJ sequences are shown as follows. **name, origin** indicates the name and cellular origin of the sequence **D-segment** shows the nucleotides assigned to D_H. In case of overlaps, nucleotides that could be encoded by either D_H or J_H segment are added to the D-segment. Stop codons are underlined. **N/P sequence** shows added N or P nucleotides, **J-segment** the nucleotides assigned to J_H. **D** and **J** are the names of the segments used for rearrangement. **O+** indicates overlaps between D and J. When a rearrangement in rf III is functional **(+)**, i.e. contains no stop codon, or a rearrangement in rf I or II contains a **stop** codon, this is indicated together with the rf **I+I**, **D-D**, or **2. DJ** indicate D_H to D_H fusion; **N+**, **P+**, **N/P+**, or **–** indicate the presence or absence of added nucleotides at the junction.

infrequent in fetal liver-derived pre B cells of λ5T mice, rf III joints were present in expected frequencies (~20–35%) in all other joints of all other cell populations (Fig. 1). We conclude from these findings that the proliferative expansion of pre-B cells in tissue culture does not alter the frequencies of representation of rf I and rf III within the H chain gene loci of these cells (compare Fig. 1a and b).

Similarly, the representation of rf II did not change significantly upon *in vitro* expansion of pre-B cells from fetal liver of λ5T or from bone marrow of normal and λ5T mice, although rf II suppression appeared less pronounced in $D_{FL}/D_{SP}-J_H$ joints of normal pre-B cell lines from bone marrow (Fig. 1b, 5 + 6)

Most important for the possible role of the surrogate L chain in the rf selection process are the following findings: (i) rf II *is* suppressed in fetal liver $D_{FL}/D_{SP}-J_H$ joints of pre-B cell clones and lines from normal, but not from λ5T mice upon proliferative expansion *in vitro* ($P=0.038$) (Fig.1b, 1 + 2) and (ii) rf II is *not* suppressed in $D_{Q52}-J_H$ joints of the same lines from the same mice (Fig. 1b, 3 + 4). Therefore, we conclude that proliferative expansion *in vitro* can establish suppression of rf II in cell populations which do not show such rf II suppression when they are isolated *ex vivo*. The findings also indicate that the usage of D_{Q52} (containing a stop codon) does not allow this establishment of rf II suppression *in vitro* and suggest that a $D_HJ_HC_\mu$ protein might be operative in the rf II suppression.

Table 3. D_H rf distribution in preB cells

1. FACS-sorted cells	
DSP/DFL normal fetal liver day 15 (36 sequences)	
rf I	24 (67%)
rf II	6 (17%)
rf III	6 (17%)
DSP/DFL λ5T fetal liver day 13 (25 sequences)	
rf I	15 (60%)
rf II	6 (24%)
rf III	4 (16%)
DSP/DFL normal bone marrow cells 3 weeks (58 sequences)	
rf I	23 (40%)
rf II	7 (12%)
rf III	28 (48%)
DSP/DFL λ5T bone marrow 14 weeks (31 sequences)	
rf I	11 (35%)
rf II	13 (42%)
rf III	7 (23%)
2. Cells from pre-B cell cultures	
DSP/DFL normal fetal liver (54 sequences)	
rf I	38 (70%)
rf II	5 (9%)
rf III	11 (20%)
DSP/DFL λ5T fetal liver (38 sequences)	
rf I	20 (53%)
rf II	11 (29%)
rf III	7 (18%)
DSP/DFL normal bone marrow etc. (34 sequences)	
rf I	15 (44%)
rf II	8 (24%)
rf III	11 (32%)
DSP/DFL λ5T bone marrow (11 sequences)	
rf I	3 (27%)
rf II	5 (45%)
rf III	3 (27%)
DQ52 normal fetal liver (55 sequences)	
rf I	26 (47%)
rf II	16 (29%)
rf II	13 (24%)
DQ52 λ5T fetal liver (43 sequences)	
rf I	25 (58%)
rf II	15 (35%)
rf III	3 (7%)

All results taken together imply that a membrane-bound Ig-like complex of truncated $D_HJ_HC_\mu$ protein and surrogate L chain suppresses the representation of H chain gene loci which are D_HJ_H rearranged in rf II.

Discussion

We found that N sequences in D_HJ_H joints are very frequent within precursor B cells from bone marrow, but very infrequent within similar cells from fetal liver. This confirms findings of other laboratories (9,15,16,21,22). Furthermore, it was found that H chain gene loci which are D_HJ_H rearranged in rf II (8) are suppressed in pre-B cells of normal mice (3,27) We have concentrated our analyses on pre-B cells which are in the process of, or have completed D_H to J_H rearrangements but which have not yet begun V_H to D_HJ_H rearrangements. These cells, which we call pre-B I cells (4), express CD45R, CD43, and c-*kit*, are clonable and will proliferate for long periods of time *in vitro* on stromal cells in the presence of IL-7 (31). When transplanted into SCID hosts, they repopulate B lineage compartments for long periods of time (31). Our results suggest, as do also the results of others (3,6,22,27,33), that at least part of the suppression of rf II is established at the early pre-B I stage of B cell development, i.e. during and directly after D_HJ_H rearrangement. It does not rule out that later stages of B cell development continue to favor rf I-rearranged H chain loci over those rearranged in rf II or III.

The two different oligonucleotide primers (D_{FL}/D_{SP} and D_{Q52}) used in the PCR reaction bind upstream of 14 out of 15 functional D segments. The frequencies of representation of D_H and J_H segments in the analyzed sequences do not necessarily reflect their representation in the pre-B cells which we have analyzed, since different primers were used in the PCR reactions for D_{Q52} and D_{FL}/D_{SP}, since it is unknown whether the 5′ regions of all D_{FL} and D_{SP} segments act with equal efficiency as acceptor sites for the primer used, and since the different lengths of $D-J_1$, $D-J_2$, $D-J_3$, and $D-J_4$ joints are likely to influence the efficiency of both PCR and cloning. In summary, however, rf II suppression is observed in D_{FL}- and D_{SP}-containing H chain gene loci joined to either J_H1, 2, 3, or 4.

The situations in which suppression of rf II is *not* observed shed light on the mechanism by which this suppression is mediated in pre-B cells.

(i) $D_{Q52}-J_H$ joints in normal mice. Since $D_{Q52}-J_H$ joints cannot be translated into a $D_HJ_HC_\mu$ protein due to a stop codon, this result suggests that the $D_HJ_HC_\mu$ protein is involved in suppression.

(ii) The non-functional D_HJ_H rearranged alleles of splenic B cells from μMT mice (27). This suggests that the $D_HJ_HC_\mu$ protein has to be inserted into (surface) membranes.

(iii) Pre-B cells from fetal liver of normal mice *ex vivo*. This suggests that early fetal liver cells have not had sufficient time to proliferate as D_HJ_H rearranged cells, and that they rearrange D_H to J_H preferentially in rf I due to short sequence homologies in D and J segments (9). The frequencies of rf I versus rf II versus rf III in early fetal liver cells resemble those of similar joints generated in fetal liver-derived pre-B cells with an extrachromosomal substrate for such rearrangements (18).

By contrast, pre-B cell clones from early fetal liver of normal mice, expanded *in vitro* by proliferation for ~20 divisions, show rf II suppression. This suggests that pre-B cells establish rf II suppression by proliferation and that fetal liver derived cells can do so *in vitro*.

(iv) Pre-B cells from λ5T mice. This suggests that the surrogate L chain $V_{pre\text{-}B}/\lambda5$ may form a disulfide-bonded, Ig-like complex with $D_HJ_HC_\mu$ protein and insert this complex into the (surface) membranes in normal mice.

Ligands fitting the complementarity-determining regions (CDR)-like structures (CDRs of variable regions of Ig H and L chains) of this complex (13) , possibly provided by stromal cells, may signal these pre-B cells to stop proliferation while pre-B cells not expressing this complex on the surface (i.e. with H chain gene loci D_HJ_H rearranged in rf I or III or pre-B cells of μMT or λ5T mice) continue to expand and thus overgrow those with rf II.

(v) Pre-B cells from bone marrow of normal mice, expanded by proliferation *in vitro*. These cells show some, but much less pronounced suppression of rf II, especially when compared with pre-B cells from bone marrow of normal mice analyzed *ex vivo* and when compared with pre-B cells from fetal liver of normal mice expanded *in vitro*. This result needs to be analyzed in greater detail

with many more sequences to see how N-region insertion could influence the structures of D_HJ_H joints. It might be that this N-region insertion leads to insertion of amino acids in the D_H to J_H joining site, i.e. within CDR3 which might interfere with the signalling function of the $D_HJ_HC_\mu$—surrogate L chain complex leading to rf II suppression in pre-B cells.

In principle our results, as well as those of other investigations (3,9,15), cannot formally rule out the alternative possibility that pre-B cells with rf I and III rearranged H chain loci are selectively expanded while those with rf II are not, but are also not inhibited or deleted. While the suppression of rf II can be explained by the expression and suppressive function of a membrane-bound $D_HJ_HC_\mu$ – surrogate L chain complex on pre-B cells we have at present no good mechanistic explanation for a possible expansion of pre-B cells with rfI and rfIII D_HJ_H rearranged H chain loci.

If $D_HJ_HC_\mu$ protein is indeed involved in suppression of pre-B I cells with rf II D_HJ_H rearranged H chain loci, then at least 15 – 20% of all pre-B I cells in fetal liver and bone marrow of λ5T mice should express $D_HJ_HC_\mu$ protein. We have begun to analyze these pre-B cells for $D_HJ_HC_\mu$ protein expression, but the levels of expression appear to be so low that immunofluorescence analyses and western blotting of protein have not yet detected $D_HJ_HC_\mu$ protein with certainty.

Although rearrangement in three rfs can in principle lead to a more diverse repertoire of antigen-binding variable regions, it is surprising that the repertoire of B cells appears to be biased towards only one of these three rfs, i.e. rf I. First, sequence homologies in D and J favor rearrangements in rf I at the molecular level, stop codons in 50% of the D segments disfavor expression of H chains in rf III Furthermore, the results of this paper extend previous findings that rf II is suppressed and that this may be achieved by a $D_HJ_HC_\mu$ – surrogate L chain complex. In fetal repertoires the lack of N region insertion further restricts the variability in CDR III. It remains to be a matter of debate (34) why the immune system would in such a way suppress its potential for generating variable regions of antibody molecules. It has been proposed that these invariant receptors could recognize self antigens in their environment and that this recognition may be important for the original expansion of precursor B and B cells to fill up the B cell compartment (35,36), reviewed and discussed in (28).

Acknowledgements

The able technical assistance of Andrea Groenewegen and Marc Dessing is gratefully acknowledged We thank Drs Jan Andersson, Klaus Karjalainen, and Jim Kaufman for critical reading of the manuscript. The Basel Institute for Immunology was founded and is supported by F Hoffmann-LaRoche Ltd, Basel, Switzerland

Abbreviations

CDR	complementarity determining region
H	heavy
L	light
λ5T mice	homozygous λ5 knockout mice
μMT mice	μH membrane exon knockout mice
N	non-templated
P	palindrome
PCR	polymerase chain reaction
rf	reading frame
TdT	terminal deoxynucleotidyl transferase

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Note added in proof

We have recently sequenced $D_{Q52} - J_H$ joints of sorted cells from bone marrow of normal and λ5T mice. They show the expected rf distribution normal mice (28 sequences): rf I, 12 (43%); rf II, 7 (25%); rf III, 9 (32%); λ5T mice (31 sequences): rf I, 11 (35%); rf II, 11 (35%); rf III, 9 (25%).